EVOLUTION IN THE Genus Drosophila

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PREFACE

Evolution is the most comprehensive, fascinating, and generalized theory of living organisms. The earlier studies utilized comparative morphology, embryology, paleontology, and even certain aspects of natural history to develop this theory. More recently studies in genetics have furnished major contributions. In this volume we have attempted to give the pertinent details of evolution as it has been revealed in investigations on one of the most successful insect genera, the genus Drosophila. We have endeavored to present and evaluate the more important data which are available from numerous sources. Because of the limitations imposed by time and space in such a book, we have had to omit reference to certain contributions. We hope this treatise presents a full analysis and synthesis of the many types of information on Drosophila which have been accumulated during the present century.

We are grateful to many of our students for permission to use their unpublished data, notably Miss Mary L. Alexander, Mr. Calvin L. Ward, Mr. T. C. Hsu, and Miss Mary Warters. We are also indebted to Dr. Herman T. Spieth for allowing us to include some of his unpublished data on the habitats of certain species of Drosophila. We express our thanks to Dr. R. P. Wagner for reading parts of the manuscript, and to Dr. M. R. Wheeler for reading all of the manuscript and for his suggestions on the phylogeny of Drosophila. The Blakiston Company kindly allowed us to use Plate III from Folsom and Wardle's *Entomology*.

We wish also to thank The University of Texas and the Rockefeller Foundation for their financial support of our investigations on the genetics of Drosophila during the past quarter of a century.

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CONTENTS

EVOLUTION IN THE
Genus Drosophila

1 INTRODUCTION: THE PROBLEM

Organic evolution is one of the more interesting and complex aspects of the evolution of the universe. Life is an evolved complexity, with inherited continuity of both similarities and dissimilarities—a system characterized not only by stability, but also by the origin of unpredictable novelty. The fascinating nature of evolution is illustrated by the fact that one of its products, man, is able to appreciate and even to understand some of its mechanisms. Delbrück (1949), examining biology as a physicist, points out that all organisms are the product of their more than billion-year evolutionary history and that understanding the fundamental characteristics of organisms depends, among other things, upon a realization of this historic background. We desire to contribute to this fundamental type of knowledge by an analysis of evolution in the genus Drosophila.

Although many men had believed in and spoken of evolution in living organisms, it remained for Darwin (1859) to develop the theory of evolution through natural selection and to bring together sufficient evidence to justify and support it. As *The Origin of Species* was published six years before Mendel (1865) presented the first unequivocal evidence on the mechanisms of inheritance, Darwin could not give genetic evidence for the inheritance of unit characters which might determine survival or death. That these details were added later does not detract from his genius and scientific acumen, for his statement on the main critical essentials was correct.

Organic evolution is not a repeatable experiment, at least not in detail. Were conditions somewhere else or at some other time suitable for organic evolution, we would expect it to occur. Perhaps many or most of the same physical and functional systems might take place, along with others that have occurred or unlike the systems we know, but it is highly improbable that the same identical complexes of systems (or even more improbable the same genotypes) would occur.

The probability that there would again take place the same grouping of some million species of organisms that are now found on the earth very closely approaches zero. Our knowledge of the evolving system must come from other types of data.

Many disciplines contribute to our knowledge of evolution. Comparative anatomy tells us the types of structures present in adult living forms, the positional relation between them, and the comparative relations between different living forms. Embryology describes the development of form and structure. Paleontology gives a partial picture of the comparative anatomy of forms that lived in the past and, through time sequences, forms that changed with time, leading up (with a high degree of probability) to present forms. In addition, we can study comparative physiology, biochemistry, and biophysics to determine similarities and differences in activity and function; these disciplines show what has evolved. We can study the cytology and genetics of organisms to determine to what extent each of the characteristics of an organism is determined or modified by environment or inheritance. We must also find out how inheritance is modified to produce a different system in an organism. Further, if evolution has and is occurring, we must show that the system of inheritance will account for both the stability and the plasticity of organisms, and establish a genetic system which makes both evolution and its systems statistically predicable. As with all scientific theories, the validity of an evolutionary theory and the mechanisms postulated to explain it are tested in terms of their effectiveness in predicting what has or will happen. We have been especially concerned with the genetic mechanisms involved in these systems and how they contribute to or determine evolutionary divergence. At the onset we are handicapped by a complete absence of paleontological data, but we have used the other disciplines where applicable.

There are a number of recent publications that add details to the theory of evolution proposed by Darwin. Fisher (1930) develops particularly the mathematical relations between the genetic system and natural selection; Haldane (1932) discusses general factors including mathematical systems; Wright (1931, 1932, 1940, 1942, 1945, 1948, 1949), in a series of articles, developed his concepts of population genetics; Dobzhansky (1937b) gives a general account of genetics in relation to evolution, chiefly in animals; Goldschmidt (1940) presents his very different concept of evolution; Stebbins (1950) presents a

general picture of evolution in plants; Simpson (1944, 1949) analyzes certain essential features of evolution from paleontological evidence; Huxley (1942) reviews many facts and examples from a great variety of forms; Mayr (1942) discusses systematics, ecology and distribution; and Darlington (1932, 1939) and White (1945) analyze chromosome evolution in plants and animals. With all of these publications available for general and comparative analysis of this system, we have restricted ourselves to the genus Drosophila, except for a few comparisons to show that the systems we are discussing are general evolutionary systems rather than specialized ones pertaining only to these organisms.

In our development of evolution, the genus Drosophila has been divided into subgenera and then species, which are often discussed as species groups. In some cases we have established subspecies and often have used strains or stocks of the several species to illustrate variation. According to our classification, subspecies are two or more genetically and usually phenotypically distinguishable populations of a species that have separate distributions but which cross freely in overlap zones or when brought into contact. These zones are characterized by a free or nearly free exchange of genes between the two populations, with a gradient of genes exchanged between the subspecies highest at the contact zone and decreasing away from it, that is, introgressive hybridization, as discussed by Anderson (1949).

The definition or definitions of a species are more difficult to formulate satisfactorily. Although we are concerned only with a definition applicable to sexual forms in Drosophila, we must in practice use two definitions. The first is that of Darwin (1859): "In determining whether a form should be ranked as a species or a variety, the opinion of naturalists having sound judgment and wide experience seems the only guide to follow." This is the morphological definition and is the one advocated by Sturtevant (1942). If desirable, we might substitute "systematist" for "naturalist," but it seems to us that the term "naturalist," implying more than a knowledge of anatomical taxonomy, has considerable merit, as indeed the modern systematics implies. This definition is necessary for asexual forms, for even in bacterial taxonomy classification depends on a description of the physical and physiological characteristics of the organism. It must apply to all paleontological classification *per se,* substituting the word "paleontologist" for "naturalist."

It is only in living sexual forms that we can test a biological definition of "species," although obviously evolution is carried out on this biological level and our analyses are mainly concerned with it. Any definition will appear arbitrary in some application. The following is a useful definition of "species" which in substance has been used by others: In sexual forms, a species consists of the members of a population or group of populations which can exchange genes freely with each other, but which can cross to members of no other form (or population) sufficiently to lose their separate genetic identity. This separation should not be stated in terms of a single factor such as the mutation rates of the two forms being compared, although it is primarily concerned with their independent evolution. This does not imply that hybridization cannot occur or contribute to the genetic evolution of the species, but that it cannot determine it nor does it imply that hybrids cannot produce a new species, a fairly frequent occurrence in the origin of polyploids of hybrid origin in plants. Furthermore, two populations may not be genetically isolated into species, but may be so geographically isolated that they evolve separately. On the basis of this definition, these last are separate species only after they have become sufficiently reproductively isolated. This does not imply that separate species are necessarily different ecologically, for they may not be able to survive together over more than a mixed overlap area, e.g., *Drosophila pseudoobscura* and *Drosophila affinis*. They do not need to be phenotypically distinct but may be sibling species, to adopt the general term used by Mayr (1942). In fact, this opinion of the species unit is necessary to allow us to separate phenotypically similar but genetically isolated forms into separate species through genetic tests. The species will not remain the same throughout time, but will either evolve into other species or die.

This definition demands that separate species in sexual forms have genetically determined isolating mechanisms which prevent their crossing with other forms enough to lose their identity. There are obviously borderline cases if evolution is occurring. We can imagine the following, which in fact is known to occur. There may be two large populations occupying adjacent but ecologically different areas which are in contact and between which cross-mating can occur freely. The genes transferred from one to the other of these forms are insignificantly different, except for those which determine their ecological adaptations. These dilute the populations locally, but are

eliminated within a short distance of the contact zone. We do not consider that these are separate species, but many competent investigators do, especially in plant material.

The main body of taxonomy and classification has been published in other volumes (Patterson, *et al.,* University of Texas Publications 4213, 4313, 4445, 4920). Many of the records of species are but fragmentary descriptions and useless for most of our analyses. We are presenting such material on species groups in the subgenera Sophophora and Drosophila as is suitable for cytological and genetic studies. To conserve space and save repetition, the genus name *Drosophila* has been generally omitted. We have been forced to deal somewhat arbitrarily with borderline cases, but have attempted to give the evidence, to enable the reader holding a different view to make his own decision. As this is an attempt to describe and analyze our present state of knowledge of evolution in this genus, we have included much data to validate our discussions.

In our study we shall summarize and analyze the distribution of the known Drosophila in their systematic groupings into subgenera, species, species groups, subspecies, and populations. We shall discuss briefly the meager information on the ecology of the genus. We shall review the gene variability discovered in populations of different species, the types of differences in phenotype between species, and the variations in chromosome patterns established and in existence as alternatives. The chromosome phylogeny of certain groups, such as the restricted *Drosophila pseudoobscura* subdivision of the obscura group and that of the virilis group, are particularly illuminating and instructive. We shall develop the concept of isolating mechanisms at some length, as Drosophila is particularly satisfactory in demonstrating these mechanisms. Since more species hybrids of Drosophila have been studied than of any other animal genus, we shall discuss these hybrids at some length, as a measure of the genetic differences between related forms. We believe that such an analysis of evolution in Drosophila can give us a much better understanding of the general problems of evolution.

2 THE GENUS DROSOPHILA

INTRODUCTION

It is not our purpose in this chapter to give an extended account of the taxonomy of the genus Drosophila, but some knowledge of its systematics is deemed necessary for an understanding of the problem of speciation in this group of flies. This genus was established in 1823 by Fallén and belongs to the family Drosophilidae. Since 1823 it has undergone several changes and revisions, of which the most recent have been made by Sturtevant (1939, 1942), Patterson and Mainland (1944), and Wheeler (1949b).

With the exception of the extreme arctic regions, Drosophila is found all over the world and has been reported from each of the six zoogeographical regions into which the world usually has been divided. In his monograph, *The North American Species of Drosophila,* Sturtevant has given the following numbers of species for his six regions: Nearctic, 28; Neotropical, 41; Palaearctic, 43; Ethiopian, 22; Oriental, 51; Polynesian, 44. He used the name Polynesian principally for the Hawaiian Islands, and included Australia and New Zealand in the Oriental region. These three areas are usually placed in the Australian region.

The number of species reported for each of the six regions has been greatly enlarged since 1921, owing mainly to an increased activity in collecting during the past two decades. Patterson and Wheeler (1949) have listed the number of species known to occur in these regions, but since then the numbers have been modified by the elimination of eleven species and by the addition of eighteen forms to the genus Drosophila, of which fourteen were recently described. These changes, which were in part suggested by Dr. M. R. Wheeler, are indicated in Table 1. Of the eleven species removed from the 1949 list, two are synonyms (*immatura, paulista*) and the remaining nine have been

assigned to other genera. Of the fourteen recently described species, thirteen have been recorded from South America and one from Russia.

The number of species now known for each of the six regions is as follows: (1) Nearctic, 141; (2) Neotropical, 200; (3) Palaearctic, 89; (4) Ethiopian, 33; (5) Oriental, 128; (6) Australian, 114. The endemic species for these regions will be given in the next chapter.

THE SUBGENERA OF DROSOPHILA

With the rapidly growing lists of newly described species during the past two decades, the need for a revision of the genus became very urgent. In 1942 Professor A. H. Sturtevant undertook to bring up to date the work on the classification of Drosophila. In his paper he gives a full discussion of the various characters which are commonly studied in the genus for use in determining and describing species. It will not be necessary to repeat in this chapter his account of these

TABLE 1

SPECIES REMOVED FROM THE GENUS

abicornis de Meijere 1915
biradiata Duda 1923
cinerella Fallén 1823
congesta Zetterstedt 1847
immatura Walker 1849
notabilis Lamb 1914
paulista Dobzhansky & Pavan 1943a
pictipes de Meijere 1911
preciosa de Meijere 1911
remota Walker 1849
spurca Zetterstedt 1847

SPECIES ADDED TO THE GENUS[1]

addisoni Pavan 1950
araicás Pavan & Nacrur 1950
araúna Pavan & Nacrur 1950
camargoi Dobzhansky & Pavan 1950
campestris Burla 1950
gracilis Duda 1924a
hirsuta Duda 1926
hypopygialis—2 Duda 1924a
imeretensis Sokolov 1948
mojú Pavan 1950
neoelliptica Pavan & Magalhaes 1950
neosaltans Pavan & Magalhaes 1950
obscuricornis—2 de Meijere 1911
pará Pavan and Burla 1950
paranaensis de Barros 1950
paulistorum Dobzhansky & Pavan 1949
tropicalis Burla & da Cunha 1949
tuchaua Pavan 1950

[1] The following new species although referred to at several places in the text, were described too late to be included in the tabulations and analyses: *D. flavomontana* Patterson 1952b; *D. borealis* Patterson 1952b; *D. euronotus* Patterson and Ward 1952; *D. wheeleri* Patterson and Alexander 1952.

characters; instead we shall give for each subgenus and species group its main diagnostic characters, together with illustrations of the reproductive systems, puparium, and egg of representatives of the more important classes. These illustrations are from a previously published article (Patterson, 1943).

Sturtevant divided the genus into six subgenera, namely, **Hirtodrosophila, Pholadoris, Dorsilopha, Phloridosa, Sophophora,** and **Drosophila;** and upon his advice Patterson and Mainland (1944) added the subgenus **Siphlodora.** Still more recently, Wheeler (1949b) established the subgenus **Sordophila** to include a single species which could not be fitted into any of the other seven subgenera. Some of the larger subgenera have been divided into *species groups,* and in turn some of these have been broken up into subgroups. The question of geographical distribution of the different species of Drosophila is considered in the next chapter and therefore, except for a few brief references, will not be considered here.

Subgenus **Hirtodrosophila,** Duda

Members of this subgenus have a long third antennal segment, usually a short narrow carina, and arista usually with but a single branch below in addition to the terminal fork. In all species examined, the ventral receptacle was in the form of loops folded flat against the ventral surface of the vagina. The eggs have four thick filaments (Figure 1). In a recent article, Frota-Pessoa (1945) gives a revision, with bibliography, of this subgenus, listing a total of twenty-six species (Table 2).

TABLE 2

Subgenus Hirtodrosophila

alabamensis Sturtevant 1918
astioidea Duda 1923
chagrinensis Stalker & Spencer 1939
cinerea Patterson & Wheeler 1942
dentata Duda 1924a
duncani Sturtevant 1918
flavohalterata Duda 1925a
fuscohalterata Duda 1925a
glabrifrons Duda 1925a
grisea Patterson & Wheeler 1942
hirticornis de Meijere 1914
innocua Malloch 1934b
jordanensis Frota-Pessoa 1945
latifrontata Frota-Pessoa 1945
longala Patterson & Wheeler 1942
longecrinita Duda 1924a
lundstroemi Duda 1938
narinosa Frota-Pessoa 1945
nigrohalterata Duda 1925a
ochracella Hendel 1936
oldenbergi Duda 1924a
orbospiracula Patterson & Wheeler 1942
prognatha Sturtevant 1916
seminigra Duda 1923
trapezina Duda 1923
unicolor—2 Malloch 1934b

So far as known, all of these species are fungus-feeders, and only three of them have been successfully bred on laboratory food. It has

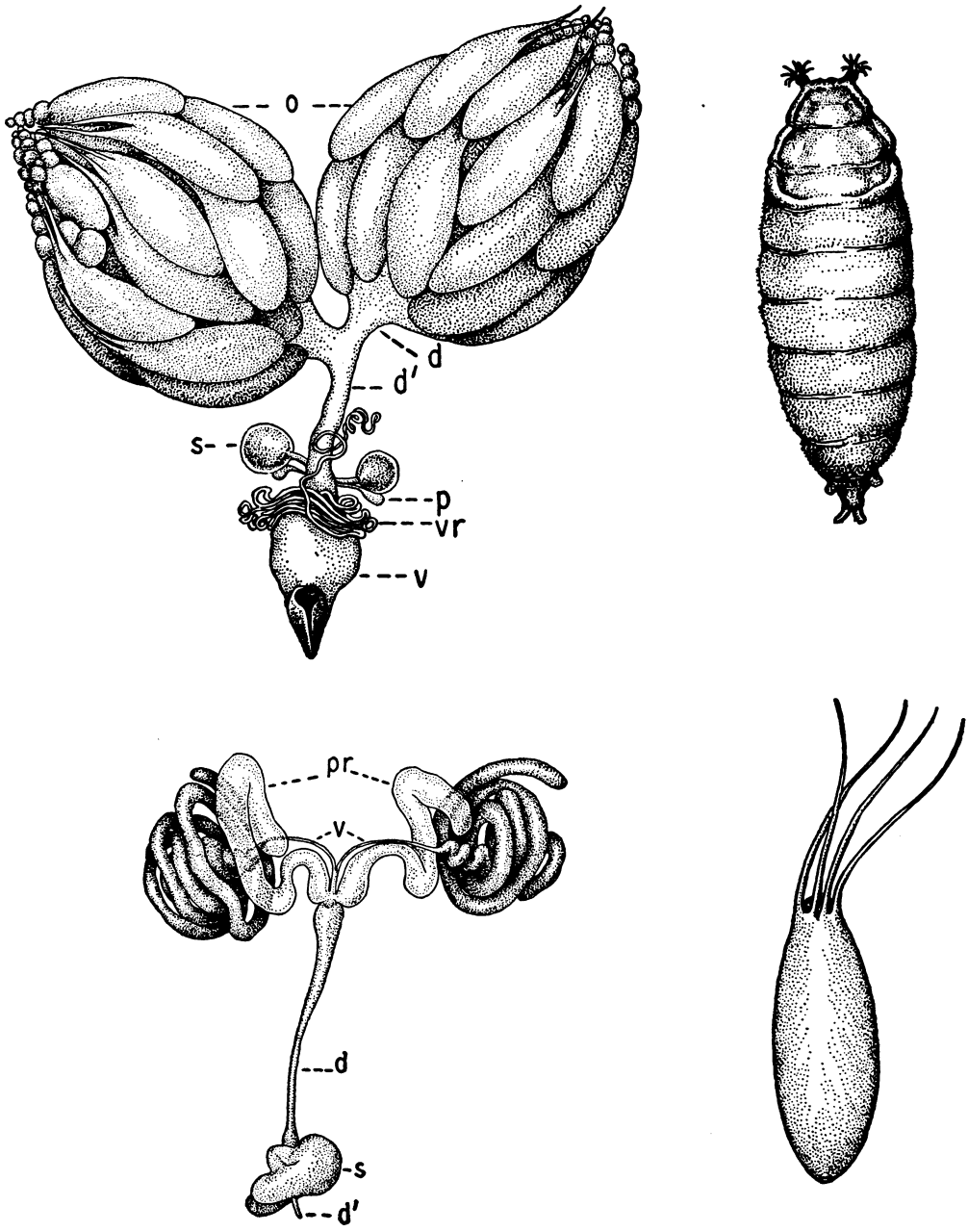

Fig. 1 Female and male reproductive systems, puparium, and egg of *D. orbospiracula.* **Note.** In this and a majority of the other text figures the key to labels on the drawings is a follows: *Female reproductive system,* o, ovaries; d, branched oviducts; d', azygous oviduct; p, parovaria; s, spermatheca; v, vagina; vr, ventral receptacle. *Male reproductive system,* t, testes; v, vasa deferentia; pr, paragonia; d, anterior ejaculatory duct; d', posterior ejaculatory duct; s, sperm pump; ed, sperm pump diverticula. (Rosenblad, 1941.)

been possible to keep laboratory stocks of *duncani* for a considerable period of time, and stocks of *orbospiracula* and *longala* were maintained in the laboratory for a few generations. Future explorations, especially in the tropics, should result in the discovery of many undescribed forms. If the difficulty of breeding them on laboratory food can be overcome, members of this subgenus should furnish interesting material for genetic and speciation studies.

Subgenus **Pholadoris**, Sturtevant

This subgenus includes black and yellowish species which have prescutellar bristles, a V-shaped shining bristle-bearing area on the front, usually six to eight curved egg filaments, a short curved ventral receptacle, noncoiled saclike testes, and skipping larvae (Figure 2).

In establishing this subgenus, Sturtevant (1942) included *D. victoria* Sturtevant and *D. coracina* Kikkawa and Peng 1938. He mentions, however, that there are a number of undescribed forms which belong to this subgenus. Wheeler (1949a) has revised the subgenus and has added four other species: *D. nitens* Buzzati-Traverso 1943, *D. mirim* Dobzhansky and Pavan 1943a, *D. lebanonensis* Wheeler 1949a, and *D. baeomyia* Wheeler 1949a. He further suggests that three other forms probably belong to the subgenus; these are *D. anuda* Curran 1936, *D. bryani* Malloch 1934b, and *D. excepta* Malloch 1934b. Not too much is known about the habits of members of this subgenus, although it has been shown that *victoria* breeds in the sap of bleeding cottonwood trees and is not too difficult to maintain on laboratory food.

Subgenus **Dorsilopha**, Sturtevant

This subgenus includes yellowish species with longitudinally striped mesonotum, preapical bristles not evident on second and third tibiae, larvae with dorsal processes, four egg filaments, and a ventral receptacle of folded loops lying against the ventral surface of the vagina (Figure 3).

The only known species is *D. busckii* Coquillett 1901. It is widely distributed and represents a cosmopolitan species. This species breeds on many different kinds of food, such as bread and milk, formalized

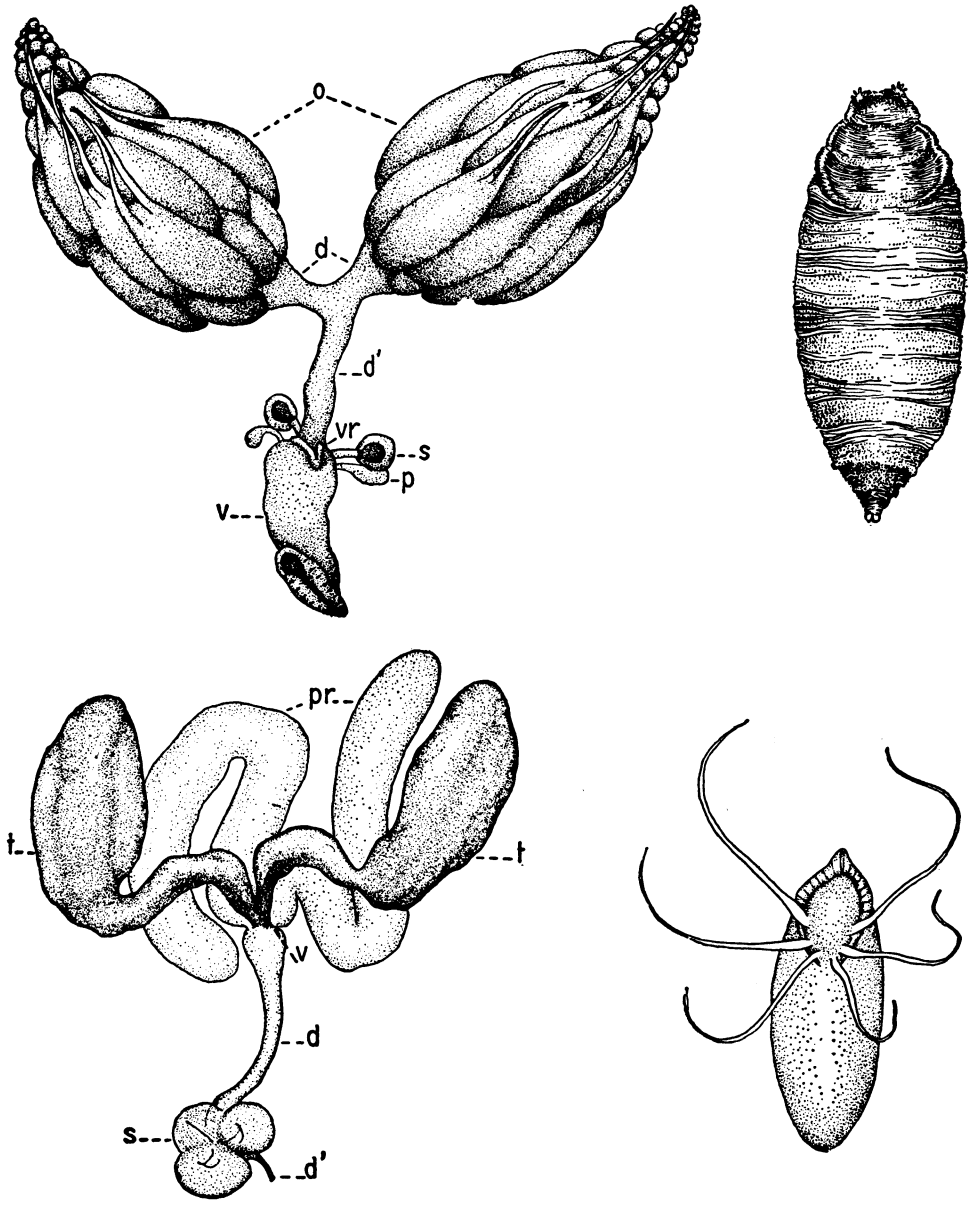

Fig. 2 Female and male reproductive systems, puparium, and egg of *D. victoria.*

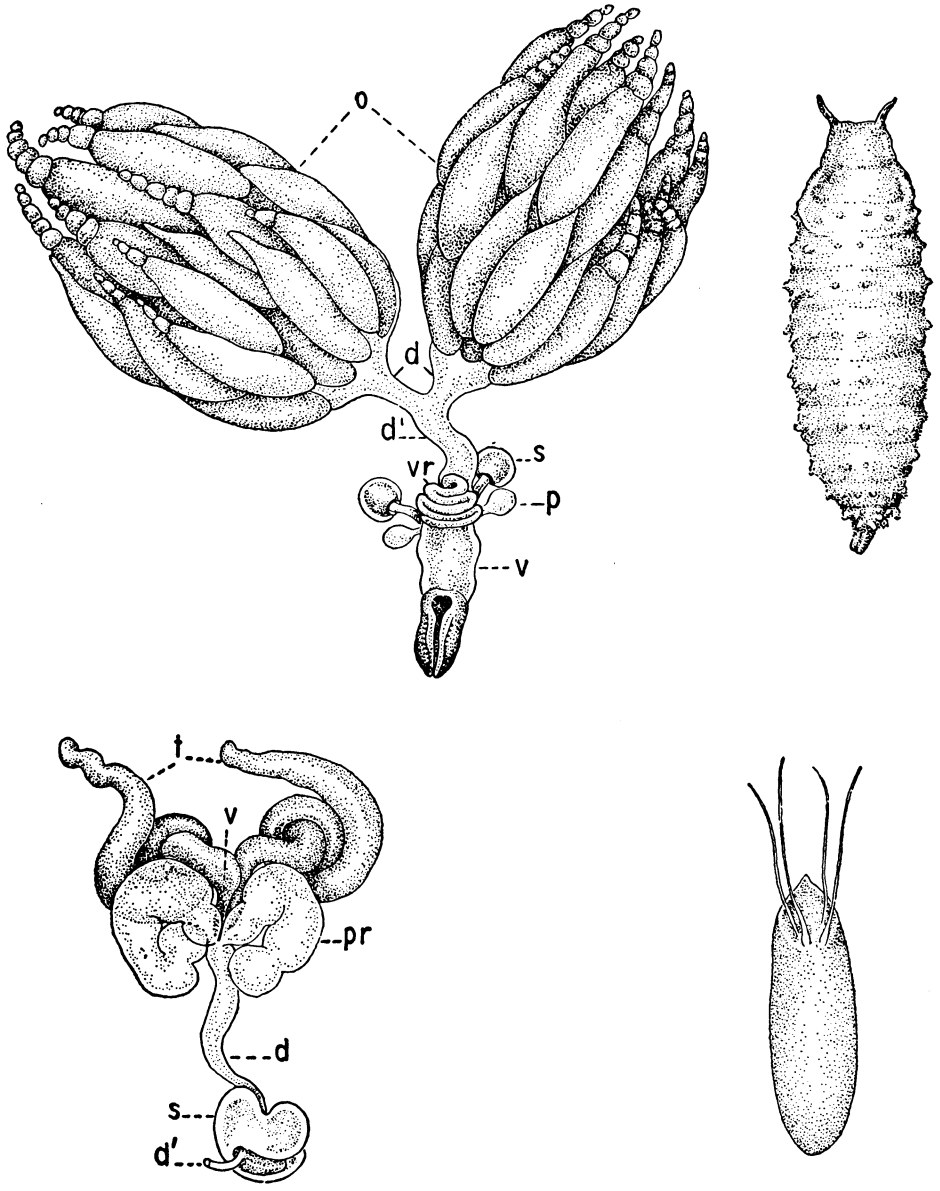

Fig. 3 Female and male reproductive systems, puparium, and egg of *D. busckii.*

meats, rotten eggs, fish and potato, and several other kinds of vegetable matter. It breeds well on moist bran in the laboratory.

Subgenus **Phloridosa,** Sturtevant

This subgenus includes shining black or brown species with short bristles and hairs on branches of arista, long coiled testes, long ventral receptacle with minor coils, eggs without filaments and the larvae feed on flowers.

The type species of this subgenus is *D. floricola* Sturtevant 1942. Other members of the subgenus are, *D. alfari* Sturtevant 1921b, *D. lutzii* Sturtevant 1916, *D. tristani* Sturtevant 1921b, and perhaps *D. mauiensis* Grimshaw 1901. Sturtevant collected *floricola* in the southern part of California, and we have taken it in eight states in Mexico. The second, third, and fourth species occur mainly in the tropics, and the fifth is from the Hawaiian Islands. The species of this subgenus live in flowers, and their larvae apparently feed on pollen. The species *floricola* was collected by Sturtevant on *Datura, Hibiscus,* melon, morning-glory and calla lily. This species is difficult to breed on laboratory food, and only a very few specimens have been so reared.

Subgenus **Siphlodora,** Patterson and Mainland

This subgenus includes yellowish brown species with prescutellar bristles present, arista with eight branches, posterior cross veins distinctly sinuate, testes coiled, ventral receptacle loosely coiled or looped and two-filament eggs (Figure 4).

The type species of this subgenus is *D. sigmoides* Loew 1872. Other members are *D. flexa* Loew 1865 and *D. subsigmoides* Patterson and Mainland 1944. The distribution of these three species is as follows: *flexa* in Cuba; *sigmoides* in Alabama, Illinois, New York, Tennessee, Maryland, and Texas; *subsigmoides* in the states of San Luis Potosí, Veracruz, Puebla, and the Distrito Federal in Mexico. These species are usually obtained by sweeping grass or weeds, and none of them has ever been bred on laboratory food.

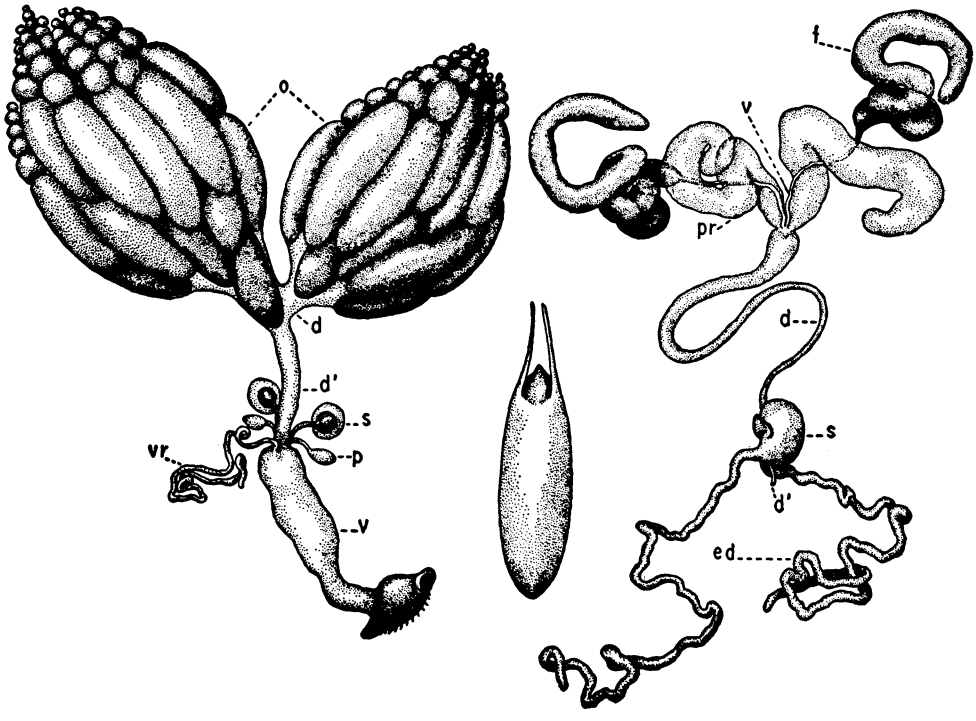

Fig. 4 Female and male reproductive systems, and egg of *D. sigmoides.*

Subgenus **Sordophila,** Wheeler

The subgenus includes yellowish species with two-filament eggs expanded at tip, ventral receptacle long and tightly coiled, spermathecae thin-walled without sclerotized centers, testes composed of a moderate number of coils, acrostichal hairs in six rows and with no prescutellar bristles present.

The only known species is *D. acanthoptera* Wheeler 1949b. A stock of this species was developed from two females and a male collected about sixty miles south of Oaxaca, Mexico. It belongs to the genus Drosophila, and within the genus it seems to be nearest to the subgenus Sophophora.

Subgenus **Sophophora,** Sturtevant

Members of this subgenus have eggs with two blunt filaments, ventral receptacle not coiled or kinky, anterior spiracle short, not over one-fifth length of puparium, second to fifth abdominal tergites

with posterior dark bands not broken in middorsal line, and cheeks relatively narrow. Sturtevant recognized four distinct species groups, and three others have recently been added.

THE SPECIES GROUPS

saltans group

These are dark species with long fine ventral receptacle, long spiral testes, one or two heavily sclerotized areas on fifth abdominal tergite of female, no sex combs, two egg filaments much expanded apically, sterno-index .3 to .4, and anterior scutellars divergent. These species are mostly found in tropical America.

Sturtevant divides the several species into two subgroups: (a) forms with grayish markings on the mesonotum, including *D. earlei* Sturtevant 1916, *D. saltans* Sturtevant 1916, *D. prosaltans* Duda 1925b, *D. rectangularis* Sturtevant 1942, and *D. sturtevanti* Duda 1925b; (b) forms without mesonotal pattern, including *D. cordata* Sturtevant 1942, *D. elliptica* Sturtevant 1942, and *D. emarginata* Sturtevant 1942. Since Sturtevant made his analysis in 1942, Pavan and Magalhaes (1950) have described two additional species under the names *D. neosaltans* and *D. neoelliptica.*

The known distributional ranges of these ten species lie mainly in the tropics of continental America, although both *earlei* and *saltans* have also been recorded from Cuba. The saltans group includes a fine series of species which offers unusual opportunities for genetic and speciation studies.

willistoni group

These are yellowish species without true sex combs, no opaque areas on tergites, long fine ventral receptacle, moderately long spiral testes, sterno-index .3, two egg filaments much expanded at apices, and anterior scutellar bristles divergent. They occur chiefly in tropical America.

This group originally included but two known species, *D. willistoni* Sturtevant 1916 and *D. nebulosa* Sturtevant 1916. Since 1921 seven additional species have been described. In 1943 Dobzhansky and Pavan referred *D. fumipennis* Duda 1925a to this group and described *D. capricorni* as new, and also *D. paulista,* which, however, was later shown (Burla *et al.,* 1949) to be a synonym of *D. willistoni.*

In 1944 Patterson and Mainland added a new member from the state of Veracruz in Mexico, under the name *D. sucinea.* In 1946b Dobzhansky described as new *D. equinoxialis,* from the state of Amazonas, Brazil. In 1947 Pavan and da Cunha described *D. bocainensis* from Brazil and placed it in the willistoni group. In 1949 Dobzhansky and Pavan added *D. paulistorum* from Brazil. Finally, Burla and da Cunha (1949) described as new *D. tropicalis* and recorded it from Brazil and Bolivia.

The members of this group seem to be restricted to the Nearctic and Neotropical regions, mainly in the latter. The only apparent exception is a report by Adams (1905) that *willistoni* had been taken in Rhodesia, South Africa; but Sturtevant (1921b) has suggested that, in view of the large number of known similar species, this record from the Ethiopian region must be regarded as doubtful.

melanogaster group

This group includes yellowish species with rather long ventral receptacle, sex combs, medium-long spiral testes, no opaque areas on tergites, sterno-index .5 to .6, and anterior scutellars convergent. The larvae do not skip. These species occur mainly in the tropical and subtropical regions of the Old World (Figure 5).

The group includes a total of eighteen species which could be arranged into several subgroups (Table 3). Three of the forms (*ananassae, melanogaster, simulans*) represent cosmopolitan species, and a fourth member (*montium*) has been reported from four of the six regions. Several of the members of this group have been used for the study of genetics and related topics. This is especially true for

TABLE 3

melanogaster group

ananassae Doleschall 1858	*montium* de Meijere 1916
auraria Peng 1937	*nipponica* Kikkawa & Peng 1938
biarmipes Malloch 1924a	*pulchrella* Tan, Hsu, & Sheng 1949
bipectinata Duda 1923	*rufa* Kikkawa and Peng 1938
ficusphila Kikkawa & Peng 1938	*serrata* Malloch 1927
illata Walker 1860	*simulans* Sturtevant 1919
lutea Kikkawa and Peng 1938	*suzukii* Kamizawa 1934
melanogaster Meigen 1830	*takahashii* Sturtevant 1927
miki Duda 1924a	*unipectinata* Duda 1924a

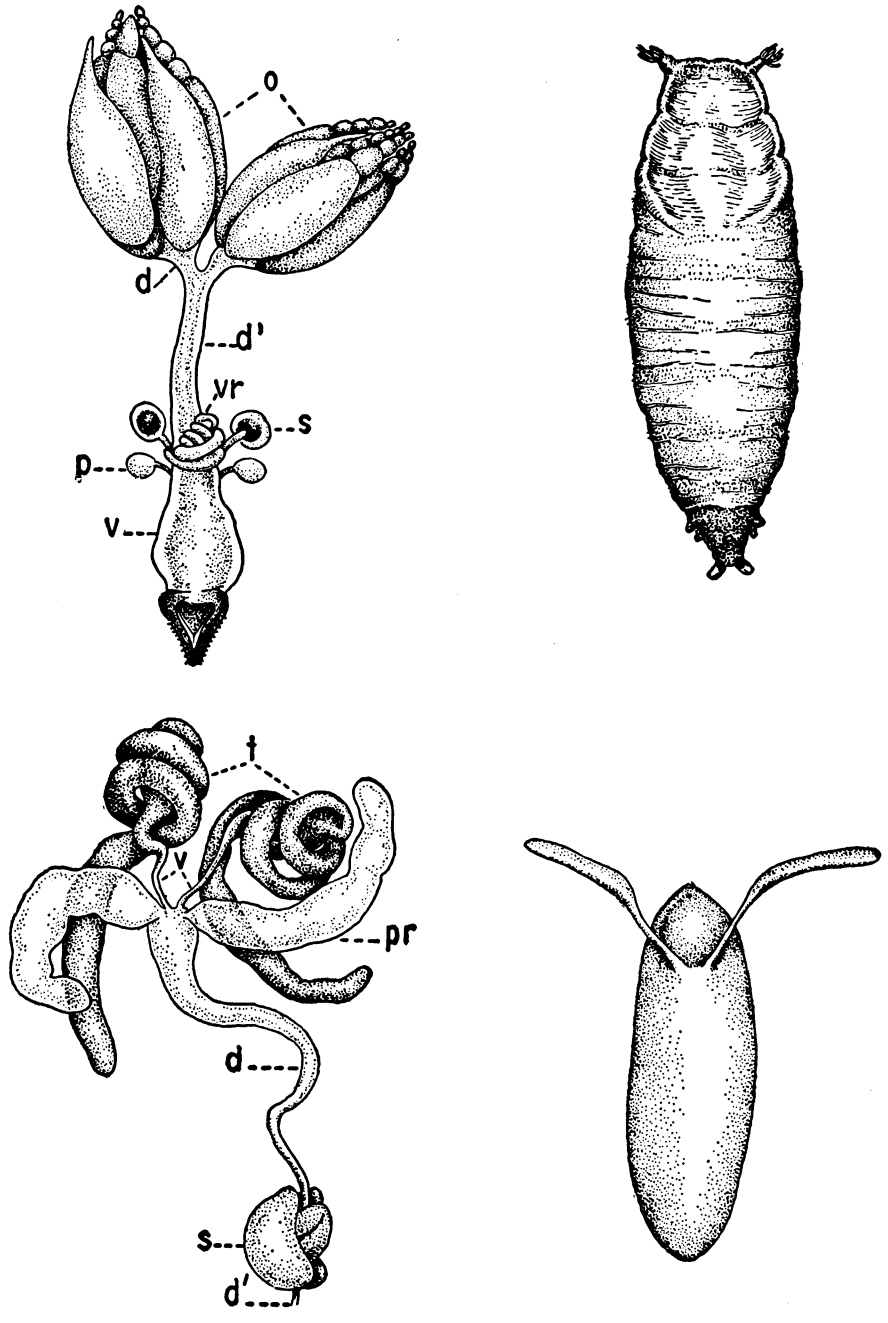

Fig. 5 Female and male reproductive systems, puparium, and egg of *D. melanogaster*.

D. melanogaster, which has been more thoroughly studied than any other species, and the results obtained have given the basis for most of the fundamental concepts of modern genetics.

obscura group

The obscura group includes dark species with the following characters: no opaque areas on tergites, sex combs present, preapicals on first tibiae usually long, sterno-index .6, anterior scutellars convergent, second oral bristle small, middle orbital bristle large, and larvae do not skip. It comes mostly from the north temperate zone.

Twenty-one species have been placed in this group, and, as first suggested by Sturtevant (1942), they can be classified under two subgroups: (a) forms with several teeth in the distal sex comb, acrostichal hairs in eight rows, ventral receptacle short, testes elliptical (Figure 6), and carina broad and flat. This subgroup includes twelve species, of which only four occur in the Western Hemisphere (Table 4). (b) Forms with one tooth in distal sex comb, acrostichal hairs in six

TABLE 4

obscura group

SUBGROUP a	SUBGROUP b
alpina Burla 1948	*affinis* Sturtevant 1916
ambigua Pomini 1940	*algonquin* Sturtevant & Dobzhansky 1936
bifasciata Pomini 1940	*athabasca* Sturtevant & Dobzhansky 1936
frolovae Wheeler 1949b	*azteca* Sturtevant & Dobzhansky 1936
miranda Dobzhansky 1935	*dobzhanskii* Patterson 1943
obscura Fallén 1823	*helvetica* Burla 1948
obscuroides Pomini 1940	*narragansett* Sturtevant & Dobzhansky 1936
persimilis Dobzhansky & Epling 1944	*seminole* Sturtevant & Dobzhansky 1936
pseudoobscura Frolowa 1929	*tolteca* Patterson & Mainland 1944
segúyi Smart 1945	
subobscura Collin 1936	
tristis Fallén 1823	

rows, ventral receptacle nearly as long as in the melanogaster group, testes rather short and spirally coiled, carina narrow and not flat. All the members of this subgroup occur in America except *helvetica,* which does not quite fit the diagnostic characters of the subgroup, in that it has two teeth instead of one in the distal sex comb.

The obscura group of species is one of the most important for studies concerning problems in speciation. Workers at the Pasadena

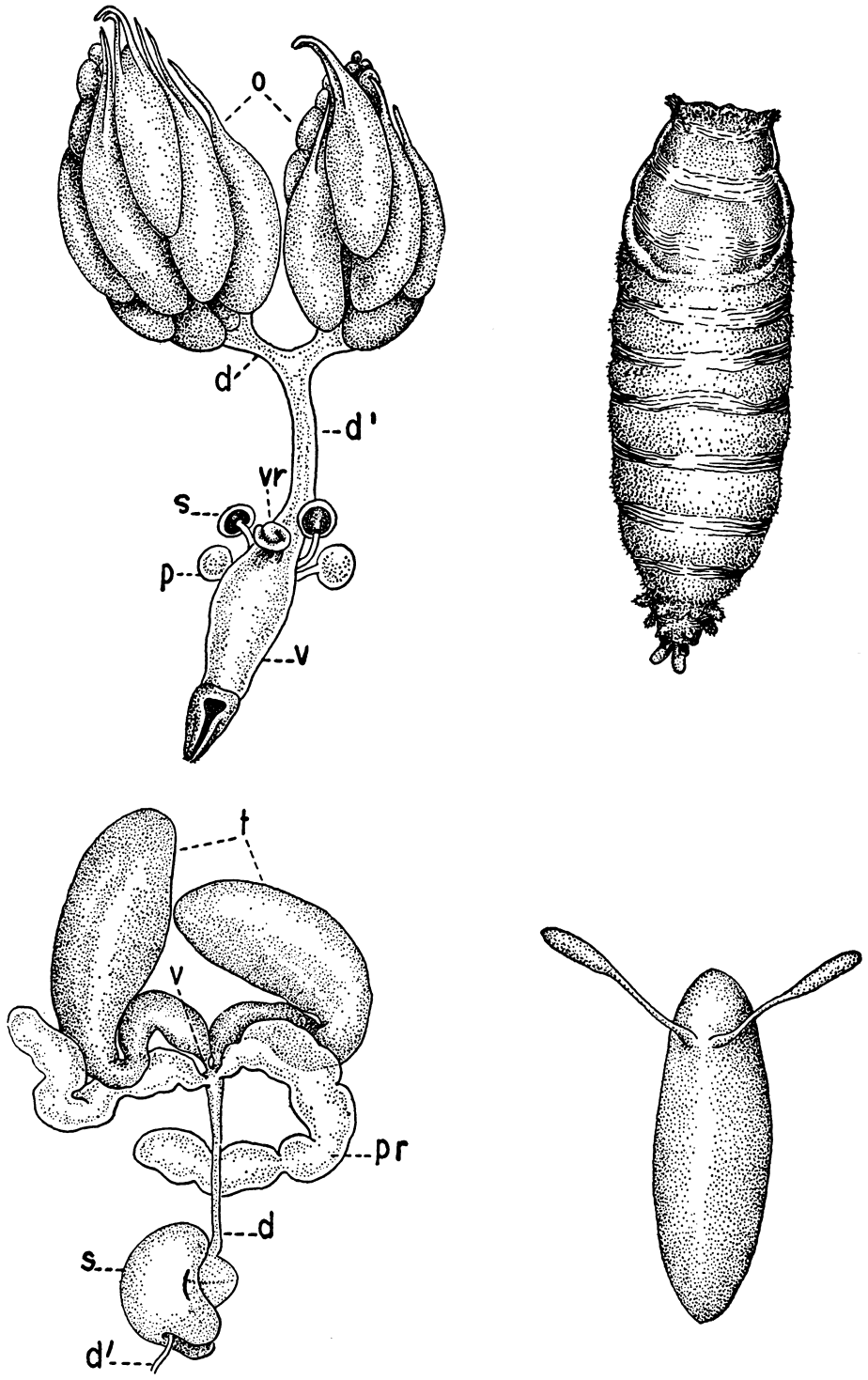

Fig. 6 Female and male reproductive systems, puparium, and egg of *D. pseudoobscura.*

and Columbia laboratories have made extensive studies on members of this group, especially on *pseudoobscura* and related forms, and have obtained results of great importance, which will be discussed in later chapters.

alagitans group

The species belonging to this group are reddish, with acrostichal hairs in six rows, divergent anterior scutellars, and clouded cross veins. Members of the group have the habit of constantly waving their wings, after the manner of *Chymomyza.*

There are two known species, *D. alagitans* Patterson and Mainland 1943 and *D. capnoptera* Patterson and Mainland 1944. All of the specimens of *alagitans* were collected in the state of Michoacán, while *capnoptera* was collected in the states of Hildago and Veracruz, Mexico. Since it has never been possible to breed either of these species on laboratory food, this group is unimportant for genetics and speciation. It was originally placed under the subgenus Drosophila (Patterson and Mainland 1944), but because of the close similarity of its members to certain species of the subgenus Sophophora we have placed it under that subgenus (see Wheeler 1949b).

nannoptera group

This is the sixth group to be considered under the subgenus Sophophora. It was established by Wheeler (1949b) to include the recently described *D. nannoptera.* This is a dull-black species with the following characters: acrostichal hairs in eight rows, with a pair of enlarged hairs in the prescutellar position, anterior scutellars convergent, testes orange and spiral, ventral receptacle long and loosely coiled, tangled with about fifty coils, eggs with two long filaments, their apical two-thirds broadly expanded into thin blades. The flies from which a stock was established came from a point about sixty miles south of Oaxaca, Mexico. This group does not breed well on laboratory food.

bromeliae group

This group includes dull-colored species with seven to nine branches in arista, acrostichal hairs in eight rows, no prescutellars present, wings clear or light, eggs with two filaments. It is usually collected in flowers.

Either two or three species belong to this group. These are *D. bromeliae* Sturtevant 1916, *D. bromelioides* Pavan and da Cunha 1947, and perhaps *D. florae* Sturtevant 1916. The first species has been recorded from Havana, Cuba; the second from São Paulo, Brazil; and the third from Cuba, Puerto Rico, and Costa Rica.

Subgenus **Drosophila** Fallén

The members of this subgenus have three or four egg filaments (two in all but one member of the melanica group), at least the anterior ones tapering, ventral receptacle long, fine and usually kinky, long spiral testes, and dark posterior bands of abdomen usually narrowed or broken in midline.

This is by far the largest and most complex of the several subgenera. Many of the species of this subgenus can be arranged into species groups; others, which clearly belong to it, are not sufficiently known to permit placing them in the groups already established. Sturtevant classified a majority of the better known species under fourteen groups. Since then several additional groups have been established by other workers.

quinaria group

This group is represented by shining yellowish species with three-filament eggs, clouded cross veins (except *innubila*), arista with from nine to eleven branches, and dark abdominal bands often broken up into spots.

A total of sixteen species have been placed in this group, although the inclusion of one of them (*nigromaculata*) is somewhat doubtful (Table 5). Twelve of these species occur in continental North America, although one of them (*transversa*) has also been reported from Europe, both by de Meijere and Burla; from Japan, by Kikkawa and Peng; and from China, by Tan, Hsu, and Sheng. It is highly probable that three different forms are involved here, and, if so, the name *transversa* should be limited to the European form and substitute names given to the American and Asiatic forms.

The series of species constituting the quinaria species group is one of the most promising for future work on speciation. In spite of the difficulty of breeding them on laboratory foods, Sears (1947) and

Blumel (1949) were able to work out the main relationships within the group and to determine the extent of interspecific hybridization.

guttifera group

This is a yellowish species with striped mesonotum, eggs with three filaments, numerous black spots on wings, second oral bristle large, and third antennal segment with longish hairs.

The group consists of a single species, *D. guttifera* Walker 1849, which is similar morphologically to members of the quinaria group, including the internal structures (Figure 7). The male reproductive tract of members of both groups has two structures of interest: the vasa deferentia (v) fuse before entering the anterior ejaculatory duct

TABLE 5

quinaria group

deflecta Malloch 1924	*phalerata* Meigen 1830
innubila Spencer 1943	*quinaria* Loew 1865
limbata van Roser 1840	*suboccidentalis* Spencer 1942
munda Spencer 1942	*subpalustris* Spencer 1942
mutandis Tan, Hsu, & Sheng 1949	*subquinaria* Spencer 1942
nigromaculata Kikkawa & Peng 1938	*suffusca* Spencer 1943
occidentalis Spencer 1942	*tenebrosa* Spencer 1943
palustris Spencer 1942	*transversa* Fallén 1830

(d), and the pair of sperm-pump diverticula are twisted structures (ed). These two characters are present in all of the American species examined of the quinaria group. *Drosophila guttifera,* like many members of the quinaria group, is a fungus-feeder.

pinicola group

This is a grayish-brown species with ventral receptacle without minor coils, middle orbital bristle one-fourth length of the other two, horns of puparium scarcely one-tenth length of puparium, and sterno-index about .5.

The group includes a single described species, *D. pinicola* Sturtevant 1942, which occurs in the southern part of California. It is usually associated with conifers and is probably a sap-feeder, and it is very difficult to breed on laboratory foods. The chief interest in this form is that it appears to represent a very primitive species.

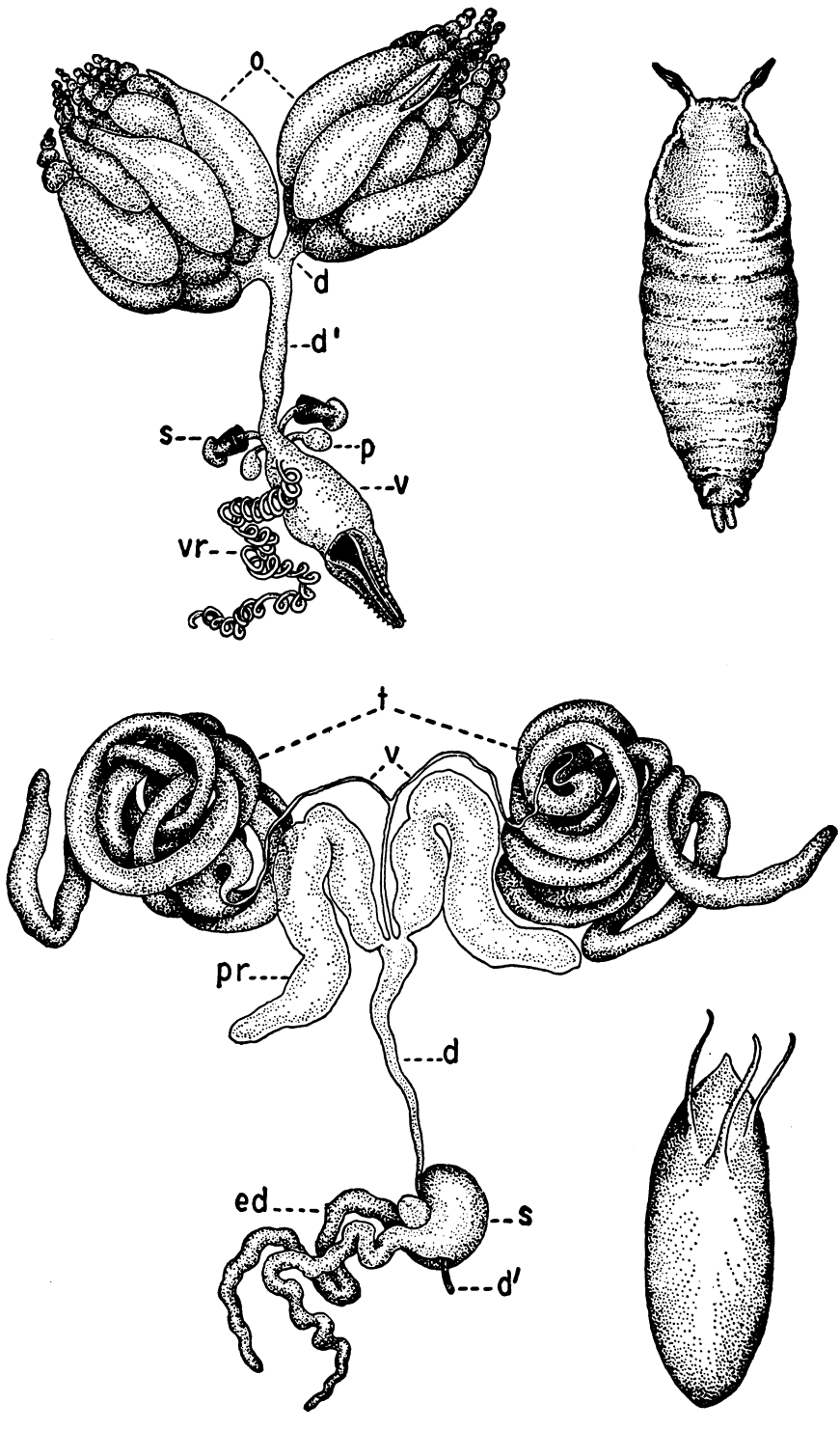

Fig. 7 Female and male reproductive systems, puparium, and egg of *D. guttifera.*

virilis group

These are blackish species with anterior scutellars divergent, posterior cross veins clouded, and sterno-index varying from .8 to .9 (Figure 8).

The group includes *D. virilis* Sturtevant 1916, *D. montana* Patterson and Wheeler 1942, *D. novamexicana* Patterson 1941b, *D. laccicola* Patterson 1944, a pair of subspecies, *D. americana americana* Spencer 1938 and *D. americana texana* Patterson, Stone, and Griffen 1940a, *D. littoralis* Meigen 1830, and *D. imeretensis* Sokolov 1948.

Drosophila virilis is common in Japan and China, where it occurs in the wild state as well as in cities. In North and South America it is rare and is found almost exclusively in domestic habitats. In contrast to the habits of *D. virilis,* the other North American members of the group have never been found in cities.

Both *littoralis* and *imeretensis* have been recorded from the Palaearctic region. The first was described by Meigen from Germany; and Dr. Hans Burla, who determined that it was a member of the virilis group, kindly sent us cultures of two strains of this species which he had collected in Switzerland. Dr. A. Buzzati-Traverso has collected *littoralis* in Italy (personal communication). The second species, *imeretensis,* was recently described by N. N. Sokolov, and we are indebted to Professor Dobzhansky for sending a translation of his article, in which it is stated that this species was first collected at Imeretia, Georgian USSR, and later was discovered in the Moscow region of northern Russia. The possible relationships of these two forms to each other and to other members of the group are discussed in Chapter 10.

testacea group

The members of this group are yellowish or brownish species, with a pair of presutural acrostichal bristles and ventral receptacle without minor coils (Figure 9).

There are two known species belonging to this group and both are fungus-feeders, *D. testacea* van Roser 1840 and *D. putrida* Sturtevant 1916. They can be distinguished from each other by the character and position of the pair of presutural bristles. In *testacea* these bristles are located near the anterior margin of the mesonotum and are erect, while in *putrida* they are located back of the anterior mar-

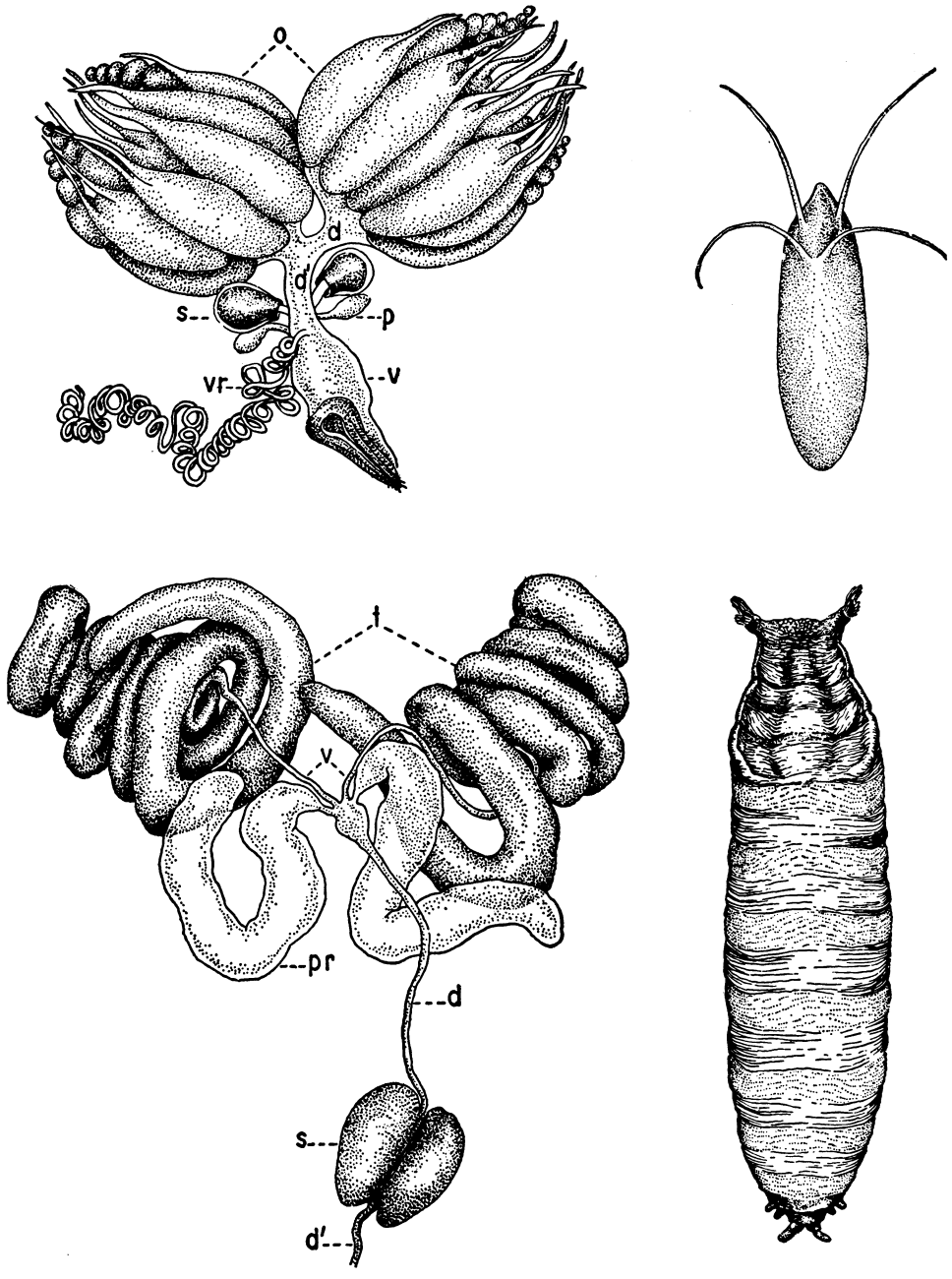

Fig. 8 Female and male reproductive systems, puparium, and egg of *D. virilis.*

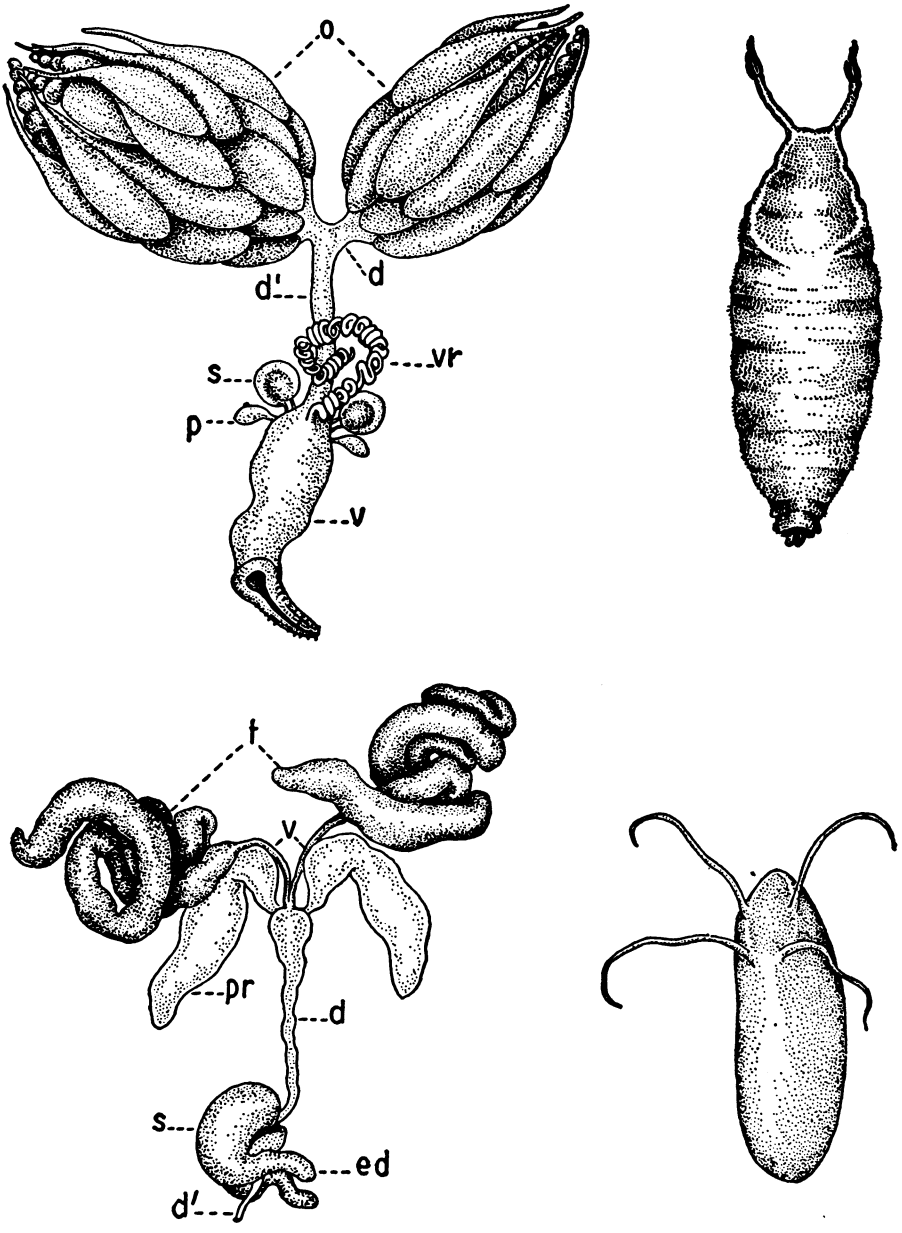

Fig. 9 Female and male reproductive systems, puparium, and egg of *D. putrida.*

gin of the mesonotum and are reclinate. *Drosophila testacea* is found both in Europe and the United States, while *putrida* is apparently restricted to the United States.

tripunctata group

This group is composed of yellowish species with one to three black spots on abdominal segments three to five, arista with twelve branches and a costal index of about 4.3 (Figure 10).

Sturtevant (1942) assigned *D. tripunctata* Loew 1862 to this group, and we have added two new forms, *D. unipunctata* Patterson and Mainland 1943 and *D. crocina* Patterson and Mainland 1944. All three of these species occur in North America. More recently, Freire-Maia and Pavan (1950) have suggested that three South American species should be placed in the tripunctata group. The three species are, *D. mediopunctata* Dobzhansky and Pavan 1943a, *D. mediosignata* Dobzhansky and Pavan 1943a, and *D. mediostriata* Duda 1925a.

funebris group

These are reddish-brown species with sterno-index about .7, horn about one-fifth length of puparium, arista with ten or eleven branches, and male abdomen mostly shining black (Figure 11).

Four species are included in this group, *D. funebris* Fabricius 1787, *D. subfunebris* Stalker and Spencer 1939, *D. macrospina* Stalker and Spencer 1939, and *D. trispina* Wheeler 1949b. Three subspecies have been recognized for *macrospina,* as follows: *D. macrospina macrospina, D. macrospina ohioensis* Spencer 1940, and *D. macrospinâ limpiensis* Mainland 1941.

As to distribution, *funebris* is practically world-wide and represents a cosmopolitan species, while the other members of the group are all found in the Nearctic region, that is, in the United States and northern Mexico. The distribution pattern of the *macrospina* series of three forms is interesting, for it shows a definite east-west gradient with respect to the degree of cross-sterility. This was found to be true between the subspecies, as well as between the geographical strains of the subspecies, particularly those of *macrospina* and *limpiensis.*

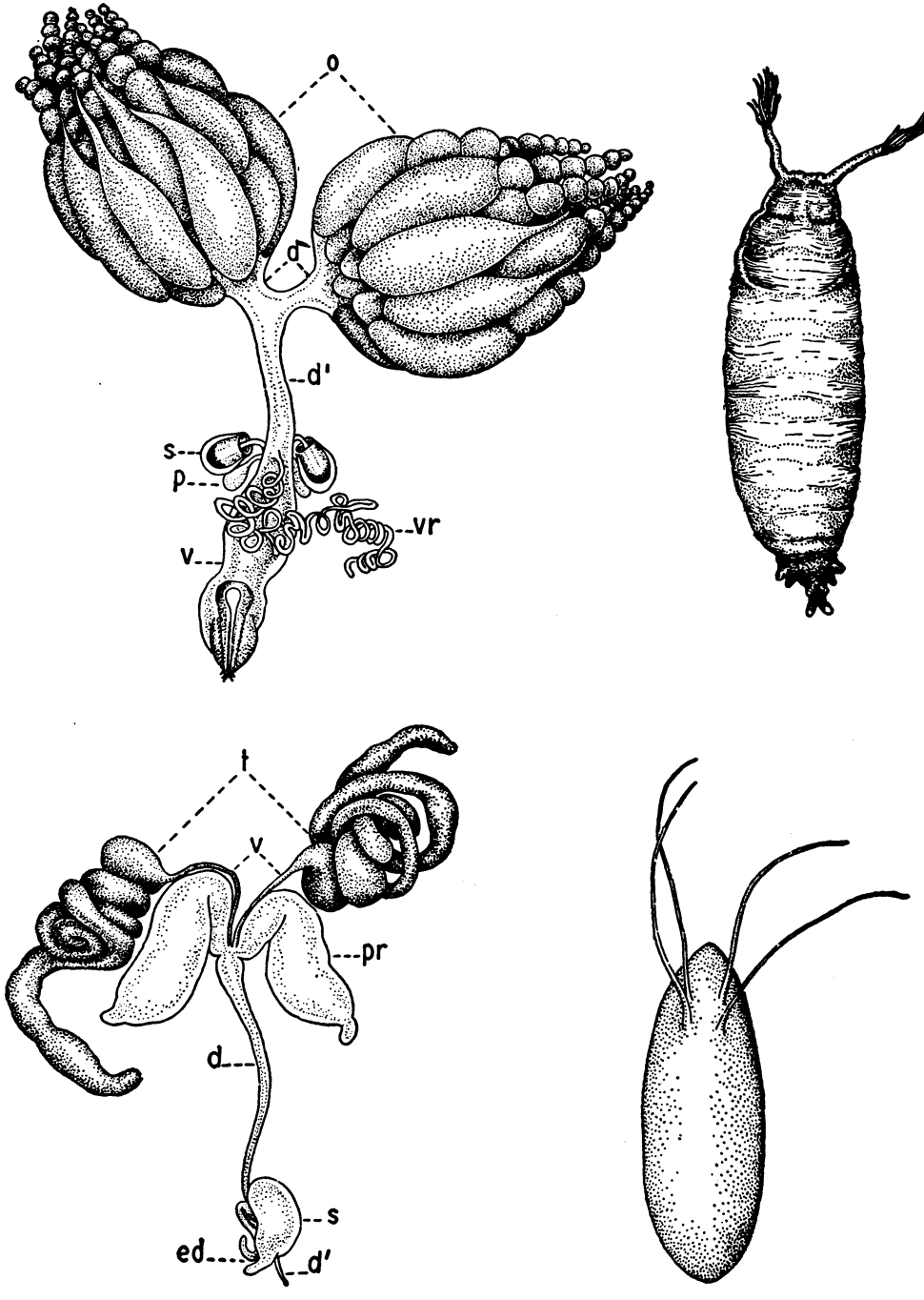

Fig. 10 Female and male reproductive systems, puparium, and egg of *D. tripunctata.*

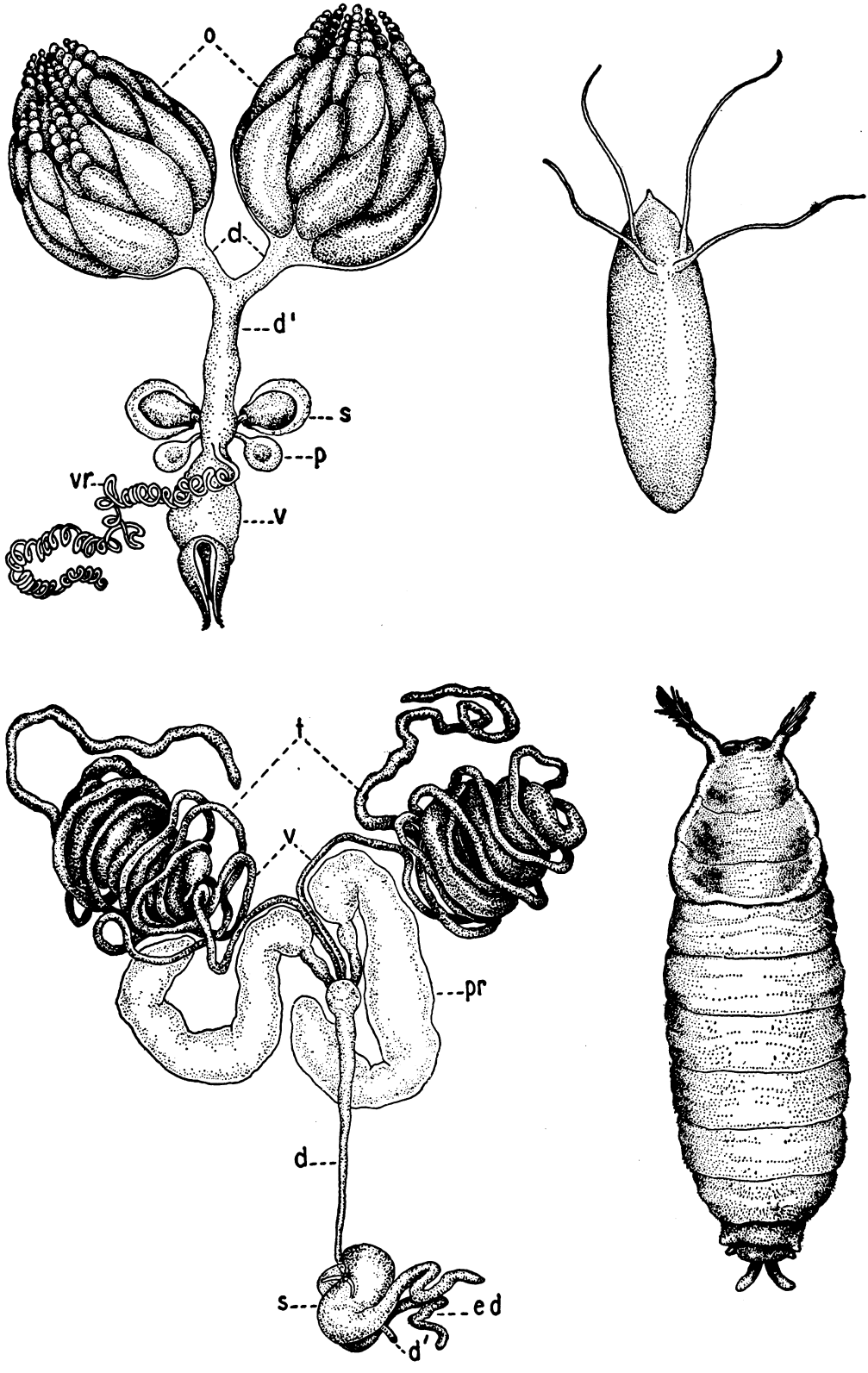

Fig. 11 Female and male reproductive systems, puparium, and egg of *D. funebris.*

repleta group

The members of this species group have a grayish mesonotum, with each hair and bristle arising from a black or dark-brown spot, arista with six to nine branches, testes usually tightly coiled, and the ventral receptacle in many forms tightly coiled like the spring of a screen door (Figure 12).

A total of fifty-two described forms have been placed in this group, including six that represent three pairs of subspecies. Forty of these forms have been classified tentatively under four subgroups (Table 6).

TABLE 6

repleta group

(*a*) hydei subgroup

bifurca Patterson & Wheeler 1942
hydei Sturtevant 1921b
hydeoides Patterson and Wheeler 1942
nigrohydei Patterson and Wheeler 1942
novemaristata Dobzhansky & Pavan 1943a
pachea Patterson & Wheeler 1942

(*b*) melanopalpa subgroup

brunneipalpa Dobzhansky & Pavan 1943a
californica Sturtevant 1923
canapalpa Patterson & Mainland 1944
fascioloides Dobzhansky & Pavan 1943a
fuliginea Patterson & Wheeler 1942
fulvimacula Patterson & Mainland 1944
f. flavorepleta Patterson & Pavan 1952c
limensis Pavan & Patterson 1947
linearepleta Patterson & Wheeler 1942
melanopalpa Patterson & Wheeler 1942
neorepleta Patterson & Wheeler 1942
nigrospiracula Patterson & Wheeler 1942
onca Dobzhansky & Pavan 1943a
repleta Wollaston 1858

(*c*) mulleri subgroup

aldrichi Patterson & Crow 1940
anceps Patterson & Mainland 1944
arizonensis Patterson & Wheeler 1942
buzzatii Patterson & Wheeler 1942
hamatofila Patterson & Wheeler 1942
hexastigma Patterson & Mainland 1944
longicornis Patterson & Wheeler 1942
mainlandi Patterson 1943
meridiana Patterson & Wheeler 1942
m. rioensis Patterson 1943
mojavensis Patterson & Crow 1940
mulleri Sturtevant 1921b
peninsularis Patterson & Wheeler 1942
racemova Patterson & Mainland 1944
ritae Patterson & Wheeler 1942
spenceri Patterson 1943
subviridis Patterson & Mainland 1943

(*d*) mercatorum subgroup

mercatorum Patterson & Wheeler 1942
m. pararepleta Dobzhansky & Pavan 1943a
paranaensis de Barros 1950

(*e*) Unclassified species

betari Dobzhansky & Pavan 1943a
brevicarinata Patterson & Wheeler 1942
fasciola Williston 1896
icteroscuta Wheeler 1949b
inca Dobzhansky & Pavan 1943a
leonis Patterson & Wheeler 1942
maculipennis Duda 1925b
mojú Pavan 1950
nigricruria Patterson & Mainland 1943
obsoleta Malloch 1923
poecilithorax Malloch 1925
ramsdeni Sturtevant 1916

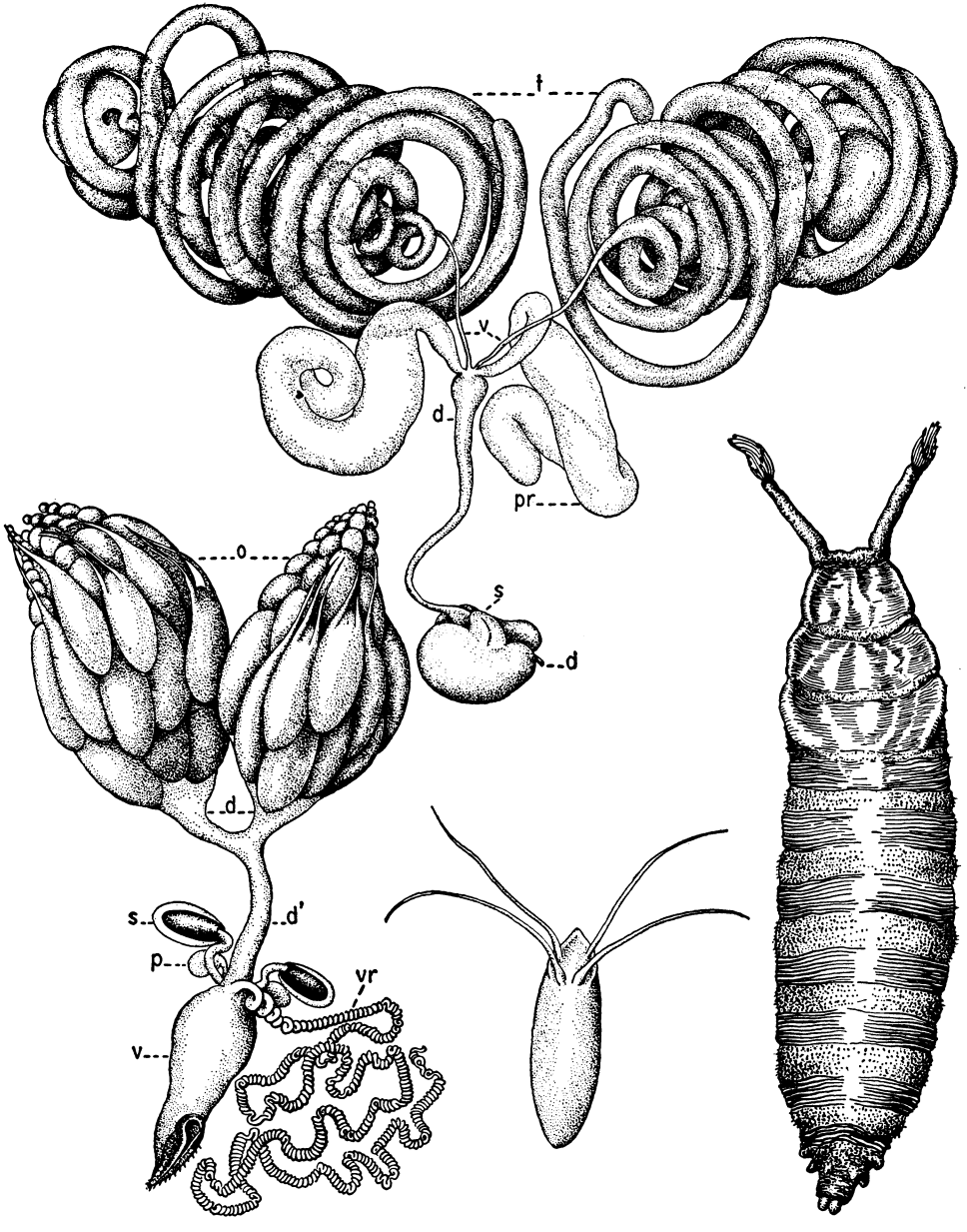

Fig. 12 Female and male reproductive systems, puparium, and egg of *D. hydeoides*.

(a) hydei subgroup. We have placed in this subgroup six hydei-like species with the spermathecae less highly sclerotized than those of the next subgroup and with an unusually long ventral receptacle, which varies from 245 to 735 coils (Table 6, a). The geographical distribution of these species, as well as that of the members of the other subgroups, are considered in the next chapter.

(b) melanopalpa subgroup. This subgroup includes fourteen repletalike forms with highly sclerotized spermathecae and with the number of coils of the ventral receptacle varying from 52 to 116 among the different forms (Table 6, b). Two of these forms are ranked as subspecies. One of these came from Mexico and in 1944 was described by Patterson and Mainland under the name *Drosophila fulvimacula;* the other was collected at Belém in northern Brazil. A stock of the latter was sent to this laboratory by Professor Dobzhansky, with the statement that it was to be named by Dr. Pavan as *Drosophila flavorepleta.* A comparison of the flies of the two stocks showed that, while they represented the same species, yet there were certain morphological differences which made it possible to separate the two with certainty. It was therefore decided to rank them as subspecies, as follows: *Drosophila fulvimacula fulvimacula* Patterson and Mainland, and *Drosophila fulvimacula flavorepleta, subsp. nov.,* Patterson and Pavan. The most striking difference between the Mexican and Brazilian forms is the color, especially on the mesonotum. The Brazilian form is much lighter in color, and has a fine light stripe extending throughout the length of the mesonotum in midline. Results obtained from cross tests showed that the two forms differ genetically. They have identical metaphase configurations. Under the rules of taxonomy, the Mexican form now becomes *Drosophila fulvimacula fulvimacula.*

(c) mulleri subgroup. These forms are characterized by relatively short ventral receptacles, which vary from fifteen to thirty-five coils. The spermathecae are not sclerotized, or only slightly so in some species, and have usually lost their normal function as sperm receptacles. Seventeen forms are included in this subgroup, and a few others probably belong here (Table 6, c).

(d) mercatorum subgroup. The three forms listed under this heading are closely related to the mullerilike species, but constitute a distinct subgroup. The subspecies *mercatorum* occurs in North

America and the Hawaiian Islands, while the other two are found in South America. Interspecific hybridization occurs between these three forms (Table 6, d).

UNCLASSIFIED SPECIES. At least some of the remaining species of the repleta group will, with further study, be assigned to one or the other of these four subgroups, and others of them may form additional subgroups (Table 6, e). Of the twelve species listed in this category, all except two have been recorded from the Nearctic and Neotropical regions. The two exceptions are *obsoleta* and *poecilithorax,* both of which were described from Australia by Malloch. A few other members of the repleta group have become distributed beyond the limits of these two regions. Both *hydei* and *repleta,* which frequently occur in domestic habitats, represent cosmopolitan species, and the subspecies *mercatorum* has also been collected in the Hawaiian Islands. *Drosophila buzzatii* Patterson and Wheeler 1942 was first recorded from the southern part of South America and later was found in Sicily by Dr. Buzzati-Traverso, who in 1943 redescribed it under the name *D. tigrina.* The distribution data clearly indicate that the repleta group is native to the Western Hemisphere, from whence a few species have spread out to other parts of the world.

The repleta group contains perhaps the finest series of species of the entire genus for the study of evolutionary genetics and presents an excellent opportunity for those who are interested in the problem of evolution.

robusta group

Members of this group are large dark species with about nine branches in the arista, a costal index of about 4.0 and the horn two-fifths the length of the puparium. The ventral receptacle has about thirty-five loose coils, and the vase-shaped spermatheca is highly sclerotized.

We have included five species in this group, as follows: *D. cheda* Tan, Hsu, and Sheng 1949, *D. robusta* Sturtevant 1916, *D. colorata* Walker 1849, *D. pullata* Tan, Hsu, and Sheng 1949, and *D. sordidula* Kikkawa and Peng 1938. The species *cheda* and *pullata* occur in China, *robusta* and *colorata* are found in the United States, with the latter also recorded from Canada, and *sordidula* has been collected at several different points in the Japanese Islands.

melanica group

This group includes blackish species with two-filament eggs, each about the same length as the egg, arista with seven to eight branches, second oral bristle not over one-half the length of the first, sterno-index, .7 to .8, ventral receptacle spiral, and in some species the sperm pump has four diverticula.

Nine different forms have been included in the melanica group. These are *D. melanica* Sturtevant 1916, *D. melanica paramelanica* Patterson 1943, *D. melanura* Miller 1944, *D. nigromelanica* Patterson and Wheeler 1942, *D. micromelanica* Patterson 1941a, *D. melanissima* Sturtevant 1916, *"D. melanissima"* Kikkawa and Peng 1938, *D. afer* Tan, Hsu, and Sheng 1949, and probably *D. pseudomelanica* Sturtevant 1916. The *melanissima* of Kikkawa and Peng is distinct from the American form of the same name and should be given a substitute name. With the exception of this Japanese species and *afer* from China, all other described members of the group are endemic to the Nearctic region (Dr. Burla has recently sent us a stock of an undescribed member of this group from France).

Taken as a whole, the members of this group represent a very heterogeneous collection of species. Three of them, *melanica, melanura,* and *paramelanica* have the unique condition of four diverticula on the sperm pump (Figure 13), structures which are absent in other members of the group. If *D. melanissima* Sturtevant belongs to the melanica group, it differs from all other members in having four- instead of two-filament eggs (Figure 14).

polychaeta group

These are reddish-brown species with three pairs of postsutural dorsocentral bristles, costal index 2.0, fourth-vein index 1.8 to 2.2, and ventral receptacle relatively short and loosely coiled.

This group includes *D. polychaeta* Patterson and Wheeler 1942 from Texas, *D. illota* Williston 1896 from St. Vincent and perhaps *D. grandis* Kikkawa and Peng 1938 from Japan. The American species was collected several times off piles of banana refuse on the wharfs of fruit companies at Galveston, Texas. The bananas had been shipped from southern Mexico and Central America, and the flies must have been introduced into Texas from one or both of these areas. This species breeds prolifically on the banana-agar-yeast food.

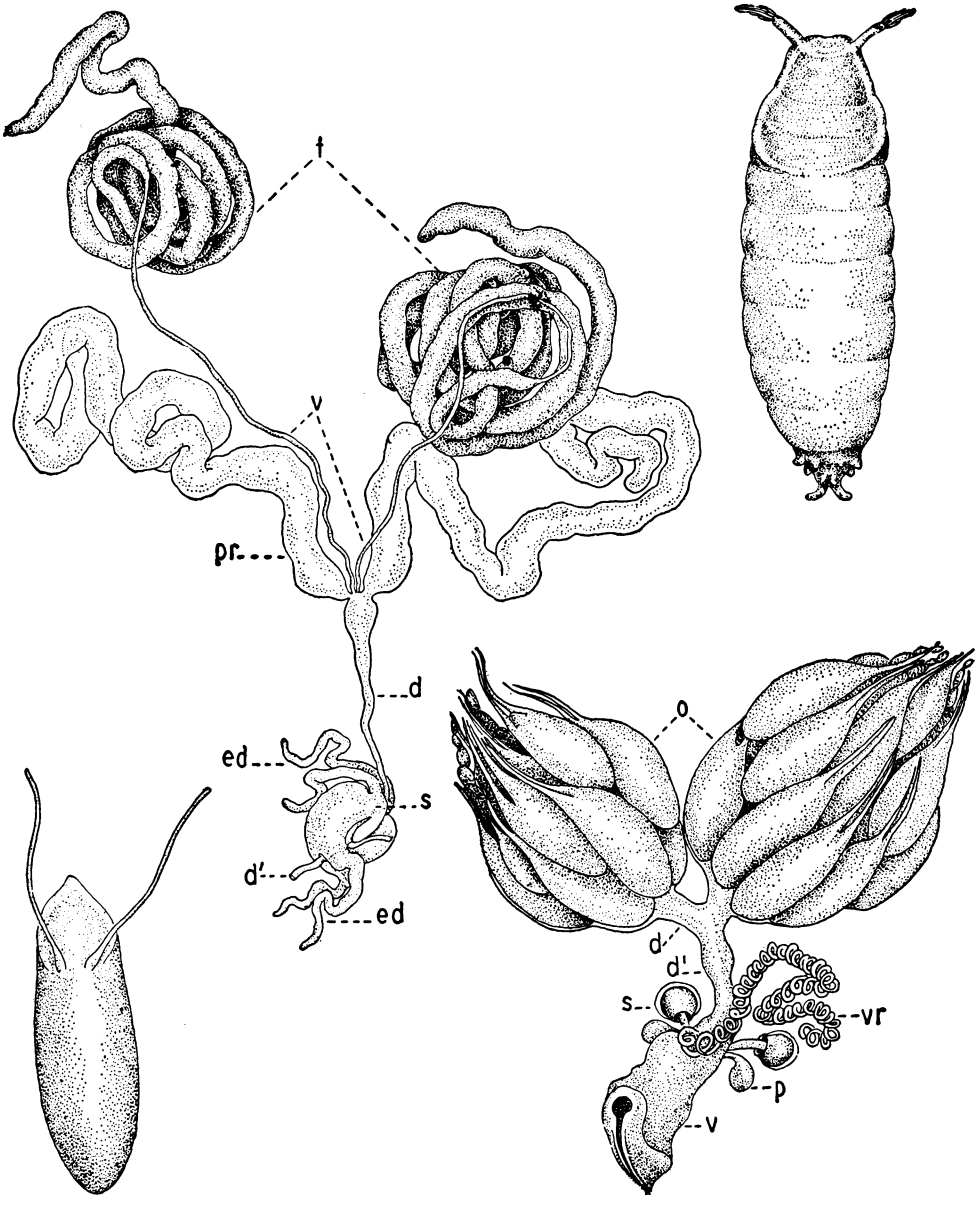

Fig. 13 Female and male reproductive systems, puparium, and egg of *D. melanica.*

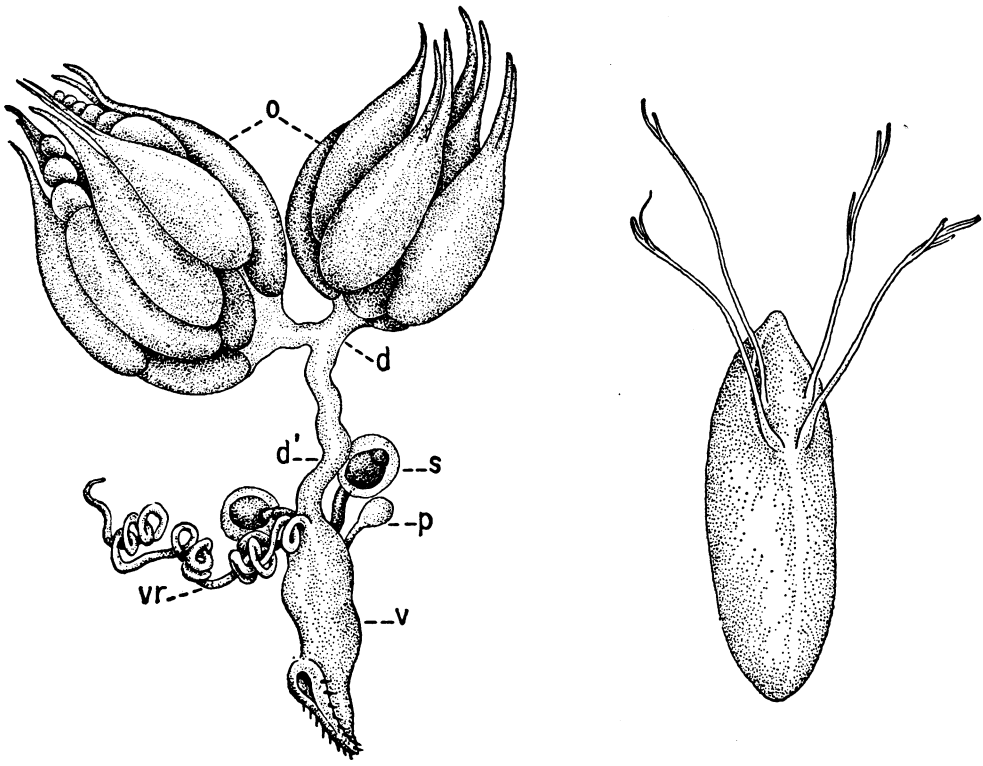

Fig. 14 Female reproductive system and egg of *D. melanissima.*

carbonaria group

A black species with middle orbital bristles almost as long as the anterior, eggs with four filaments, and puparium with a strongly roughened surface (Figure 15).

This group includes only *D. carbonaria* Patterson and Wheeler 1942, which has been collected at several points in Texas, Arizona, and Oklahoma. Professor A. H. Sturtevant collected a single female of this species in Death Valley, California (personal communication, September 4, 1947). It has also been taken in northern Mexico in the states of Chihuahua, Nuevo León, and Tamaulipas. In the main, its distribution area coincides with that of the common mesquite tree, *Prosopis glandulosa* Torr.

cardini group

These are reddish- or yellowish-brown species, shining, with cheeks narrow, sterno-index about .5, costal index about 3.9, and sperm pump with two posterior twisted diverticula (Figure 16).

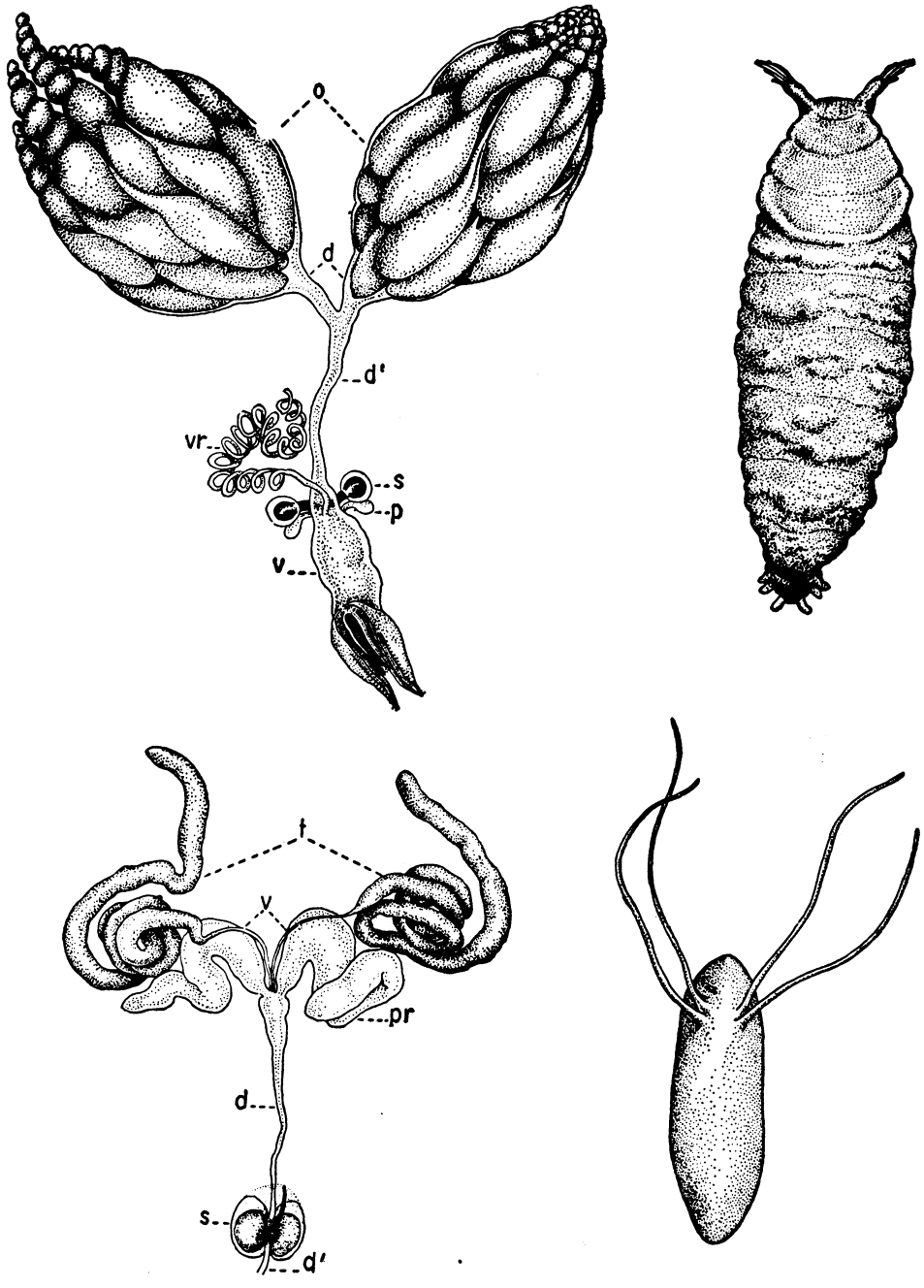

Fig. 15 Female and male reproductive systems, puparium, and egg of *D. carbonaria.*

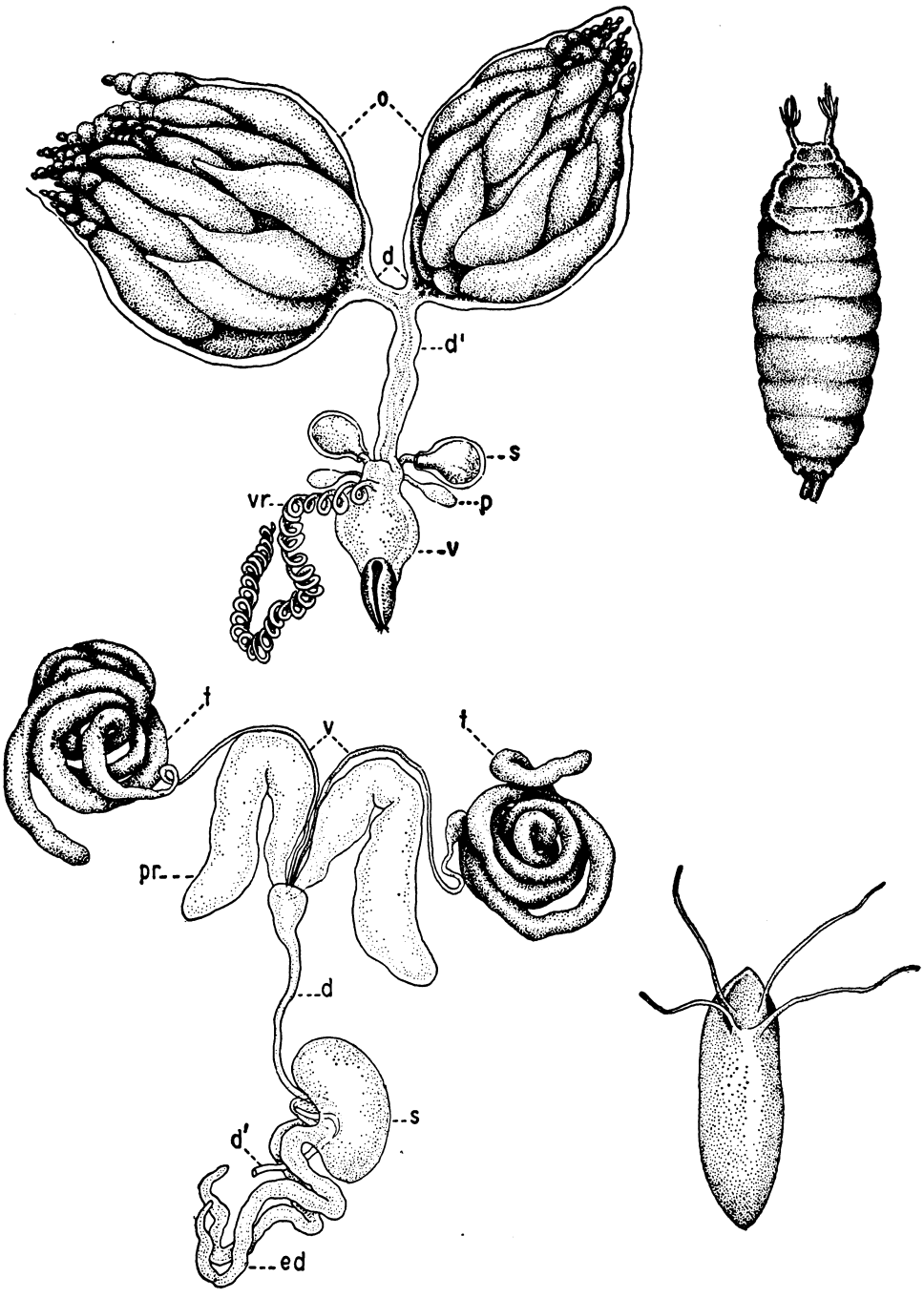

Fig. 16 Female and male reproductive systems, puparium, and egg of *D. cardini.*

Some confusion exists in the literature concerning the taxonomy of this group of species. Dr. M. R. Wheeler (1949b) has made a study of the group, and the following list is in part based on his analysis: *D. albirostris* Sturtevant 1921b, *D. bandeirantorum* Dobzhansky and Pavan 1943a, *D. cardini* Sturtevant 1916, *D. cardinoides* Dobzhansky and Pavan 1943a, *D. metzii* Sturtevant 1921b, *D. neocardini* Streisinger 1946, *D. polymorpha* Dobzhansky and Pavan 1943a, *D. prosimilis* Duda 1925b, perhaps *D. campestris* Burla 1950, and *D. similis* Williston 1896.

All ten of these species have been recorded for the Neotropical region, but the ranges of some of them extend northward into the lower part of the Nearctic. This is true of *similis,* which we have collected in Florida and in the state of San Luis Potosí, Mexico. This is apparently also true for *neocardini* and *cardinoides.* A fuller account of the distribution of members of this group is given in the next chapter.

immigrans group

Members of this group are yellowish species with a row of short thick spines on the first femur, costal index 3.0, horn about one-half length of puparium, ventral receptacle short with about twenty-five loosely arranged coils, and a sperm pump with two twisted posterior diverticula (Figure 17).

There is also some confusion concerning the taxonomy of the immigrans group. We have included seventeen species, all of which probably belong to this species group (Table 7). Each of the zoogeographical regions is represented by at least one species of the group. However, a majority of the seventeen species has been recorded for the Australian and Oriental regions, with ten endemic to the latter region. One species (*immigrans*) is cosmopolitan.

TABLE 7

immigrans group

annulipes Duda 1924a
balneorum Sturtevant 1927
hexastriata Tan, Hsu, & Sheng 1949
immigrans Sturtevant 1921b
komaii Kikkawa & Peng 1938
maculifrons Duda 1925b
monochaeta Sturtevant 1927
nasuta Lamb 1914
nixifrons Tan, Hsu, & Sheng 1949
ruberrima de Meijere 1911
rubra Sturtevant 1927
setifemur Malloch 1924b
signata Duda 1923
spinofemora Patterson & Wheeler 1942
subfasciata de Meijere 1914
virgata Tan, Hsu, & Sheng 1949
willowsi Curran 1936

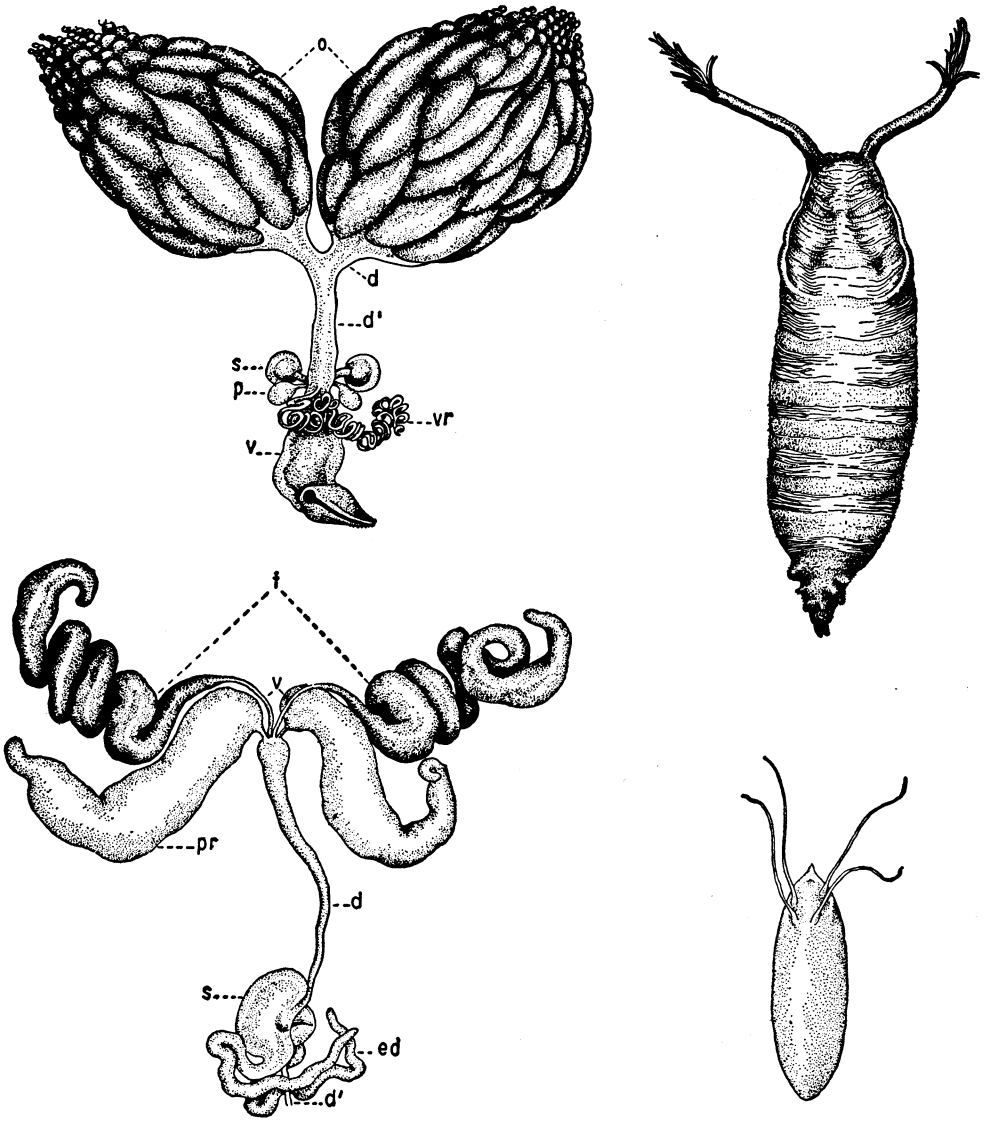

Fig. 17 Female and male reproductive systems, puparium, and egg of *D. immigrans.*

macroptera group

The macroptera group includes reddish-gray species with anterior scutellars divergent, cross veins clouded, sterno-index .6 to .7, ventral receptacle rather short with irregular coiling, and two long tangled diverticula on the sperm pump.

The following species have been included in this group: *D. aurea* Patterson and Mainland 1944, *D. alafumosa* Patterson and Mainland

1943, *D. macroptera* Patterson and Wheeler 1942, *D. magnabadia* Patterson and Mainland 1943, and *D. submacroptera* Patterson and Mainland 1943. All five species occur in Mexico, although the range of one of them (*macroptera*) extends northward into the United States. The latter species has been bred on laboratory food for a few generations, but the group as a whole is not important for genetic and speciation studies.

rubrifrons group

This group contains brownish species with front reddish-brown to maroon, sterno-index .6, anterior scutellars divergent, cross veins clouded, costal index 4.0, ventral receptacle irregularly coiled, and sperm pump with two short diverticula (Figure 18).

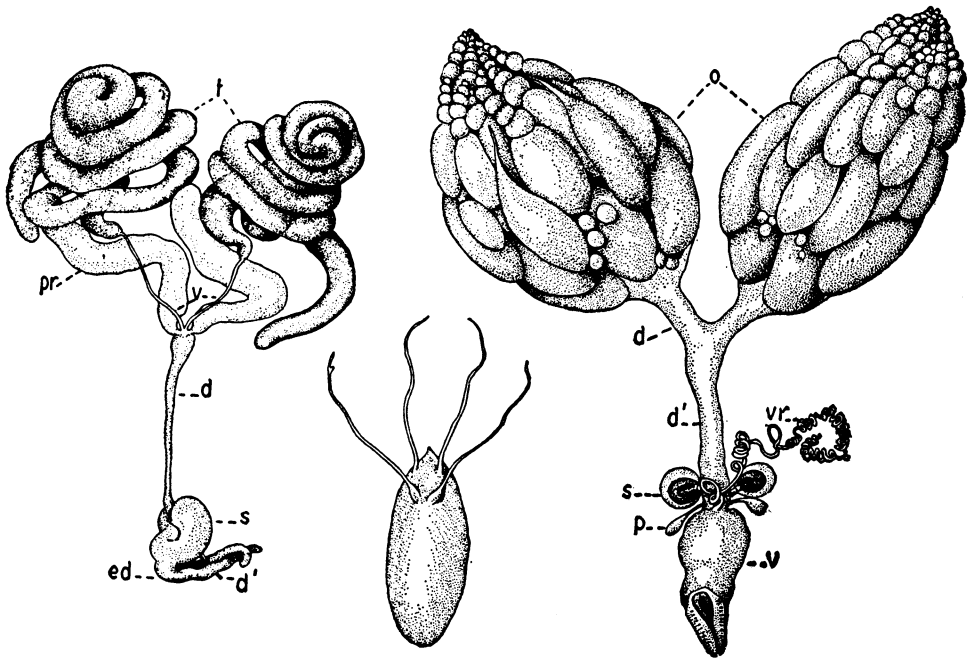

Fig. 18 Female and male reproductive systems and egg of *D. rubrifrons.*

The five species belonging to this group are, *D. rubrifrons* Patterson and Wheeler 1942, *D. rubidifrons* Patterson and Mainland 1944, *D. spadicifrons* Patterson and Mainland 1944, *D. uninubes* Patterson and Mainland 1943, and *D. nubiluna* Wheeler 1949b. These five species all occur in the Nearctic region, either in the United States or in the northern part of Mexico. We have never been able to breed

any of these species on laboratory food, and they are therefore unimportant for the study of speciation.

annulimana group

This group consists of large dark species with acrostichal hairs in eight rows, ventral receptacle spiral, usually with many coils, sperm pump usually with two diverticula and eggs with four filaments.

Pavan and da Cunha (1947) have established this group to include the following species: *D. annulimana* Duda 1925b, *D. gibberosa* Patterson and Mainland 1943, *D. ararama* Pavan and da Cunha 1947, *D. arassari* da Cunha and Frota-Pessoa 1947, and *D. arapuan* da Cunha and Pavan 1947. More recently, Pavan and Nacrur (1950) have described two additional species belonging to this group, *Drosophila araicás* and *Drosophila araúna*. Formerly, *annulimana* and *gibberosa* were placed in the repleta group as aberrant forms. Six of the species have been recorded from South America, chiefly from Brazil, but *gibberosa* occurs in Mexico. We have maintained a stock of the latter species in the laboratory since its discovery in 1943.

melanderi group

These are yellowish species with six or seven branches in the arista, only one prominent oral bristle, acrostichal hairs in six rows, no prescutellars, wings clear and costal index 3.0.

Only two species have been placed in this group. These are, *D. melanderi* Sturtevant 1916 and *D. magnafumosa* Stalker and Spencer 1939. Both are endemic to the Nearctic region, having been recorded from the United States only. Neither one of these species has ever been bred in the laboratory.

bizonata group

The bizonata group includes shining species with eight to ten branches in the arista, second oral bristle nearly as long as first, acrostichal hairs in six rows, no prescutellars, wings clear, cross veins slightly clouded, and costal index 3.0 to 3.5.

The three species belonging to this group are *D. bizonata* Kikkawa and Peng 1938, from Japan, *D. heterobristalis* and *D. meitanensis* Tan, Hsu, and Sheng 1949, both from China. The main difference between the Japanese species and the two from China is seen in the number of egg filaments; the first has four filaments, and the others have two.

guaraní group

This group includes brownish species with divergent anterior scutellars, arista with eleven to thirteen branches, strongly clouded cross veins, and eggs with four filaments.

At least six species belong to this group, as follows: *D. guaraní* Dobzhansky and Pavan 1943a, *D. griseolineata* Duda 1925b, *D. guarú* Dobzhansky and Pavan 1943a, *D. guaramunú* Dobzhansky and Pavan, 1943a, *D. guarajá* King 1947a, and *D. subbadia* Patterson and Mainland 1943. Dobzhansky and Pavan suggest that *D. ornatifrons* Duda 1925b is related to these six species, and if so it should be included in the group. With the exception of *subbadia,* the other six species have been recorded from South America, while *subbadia* has been collected at two points in the state of Michoacán, Mexico. King (1947b) has divided this group into two subgroups of three species each, with *ornatifrons* not included. The other six species have been bred successfully on laboratory food.

pallidipennis group

A large yellowish species with eight to ten branches in arista, acrostichal hairs in eight regular rows, anterior scutellars divergent, wings almost clear, costal index about 5.7, ventral receptacle spiral with about fifty coils, sperm pump with two posterior diverticula, and eggs with four filaments.

This group includes a single species which is composed of a pair of subspecies. These are, *D. pallidipennis pallidipennis* Dobzhansky and Pavan 1943a and *D. pallidipennis centralis* Patterson and Mainland 1944. The first subspecies was collected by Dr. C. Pavan at Iporanga and in the city of São Paulo, state of São Paulo, Brazil. The second subspecies was collected by Dr. G. B. Mainland at Jalapa, Veracruz, and by Dr. M. R. Wheeler in Oaxaca, Mexico. Intraspecific hybrids have been obtained between these two forms (Patterson and Dobzhansky, 1945).

dreyfusi group

This group includes yellowish-brown species with one prominent oral bristle, nine to ten branches in arista, acrostichal hairs in eight rows, no prescutellars, anterior scutellars divergent, wings brown, ventral receptacle spiral with about one hundred coils, testes with six coils, and eggs with four filaments.

Thus far only two species have been included in this group, *D. dreyfusi* Dobzhansky and Pavan 1943a and *D. camargoi* Dobzhansky and Pavan 1950, both collected in Brazil.

Unclassified Species

The total number of forms (species and subspecies) listed above for the several subgenera and species groups is 274, of which 267 represent full species. It has been determined that the total number of described species for the genus Drosophila amounts to about 613 full species (Chapter 3, Table 8). Our list therefore contains over 43 per cent of the total number. The characters of most of the species not listed are not well enough known to permit assigning them to such groups, and in many cases even the subgenus to which they may belong is uncertain. In Chapter 3, Table 15, we list twenty-six additional species, to which we will have occasion to refer in subsequent chapters. All except two of these were described after 1921, and, according to their describers, a majority of them belong to the subgenus Drosophila. Naturally, the descriptions of species reported prior to this date do not follow the modern classification.

The various forms presented in this chapter constitute the chief material upon which will be based our discussion and analysis and that have a bearing on the question of evolution in the genus Drosophila.

3 GEOGRAPHICAL DISTRIBUTION AND SPECIATION

The subject of geographical distribution is one of great importance in connection with any discussion of the problem of speciation. The species of many groups of animals tend to expand in all directions from centers of dispersal. In time this might result in their universal distribution, were it not for the presence of inhibitory barriers. Physical barriers like stretches of water, mountain ranges, and deserts serve as checks to dispersion, as do also temperature and humidity, by acting directly upon vegetation which constitutes the food media of many animals. The dispersion of many forms follows the ranges of their food plants. Winged insects like Drosophila have certain advantages, since they have the power of flight and are frequently borne by the wind. They are thus able to surmount various types of physical barriers. It is therefore not surprising to find that Drosophila species are found all over the world, with the exception of the extreme arctic regions.

Students of distribution usually divide the world into six zoogeographical regions. As outlined by Folsom and Wardle (1934), these are as follows: (1) Nearctic region, which occupies almost all of the entire continent of North America, including Greenland; (2) Neotropical region, including South America, Central America, the West Indies, and most of the coasts of Mexico; (3) Palaearctic region, which embraces the whole of Europe, northern Africa as far south as the Sahara, Asia down to the Himalayas, and the Japanese Islands; (4) Ethiopian region, composed of Africa south of the Sahara, southern Arabia, and Madagascar and adjacent islands; (5) Oriental region, which includes India, Ceylon, Tropical China, and the western Malay Islands; (6) Australian region which embodies Australia, New Zealand, the eastern Malay Islands, and groups of Pacific islands, including the Hawaiian Islands (Figure 19).

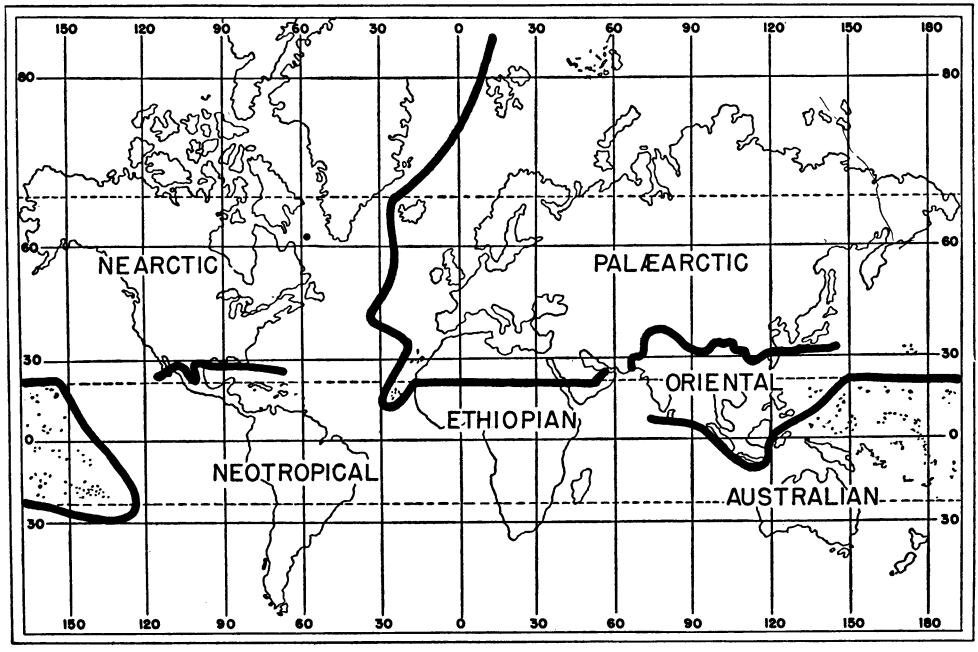

Fig. 19 Faunal Realms. (From Folsom and Wardle, after Sclater and Wallace.)

Some writers prefer to use only five faunal realms or regions by combining the Nearctic and Palaearctic regions into the Holarctic. Moreover, some students of distribution subdivide each of the six regions into smaller units. But for our purpose it seems unnecessary to consider in detail such subdivisions because the distribution data on Drosophila are often incomplete.

Sturtevant (1921b), in his treatment of the distribution of Drosophila species, included Australia and New Zealand in the Oriental region, chiefly because of the lack of reliable data. He used the term Polynesian region to include the Drosophila of the Hawaiian Islands. Geographically, these islands are well isolated from the great continents, as well as from most of the other island groups of the Pacific. They contain a rather large number of unique species which, so far as is known, do not exist elsewhere. As Sharp (see Folsom and Wardle, 1934, page 418) has pointed out, if this is so,

> . . . it follows that either they must have existed formerly elsewhere and migrated to the islands, or that they must have been produced within the islands. This last seems the simpler and more probable supposition,

and it appears highly probable that there has been a large amount of endemic evolution within the limits of these islands.[1]

For the purpose of presenting the distribution data on Drosophila species, we have summarized these in the tables by following the specific name of the species with that of the describer and the date of publication of the description. The region or regions from which a given species has been reported is shown in parenthesis by number (or numbers), which corresponds to the number used for the different regions. For example, *D. alabamensis* was described by Sturtevant in 1918, and occurs in the Nearctic region.

GENERAL DISTRIBUTION WITHIN THE SIX REGIONS

We have recently compiled a list of the known forms of the genus Drosophila (Patterson and Wheeler, 1949). With a few changes, this list now contains 613 full species. The number recorded for each of the six geographical regions is given in the upper line of Table 8.

TABLE 8

Distribution of Drosophila species within the six regions (modified after Patterson and Wheeler)

Regions	(1) Nearc.	(2) Neotrop.	(3) Palae.	(4) Ethiop.	(5) Orien.	(6) Aust.	Total
Number per region	141	200	89	33	128	114	613
Number endemic	95	153	70	26	109	100	553

The Neotropical region leads the list with 200, followed in order by Nearctic, Oriental, Australian, Palaearctic, and Ethiopian. Each of these figures includes some species that occur in two or more regions, and if these are subtracted it gives the numbers that are *endemic* for each of the several regions. These are listed in the second line of Table 8. The number of endemics is 553, or over 90 per cent of the total.

Eleven out of a possible fifty-seven combinations are represented among the sixty nonendemic species (Table 9). The largest number

[1] Folsom, J. W., and R. A. Wardle. *Entomology with special reference to Its Ecological Aspects,* page 418. Blakiston, Philadelphia. 1934.

TABLE 9

Number occurring in two or more regions among 611 full species (modified after Patterson and Wheeler)

1+2	3+5	5+6	2+3	1+2+6	1+3	4+5	1+3+5	1+2+3+5	2+3+5+6	1—6	Total
35	7	3	1	1	1	1	1	1	1	8	60

of thirty-five is for the 1 + 2 combination of the Nearctic-Neotropical regions. For the most part, this is the result of the overlapping faunae of these two adjacent regions in the transition area in Mexico.

The next largest number includes the eight cosmopolitan species (1 to 6), which group consists of *ananassae, busckii, funebris, hydei, immigrans, melanogaster, repleta,* and *simulans.* These eight species are frequently found in domestic habitats.

The seven species of the 3 + 5 combination are in the main from adjacent areas of the eastern Palaearctic and the Oriental tropics of China. The three species of the 5 + 6 combination come from the closely associated Oriental and Australian regions in or near the East Indies. The seven remaining combinations are each represented by a single species. It is probable that the two species recorded in four of the six regions are on the way to becoming cosmopolitans.

DISTRIBUTION OF THE SUBGENERA

In Tables 10 to 15 are listed 303 forms, all classified as to subgenera, and those belonging to the subgenera Sophophora and Drosophila are for the most part classified as to species groups. The twenty-six species of the subgenus Hirtodrosophila are widely scattered geographically (Table 10). There are seven species endemic to each of the Nearctic and Neotropical regions, with one form common to both regions (Figure 20). There are four species endemic to each of the Oriental and Australian regions, with a single species common to both regions. Finally, two species of the subgenus are endemic to the Palaearctic region. Thus twenty-four of the twenty-six species are endemic. The only regions without a representative of this subgenus is the Ethiopian. Apparently, all of these species are fungus-feeders and are difficult to breed on laboratory foods. *Drosophila duncani* is the only one that has been maintained for any length of time in the laboratory.

The nine members of the subgenus Pholadoris are also widely scattered, with no representative for the Ethiopian region (Table 10). Two of the species (*baeomyia* and *victoria*) are endemic to the Nearctic region, and two others (*lebanonensis* and *nitens*) are restricted to the Palaearctic region. A single species *(mirim)* is endemic to the Neotropical region. Only one species occurs in two regions, *coracina,* which has been reported from both Japan and China. The three remaining species have all been recorded from the Australian region.

TABLE 10

Geographical distribution of the species of six Subgenera

Subgenus Hirtodrosophila

alabamensis Sturtevant 1918 ....(1)
astioidea Duda 1923 .....(6)
chagrinensis Stalker & Spencer 1939 .....(1)
cinerea Patterson & Wheeler 1942 .....(1)
dentata Duda 1924a .....(5)
duncani Sturtevant 1918 .....(1)
flavohalterata Duda 1925a .....(2)
fuscohalterata Duda 1925a .....(2)
glabrifrons Duda 1925a .....(2)
grisea Patterson & Wheeler 1942.(1)
hirticornis de Meijere 1914 .....(5)
innocua Malloch (1934b) .....(6)
jordanensis Frota-Pessoa 1945 ...(2)
latifrontata Frota-Pessoa 1945 ..(5)
longala Patterson & Wheeler 1942.(1)
longecrinita Duda 1924a ...(5 + 6)
lundestroemi Duda 1938 .....(3)
narinosa Frota-Pessoa 1945 .....(2)
nigrohalterata Duda 1925a ..(1 + 2)
ochracella Hendel 1936 .....(2)
oldenbergi Duda 1924a .....(3)
orbospiracula Patterson & Wheeler 1942 .....(1)
prognatha Sturtevant 1916 .....(2)
seminigra Duda 1923 .....(6)
trapezina Duda 1923 .....(5)
unicolor Malloch 1934b .....(6)

Subgenus Pholadoris

anuda Curran 1936 .....(6)
baeomyia Wheeler 1949a .....(1)
bryani Malloch 1934b .....(6)
coracina Kikkawa & Peng 1938 .....(3 + 5)
excepta Malloch 1934b .....(6)
lebanonensis Wheeler 1949a ....(3)
mirim Dobzhansky & Pavan 1943a .....(2)
nitens Buzzati 1943 .....(3)
victoria Sturtevant 1942 .....(1)

Subgenus Dorsilopha

busckii Coquillett 1901 .....(1–6)

Subgenus Phloridosa

alfari Sturtevant 1921b .....(2)
floricola Sturtevant 1942 ...(1 + 2)
lutzii Sturtevant 1916.....(1 + 2)
mauiensis Grimshaw 1901 .....(6)
tristani Sturtevant 1921b.....(2)

Subgenus Siphlodora

flexa Loew 1865 .....(2)
sigmoides Loew 1872.....(1)
subsigmoides Patterson & Mainland 1944 .....(1 + 2)

Subgenus Sordophila

acanthoptera Wheeler 1949b....(2)

The subgenera Dorsilopha and Sordophila are both monotypic, the former being represented by the cosmopolitan *busckii* (Figure 20) and the latter by *acanthoptera,* which was collected in the southern part of Mexico in the Neotropical region.

Of the five members belonging to the subgenus Phloridosa, *alfari* and *tristani* are both endemic to the Neotropical region, while *floricola* (Figure 20) and *lutzii* have been reported from both the Nearctic and Neotropical regions. The fifth member, *mauiensis,* was reported from Hawaii of the Australian region, but Sturtevant regards this species as a doubtful member of the subgenus (Table 10).

Only three known species have been included in the subgenus Siphlodora (Table 10). *Drosophila flexa* was described by Loew from Cuba of the Neotropical region. He also described *sigmoides,* which apparently is restricted to the Nearctic region. The third species, *subsigmoides,* has been collected in both the Nearctic and Neotropical regions of Mexico (Figure 20).

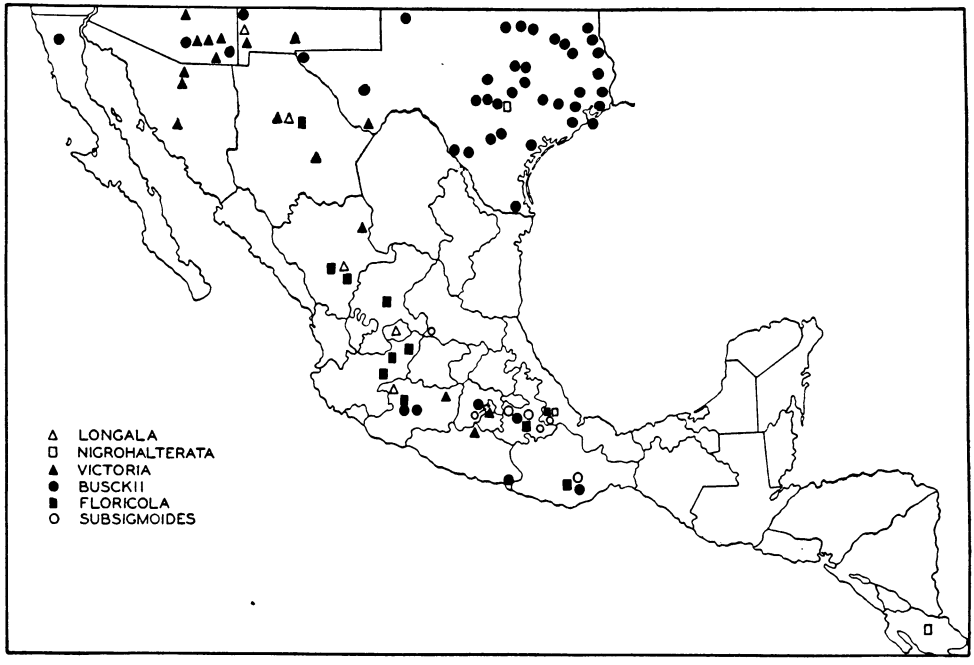

Fig. 20 Distribution of the known records of members of five subgenera of the genus Drosophila. (Figs. 20–22 and 26–33 modified after Patterson and Mainland.)

Table 10 lists a total of forty-five known species which belong to the first six subgenera. Ten of these are endemic to the Nearctic region, twelve to the Neotropical, four to the Palaearctic, four to the Oriental, and eight to the Australian. In addition, one species is common to the Oriental and Australian regions, another to the Palae-

TABLE 11

Geographical distribution of members of the subgenus Sophophora

saltans group

cordata Sturtevant 1942 .....(2)
earlei Sturtevant 1916 .....(2)
elliptica Sturtevant 1942 .....(1)
emarginata Sturtevant 1942 ....(2)
neoelliptica Pavan & Magalhaes 1950 .....(2)
neosaltans Pavan & Magalhaes 1950 .....(2)
prosaltans Duda 1925b .....(2)
rectangularis Sturtevant 1942 .....(1 + 2)
saltans Sturtevant 1916 .....(2)
sturtevanti Duda 1925b .....(2)

willistoni group

bocainensis Pavan & da Cunha 1947 .....(2)
capricorni Dobzhansky & Pavan 1943a .....(2)
equinoxialis Dobzhansky 1946 ...(2)
fumipennis Duda 1925a .....(2)
nebulosa Sturtevant 1916 ...(1 + 2)
paulistorum Dobzhansky & Pavan 1949 .....(2)
sucinea Patterson & Mainland 1944 .....(1 + 2)
tropicalis Burla & da Cunha 1949 .....(2)
willistoni Sturtevant 1916 ...(1 + 2)

melanogaster group

ananassae Doleschall 1858 ....(1–6)
auraria Peng 1937 .....(3 + 5)
biarmipes Malloch 1924a .....(5)
bipectinata Duda 1923 .....(3 + 5)
ficusphila Kikkawa & Peng 1938.(3)
illata Walker 1860 .....(6)
lutea Kikkawa & Peng 1938 .....(3)
melanogaster Meigen 1830 ...(1–6)
miki Duda 1924a .....(3)
montium de Meijere 1916 .....(2 + 3 + 5 + 6)
nipponica Kikkawa & Peng, 1938.(3)
pulchrella Tan, Hsu, & Sheng, 1949 .....(5)
rufa Kikkawa & Peng 1938 .....(3)
serrata Malloch 1927 .....(6)
simulans Sturtevant 1919 .....(1–6)
suzukii Kamizawa 1934 ....(3 + 5)
takahashii Sturtevant 1927 ..(3 + 5)
unipectinata Duda 1924a .....(5)

obscura group

affinis Sturtevant 1916 .....(1)
algonquin Sturtevant & Dobzhansky 1936 .....(1)
alpina Burla 1948 .....(3)
ambigua Pomini 1940 .....(3)
athabasca Sturtevant & Dobzhansky 1936 .....(1)
azteca Sturtevant & Dobzhansky 1936 .....(1 + 2)
bifasciata Pomini 1940 .....(3)
dobzhanskii Patterson 1943 .....(1)
frolovae Wheeler 1949b .....(1)
helvetica Burla 1948 .....(3)
miranda Dobzhansky 1935b ....(1)
narragansett Sturtevant & Dobzhansky 1936 .....(1)
obscura Fallén 1823 .....(3)
obscuroides Pomini 1940 .....(3)
persimilis Dobzhansky & Epling 1944 .....(1)
pseudoobscura Frolowa 1929.(1 + 2)
seguyi Smart 1945 .....(4)
seminole Sturtevant & Dobzhansky 1936 .....(1)
subobscura Collin 1936 .....(3)
tolteca Patterson & Mainland 1944 .....(1)
tristis Fallén 1823 .....(3)

alagitans group

alagitans Patterson & Mainland 1944 .....(1)
capnoptera Patterson & Mainland 1944 .....(1 + 2)

nannoptera group

nannoptera Wheeler 1949b .....(2)

bromeliae group

bromeliae Sturtevant 1921b .....(2)
bromelioides Pavan & da Cunha 1947 .....(2)
florae Sturtevant 1916 .....(2)

arctic and Oriental, and four to the Nearctic and Neotropical. The Ethiopian region has one representative, if we include the cosmopolitan *busckii.*

DISTRIBUTION OF THE SPECIES GROUPS

Tables 11 to 15 list 252 species, of which 64 belong to seven species groups of the subgenus Sophophora and 162 to twenty-two species groups of the subgenus Drosophila. At the end of Table 15 are listed twenty-six species which are unassigned as to species groups. A majority of these are known to belong to the subgenus Drosophila.

Subgenus Sophophora

For this subgenus sixty-four known species are listed under seven species groups (Table 11).

saltans group

The ten members of the saltans group occur exclusively in the Nearctic and Neotropical regions, but only one (*elliptica*) is endemic to the Nearctic region, while eight others are endemic to the Neotropical. The tenth member (*rectangularis*) occurs in both of these regions (Figure 21).

willistoni group

This species group consists of nine known species. As indicated in the table, six of these are endemic to the Neotropical, and the other three occur in this and the Nearctic region. From the available information, this group probably originated in the tropics with three of its members (*nebulosa, sucinea, willistoni,* Figure 21) extending their ranges into the lower part of the Nearctic region, and with a single record of *nebulosa* from northwestern Nebraska (Dr. D. D. Miller's record). In any event, the members of this and the preceding group are clearly New World forms.

melanogaster group

We have included eighteen species in the melanogaster group. Five species are endemic to the eastern Palaearctic, and three others to the Oriental region, while four species occur in both regions. Two

species (*illata, serrata*) are from the Australian region. Three of the remaining four are cosmopolitan (*ananassae, melanogaster, simulans*) in the sense that each has been recorded from all six geographical

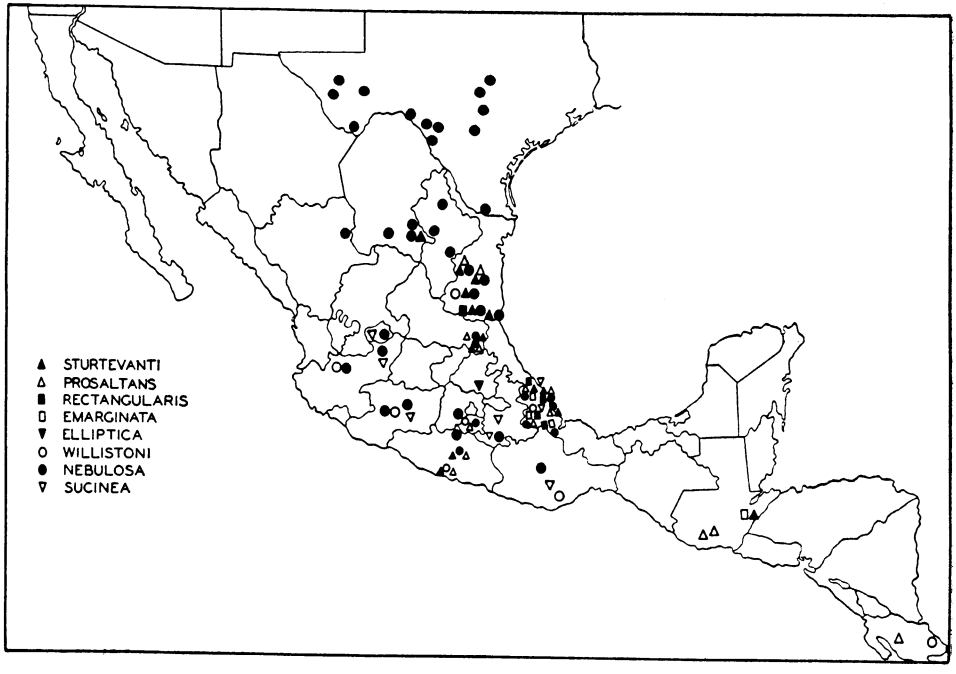

Fig. 21 Distribution of the known records of members of the saltans and willistoni groups of the subgenus Sophophora.

regions. Finally, *montium* also has a wide distribution, having been reported from Java, India, various parts of the Japanese Islands, Manchuria, Korea, Tropical China, Formosa, Hawaii, and South America. From the available records, it seems clear that this group was, as Sturtevant (1942) has suggested, originally native to the tropical and subtropical regions of the Old World, but several members have become much more widely distributed.

obscura group

This is a large group consisting of twenty-one species. We have pointed out in Chapter 2 that this array of species can be divided into two subgroups, chiefly on the basis of the number of teeth on the distal sex comb and the number of rows of the acrostichal hairs. One group of obscuralike forms has several teeth on this sex comb, and eight acrostichal rows, while the other has but a single tooth and

six acrostichal rows, and is composed of affinislike forms. Members of the latter subgroup are found in North America, with the exception of *helvetica* from Switzerland which morphologically is somewhat intermediate between the two subgroups. Of the other eight species of the affinislike forms, seven are endemic to the Nearctic region, and one (*azteca*) occurs in this and the Neotropical region. The obscura-like forms are divided between North America and the Old World. Four of them occur in North America, with *persimilis, miranda,* and *frolovae* endemic to the Nearctic region and *pseudoobscura* occurring in this and the Neotropical region (Figure 35). Seven of the eight Old World species of this subgroup are endemic to the Palaearctic region. A single species (*seguyi*) has been recorded from Africa of the Ethiopian region.

alagitans group

Only two species have been assigned to this species group (Table 11). Both occur in Mexico, but *alagitans* is restricted to the Nearctic region, while *capnoptera* is found in both the Nearctic and Neotropical areas of that country (Figure 32).

nannoptera group

The species *nannoptera* is the only one included in this group, which is therefore monotypic. It has been recorded from the Neotropical region of southern Mexico.

bromeliae group

As indicated in the preceding chapter, this group includes *bromeliae, bromelioides,* and probably *florae.* All three are endemic to the Neotropical region (Table 11).

Subgenus **Drosophila**

Tables 12 to 15 list 186 species belonging to this subgenus, 162 of which are included under twenty-two species groups.

quinaria group

The sixteen members of the quinaria group are largely from continental North America, with eleven species endemic to the Nearctic region (Table 12, Figure 22). The species *transversa* has also been

TABLE 12

Geographical distribution of nine species groups of the subgenus Drosophila

quinaria group

deflecta Malloch 1924 .....(1)
innubila Spencer 1943 .....(1)
limbata van Roser 1840 .....(3)
munda Spencer 1942 .....(1)
mutandis Tan, Hsu & Sheng 1949.(5)
nigromaculata Kikkawa & Peng 1938 .....(3)
occidentalis Spencer 1942 .....(1)
palustris Spencer 1942 .....(1)
phalerata Meigen 1830 .....(3)
quinaria Loew 1865 .....(1)
suboccidentalis Spencer 1942 ...(1)
subpalustris Spencer 1942 .....(1)
subquinaria Spencer 1942 .....(1)
suffusca Spencer 1943 .....(1)
tenebrosa Spencer 1943 .....(1)
transversa Fallén 1830 ..(1 + 3 + 5)

guttifera group

guttifera Walker 1849 .....(1)

pinicola group

pinicola Sturtevant 1942 .....(1)

virilis group

americana Spencer 1938 .....(1)
a. texana Patterson, Stone & Griffen 1940 .....(1)
lacicola Patterson 1944 .....(1)
littoralis Meigen 1830 .....(3)
imeretensis Sokolov 1948 .....(3)
montana Patterson & Wheeler 1942 .....(1)
novamexicana Patterson 1941b ..(1)
virilis Sturtevant 1916 .....(1 + 2 + 3 + 5)

testacea group

putrida Sturtevant 1916 .....(1)
testacea van Roser 1840 ....(1 + 3)

tripunctata group

crocina Patterson & Mainland 1944 .....(2)
mediopunctata Dobzhansky & Pavan 1943a .....(2)
mediosignata Dobzhansky & Pavan 1943a .....(2)
mediostriata Duda 1925a .....(2)
tripunctata Loew 1862 .....(1)
unipunctata Patterson & Mainland 1943 .....(1 + 2)

funebris group

funebris Fabricius 1787 .....(1–6)
macrospina Stalker & Spencer 1939 .....(1)
m. limpiensis Mainland 1941 ....(1)
m. ohioensis Spencer 1940 .....(1)
subfunebris Stalker & Spencer 1939 .....(1)
trispina Wheeler 1949b .....(1)

robusta group

cheda Tan, Hsu, & Sheng 1949 ..(5)
colorata Walker 1849 .....(1)
pullata Tan, Hsu, & Sheng 1949 ..(5)
robusta Sturtevant 1916 .....(1)
sordidula Kikkawa & Peng 1938..(3)

melanica group

afer Tan, Hsu, & Sheng 1949 ...(5)
melanica Sturtevant 1916 .....(1)
m. paramelanica Patterson 1943..(1)
melanissima Sturtevant 1916 ....(1)
"melanissima" Kikkawa & Peng 1938 .....(3)
melanura Miller 1944 .....(1)
micromelanica Patterson 1941a ..(1)
nigromelanica Patterson & Wheeler 1942 .....(1)
pseudomelanica Sturtevant 1916.(1)

reported from this region, as well as from Europe, Japan, and China. Since Fallén described *transversa* from Europe, his form must be regarded as the true *transversa,* while those listed under the same name

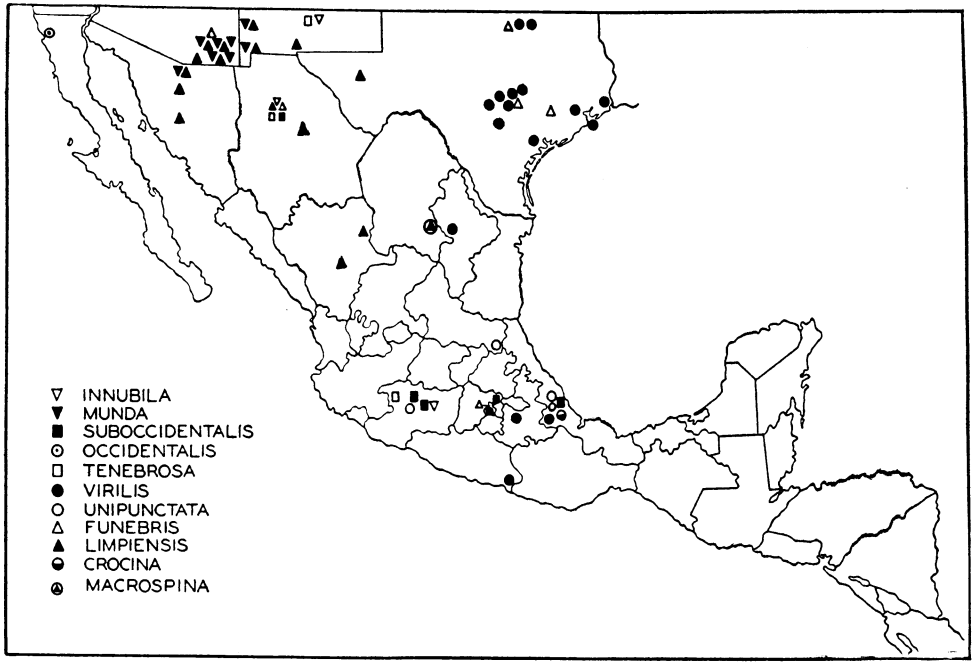

Fig. 22 Distribution of the known records of members of the quinaria, virilis, tripunctata, and funebris groups of the subgenus Drosophila.

from North America and from China and Japan probably represent similar but distinct species. However, two other species (*limbata, phalerata*) are endemic to Europe, and a third one (*nigromaculata*) from Japan is also from the Palaearctic region. The one from China (*mutandis*) is apparently endemic to the Oriental region.

guttifera group

The only known member of this group is *D. guttifera,* which is apparently restricted to the United States. We have records showing that it has been collected in the following states: Indiana, Massachusetts, New Jersey, Nebraska, District of Columbia, Virginia, North Carolina, South Carolina, Tennessee, Florida, Alabama, Louisiana, and Texas. Its distribution range is therefore in the eastern half of the United States.

pinicola group

This group consists of a single described species, *D. pinicola.* Sturtevant records it from various points in southern California.

virilis group

The eight described forms which constitute this group are, *virilis, montana, lacicola, americana,* represented by the subspecies *americana americana* and *americana texana, novamexicana, littoralis,* and *imeretensis* (Table 12). In 1942, a preliminary report was published on the geographical distribution of the forms then known to occur in the United States (Patterson, 1942d). Figure 23 presents a revised

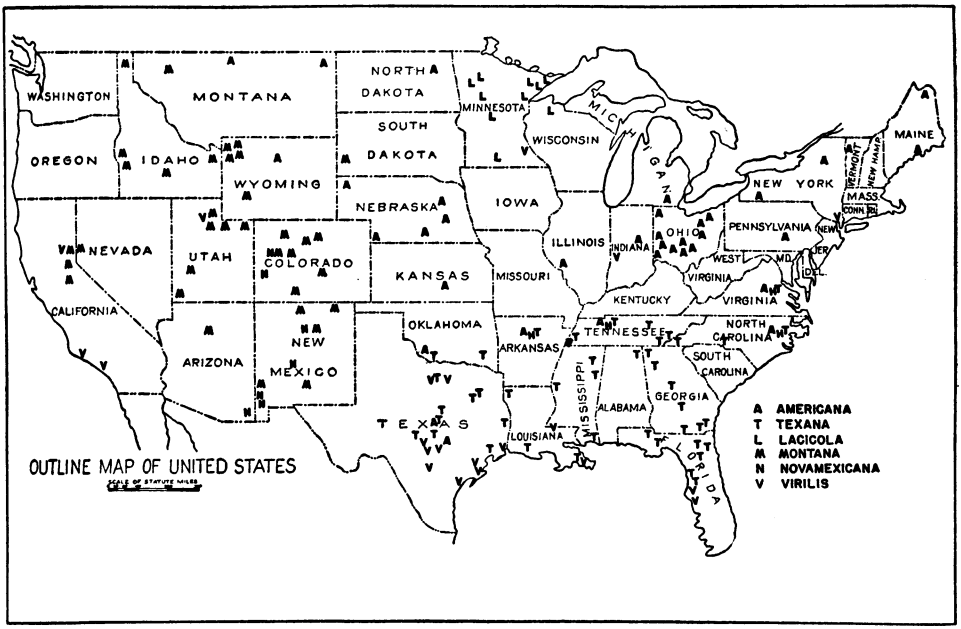

Fig. 23 Distribution of known records of members of the virilis group in the United States.

distribution map of all known records for the group for this same area.

Drosophila virilis occurs in the wild state in Japan and China, and is fairly common in both countries (Kikkawa and Peng, 1938; Tan, Hsu, and Sheng, 1949). It also occurs in North America, where it is rare and is found almost exclusively in domestic habitats. We have

records for this species from New York, Indiana, Tennessee, Florida, Louisiana, Texas, Utah, and California. In Mexico *virilis* has been collected at Monterrey in the state of Nuevo León, at Cordoba in the state of Veracruz, at Mexico City in the Distrito Federal, at Texmelucan in the state of Puebla, and at Ometepec near the southern tip of the state of Guerrero (Figure 22).

Dr. Marta Wedel of the Instituto de Genetica, Buenos Aires, Argentina, in a letter dated April 26, 1950, reported the discovery of *virilis* among some material received from the states of Chaco and Mendoza, in the interior of the Republic of Argentina. Dr. Wedel kindly sent a culture of the strain from Chaco, and the results obtained from cross tests demonstrated that it represents a geographic strain of *D. virilis* Sturtevant. This is the first record of the occurrence of this species in South America. It was probably introduced into that country through some human agency. These records show that *virilis* occurs in both the Nearctic and Neotropical regions.

In contrast to *virilis,* the other members of the group from the United States have invariably been found outside the limits of towns and cities in wild habitats. None of them has ever been taken south of the border between the United States and Mexico. It is probable that the distribution ranges of *americana, montana,* and *lacicola* extend up into Canada, since all three species have been collected near the border.

The known distribution area of *lacicola* in the United States is restricted to the states of Wisconsin and Minnesota. This species has always been collected at or near fresh-water lakes.

The distribution range of *montana* lies within the Rocky Mountain System, where it occurs at elevations of from forty-five hundred to ten thousand feet, with the large majority of the specimens being collected at sixty-five hundred feet or above. It has been collected in New Mexico, Arizona, California, Colorado, Nevada, Utah, Wyoming, South Dakota, Idaho, Montana, Oregon, and Washington.

The species *novamexicana* has been collected at six different localities, as follows: at one point in southeastern Arizona, at four different places in New Mexico, and at one point in western Colorado (Figure 23). Its distribution area overlaps that of *montana,* but the latter species occurs at a higher altitude.

The two subspecies exhibit interesting distribution patterns. The distribution area of *texana* includes all of the states bordering on the

Gulf of Mexico, as well as some that face the Atlantic Ocean. Thus *texana* has been recorded from Texas, Louisiana, Mississippi, Alabama, Florida, and Georgia. North of this area is another tier of states in which the distribution range of *texana* comes in contact with that of *americana,* forming an overlapping zone. This latter group of states includes Oklahoma, Arkansas, Tennessee, North Carolina, and Virginia (Figure 23). It will be observed that the distribution area of *texana* coincides with the major part of the Lower Austral life zone of Merriam.

The distribution range of *americana* extends northward in two directions from the zone of contact with the range of *texana.* In the eastern branch *americana* has been collected in Illinois, Michigan, Indiana, Ohio, Pennsylvania, New York, Vermont, and Maine. It has been taken in the western branch in Kansas, Nebraska, South Dakota, North Dakota, Wyoming, and Montana. In a degree, the eastern and western *americanas* represent two different genotypes.

There is not too much known about the distributions of the two Palaearctic species, *littoralis* and *imeretensis.* As was pointed out in the preceding chapter, *imeretensis* was first collected at Imeretia, which is located in the southern part of Georgian USSR, between the Black and Caspian Seas. It also occurs farther north in the Moscow region. The known distribution range of *littoralis* lies in Europe, where it was originally recorded from Germany (Meigen, 1830). Other records known to us are from Merlingen, Vitznau, and Kulm Aargau in Switzerland; from Oloron in France (Burla); from Styria in Austria (Mainx); and from northern Italy (Buzzati-Traverso).

testacea group

The two species belonging to this group are *testacea* and *putrida.* The former occurs in Europe and in Nearctic North America, while the latter has been recorded from the United States alone. In Figure 24 are plotted all of the known records of these two species for the United States. The distribution area of *putrida* is similar to those which have been observed for a number of other Drosophila species. It closely coincides with the distribution of the deciduous forests of eastern United States. This area begins in New England and extends in a southwesterly direction into eastern Oklahoma and Texas, with only a few records of *putrida* found west of the Mississippi River and north of these two states. As a matter of fact, we have collected this

species at only five points (all in Texas) west of the ninety-ninth meridian. The number of records for *testacea* in this country is much smaller than that of *putrida,* but they are sufficient to show that its distribution area lies in the northern half of the United States. It exends from Maine to Idaho in an east-west direction along the northern border of this country, and southward as far as the Great Smoky Mountains in Tennessee. Both of these species probably occur in Canada.

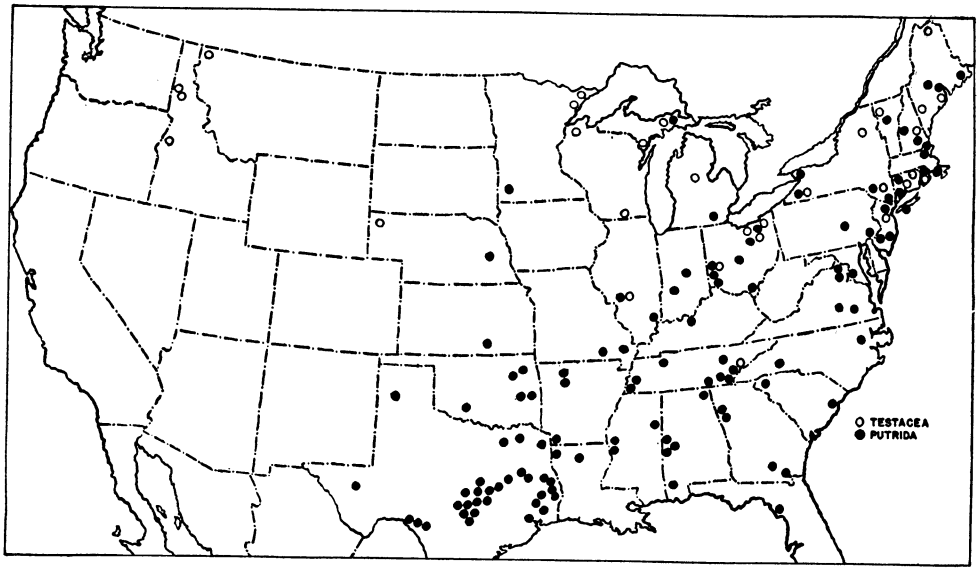

Fig. 24 Distribution of known records of members of the testacea group in the United States. (From Patterson and Wagner.)

tripunctata group

Members of the tripunctata group have been recorded from both North and South America (Table 12). The species *tripunctata* is endemic to the Nearctic region, but has not been reported from outside the United States. Two other species, *crocina* and *unipunctata,* occur in Mexico; the first is from the state of Veracruz in the Neotropical region, and the second from the states of Veracruz, San Luis Potosí, and Michoacán in both the Nearctic and Neotropical regions (Figure 22). The three remaining species, *mediopunctata, mediosignata,* and *mediostriata,* were all described from Brazil in the Neotropical region.

funebris group

In discussing the geographical distribution of the funebris group, we shall consider all six members as outlined in the preceding chapter. With the exception of *funebris,* which is a cosmopolitan species, the other forms are endemic to the Nearctic North America. Their known records are plotted in Figure 25. The distribution records of *funebris* are fairly evenly scattered over the United States, where it occurs in both domestic and wild habitats. The distribution range of *subfunebris* is restricted to a small area along the coast of California, and that of *trispina* is known for a single locality near Earp in the same state.

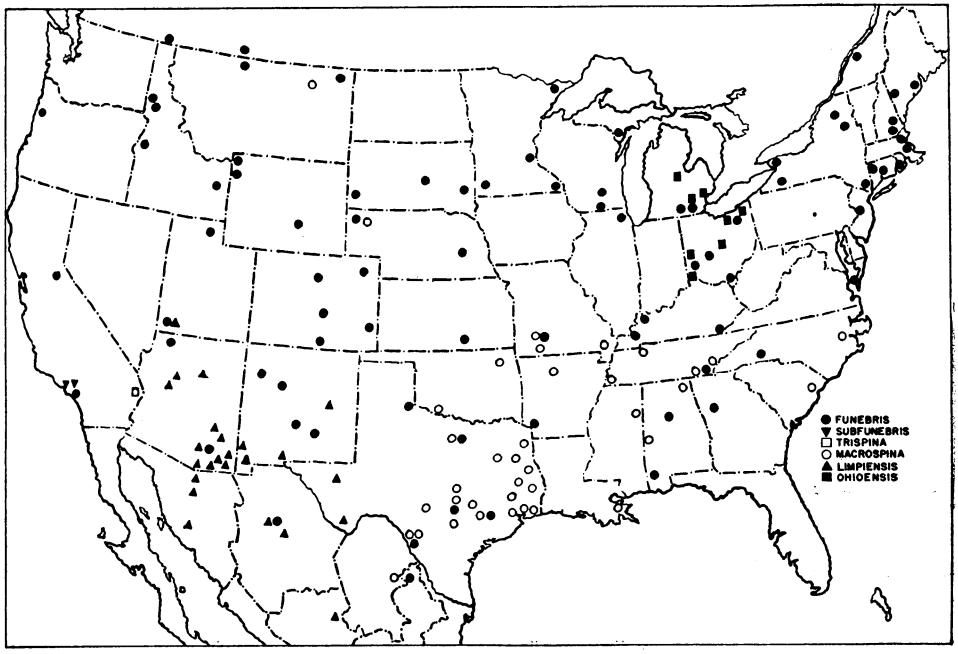

Fig. 25 Distribution of known records of members of the funebris group in the United States. (Modified after Patterson and Wagner.)

The three subspecies forming the *macrospina* complex show a very interesting distribution pattern. The distribution range of the subspecies *limpiensis* lies west of the 103rd meridian. It has been recorded from the southern part of Utah, Arizona, New Mexico, and west Texas in the United States, and in Mexico has been collected in the states of Sonora, Chihuahua, and Durango (Figure 22). The

western limit of the distribution range of the subspecies *macrospina* is located at about the hundredth meridian, and extends eastward to the Atlantic coast across the southern half of the United States. It has also been collected in the state of Coahuila in Mexico and at two widely separated points north of its main range, one in northwestern Nebraska and the other in northern Montana. The third subspecies, *ohioensis,* was established by Spencer (1940). Its known distribution centers in Ohio and Michigan.

The geographical distribution of the members of this group is of interest for two reasons. In the first place, the *macrospina* series exhibits a definite geographical east-west gradient with respect to the degree of cross-sterility (Mainland, 1942b). This was found to be true not only between the subspecies, but also between geographical strains of the subspecies, especially those of *macrospina* and *limpiensis.* This point will be discussed in a later chapter. In the second place, the group in several respects resembles the virilis species group. The group as such clearly evolved in Nearctic North America, as did most of the virilis group. Moreover, it also has one member that is found beyond the limits of this region, although *funebris* is world-wide in its distribution, while *virilis* occurs in but four of the six geographical regions.

repleta group

Fifty-two different forms have been placed in this species group, including three pairs of subspecies. Forty-nine full species are listed in Table 13. An inspection of this list shows that fifteen species are endemic to the Nearctic region and fifteen to the Neotropical, and that thirteen are common to both regions. This makes a total of forty-three recorded for these two regions. Of the remaining six, two are cosmopolitan (*hydei, repleta*); one occurs in both the Neotropical and Palaearctic (*buzzatii*) regions; one is from the Nearctic, Neotropical, and Australian regions (*mercatorum*); and two have been reported by Malloch from Australia (*obsoleta, poecilithorax*). Therefore, all but two of the forty-nine species listed in the table are represented in the Nearctic and Neotropical regions, indicating clearly that, as a group, this complex of species was originally native to and evolved in the New World. Only a few of them have spread out from here and are now found in other parts of the world.

But this does not tell the entire story. In order to make this point

clear, we are introducing five distribution maps from a previous publication (Patterson and Mainland, 1944), which illustrates their concentration in and around the transition tract between the Nearctic and Neotropical regions in Mexico (Figures 26 to 30).

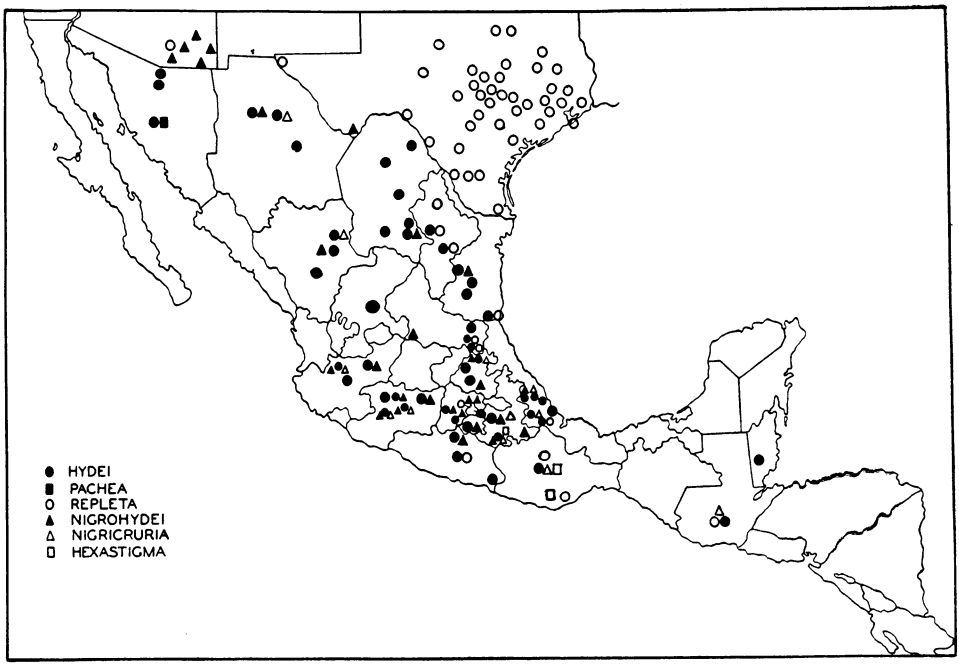

Fig. 26 Distribution of the known records of six members of the repleta group.

We have divided the majority of the members of this group into four subgroups, of which the first is known as the hydei subgroup and consists of six species. Aside from the cosmopolitan *hydei,* the distributions of the other five members are as follows: (1) *bifurca,* as shown in Figure 27, has been reported from Texas, Arizona, and several of the states in Mexico, chiefly from the Nearctic region; (2) *hydeoides* is restricted to the Nearctic part of Mexico, where it has been collected in the states of Neuvo León, Michoacán, Guerrero, Puebla, and the Distrito Federal (Figure 27); (3) *nigrohydei* is found in southeastern Arizona, West Texas, and twelve states and the Distrito Federal in Mexico (Figure 26); (4) *pachea* was collected at a single locality in Sonora, Mexico (Figure 26); and (5) *novemaristata* was described from Peru by Dobzhansky and Pavan (1943a).

TABLE 13

Geographical distribution of members of the repleta group

aldrichi Patterson & Crow 1940 .....(1 + 2)
anceps Patterson & Mainland 1944 .....(1)
arizonensis Patterson & Wheeler 1942 .....(1)
betari Dobzhansky & Pavan 1943a .....(2)
bifurca Patterson & Wheeler 1942 .....(1 + 2)
brevicarinata Patterson & Wheeler 1942 .....(2)
brunneipalpa Dobzhansky & Pavan 1943a .....(2)
buzzatii Patterson & Wheeler 1942 .....(2 + 3)
californica Sturtevant 1923 ..(1 + 2)
canapalpa Patterson & Mainland 1944 .....(1)
fasciola Williston 1896 .....(2)
fascioloides Dobzhansky & Pavan 1943a .....(2)
fuliginea Patterson & Wheeler 1942 .....(1)
fulvimacula Patterson & Mainland 1944 .....(1 + 2)
f. flavorepleta Patterson & Pavan 1951 .....(2)
hamatofila Patterson & Wheeler 1942 .....(1 + 2)
hexastigma Patterson & Mainland 1944 .....(1)
hydei Sturtevant 1921b .....(1–6)
hydeoides Patterson & Wheeler 1942 .....(1)
icteroscuta Wheeler 1949b .....(1)
inca Dobzhansky & Pavan 1943a.(2)
leonis Patterson & Wheeler 1942 .....(1 + 2)
limensis Pavan & Patterson 1947.(2)
linearepleta Patterson & Wheeler 1942 .....(1 + 2)
longicornis Patterson & Wheeler 1942 .....(1 + 2)
maculipennis Duda 1925b .....(2)
mainlandi Patterson 1943 .....(1)
melanopalpa Patterson & Wheeler 1942 .....(1)
meridiana Patterson & Wheeler 1942 .....(1)
m. rioensis Patterson 1943 ..(1 + 2)
mercatorum Patterson & Wheeler 1942 .....(1 + 2 + 6)
m. pararepleta Dobzhansky & Pavan 1943a .....(2)
mojavensis Patterson & Crow 1940 .....(1)
mojú Pavan 1950 .....(2)
mulleri Sturtevant 1921b ...(1 + 2)
neorepleta Patterson & Wheeler 1942 .....(2)
nigricruria Patterson & Mainland 1943 .....(1 + 2)
nigrohydei Patterson & Wheeler 1942 .....(1 + 2)
nigrospiracula Patterson & Wheeler 1942 .....(1)
novemaristata Dobzhansky & Pavan 1943a .....(2)
obsoleta Malloch 1923 .....(6)
onca Dobzhansky & Pavan 1943a.(2)
pachea Patterson & Wheeler 1942 .....(1)
paranaensis de Barros 1950 .....(2)
peninsularis Patterson & Wheeler 1942 .....(1)
poecilithorax Malloch 1925 .....(6)
racemova Patterson & Mainland 1944 .....(1 + 2)
ramsdeni Sturtevant 1916 .....(2)
repleta Wollaston 1858 .....(1–6)
ritae Patterson & Wheeler 1942 .....(1 + 2)
spenceri Patterson 1943 .....(1)
subviridis Patterson & Mainland 1943 .....(1)

The melanopalpa subgroup contains thirteen repletalike species. Four of these are from South America: *limensis* is from Peru; *onca, brunneipalpa,* and *fascioloides* were described from the state of São

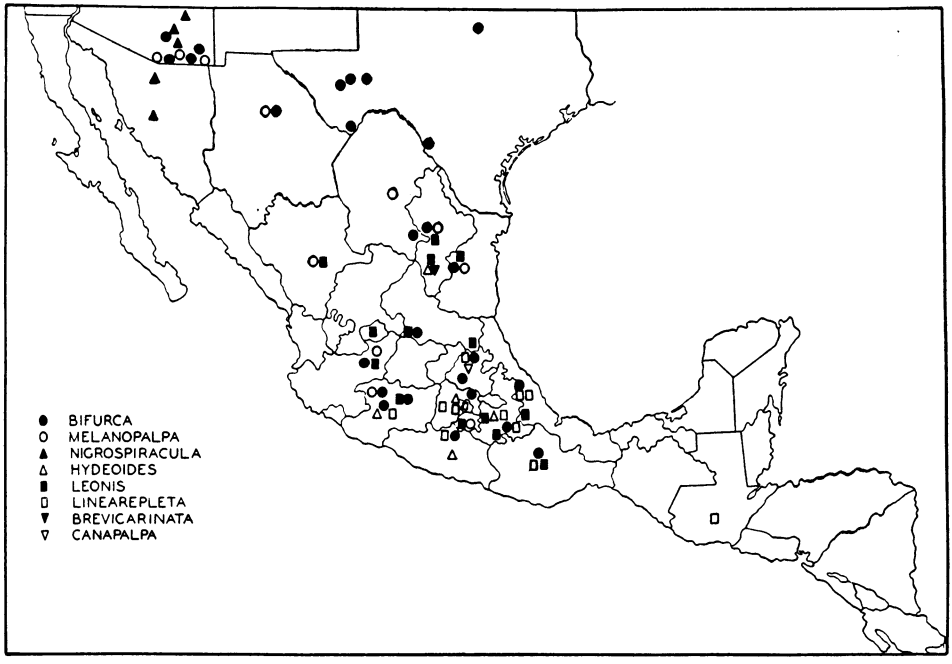

Fig. 27 Distribution of the known records of eight members of the repleta group, mainly from Mexico.

Paulo, Brazil. Exclusive of the cosmopolitan *repleta,* the distribution of the remaining species is as follows: *californica* has been recorded from California and from the state of Puebla, Mexico, and *fuliginea* from New Mexico; *neorepleta* is from Guatemala; *canapalpa* is known only from the state of Hidalgo; *nigrospiracula* is recorded from southern Arizona and from the state of Sonora (Figure 27); the subspecies *fulvimacula* has been collected in these five states of Mexico, San Luis Potosí, Hidalgo, Veracruz, Guerrero, and Oaxaca (Figure 30); the other subspecies, *flavorepleta,* has been reported from Brazil; *melanopalpa* is from southern Arizona, and the Mexican states of Chihuahua, Durango, Coahuila, Nuevo León, Tamaulipas, Jalisco, Michoacán, and Morelos (Figure 27); and *linearepleta* is from Guatemala and seven states of Mexico (Figure 27).

There has been included in the mulleri subgroup a total of seventeen different forms, including one pair of subspecies. We may first

consider the distribution of five similar species, namely, *mulleri, aldrichi, arizonensis, mojavensis,* and *spenceri* (Figure 28). For the most part, these species breed on cacti, and their ranges are deter-

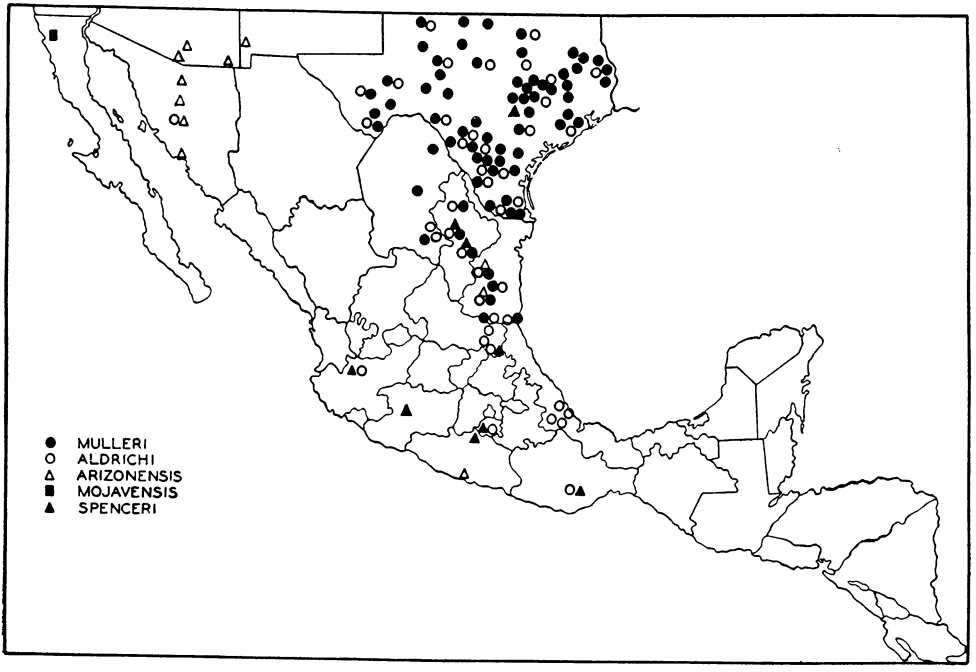

Fig. 28 Distribution of the known records of five members of the mulleri subgroup.

mined to a considerable extent by the distribution of these plants. The distribution ranges of *mulleri* and *aldrichi* coincide over a considerable portion of Texas and the eastern part of northern Mexico. Both species have also been collected in Oklahoma, and in addition *mulleri* has been taken in Arkansas, Louisiana, and at the southern tip of Florida. In their southward extension into Mexico, the range of *aldrichi* reaches beyond the southern limits of that of *mulleri.* The main distribution area of *arizonensis* lies in the southwestern corner of New Mexico, the southeastern corner of Arizona, and the state of Sonora in Mexico. It has also been taken at two widely separated localities, one in the state of Tamaulipas, the other in Guerrero on the coast. The distribution of *mojavensis* is largely limited to the deserts in California, but its range extends south into the northern part of Baja California. The distribution area of *spenceri* also overlaps those of *mulleri* and *aldrichi,* especially that of the latter species. Two

specimens of this species were collected at Georgetown, in Texas. On the basis of current knowledge, *mojavensis* is geographically isolated from the other four species; and to a considerable extent this is true also for *arizonensis,* except for the two localities noted above. Although the distribution ranges of the other three species overlap, they are nevertheless able to retain their identity through reproductive isolation.

The distribution of six other species classed under the mulleri subgroup is shown in Figure 29. *Drosophila buzzatii* may be consid-

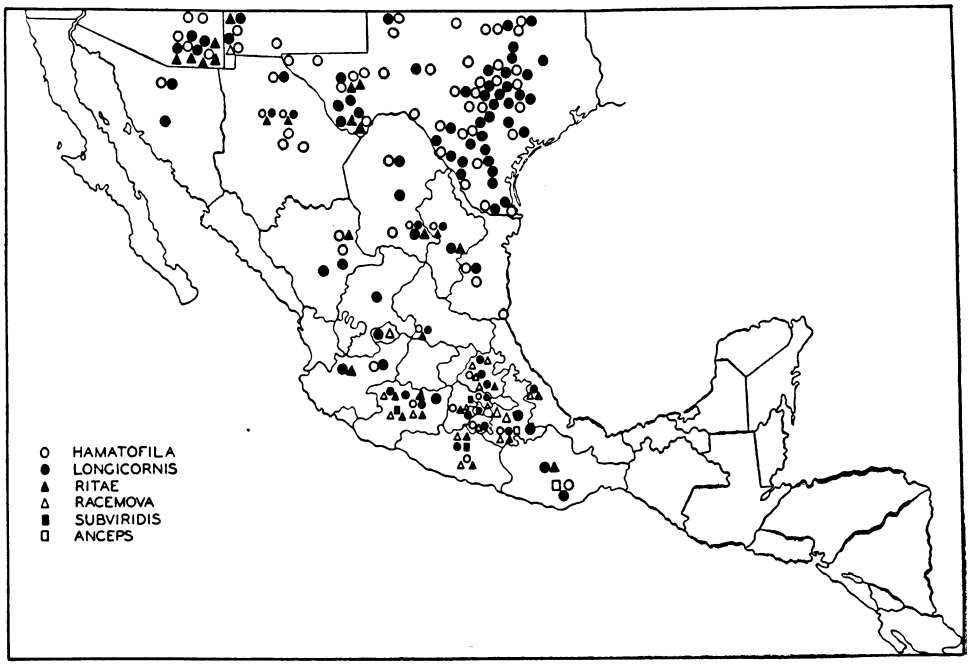

Fig. 29 Distribution of the known records of six members of the mulleri subgroup.

ered here. This species was described in 1942 (Patterson and Wheeler) from stocks derived from flies collected in Argentina and in Sicily. It has since been reported from Brazil and from other points in Europe. It therefore occurs in both the Neotropical and Palaearctic regions. The distribution ranges of *hamatofila* and *longicornis* cover approximately the same general area, which includes most of Texas, the southern parts of New Mexico and Arizona, and as far south as the Mexican states of Michoacán and Guerrero. The distribution range of *ritae* covers much of the same area, except in Texas, where it oc-

curs in the extreme western part of the state. The distribution areas of the other three species (*racemova, subviridis, anceps*) are very much restricted, being confined largely to a tract extending along the boundary between the Nearctic and Neotropical regions across the country from the Gulf of Mexico to the Pacific Ocean, at about the level of 19° north latitude.

The distribution of two of the four remaining species of the mulleri subgroup requires but little comment; *mainlandi* has been recorded from California, and *peninsularis* from Florida. The species *hexastigma* is known from the states of Puebla and Oaxaca, in southern Mexico (Figure 26). The distribution of the pair of subspecies, *meridiana meridiana* and *meridiana rioensis,* is still to be considered. Their distribution ranges are shown in Figure 30. Their distribution areas overlap to a considerable extent, although that of *meridiana* occurs mainly in Texas and northern Mexico, while that of *rioensis* lies mainly in Mexico. It will be observed from the map that *rioensis* largely replaces *meridiana* in the southern part of their ranges.

The mercatorum subgroup consists of three forms, a pair of subspecies, *mercatorum mercatorum* and *mercatorum pararepleta,* and *D. paranaensis.* The two forms of *mercatorum* present a very interesting distribution pattern. The subspecies *mercatorum* was first collected in produce houses at Santa Barbara and Bell in California, by Dr. G. B. Mainland in 1940. It was later found in the same type of habitat at Tucson, Arizona, and in New Orleans, Louisiana. Material sent by Dr. E. C. Zimmerman from Hawaii proved to be the same species. It has also been collected at several points in Mexico. Finally, upon examination, specimens from Guatemala and Costa Rica in Central America proved to be the same species (Figure 30). The subspecies *pararepleta* occurs in Brazil and has been taken as far north as Belém in the state of Para (Dobzhansky and Pavan, 1943a). It is interesting to note that the subspecies *mercatorum,* while expanding north from central Mexico, changed its habitat from wild to domestic. It was introduced in Hawaii and must have reached there through shipments from California. The known distribution ranges of these two forms are widely separated geographically, but future collecting may bring them closer together. *Drosophila paranaensis* has been recorded from several different points in Brazil.

UNCLASSIFIED SPECIES. There are twelve species of the repleta group which could not be placed in any of the established species

groups. Their distributions are as follows: *inca* and *maculipennis* (Duda) both occur in Peru; *betari* occurs in São Paulo, Brazil; *mojú* is from Belém, Brazil, *fasciola* was described from St. Vincent by

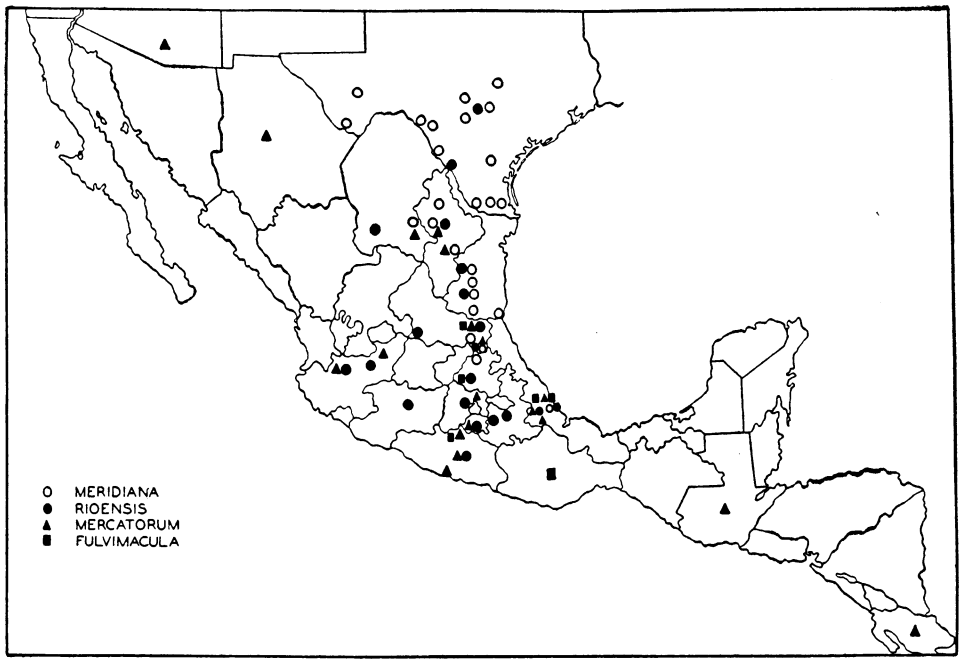

Fig. 30 Distribution of the known records of four members of the repleta group.

Williston; *ramsdeni* was reported from Cuba; *obsoleta* and *poecilithorax* are from Australia. All other four species occur in Mexico: *brevicarinata* from Nuevo León (Figure 27), *icteroscuta* from Michoacán, *leonis* from central Mexico (Figure 27), and *nigricruria* ranges from northern Mexico to Guatemala (Figure 26).

robusta group

The five known species assigned to this group occur in three different geographical regions (Table 12). Two of them, *robusta* and *colorata,* are endemic to the Nearctic region, where their ranges appear to be restricted to the eastern half of the United States. Two other members, *cheda* and *pullata,* have been reported from China in the Oriental region. The fifth species, or *sordidula,* has been collected in several of the Japanese Islands in the Palaearctic region.

Sturtevant (1942) has expressed some doubt as to whether this species should be placed in the *robusta* group, but the fact that since then two other members of the group have been found in China would seem to justify its inclusion.

melanica group

Seven of the nine members of this group are endemic to Nearctic North America, including the subspecies *melanica melanica* and *melanica paramelanica* (Table 12). The distribution area of the subspecies *paramelanica* occupies the northeastern part of the United States down to about the 37° north latitude. From here on south and southwest it is replaced by the subspecies *melanica,* which ranges on down into northern Mexico and west into Colorado, New Mexico and Arizona, with a single record from the southwestern corner of Utah (Figure 31). The species *melanura* was described by Miller

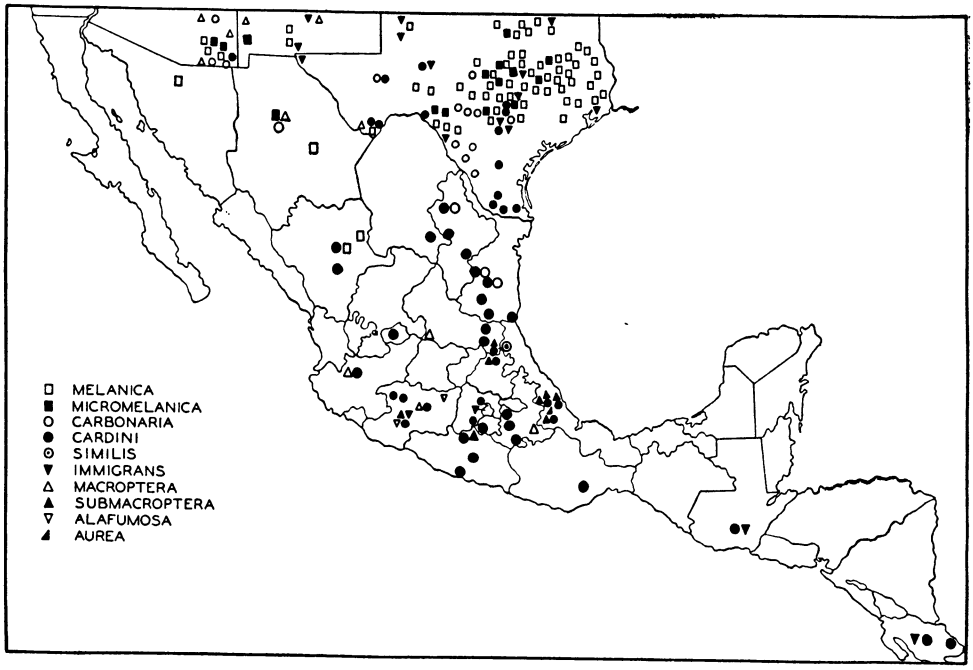

Fig. 31 Distribution of the known records of members of the melanica, carbonaria, cardini, immigrans, and macroptera groups.

(1944) from New York, and Dr. Wheeler and Mr. Hsu collected it at Guarette, Maine.

The main distribution range of *micromelanica* lies in southwestern

United States, with one record from the state of Chihuahua, Mexico (Figure 31). It has also been found in the Great Smoky Mountains of Tennessee and at five different localities in Florida. The distribution of *nigromelanica* is widely scattered, as is indicated by the following list of states where it has been found: Illinois, Indiana, Ohio, Pennsylvania, New York, Rhode Island, Connecticut, Virginia, North Carolina, South Carolina, Florida, Alabama, Mississippi, Arkansas, Missouri, Oklahoma, and Texas. The typical habitat of this species is the deciduous forest within its distribution range, but it is never very abundant in any given locality.

The distribution range of *pseudomelanica,* which is a somewhat doubtful member of the group, is rather limited. Sturtevant (1921b), in connection with his description of this species, records it from Maryland and Virginia, and we do not know of any other records. The rare species, *melanissima,* has a widely scattered distribution. Sturtevant (1921b) records it from North Carolina, Georgia, Florida, and Alabama. In the last state he collected this species from moist sawdust made from a living pine tree by a boring beetle, and suggests that it was breeding in this sawdust. We have also collected this species in both Georgia, Mississippi, and Florida, and in addition in the pine woods of East Texas, where in one locality it was collected from a pile of pine sawdust.

The two species of this group not found in North America are *"melanissima"* of Kikkawa and Peng from Japan, and *afer* from China. There is now no doubt that the first species is distinct from the American species of the same name. Kikkawa and Peng (1938) record it from Kyôto and Kôbe, on the main island Hondo of Japan. The second species is recorded from Hangchow and Meitan in Oriental China by Tan, Hsu, and Sheng (1949).

polychaeta group

Of the three members of this group (Table 14), *polychaeta* was collected at Galveston, Texas; but, as was pointed out in the preceding chapter, it is doubtful whether it should be regarded as endemic to the Nearctic region. *D. illota* was described by Williston from St. Vincent in the Neotropical region. The third member, *grandis,* was reported from Kôhu and Kyôto, in the Japanese Island of Hondo, and is from the eastern Palaearctic region.

carbonaria group

This group contains but a single species, *carbonaria* (Table 14). In the United States it has been recorded from several different localities in Texas, Oklahoma, and Arizona; and a single female has been found in Death Valley, California (Sturtevant, collector). In Mexico we have collected this species at Santa Clara in Chihuahua, Sabinas Hidalgo in Nuevo León, and Güemez and Hidalgo in Tamaulipas (Figure 31). Its distribution area is therefore limited to southwestern United States and northern Mexico, and falls within the range of the common mesquite tree (*Prosopis glandulosa* Torr.). This is not surprising, since Dr. M. R. Wheeler found it breeding in the sap from bleeding mesquite trees at his home here in Austin. It is one of the few instances in which the specific food upon which a given species of Drosophila lives has been definitely determined, and serves to indicate that its range may be determined by the distribution of the food media. In spite of the fact that we have made extensive collections over the range of *carbonaria* and surrounding regions, no similar species has ever been found, thus indicating that it belongs to a distinct monotypic group.

cardini group

The difficulty concerning the taxonomy of members of the cardini group was referred to in Chapter 2. On the basis of Wheeler's study, we have listed ten species for the group (Table 14). The exact distribution of some of these is somewhat uncertain. The localities plotted on the map of distribution to indicate *cardini* (Figure 31) do not all belong to that species, but we do have known records for it from Texas, Arizona, and perhaps Florida in the United States, and from Monterrey, Atlixco, Durango, Oaxaca, and Mexico City in Mexico. Moreover, we have good records for *similis* from Florida and from the state of Veracruz, and one for *neocardini* at Atlixco. Perhaps most of the other indicated localities on the map represent *cardinoides,* including the one in Guatemala. Both *neocardini* and *cardinoides* occur in Brazil, as do also *bandeirantorum, polymorpha, campestris,* and *prosimilis,* which was originally described from Peru by Duda. *D. albirostris* occurs in Panama, and Sturtevant described *cardini* and *metzii* from Cuba, where he also reported the presence of *similis.* The last-named species, however, was described from St. Vincent by Williston.

TABLE 14

Geographical distribution of members of the polychaeta, carbonaria, cardini, immigrans, macroptera, rubrifrons, and annulimana species groups

polychaeta group

grandis Kikkawa & Peng 1938 ...(3)
illota Williston 1896 .....(2)
polychaeta Patterson & Wheeler 1942 .....(1)

carbonaria group

carbonaria Patterson & Wheeler 1942 .....(1)

cardini group

albirostris Sturtevant 1921b .....(2)
bandeirantorum Dobzhansky & Pavan 1943a .....(2)
campestris Burla 1950 .....(2)
cardini Sturtevant 1916 .....(1 + 2)
polymorpha Dobzhansky & Pavan 1943a .....(2)
metzii Sturtevant 1921b .....(2)
neocardini Streisinger 1946 ..(1 + 2)
polymorpha Dobzhansky & Pavan 1943a .....(2)
prosimilis Duda 1925b .....(2)
similis Williston 1896 .....(1 + 2)

immigrans group

annulipes Duda 1924a .....(5)
balneorum Sturtevant 1927 .....(5)
hexastriata Tan, Hsu, & Sheng 1949 .....(5)
immigrans Sturtevant 1921b ..(1–6)
komaii Kikkawa & Peng 1938 .....(3 + 5)
maculifrons Duda 1925b .....(2)
monochaeta Sturtevant 1927 ....(5)
nasuta Lamb 1914 .....(4 + 5)
nixifrons Tan, Hsu, & Sheng 1949.(5)
ruberrima de Meijere 1911 .....(5)
rubra Sturtevant 1927 .....(5)
setifemur Malloch 1924b .....(6)
signata Duda 1923 .....(5)
spinofemora Patterson & Wheeler 1942 .....(6)
subfasciata de Meijere 1914 .....(5)
virgata Tan, Hsu, & Sheng 1949 ..(5)
willowsi Curran 1936 .....(6)

macroptera group

alafumosa Patterson & Mainland 1943 .....(1)
aurea Patterson & Mainland 1944.(2)
macroptera Patterson & Wheeler 1942 .....(1)
magnabadia Patterson & Mainland 1943 .....(1)
submacroptera Patterson & Mainland 1943 .....(1 + 2)

rubrifrons group

nubiluna Wheeler 1949b .....(1)
rubidifrons Patterson & Mainland 1944 .....(1)
rubrifrons Patterson & Wheeler 1942 .....(1)
spadicifrons Patterson & Mainland 1944 .....(1)
uninubes Patterson & Mainland 1943 .....(1 + 2)

annulimana group

annulimana Duda 1925b .....(2)
araicás Pavan & Nacrur 1950 ...(2)
arapuan Pavan & da Cunha 1947.(2)
ararama Pavan & da Cunha 1947.(2)
arassari da Cunha & Frota-Pessoa 1947 .....(2)
araúna Pavan & Nacrur 1950 ....(2)
gibberosa Patterson & Mainland 1943 .....(1 + 2)

The difficulty of giving a satisfactory account of the distribution of the cardini group would be intrinsic for any large species group occurring in the tropics, because of the scarcity of good records. The evidence all indicates that this group was originally native to tropical South America. From there it expanded, chiefly northward, with part of the population reaching Florida by the way of the West Indies, and part coming to Texas through Central America and Mexico.

immigrans group

The seventeen species assigned to this group, together with their regional distributions, are listed in Table 14. They have mostly been reported from the Oriental region; and if the cosmopolitan *immigrans* is included, a total of thirteen species are represented in that region. Two of these are also found in other regions: *komaii,* from China and from Japan of the eastern Palaearctic; and *nasuta,* originally described from Seychelles of the Ethiopian region by Lamb, which has also been recorded from Formosa. Three species, *setifemur, willowsi,* and *spinofemora,* are from the Australian region; and *maculifrons* was described by Duda from Peru and Brazil of the Neotropical region. The last species and the cosmopolitan *immigrans* are the only members which so far have been recorded from the Americas, with *immigrans* as the sole representative of the group for the Nearctic region.

macroptera group

The five species of this group are *macroptera, submacroptera, aurea, alafumosa,* and *magnabadia* (Table 14). The distribution localities of the first four are given in Figure 31, and those of the last one in Figure 33. *D. macroptera* occurs in Utah, Colorado, Arizona, New Mexico, and Texas in the United States. In Mexico it was found in the states of Chihuahua, San Luis Potosí, Puebla, Michoacán, and Jalisco. The distribution area of *submacroptera* is more restricted. It has been recorded from San Luis Potosí, Hidalgo, Veracruz, Michoacán, and Guerrero, and therefore occurs in both the Nearctic and Neotropical regions of Mexico. The distribution range of *alafumosa* is confined to the Nearctic part of Michoacán. *D. aurea* occurs only in the state of Veracruz. Finally, *magnabadia* has been collected in the Distrito Federal, Puebla, and the northern part of Michoacán.

rubrifrons group

The five members of the rubrifrons group are *rubrifrons, rubidifrons, spadicifrons, uninubes,* and *nubiluna.* The distribution of the first three species are shown in Figure 32, while that of *uninubes* is

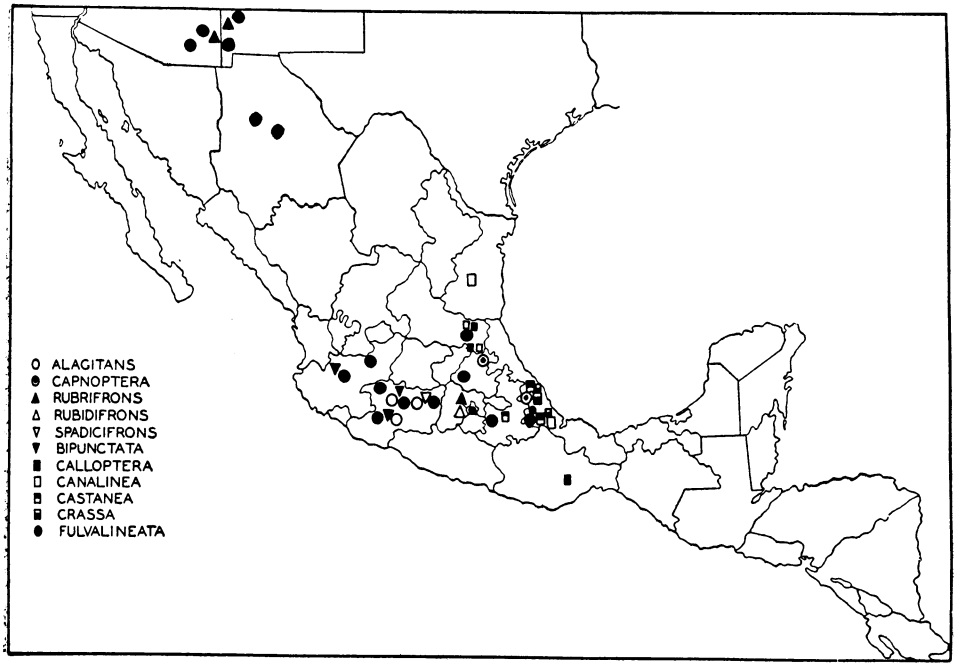

Fig. 32 Distribution of the known records of members of the alagitans and rubrifrons groups, and six other species of the subgenus Drosophila.

given in Figure 33. The distribution of *nubiluna* is not plotted on any of the figures, but the type material consisted of a pair of flies taken about nineteen miles east of Morelia in Michoacán. Of the other four species, *rubidifrons* was collected in the state of Mexico, *spadicifrons* in Michoacán, and *uninubes* in the states of Aguascalientes, Veracruz, Michoacán, Zacatecas, and Puebla (Figure 33). The distribution of *rubrifrons* covers a wider area, as it has been collected in the Distrito Federal in Mexico, and in New Mexico and Arizona in the United States. All of these species are found in the Nearctic region, with *uninubes* also present in the Neotropical (Table 14).

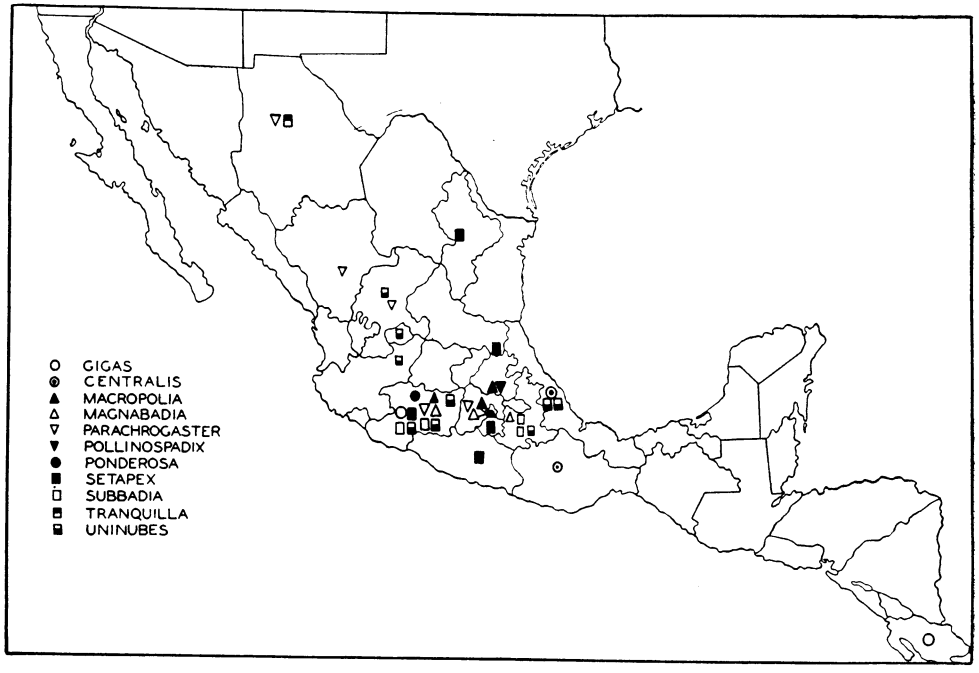

Fig. 33 Distribution of the known records of eleven species of the subgenus Drosophila.

annulimana group

The names and regional distribution of the seven members of this group are listed in Table 14. The distribution areas of *annulimana, arapuan, ararama,* and *arassari* all occur in Brazil, but the first species was described by Duda from Bolivia. The recently described *araicás* and *araúna* are from Brazil. The distribution range of *gibberosa* falls within the limits of Mexico, where it has been recorded from the states of San Luis Potosí, Veracruz, Tamaulipas, Mexico, Morelos, Michoacán, and Guerrero. This species is widely separated geographically from the other six members. It is possible that future collections from the intervening region will reveal the presence either of some of these species, or new members of the group.

melanderi group

The two members of this group are *magnafumosa* and *melanderi* (Table 15). The distribution range of the first species seems to center in the Smoky Mountains National Park, Tennessee, for it was described from a single specimen collected there off fungus (Stalker

and Spencer, 1939), and we have since collected a total of nineteen specimens from the same park. The second species was described from the state of Washington (Sturtevant, 1916). In July 1947, Dr. M. R. Wheeler and Mr. F. A. Cowan collected this same species in the Superior National Forest near Ely, Minnesota, and in the Glacier National Park in the northwestern corner of Montana. These records would indicate that the range of *melanderi* extends along the northern border of the United States from Washington to Minnesota.

bizonata group

Of the three species placed in this group (Table 15), *bizonata* was collected by Kikkawa and Peng (1938) at two localities in the Japanese island of Hondo, so that it is a Palaearctic species. The other two (*heterobristalis, meitanensis*) have been recorded from Meitan, China, thus placing them in the Oriental region (Tan, Hsu, and Sheng, 1949).

guaraní group

Either six or seven species belong to this group, as indicated in Chapter 2. The doubtful member is *ornatifrons.* The list includes *griseolineata, guarajá, guaramunú, guaraní, guarú, ornatifrons,* and *subbadia* (Table 15). The first six species in the list have all been recorded from Brazil of the Neotropical region. The seventh member (*subbadia*) is also from the Neotropical, but has been found in the states of Michoacán and Puebla, Mexico (Figure 33). No other member of this group has been reported for the intervening regions.

pallidipennis group

This group includes the pair of subspecies *pallidipennis pallidipennis* and *pallidipennis centralis.* The first subspecies was collected by Dr. Pavan at two localities in Brazil, and the second subspecies has been recorded from the states of Veracruz and Oaxaca in Mexico (Figure 33). Both, therefore, occur in the Neotropical region. Nothing is known about their ranges of distribution, but strains of one or both may occur in the intervening regions between Brazil and Mexico. The close relationship between these two forms has been demonstrated by cross tests, which gave fertile F_1 females and sterile F_1 males (Patterson and Dobzhansky, 1945).

TABLE 15

Geographical distribution of members of the melanderi, bizonata, guaraní, pallidipennis, and dreyfusi groups, and species unassigned as to groups

melanderi group

magnafumosa Stalker & Spencer 1939 .....(1)
melanderi Sturtevant 1916 .....(1)

bizonata group

bizonata Kikkawa & Peng 1938 ..(3)
heterobristalis Tan, Hsu, & Sheng 1949 .....(5)
meitanensis Tan, Hsu, & Sheng 1949 .....(5)

guaraní group

griseolineata Duda 1925b .....(2)
guarajá King 1947a .....(2)
guaramunú Dobzhansky & Pavan 1943a .....(2)
guaraní Dobzhansky & Pavan 1943a .....(2)
guarú Dobzhansky & Pavan 1943a .....(2)
ornatifrons Duda 1925b .....(2)
subbadia Patterson & Mainland 1943 .....(2)

pallidipennis group

pallidipennis Dobzhansky & Pavan 1943a .....(2)
p. centralis Patterson & Mainland 1944 .....(2)

dreyfusi group

camargoi Dobzhansky & Pavan 1950 .....(2)
dreyfusi Dobzhansky & Pavan 1943a .....(2)

Unassigned species

addisoni Pavan 1950 .....(2)
andina Dobzhansky & Pavan 1943a .....(2)
calloptera Schiner 1868 .....(2)
canalinea Patterson & Mainland 1944 .....(1 + 2)
caponei Pavan & da Cunha 1947.(2)
castanea Patterson & Mainland 1944 .....(1 + 2)
fulvalineata Patterson & Wheeler 1942 .....(1 + 2)
fumosa Pavan & da Cunha 1947.(2)
gracilis Duda 1924 .....(5)
guyénoti Burla 1948 .....(3)
hirsuta Duda 1926 .....(5)
histrio Meigen 1830 .....(3 + 5)
hypopygialis—2 Duda 1924 ....(5)
macropolia Patterson & Mainland 1944 .....(1)
mesophragmatica Duda 1925b ..(2)
obscuricornis—2 de Meijere ....(5)
pará Pavan & Burla 1950 .....(2)
parachrogaster Patterson & Mainland 1943 .....(1)
pulla Pavan & da Cunha 1947 ...(2)
subtilis Kikkawa & Peng 1938 ...(3)
tranquilla Spencer 1943 .....(1)
triangula Wheeler 1949b .....(1)
trivittata Strobl 1893 .....(3)
tuchaua Pavan 1950 .....(2)
tumiditarsus Tan, Hsu, & Sheng 1949 .....(5)
vibrissina Duda 1924a .....(3)

dreyfusi group

Only two species have been included in this group, *D. dreyfusi* Dobzhansky and Pavan 1943a and *D. camargoi* Burla 1950, both recorded from Brazil.

UNCLASSIFIED SPECIES

Table 15 lists twenty-six species, none of which could be fitted into any of the established groups. At least twenty of these, a majority of which have been described since 1921, are known to belong to the subgenus Drosophila. The subgenera to which the other six species may belong are unknown. These are *gracilis, guyénoti, hirsuta, hypopygialis, obscuricornis,* and *trivittata.* An analysis of the records shows that four of the twenty-six species are endemic to the Nearctic and nine to the Neotropical, with two common to both regions. There are four endemic to the Palaearctic and five to the Oriental, with one common to both regions. The recorded localities of the following species are plotted on the map shown in Figure 32: *calloptera, canalinea, castanea,* and *fulvalineata.* The localities of the following species are given in Figure 33: *macropolia, parachrogaster,* and *tranquilla.*

DISCUSSION

Any analysis of the large genus Drosophila is facilitated by certain practical subdivisions. Sturtevant (1942) divided the genus into six subgenera on the basis of the analysis and correlation of a large number of characters. Patterson and Mainland (1944) created another subgenus and Wheeler (1949b) still another. These subgenera, even though they may not represent equivalent branches of the actual phylogenetic tree, are sufficiently distinct and critical to facilitate greatly our analysis of the genus. The two large subgenera, Sophophora and Drosophila, are further divided into species groups with some extra species which do not fit into these groups. The several species groups obviously do not express the same degrees of relationships, for in some all members of the group will cross to produce hybrids (e.g., the virilis group, Patterson and Stone, 1949), while in others isolation is so complete between members that no hybrids, and in fact,

practically no cross-mating occurs. Despite their differences, the species groups are useful and convenient entities.

DISTRIBUTIONS AND RELATIONSHIPS

In order to clarify the distributions and relationships in the large genus Drosophila, much of the available information has been summarized in Figures 19 and 34, and in Tables 8 to 15. Figure 34 shows the breakdown of the genus into its several subgenera. The two large well-studied subgenera, Sophophora and Drosophila, are further subdivided into species groups. These divisions follow Sturtevant (1942) with several later additions. Figure 19 shows the six zoogeographical regions of the world, following Folsom and Wardle (1934). Table 8 gives the number of species of the 613 listed known from only one region, while Table 9 gives those present in two or more regions.

Tables 10 to 15 give the better known and described species that have been assigned to subgenera and species groups of the Sophophora and Drosophila (Patterson and Wheeler, 1949). These tables include all species that occur in more than one zoogeographical region, with two exceptions, namely, *D. curvinervis* Duda 1924a and *D. lurida* Walker 1860. Both occur in regions 5 and 6, but neither has been assigned to a subgenus.

A great majority (553 out of 613) of the species are endemic to a single one of the zoogeographical regions. Even if further collecting explorations modify this distribution, there is little doubt that it represents the actual type of distribution.

The distribution is, of course, nonrandom, for it reflects relationships and evolutionary history. Certain factors in population distribution need to be reviewed. If all species have the same reproductive potential, the area covered by a species would be directly proportional to its age, were it not for barriers to movement or to occupation of a territory. These barriers may be geographical, such as mountain ranges or the sea, which mechanically block free passage. Very often they are genetical and ecological, depending on the adaptive abilities of the organism in relation to available environments. Finally, they may be competitive and ecological, in the sense that two species having identical ecological requirements cannot occupy the same area. As water barriers reduce the rate and amount of migration, it is im-

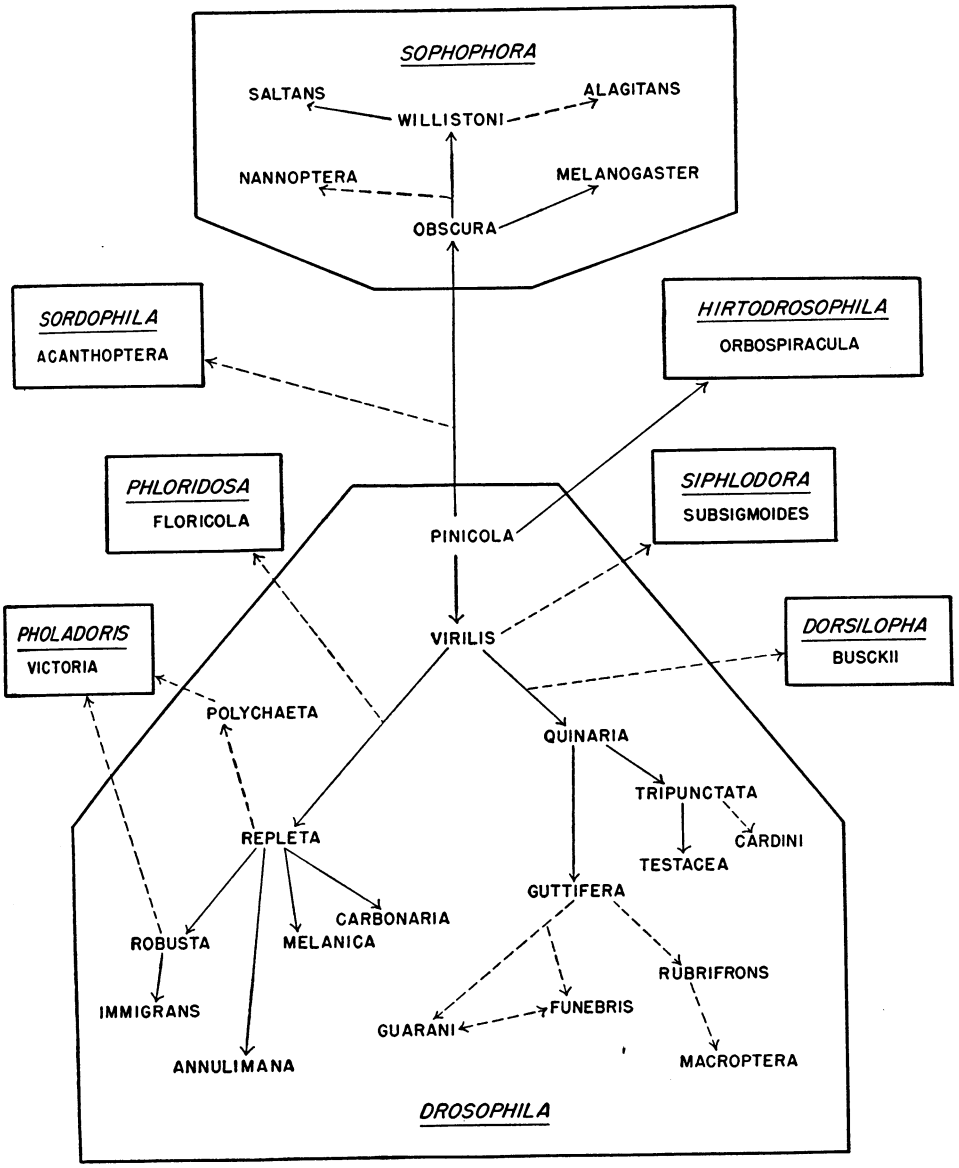

Fig. 34 Diagram of the relationships in Drosophila. (Modified after Sturtevant.)

portant to consider the changes in such barriers—for example, the Bering bridge connecting Asia and North America. Recently Simpson (1947b) has summarized the evidence as follows:

> There is evidence of intense interchange of land mammals between Eurasia and North America in the early Eocene, late Eocene, early Oligocene, late Miocene, middle to late Pliocene, and Pleistocene. There was probably some, but not much, interchange in the early and middle Miocene and early Pliocene. There was little interchange, perhaps none, in the middle Eocene, middle and late Oligocene, and Recent.
>
> Intensity of faunal interchange, as it appears in the record, is affected by factors other than the presence, extent, and duration of a land bridge, so that the continental connection cannot be assumed to have fluctuated directly with the interchange record. It is probable that there was a land migration route between Eurasia and North America during most of the Tertiary but that this was interrupted (submerged) for considerable lengths of time in the middle Eocene and again in the middle to late Oligocene. It is possible that other, probably briefer, interruptions occurred (perhaps, for instance, during the first half of the Pliocene), but these cannot at present be well substantiated or dated from the mammalian evidence.
>
> There is no clear evidence for a direct trans-Atlantic connection between Europe and North America at any time during the Cenozoic. The whole record is consistent with the existence of a single route which probably was (approximately) the Bering bridge, between northeastern Asia and northwestern North America. After the early Eocene, the evidence clearly favors this view, and does so increasingly in later epochs. The evidence for the early Eocene is less clear, but it neither favors nor demands an Atlantic bridge and it is consistent with a north Pacific bridge.

Even though ecological factors may have prevented movement of Drosophila more than of mammals, this is more than compensated for by the fact that species of Drosophila fly and are carried by the wind (Glick, 1938), in addition to their transportation in debris over water. There are species of Drosophila in the Hawaiian Islands, including a great number and diversity of endemic forms, which in themselves present a very interesting problem in evolution. Zimmerman (1948) points out that these islands have never been connected by land bridges to Asia and are late Pliocene or Pleistocene in origin. Nevertheless, many exotic endemic forms have evolved in this geologically short time. Despite the land bridge which existed between Asia and North America, and despite the motility over even exten-

sive sea barriers, most species of Drosophila are restricted to one zoogeographical region, often to a limited area. If we consider that endemic species represent the normal situation, then the wide distribution of some forms gains added significance. The distribution of forms must be considered in connection with the relationships and ecological factors. The existence of a well-developed subgenus or of a species group will often indicate a somewhat greater antiquity than single terminal species without a developed group. However, these latter may be old but relict species. Special adaptation to a certain ecological habitat must take some time. This does not require complete coexistence in time, as the adaptation may have occurred long after the environment was developed.

Sturtevant (1942) decided that *pinicola* is the species closest to the primitive type, inasmuch as it resembles the other subgenera and species groups more closely than any other form. This use of resemblance allows a reasonable deduction, since we assume that the genus diverged from a common ancestor. It is highly improbable that convergent evolution would have so affected this large a group.

The virilis and obscura species groups are considered nearest to *pinicola* in relationship. If this is so, age is not directly correlated with area (Willis, 1922), but area is related both to age and to other factors. Indeed, many very old forms are represented as relict populations (Epling, 1944, discusses this for plants and Matthew, 1915, for mammals). On the other hand, species or groups which cover wide areas (many regions) in the world must be comparatively old as well as generally well adapted.

Tables 10 to 15 give the 271 species that are best known and grouped into subgenera and species groups. Both the Sophophora and the Drosophila are world-wide in distribution, as each has member species in all zoogeographic regions. In fact, several species in each subgenus are themselves cosmopolitan. These two subgenera give obvious evidence of age. The evidence for Dorsilopha is somewhat different, as it is a monotypic subgenus, with *D. busckii* as the only known member. It is cosmopolitan and is somewhat more closely related to the melanogaster species group of the Sophophora than to other forms (Sturtevant, 1942), but possesses some distinctive characteristics. The evidence for the antiquity of Hirtodrosophila and Pholadoris depends on the presence of members of the subgenera in all regions except the Ethiopian, which has not been extensively

collected. No species in these subgenera is known to occur in more than two regions.

The distributional evidence does not indicate a similar antiquity for the three remaining subgenera, Phloridosa, Siphlodora, and Sordophila. They are small (5, 3, and 1 species) and all occur in the New World, with one exception. The species *Drosophila mauiensis* Grimshaw 1901, which occurs in the Australian region, was tentatively placed in the Phloridosa by Sturtevant (1942). These may still be small but old relict subgenera, in which no species achieved the necessary ecologically generalized genotype to become cosmopolitan. They may have been old but displaced by later and more progressive forms into their present limited distribution, as has occurred in mammals (Matthew, 1915).

The Sophophora with three species all in the melanogaster group (*melanogaster, simulans, ananassae*), the Drosophila with four (*repleta, hydei, immigrans, funebris*), and Dorsilopha with one (*busckii*), all cosmopolitan, give the greatest evidence for antiquity of the subgenera on the basis of the area occupied. There is additional evidence in that these species are all members of well-differentiated species groups and in several cases have numerous derived groups (assuming, with Sturtevant, that *pinicola* is close to the primitive original type and that the diagram, Figure 34, is approximately correct). Here *busckii* is the only exception, for it is monotypic. This species seems to have acquired a progressive generalized adaption, occupied the world, and failed to produce related species.

If we now combine the general distributional data, including regions occupied by species as members of certain species groups, with Sturtevant's relationships, and realizing that all divisions do not represent the same degree of relationship, we can form the following tentative picture of evolution in these two major subgenera.

The melanogaster group has three cosmopolitan species, and one (*montium*) that occupies regions 2, 3, 5, and 6. Of the other fourteen known members of this species group, the majority are found in the Asiatic Palaearctic and the Oriental, with a few in the European Palaearctic. No other member is found in the New World or the Ethiopian region, Africa, except the four widely distributed forms. This distribution makes more probable the assumption that the group developed in Asia and subsequently expanded.

According to Sturtevant (1942), the primitive member of the

Sophophora is the obscura group. There are two distinct and separate areas inhabited by members of this group, with a wide unknown gap between them. Its place of origin is not clear; but in Europe, as represented by *D. obscura,* its distribution range extends as far east into central Asia as Samarkand and Alma-Ata (Dubinin, Sokolov, and Tiniakov, 1937).

A wide gap exists between these records and those of North America. Furthermore, Kikkawa and Peng (1938) and Tan, Hsu, and Sheng (1949) did not find representatives of the group in eastern Asia. The relation of the species *D. segúyi* Smart 1945 to the other members of the group is uncertain, but it seems to belong with the European subgroup of species. No species of this group is known to be common to both areas. All species are endemic to one region or are even more restricted, except *pseudoobscura* and *azteca,* which occur in an area of temperate forest in the Neotropical region, quite close to the general temperate Nearctic forest habitat. The only species in the group known to us to have the primitive chromosome configuration is *subobscura,* which is found in Europe. On the other hand, all other species groups in the Sophophora (*melanogaster* excepted) are New World forms, now mostly tropical. The simplest explanation is that there existed a primitive species (or several) which occupied the Holarctic realm. This gave rise to the ancestors of the related species groups in the New World and to the ancestors of the melanogaster group in East Asia. Subsequent geological and ecological changes, perhaps including the competition of progressive melanogaster and immigrans groups, caused the separation of the obscura complex into the two areas it now occupies.

The present distributions of the willistoni, saltans, alagitans, and nannoptera groups are restricted to the New World, and most of them are tropical. No estimate of their age can be made, except that certainly the first two have developed extensive species groups. Here again they may be old, but have not developed a generalized environmentally adapted member, and so may have been restricted to the present tropical environment.

Drosophila is the most complex of the several subgenera. There is one characteristic of all forms, and that is that the major part of each of the species groups is now distributed in the New World. We have mentioned that *pinicola* is found in the Nearctic region. It is a monotypic species group, and presumably represents an archaic form,

although its chromosomes are a complex derived type (see Chapter 4). Its closest relative in the subgenus is *virilis,* which has developed a Holarctic species group, although the other members are restricted to the Nearctic or Palaearctic. *Drosophila virilis* is found in four regions (1, 2, 3, 5), but its heavy population occurs in eastern Asia, where it is found in forest areas as well as in association with man. Mr. T. C. Hsu reports that Tan, Hsu, and Sheng (1949) collected this species in forests of mixed oak and pine, and other less abundant species of trees. In the Nearctic it has been taken almost exclusively in domestic habitats. We can assume that at least *virilis* of this group is old. In any case, we know that *virilis,* or a closely allied form, was ancestral to the group.

Although there is no proof of the development of *lacicola* after the Pleistocene glaciation in northern United States, yet the range of this species is now restricted to the lake area which was covered by the ice sheet. It may have developed earlier and occupied this territory after the retreat of the ice. The members of this group are not ordinarily sympatric in the strict ecological sense, although *virilis* is geographically sympatric with several other forms. Not even *virilis* has achieved a general adaptability, and hence has failed to become worldwide in distribution. The species *virilis* was collected by Dr. M. R. Wheeler on a boat plying between San Francisco and Australia, thus illustrating motility using man's agencies.

The quinaria group is clearly Holarctic in distribution, with the main localization of the species group in North America. No species is common to Europe, Asia, and North America, unless *"transversa"* is actually one species. The distribution of the group does not make clear its age. It should be pointed out that the robusta and melanica groups have a similar pattern of distribution. Essentially, all three groups are Holarctic. The guttifera group is closely related to that of quinaria, and its single known member is Nearctic.

The small testacea group has a Holarctic distribution. In this case *putrida* occurs in the Nearctic, while *testacea* is found both in North America and Europe.

The tripunctata group is restricted to this hemisphere, and Sturtevant places it between the virilis and funebris groups. If this is correct, then the group must be old, as judged by its position in the phylogeny of the subgenus. The revised phylogeny (Figure 34) shows a more complex sequence relation.

The funebris group resembles that of virilis, but has all North American members except *funebris,* which is cosmopolitan. The immigrans group resembles most closely the quite distant melanogaster group in distribution. It is essentially East Asiatic, except the cosmopolitan *immigrans.* The Neotropical form assigned to this group is a very doubtful member. The bizonata group is small and has an Asiatic distribution like that of the immigrans group, without a generalized world-wide form.

The rubrifrons and macroptera groups are end branches from the main stem through the quinaria complex, according to Hsu (1949) and Wheeler (1949b). They are New World forms, with their main distribution in the southern part of the Nearctic and near-by Neotropical regions.

The polychaeta group is tropical and is known from the Nearctic, Neotropical, and Asiatic areas. The native habitat of *polychaeta* is unknown. It has never been established in Texas, as judged by our collecting records. The specimens caught on the Galveston wharfs on fruit dumps were undoubtedly recent imports. Dr. A. H. Sturtevant informs us that he identified one among dried specimens of Drosophila sent to him from Guam as *D. polychaeta.* Too little is known of this group to assign it a relative age or origin.

The cardini, annulimana, and guaraní groups are all basically Neotropical, and only a few species extend into the southern Nearctic. These seem to fit into the tentative relationship tree as derived forms, or side branches.

The distribution of the monotypic *carbonaria* is within that of the mesquite, where it breeds in the sap. Thus *carbonaria* represents a specialized derivitive of the repleta complex which has adopted a typical restricted New World environmental niche. The unassigned species of this subgenus are not amenable to simple analysis. The majority are Nearctic or Neotropical, but a few occur elsewhere. They simply add to the opinion, which may be biased by the available collecting data, that the main modern expansion of the two subgenera Sophophora and Drosophila lies in the New World.

The major division of the subgenus Drosophila, judged by size and complexity, is the repleta group. We might regard the subgenus as roughly centered in two complexes, the virilis-quinaria complex and the repleta complex. In this latter, the repleta group shows better than any other a peculiarly New World character. Unlike most other groups

and species, it is not a restricted forest form, but extends into the typical desert habitat, utilizing, among other things, the succulents such as the cacti. As mentioned before, *carbonaria* is adapted to the arid mesquite association.

The distribution of several members of the repleta group needs comment. The subspecies *mercatorum* has a normal wild habitat in northern Mexico, but its range extends into the United States in a domestic habitat associated with man. It is found also in Hawaii, where it is obviously a recently introduced form. Its location in Hawaii can therefore be assumed to be by recent human transport. The subspecies *pararepleta* occurs in Brazil and, when crossed to the subspecies *mercatorum,* produces hybrids.

A somewhat similar situation exists for *buzzatii,* which is native to South America, where it is found in Argentina and Brazil. It has also been obtained in Sicily and Lebanon. It seems probable that it was introduced into European countries with the cacti, when these plants were brought in from South America. The European strains are perfectly cross-fertile with those from South America. Finally, there are two species of the repleta group which have been reported from the Australian region, *D. obsoleta* Malloch 1923 and *D. poecilithorax* Malloch 1925. It seems probable that these are New World forms which were transported to Australia along with the introduced cactus (or else are misidentifications of the world-wide *repleta* and *hydei,* which is less probable). One may surmise that all of these are New World forms which have quite recently been transported to other regions by the agency of man.

The wide and complex development of the repleta group in the New World represents the expansion of ancestral generalized forms—perhaps *repleta* or *hydei,* or both—into a large, available, open environment for which there was little or no competition from other species of Drosophila.

ECOLOGY

Dobzhansky and several coworkers have demonstrated the very important fact that there exist genetic differences within the strains of *pseudoobscura* and *persimilis* which have different ecological selective values. The general issue of mutation and selection will be discussed later, but these studies in "gene ecology" give us a good basis for discussing ecological and habitat selection by Drosophila.

Dobzhansky and Epling (1944) have summarized the information available up to that time (see their bibliography for basic references), together with new evidence at their disposal. The first evidence is from the data on chromosome rearrangements which have a nonrandom distribution. Certain features of the discontinuous distributions of gene arrangements in the third chromosome and replacement by other arrangements suggest differences in ecological selective values. The use of gene arrangements in the third chromosome for genotype markers needs some comment. Dobzhansky and Epling (1948) have shown that several of the inversions often found in the third chromosome of *pseudoobscura* when heterozygous with standard reduces the amount of gene recombination to a small, negligible fraction of the normal recombination values. This restriction to gene recombination would hold for most of the heterozygous combinations of inversions. Therefore these inversions restrict the gene flow in the population. A mutation which occurs in a particular gene arrangement (inversion or standard) will not recombine freely, except with genes in the same arrangement. This is particularly true of a gene near an "inversion breakage point," when the sequence differs between two chromosomes. The high frequency of different gene arrangements in the third chromosome in most localities in the distribution range of *pseudoobscura* will therefore lead to a nonrandom distribution of genes in the chromosomes, often even in the same population. The discontinuous distribution of the Santa Cruz gene arrangement, which is an old arrangement in the species, suggests that it has been displaced in the intermediate regions by an arrangement with a group of genes having a better selective value in that region (see Epling's discussion in Part III; Dobzhansky and Epling, 1944).

Dobzhansky has published direct evidence on this point (See Part II). He has shown a seasonal cycle in frequency of several gene arrangements at certain collecting localities where this phenomenon was studied. Dobzhansky (1947a, 1947b, 1947c, 1948a, 1948b) has demonstrated both altitudinal and seasonal variations in gene arrangements (and so in genotype variation) in populations of *pseudoobscura* and *persimilis* due to natural selection. Furthermore, the same gene arrangement, standard or the inversions, from different localities does not necessarily have the same selective value. Heuts (1947, 1948) has shown that certain gene arrangements differ in their selective value in relation to humidity. Wallace (1948) has shown that a

certain peculiar genotype, the sex ratio factor(s), which is found in a characteristic gene arrangement in *pseudoobscura,* and another characteristic arrangement in *persimilis,* has certain definite selective characteristics which may be related to its distribution. Finally, Dobzhansky and Levene (1948) have demonstrated that the frequencies of heterozygous and homozygous rearrangements in the populations differ from the Hardy-Weinberg frequency rule in such a way as to prove the general selective advantage of the heterozygotes in a number of instances, thus presenting evidence to explain the retention of several gene arrangements in the same local population.

A similar situation which demonstrates the nonrandom distribution of genes related to ecological fitness in the population has been demonstrated by Dubinin and Tiniakov (1945, 1946a, 1946b, 1946c, 1947) in *funebris.* These authors were able to show that the standard gene sequence and certain naturally occurring inversions had different selective values, both in heterozygous and homozygous. The selective values varied both by season, during hibernation, and in relation to ecological environment of city, town, and country.

Carson and Stalker (1947) and Stalker and Carson (1947, 1948) demonstrated both geographical and altitudinal variations in the gene arrangement and the morphology of *robusta.* These were both presumably related to differences in selective values in different ecological environments.

Thus it is very clear that genetic differences exist and can be demonstrated in populations of the same species which fit certain segments of the general population (sometimes local populations, sometimes chromosome races) to a certain ecological environment, and so in turn affect the geographical distribution of species and even of chromosomal or other races within the species.

Dobzhansky and Epling (1944) have discussed the general ecology of the pseudoobscura subgroup in some detail with respect to habitat and food, attractive radius of banana traps, diurnal periodicity, seasonal cycles, and migrations.

In general, this subgroup is a forest form and enters open meadows only to a limited extent. They are plentiful in pine and oak growths, but scarce in grassy meadows. The authors note that A. H. Sturtevant found numerous larvae of *pseudoobscura* and *persimilis* in the fermenting sap of a wild grape vine, *Vitus californica,* and that G. B. Mainland had observed adult *pseudoobscura* on the fruit of a species

of *Opuntia* (which contained unidentified larvae). Dissections of the crops from flies which had fed only on the available natural foods showed the presence of many kinds of bacteria, yeasts, and mold spores.

The attractive radius of banana bait in a trap is from twenty to forty meters. This indicates that flies could be expected to obtain stimuli and congregate over this distance on suitable available food, even in poor habitats. Dobzhansky and Epling give considerable evidence that *pseudoobscura* and *persimilis* are active shortly after sunrise and shortly before sunset. In summer, particularly, the flies arrive and depart from the traps at very definite times, unless the day is cloudy. They conclude that the activity, for the most part, is determined by light conditions. However, temperature sets some limits to activity, for flies were caught in the temperature range of 50° to 90° F. This diurnal habit, with activity maxima in the morning and evening, seems a more general habit of the genus, judged by the extensive collection records of Patterson and his coworkers.

In general, *pseudoobscura* has a seasonal cycle which varies with a particular environment. In warmer climates, there is a spring maximum in March and April; while in cooler climates, including higher elevations in the same general area, the peak occurs in June and July. In warmer localities, *pseudoobscura* may be present all winter and nearly disappear in the heat of summer (Table 17); in colder localities, this may be reversed. *Drosophila persimilis* is much more tolerant of cold and more abundant higher in the mountains. It becomes comparatively more abundant in lower localities in the winter. Also, *pseudoobscura* occurs in the Colorado and Mojave deserts in the winter and spring, where it is not limited to a forest habitat.

Dobzhansky and Epling point out that Drosophila may be transported passively by winds and storms. Furthermore, Glick (1938) captured Drosophilidae at altitudes ranging from two hundred to three thousand feet in an airplane, and collected insect larvae and eggs high in the air. Dobzhansky and Epling state that *persimilis* is found in the Coso and Panamint ranges, across the desert from their common range in the Sierra Nevada. They believe that these are the result of such passive transport, rather than remnants of an older widespread population. The authors report results of experiments on the rate of dispersion through movement of the flies. The data indicate that the speed of travel is related to temperature. Their estimates of the dis-

tance traveled in a day, apparently as a result of random motion, is 15 to 26 meters at 50° F., 34 to 46 meters at 60° F., about 80 meters at 70° F., and 144 to 188 meters at 80° F. These distances are considerably greater than those estimated by Timofeeff-Ressovsky (1940a, b, c) for *melanogaster* and *funebris,* but no temperature was recorded for the results given.

Only one additional comment on ecological preference needs to be made. Judging from the distribution map (Figure 35), *persimilis* and *miranda* are far more restricted to the moist coastal regions, whereas *pseudoobscura* invades a much wider area, including many more varied habitats.

Sturtevant (1942) made some general comments on the food habits of Drosophila, based in part on information on larval habits which he described briefly in 1921. He points out that it is possible to recognize among species of Drosophila several more or less distinct kinds of feeding and breeding habits, and lists the following types: general scavengers, fruit-feeders, sap-feeders, fungus-feeders, and species which utilize flowers.

The general scavengers feed mainly on decaying vegetable matter and occasionally on stale animal matter. This group includes among others such cosmopolitan species as *busckii, funebris, repleta, hydei,* and *ananassae.* In the temperate zone the fruit-feeders, which are also introduced forms associated with man, include three other cosmopolitan species, *melanogaster, simulans,* and *immigrans.* The sap-feeders are found on various kinds of bleeding trees or vines, with Pinus, Quercus, Betula, Populus, and Vitis as the most favored. This group includes Pholadoris, members of the obscura group of Sophophora, and the melanica, robusta, tripunctata, and pinicola groups of Drosophila, as well as *californica* and *occidentalis* of the same subgenus. All of these forms are known to be attracted to fruits and, when available, to breed on them. The fungus-feeders include a great variety of species, including certain members of the quinaria, testacea, and guttifera groups, and perhaps all of the subgenus Hirtodrosophila. Some of the members of the quinaria group (*deflecta, palustris, subpalustris*) are known to feed on decaying water plants, especially Nymphaea and Sagittaria, and probably breed in the decaying parts of such succulents. A similar feeding habit is found among forms utilizing the decaying parts of desert succulents. Finally, several species live on flowers, including *florae, alfari, lutzii, tristani,* and

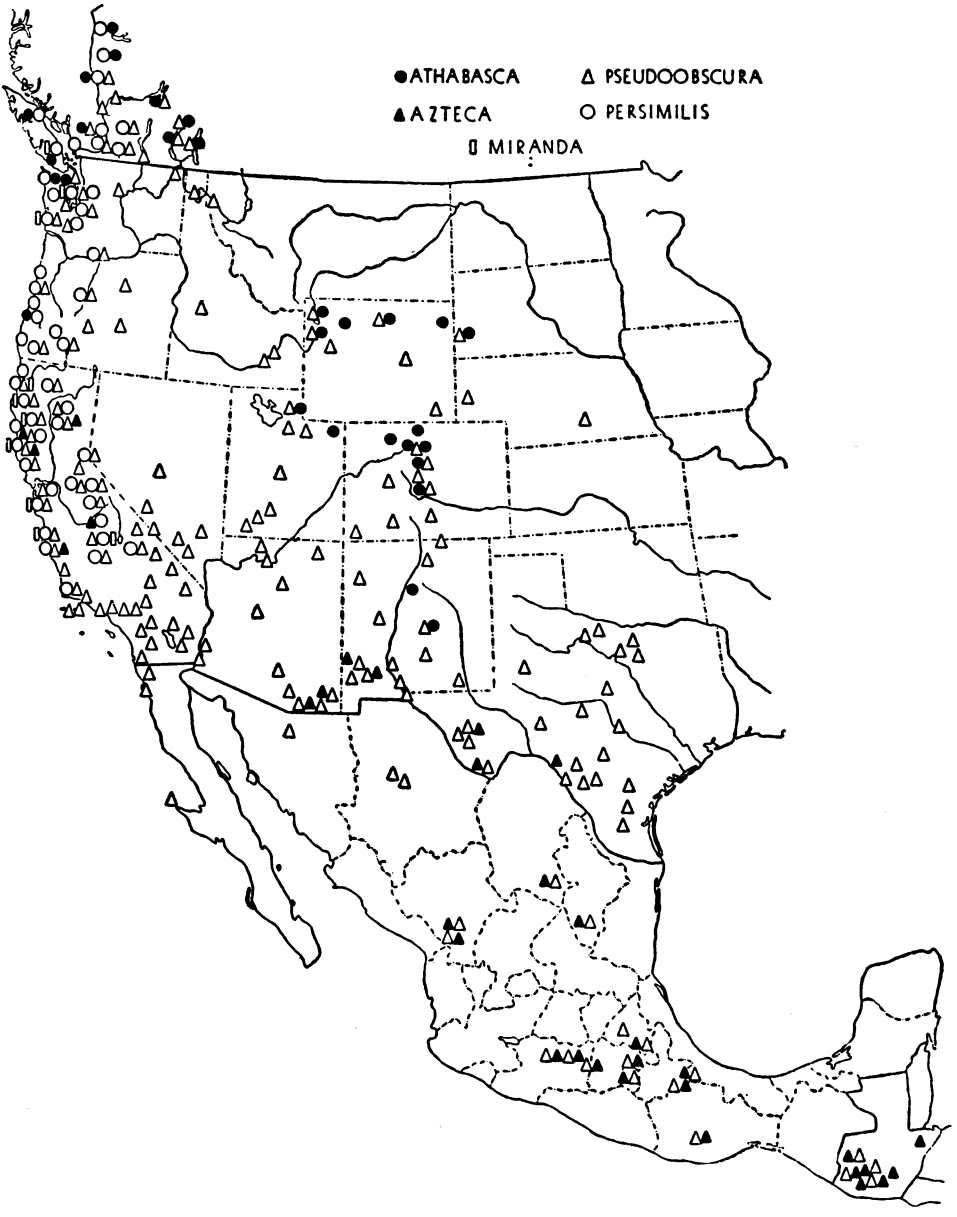

Fig. 35 The known geographical distribution of *Drosophila pseudoobscura, D. persimilis, D. miranda,* and *D. azteca,* together with the southwestern portion of the distribution area of *D. athabasca.* (From Dobzhansky and Epling.)

floricola. Their chief larval food is apparently pollen. Some of the flowers commonly used are those of Datura, Hibiscus, Gossypium, morning-glories, and melon.

Certain detailed information can be added to Sturtevant's general account of the food habits of Drosophila larvae. We are familiar with a number of records of adults that have developed from larvae recovered from some natural foods. These are presented in Table 16. Despite the fragmentary and incomplete nature of this record, it gives certain information on some of the kinds of normal foods. In addition to the genus Drosophila, larvae of two related members of the Drosophilidae, *Gitona americana* and *Scaptomyza adusta,* have been recovered from the fruit of several *Opuntia* species by Mainland and Wheeler.

TABLE 16

Recent determinations of natural foods of Drosophila larvae

Species	Type of Food	Authority
victoria	sap of bleeding *Populus*	Wheeler
floricola	pollen of *Datura, Gossypium, Hibiscus*	Sturtevant
pseudoobscura	sap of bleeding *Vitis californica*	Sturtevant
persimilis	sap of bleeding *Vitis californica*	Sturtevant
affinis	sap of bleeding wild grapevine	Wheeler
melanogaster	fruit of *Opuntia lindheimeri*	Mainland & Wagner
munda	bracket fungus on *Salix*	Mainland & Wagner
palustris	decaying *Sagittaria latifolia*	Spencer
subpalustris	decaying *Sagittaria latifolia*	Spencer
suboccidentalis	blueberry	Spencer
lacicola	decaying aspen	Spieth
limpiensis	bracket fungus on *Salix*	Mainland & Wagner
hydei	fruit of *Opuntia lindheimeri*	Patterson
nigrospiracula	fruit and flowers (decaying) of *Cereus giganteus, C. schottii, C. thurberi*	Mainland & Wagner
aldrichi	fruit of *Opuntia lindheimeri*	Patterson
arizonensis	fruit and flowers (decaying) of *Cereus giganteus, C. schottii, C. thurberi*	Mainland & Wagner
meridiana	fruit of *Opuntia lindheimeri*	Patterson
mojavensis	*Echinocactus acanthodes*	Spencer
mulleri	fruit of *Opuntia lindheimeri*	Patterson
melanissima	moist pine sawdust	Sturtevant & Patterson
carbonaria	sap of bleeding *Prosopis glandulosa*	Wheeler
robusta	slime flux on American elm	Spieth

Patterson (1943) made a study of seasonal variations in Drosophila population on the Aldrich Farm near Austin, Texas. The area chosen to study was one well suited to Drosophila and yielded thirty-one species for a total of 141,126 individuals from July, 1938, through May, 1941 (Table 17). This area was described as follows:

> The main collecting area is about 300 yards wide, and is located in a narrow, rather shallow wooded valley. Two small ponds are located at the upper end of the plot and separated from each other by an earthen dam. The upper pond contains water except during extreme dry periods, while the lower one is spring-fed, and from its lower end a small stream flows southeastward to join a creek which finally reaches the Colorado River: These ponds are covered with several kinds of aquatic plants, and the floor of the valley supports a luxuriant growth of succulent vegetation. Many different kinds of trees are found in the valley and on the adjacent bluffs, and farther back the common prickly pear, *Opuntia lindheimeri,* is abundant. There are also found here in season many different wild fruits, and during the rainy periods more than a dozen species of fungi are present, growing on decaying willow trees and rotting logs. Such an environmental complex is able to support a large and varied population of Drosophila. In addition to all of these advantages, records for the U. S. Weather Bureau for the Austin area are available, and these cover the daily variations in temperature, humidity and rainfall.

The records are scattered through 1938 and again in 1939–1940, with only part of 1941 recorded. These records are more complete with a number of collections each month from March, 1939, to June, 1940. This latter record shows the seasonal cycle very completely for one year. There are listed a number of species so rare as to tell us nothing about population cycles. These are: *duncani, nigrohalterata, nebulosa, ananassae, transversa, guttifera, virilis, americana, funebris, repleta, micromelanica, carbonaria,* and *cardini.* It should be noted that twenty-nine of the thirty *repleta* were captured in 1938 and that forty-two of the sixty-six *micromelanica* were taken in one month, May, 1940. Obviously the rare species are erratic in the numbers caught, but even so certain differences in collection records are significant.

Drosophila busckii has a very definite cycle with a spring peak, which showed in 1939 and 1941 especially, but so few occurred in 1940 that the peak is not significant. *Drosophila robusta* had a cycle that was in some respects similar to that of *busckii,* and the high

TABLE 17

Collection records from the Aldrich Farm—March, 1939, through February, 1940 (from Patterson)

Species	March	April	May	June	July	August	September	October	November	December	January	February
busckii	52	48	69	3	0	0	0	0	0	1	1	0
mel.-simulans	73	2,701	13,215	8,266	2,764	11,091	13,170	8,425	1,579	967	112	1
pseudoobscura	99	715	297	1	0	0	0	0	20	23	9	55
affinis-algonquin	54	186	39	4	6	0	5	364	134	177	38	276
putrida	5	67	28	4	135	1	50	153	27	10	1	1
tripunctata	3	0	6	3	30	7	6	12	4	1	0	1
macrospina	0	802	491	304	215	324	1,243	232	21	27	1	10
hydei	73	3,752	7,443	841	496	416	469	146	35	131	3	1
longicornis	97	2,181	1,383	29	4	0	1	7	16	252	45	11
hamatofila	0	325	206	4	3	0	0	5	6	50	22	26
mulleri-aldrichi	30	112	209	460	302	259	1,996	5,715	1,385	1,201	36	0
meridiana	0	0	0	0	0	281	203	445	23	13	4	1
robusta	0	1	103	56	12	2	0	0	0	2	0	0
melanica	3	73	117	40	44	152	190	54	8	6	3	8
Mean temperature	63.2	69.0	78.0	82.8	85.1	84.0	81.2	71.5	56.4	55.0	40.6	52.8
Mean humidity	58.0	52.0	60.0	62.6	58.6	65.3	58.3	56.3	66.6	63.0	65.3	69.0
Rainfall, inches	1.04	1.87	3.01	1.00	3.51	1.62	1.64	1.62	2.26	0.80	0.63	3.73

year was 1939. However, this was not apparent in 1940 or 1941. *Drosophila immigrans* has the same discontinuous cycle, but an effective population was not built up until the spring of 1941. This constituted one of the more extreme cases of discontinuous populations observed during the period from 1938 to 1941. One individual was collected at the Aldrich Farm in December, 1939, and another in Eagle Pass, Texas, in March, 1940. These were the only records among 453,572 Drosophila captured in two years of intensive collecting in Texas. As contrasted to this, 954 specimens were collected in April and May, 1941, at the Aldrich Farm alone. These were not isolated populations, as *immigrans* was found in other localities in the state as well as elsewhere in the United States. These findings would indicate that the several conditions necessary for the development of an extensive *immigrans* population existed for only one year of these records, but that residual populations must have been widespread.

The other species have been further analyzed for the year March, 1939, to February, 1940, to determine the relative populations per month, as parts of the total population for the year. If the average number of flies per collection during a month is taken as representative of the density of the population, then the average for one month divided by the sums of the averages for all months equals the percentage of the total fly population for the year present in that month, as far as one can measure it. These percentages are given as histograms in Figure 36 for nine single species and three sets of sibling species. In addition, the rainfall in inches and the temperature (high, low, mean) for each month are given.

In general, the populations of *tripunctata, putrida,* and *melanica* were small. Despite the effect of sampling error under those circumstances, *melanica* shows a definite peak in the spring and fall. As Patterson (1943) points out, both *tripunctata* and *putrida* being at least in part fungus-feeders, their populations follow the fungus populations, which are much influenced by rainfall.

Drosophila macrospina has a double cycle, with a dense spring and fall population and a small winter and summer population. This may be correlated with the distribution of this species in the south and only to a limited extent toward the north, especially as the subspecies *ohioensis.* It is in contrast with *pseudoobscura,* a species prominent in the higher elevations of the west, which has a single peak in April (or March).

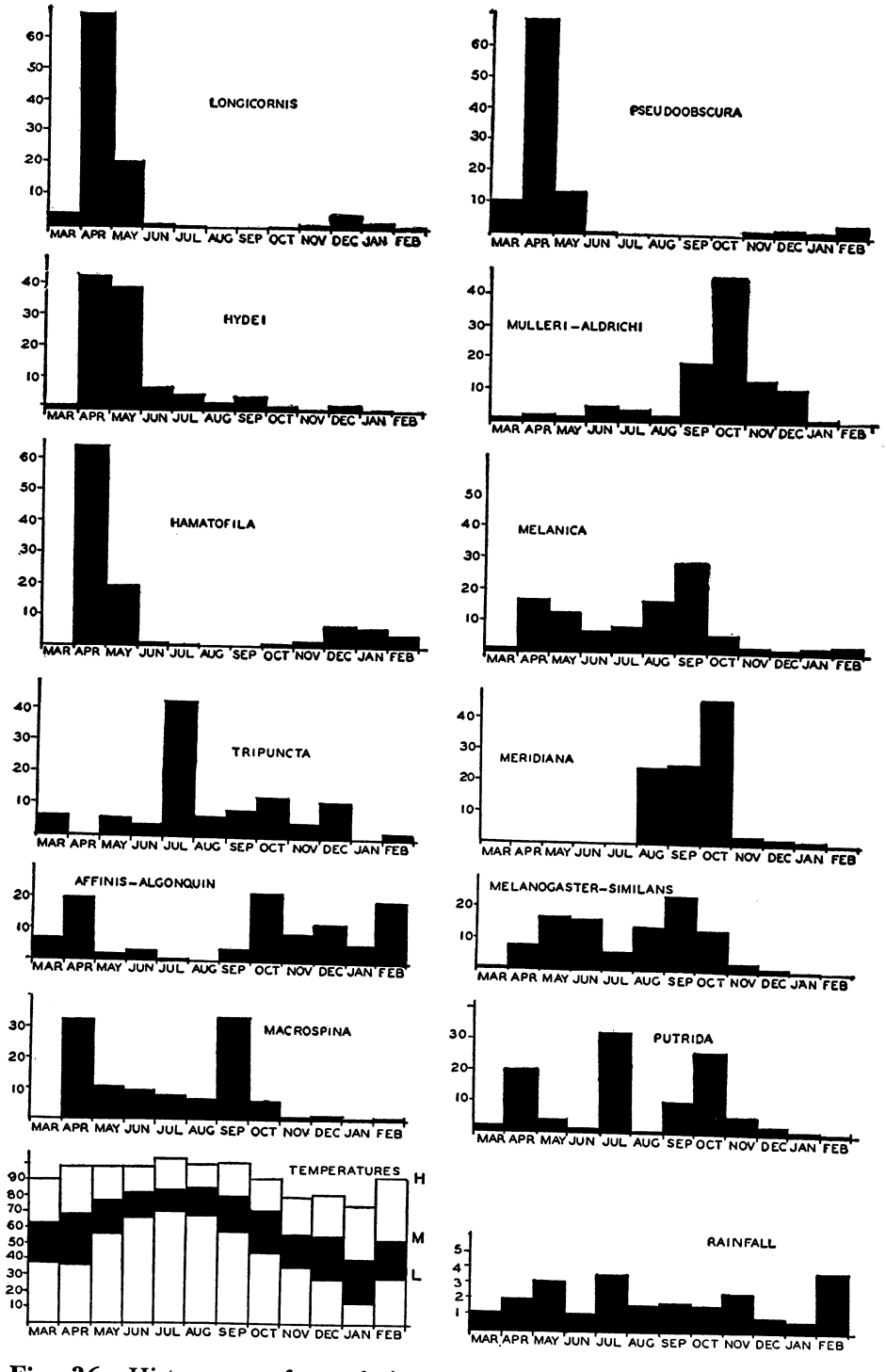

Fig. 36 Histograms of population fluctuations, March, 1939 to February, 1940. (From Patterson.)

Drosophila hydei, which is a cosmopolitan species and a member of the general repleta group, has a more restricted subgroup of its own, but no other member of that group is represented in the data. It has a very decided spring population peak and a minor fall increase, which showed up for several years. This suggests that an available food supply is the important factor, but that temperature may also affect the population, although this too may be related to the food supply.

The species *longicornis, hamatofila,* and *meridiana* are members of the mulleri subgroup of the general repleta group, but are not so closely related to *mulleri* as is *aldrichi.* Of these, *meridiana* has a definite fall peak, as does also the *mulleri-aldrichi* pair of sibling species. *Drosophila meridiana* developed a large population in only one of the years studied; but the other two, *longicornis* and *hamatofila,* have diverged from the other three members of the mulleri group mentioned above in that their large population peak is in the spring, although they show another small peak in the fall. This is a good example of ecological divergence that reduces the competition between closely related forms.

The species *affinis* and *algonquin* are very closely related members of the affinis subgroup of the obscura complex. In general, *affinis* replaces spacially *pseudoobscura,* a member of the pseudoobscura subgroup, so that *affinis* is found east of the ninety-eighth meridian, whereas *pseudoobscura* occurs west of this line, with a restricted overlap area. The *affinis-algonquin* pair have both spring and fall population peaks, in contrast to *pseudoobscura,* which has only a spring peak at the Aldrich Farm. An examination of Table 17 shows that there is a definite suggestion of competition between the forms, for in 1941 the siblings *affinis-algonquin* were much larger than for the two previous years, while *pseudoobscura* was much reduced in numbers.

The sibling species *melanogaster* + *simulans* are cosmopolitans which find this natural habitat much to their liking and are the most numerous of all the forms at the Aldrich Farm. There is a spring and a fall population peak. In this case the two peaks represent the effect of adaptive divergence between the very closely related *melanogaster* and *simulans,* for the former accounts for a much higher proportion of the population in the spring and the latter for a proportionally greater part of the fall population. Patterson checked the growths of

the two populations in the spring of 1940 and found that, from a beginning of one *melanogaster* male in the March 2 collection, the population increased to 122 males by April 9. On April 11 one *simulans* male appeared in the collection, and the population built up slowly to produce fourteen males by May 12. In the meantime the *melanogaster* population was increasing and by May 13 had reached a peak of 1,267 males, but began to decline soon thereafter. By June 3 *simulans* had become the larger population, with 122 males as compared with thirty-six for *melanogaster*. This situation held again on June 5, when 370 *melanogaster* and 435 *simulans* were collected. Collection records from other localities in Texas and from other states showed this phenomenon to be general, namely, that *melanogaster* predominated in the first half of the year but lost the dominance to *simulans* by fall. This condition must reverse again during the cold of winter.

The sibling species *mulleri* + *aldrichi* have their population peaks in the fall, as does also *meridiana*. All three live and breed on the cactus, especially on the fruits of the genus *Opuntia*. The fall season corresponds to the major abundance of these cactus fruits. Two general mechanisms may contribute to allowing all three related species to occupy the same area. In the first place, the food supply may build up fast enough to accommodate all of these forms that can develop from the remnants of populations which survive the summer low. This does not seem to be the only reason, for some very interesting work by Wagner (1944, 1949) has demonstrated a fundamental difference in food requirements between members of the mulleri subgroup. Wagner isolated a number of yeasts and bacteria growing in the cactus fruit where these Drosophila are commonly found. He discovered that the members of the mulleri subgroup could not develop on sterile cactus fruit or on a basal synthetic medium, but would develop on such a medium plus some of the yeasts. However, the yeast strains tested differed, for some would support the growth of all species of Drosophila tested, while others would support only certain of the species. Table 18 shows the difference in abilities of the several yeasts tested to supply the necessary substances for normal development of the different species. Thus a fundamental difference in nutrition must also contribute to allowing these Drosophila to inhabit the same area, despite their competition for certain foods. For such a small closely related group there is a considerable difference in nutri-

tion requirements, especially in view of the few types of yeast utilized to show these differences.

Patterson and Wagner (1943) and Patterson and Mainland (1944) have discussed in detail the collecting records and distribution of Drosophila species in the United States and Mexico. Certain general

TABLE 18

Duration of the larval period in days for five species of Drosophila cultured on various wild Yeasts (from Wagner)

Drosophila Species	Yeast Strains							
	Y–1	Y–2	Y–3	Y–4	Y–5	Y–6	Y–7	Y–8
mulleri	7	6	7	6	6	6	6	6
aldrichi	0	8	0	8	8	8	7	0
mojavensis	7	7	7	7	7	7	0	0
arizonensis	6	6	6	6	6	.	6	.
buzzatii	8	0	8	8	8	.	0	.

considerations need to be presented here. It is possible to say that a species with universal distribution is generally adapted and can live on and compete for the available food in the whole area. These may be able to use the environment provided by man, but the occurrence of such cosmopolitans as *melanogaster* and *hydei* in cactus shows that they have general adaptability. If a species is of restricted distribution, any or all of several factors may contribute to this localization. For example, it may be a young species, and so not yet expanded into a large area (age and area principle); it may have temperature or humidity restrictions; it may require a special food and be restricted to the range of that food; or it may not be able to compete with other species under different environmental conditions. We note that there is a complex of species which inhabits the eastern half of the United States and a different complex of species in the western section, with the division at about the 99th meridian. However, two members of the Holarctic groups, *testacea* (Figure 24), which also occurs in Europe, and *athabasca* (Figure 35), which has related members of the obscura complex in Europe, extend across the northern part of the United States and probably into Canada. In these cases *testacea* occurs with its related species *putrida,* but *athabasca* tends to replace *pseudoobscura,* which ranges to the south.

Representatives of the eastern species are *putrida,* which overlaps its related *testacea* in the north, and *athabasca,* which has the other

members of its group in Mexico, as well as *robusta, affinis,* and *algonquin.* The last two members of the obscura complex replace *pseudoobscura* and its relatives, which occupy the western area. Their range includes that of several other related forms in the affinis subgroup which have restricted distributions, such as *narragansett* and *seminole.* In the quinaria group, *transversa* occurs all through the eastern region. Among the species with a more limited distribution, *palustris* and *subpalustris* have a range within that of *quinaria* in the northeast. The latter species in turn is replaced to the south by *guttifera,* a related form, although not included in the group.

In several of these instances replacement is by other subspecies. For example, *melanica* is replaced by the subspecies *paramelanica* in the north. In this case *nigromelanica* has a southern distribution more like that of *melanica,* but to some extent occurs in the north also. It could be claimed that the western *melanica* (Figure 31) is a different subspecies because of difference in coloration. The western strain of *micromelanica* has been proved to be genetically different from the Texas strain, as they give some sterile hybrids (Sturtevant and Novitski, 1941a). Both of these might be regarded as cases of southern versus western subspecies. Moreover, *melanissima* is a southeastern form, probably restricted to the southern pine region, owing to its food habits.

The subspecies *texana,* with its range in the southeastern part of the United States, is replaced by *americana* in the north central states, which in turn is replaced by the distinct species *lacicola* in the northern lake region. In the funebris group the subspecies *macrospina* (Figure 24) is replaced by *ohioensis* to the north, as well as by *limpiensis* to the west of the Pecos River in Texas.

In the western part of the United States, unlike the eastern portion, there are no cases of subspecies replacing each other, with the possible exception of *occidentalis* and *suboccidentalis.* In this case *occidentalis* is nearly restricted to California, while *suboccidentalis* occurs in the Rocky Mountain area and extends on down into Mexico.

In the obscura complex, there is an overlapping distribution within the pseudoobscura subgroup (Figure 35); for both *persimilis* and the rare *miranda* occur among the Pacific Coast mountain ranges within the range of *pseudoobscura,* which extends east into Texas, where it is replaced by *affinis* and *algonquin,* and north to Canada, where it is

replaced by *athabasca*. The range of *athabasca* extends south also along the high Rockies into New Mexico, where it overlaps *pseudoobscura*. Finally, the range of *pseudoobscura* extends south through Mexico into Guatemala, but overlaps and is replaced in part by *azteca* of the affinis subgroup. It is interesting to note that there is less overlapping between members of the two American subgroups of the obscura complex than there is between members within subgroups.

Among the western members of the virilis group, *novamexicana* (Figure 23) represents a desert stream form, restricted to New Mexico, Colorado, and Arizona, whereas *montana* occurs in the Rockies, chiefly at altitudes above six thousand feet. In the funebris group (Figure 25), *limpiensis* is replaced by the very restricted species *trispina* and *subfunebris* in southern California.

In the quinaria group, *suboccidentalis* occurs in the Rockies but extends down into Mexico (Figure 22). *Drosophila subquinaria* also occurs in the Rockies, but extends only into New Mexico, where it is replaced by *innubila* in part and, to the west of *innubila,* by *munda.* There is some overlapping in each of these cases. The extension of northern forms, such as *americana* and *algonquin,* and of Mexican forms, such as *nebulosa,* into central Texas must be due to the mixed complex conditions of the transition zone between the general ecological environment of eastern United States, western United States, and Mexico. The Mexican species grade into the southwestern complex of species and even include some forms from the eastern and western complexes.

The maps, Figures 20 to 22 and 26 to 31, show the distribution of a number of species in Mexico, but do not necessarily include plots of the common forms in the United States. For example, *macrospina* (Figure 22) and *hydei* (Figure 26) are not indicated in Texas, where they are quite common, although the former is restricted to the section east of the Pecos River.

The topography, climate, plant, and animal associations are so mixed that it is difficult to explain the distribution of Drosophila in Mexico. Inasmuch as Dr. G. B. Mainland had made several collecting trips into Mexico, he prepared the following account of the ecology, which appeared in a publication by Patterson and Mainland in 1944:

> Between the east coast and the Sierra Madre Oriental lies the eastern coastal plain. In the northern portions of the plain is to be found a continuation of chaparral association characteristic of south Texas. Southward

the chaparral is gradually replaced, first, by a tropic xeric brush land, and secondly, by a tropical mesic vegetation, which in turn gives way to a moderately dense tropical jungle. Short rivers which arise in the Sierras traverse this plain and along their banks are to be found plant assemblies typical of the jungle much farther south. Near their mouths many of these rivers flow through extensive swamp-lands.

In the north, the chaparral of the eastern plain is gradually replaced by a mesquite association as one approaches the Sierras. The mountains, except for isolated canyons, have a sparse xeric vegetation along their lower reaches. With increasing elevations of the Sierras is to be found first, a temperate mesic association characterized by oaks, which is then replaced by a montane pine forest near the summits. On the western slopes facing the plateau, the pine association of the Sierras gives way to the arid grassland and xerophytes characteristic of the Chihuahuan desert.

Southward, in the region of Veracruz, the lower portions of the Sierra Madre Oriental are covered by a light to moderately dense jungle. With increasing altitudes, the jungle is gradually replaced by a tropical mesic forest, which is also supplanted, in turn by a temperate mesic oak forest. At still higher elevations, the temperate mesophytes give way to the montane pine forest. Where altitude permits, the pine woodlands merge into a subalpine forest characterized by fir. On the western slopes of the Sierras the pine woodland often merges directly into the arid to semiarid grassland of the Mexican plateau.

Between the Sierra Madre Oriental and the Sierra Madre Occidental lies the highland Mexican plateau. Dominantly it varies from an arid to semiarid grassland; however, scattered here and there are to be found extensive areas dominated by cacti, e.g., *Opuntia, Echinocactus, Cereus,* etc. Northward the plateau gradually assumes the characteristics of the Chihuahuan desert. This Mexican highland is also characterized by isolated peaks and mountain ranges which maintain a mesic vegetation. In some parts of the plateau the lower portions of the mountains are covered with a temperate oak woodland, which changes into the montane pine timberland with increasing elevations. In others, the oak association is omitted, and the pine forest is the first to be found on the mountains. Wherever the peaks are high enough, the pines merge into the subalpine fir association.

The ranges of the Sierra Madre Occidental are quite variable in their flora due to the truncate nature of this mountain system. In the southern portions of the Sierras, most of the peaks of the parallel ranges are covered by the montane pine association; however, in some cases the subalpine fir association is encountered. Normally, with decreasing elevation, a temperate oak woodland is next found. Albeit, due to variations in

the amount of precipitation, xeric associations of grasslands or cacti or both may merge with the pines above. At still lower elevations are to be found either a mesic tropical open woodland or a tropical desert. In the valleys conditions are normally quite xeric except along the streams where luxuriant tropical vegetation grows.

In Sonora, the Sierra Madre Occidental has its peaks covered with a temperate pine forest which gives way at lower elevations to the temperate oak association. The oak woodland gradually or abruptly merges into the typical Sonoran desert xerophytes which extend to the Gulf of California.

The vegetation of the Sierra Madre del Sur in the states of Guerrero, Puebla, and Oaxaca is difficult to characterize. In the higher reaches of the mountains the temperate montane pine association is present. The pine forest normally merges into either an arid grassland or the tropical xeric Oaxacan association containing many species of cacti and plants peculiar to this region. Along streams in suitable places, tropical mesophytes occur.

In the foregoing the general trends and replacements of the various plant associations have been indicated. However, it should be pointed out that abrupt changes in associations due to topography and exposure are one of the major characteristics of the Mexican flora. Often there are areas, small to rather extensive, throughout which the plants are exceedingly different from those in the surrounding areas for miles around. These islands of vegetation may yield the answer to some of the apparently peculiar distributions which have been encountered for some of the species of Drosophila.

Dobzhansky and Pavan (1950) have published data on the seasonal fluctuations in species of Drosophila in Brazil. They give information on the difference in attractiveness of several types of fruits and point out that a large number of fungus-feeders exist in the tropics which are not attracted to fermenting banana bait, as are the ordinary fruit-feeders. There is a definite cycle of frequencies of some species correlated with the seasons, which are more nearly a question of wet versus dry or less wet rather than of hot and cold. For example, the willistoni group varied from 77.3 per cent in a sample of 6,134 individuals in January, 1949, to 14.7 per cent in a sample of 1,058 individuals in July of that year at Vila Atlantica. In other years the percentage of the total represented by this group fell even lower (e.g., 1.3 per cent in September, 1946). During the decrease in the proportion of the willistoni group in 1949, *capricorni* and the medio group increased in numbers. A similar seasonal shift in the willistoni group occurred somewhat later in the year at Pirassununga,

but here the medio group again rose, although the percentage of *capricorni* remained very small and the large increase was made by *guaramunú.*

The willistoni group contains the dominant forms in Brazil. They occur in the greatest frequency more often and in the largest number of samples taken. However, *nebulosa* is the dominant species in four regions, and members of the medio group are also quite common. Unlike the willistoni group, where several members often occur together, the medio group is usually represented by one of its members; for, according to these authors, they are ecologically well differentiated. The least frequently encountered species in the 156 samples analyzed was *ananassae,* which presumably is a migrant from the asiatic tropics. The different environments in Brazil illustrate the relation of diversity and multiplication of species where ecologically diverse environments are available. Only five indigenous species are found in the desert regions, but more than twenty-eight species and species groups are found in the tropics. The authors conclude that tropical regions are the richest in Drosophila fauna; but our data show that the broken and mixed transition zone, between the temperate and tropical regions in Mexico, contains by far the greatest number of known species in a limited region.

The region roughly from 19° to 22° north latitude in southern Mexico is of most interest for Drosophila distribution, for here are to be found eighty-nine of the 391 species known to occur in the Americas, including 50 per cent of those common to both the Nearctic and Neotropical regions (Table 19). The presence of over one-fourth of all the species in these two regions in so small an area, even at the juncture of the Nearctic and Neotropical, is a remarkable phenomenon. There exists here a wide variety of environments in a broken and irregular arrangement, with almost all types of ecological niches, ranging from subalpine to tropical rain forest within a few miles. This area might well be classed as a "hybrid habitat" (Anderson, 1948).

This area coincides with the turn in the chain of forms discussed by Kinsey (1936) in Cynips. Although Drosophila shows no such simple chains of forms as the Cynips, yet the analysis is simplified if this area with its species is used as a basis to discuss the Mexican forms.

The repleta group has about half of its known members in this zone; and, if we consider only North America, twenty-three out of

thirty-three species—or twenty-four out of thirty-five forms, counting the two pairs of subspecies—occur here. Members of all three subgroups, hydei, melanopalpa, and mulleri, are represented in this zone. Some of the mulleri subgroup extend their ranges up the west coast (e.g., *arizonensis* and *mojavensis*). The ranges of *aldrichi, racemova, spenceri, anceps,* and *hexastigma* extend to the south into the Oaxacan district. However, the main ranges of *aldrichi,* together with those of *meridiana, rioensis,* and *mulleri,* extend up the eastern coastal plains, following the cactus belt into Texas. *Drosophila spenceri* also extends up this coastal plain, but is very rarely encountered in Texas. Three other members of the subgroup, *longicornis, mercatorum* and *hamatofila,* are widespread in Mexico and the southwest, occurring in the coastal regions and the plateau as well as to the south.

The majority of the members of the melanopalpa and hydei subgroups are plateau and montane forms, and only occasionally occur in the coastal plains. The species *pachea,* which was found at only one locality, and *nigrospiracula* are Sonoran desert species. Many of these species of the repleta group are associated with the cacti, but it is difficult to demonstrate a complete dependence in any case. Obviously there is a wide difference in ecological and competitive adaptability in all the subgroups. There are no subspecies displacements such as those found in eastern United States.

The difficulty of evaluating an environment in a general locality is shown best from the collections reported by Patterson and Wagner (1943) from the Sonoran desert. Traps set along streams and irrigation ditches with growths of cottonwood and willow yield different populations of flies from those set among cacti and other succulents in the desert proper, even if placed only a few hundred feet apart. The lists below are taken from their data.

Desert Proper	Desert Streams
hydei	*hydei*
pseudoobscura	*pseudoobscura*
arizonensis	*limpiensis*
hamatofila	*victoria*
longicornis	*munda*
nigrospiracula	

These discontinuous habitats account for such isolated species as *pachea* and *grisea.* The latter was found only in the Chiricahua Mountains in Arizona, where it was 16 per cent of the population.

The other Drosophila in Mexico show an even more pronounced

TABLE 19

Species recorded from transition zone in Mexico

Hirtodrosophila
- *D. longala* (1)
- *D. nigrohalterata* (1, 2)

Pholadoris
- *D. baeomya* (1)
- *D. victoria* (1)

Dorsilopha
- **D. busckii* (1–6)

Phloridosa
- *D. floricola* (1, 2)
- *D. lutzii* (1, 2)

Siphlodora
- *D. subsigmoides* (1, 2)

Sophophora
1. saltans group:
   - *D. elliptica* (1)
   - *D. emarginata* (2)
   - **D. prosaltans* (2)
   - *D. rectangularis* (1, 2)
   - **D. sturtevanti* (2)
2. willistoni group:
   - **D. nebulosa* (1, 2)
   - *D. sucinea* (1, 2)
   - **D. willistoni* (1, 2)
3. melanogaster group:
   - **D. ananassae* (1–6)
   - **D. melanogaster* (1–6)
   - **D. simulans* (1–6)
4. obscura group:
   - *D. azteca* (1, 2)
   - *D. dobzhanskii* (1)
   - *D. frolovae* (1)
   - *D. pseudoobscura* (1, 2)
   - *D. tolteca* (1)
5. alagitans group:
   - *D. alagitans* (1)
   - *D. capnoptera* (1, 2)

Drosophila
1. quinaria group:
   - *D. innubila* (1)
   - *D. suboccidentalis* (1)
   - *D. tenebrosa* (1)
2. virilis group:
   - **D. virilis* (1, 2, 3, 5)
3. tripunctata group:
   - *D. crocina* (2)
   - *D. unipunctata* (1, 2)
4. funebris group:
   - **D. funebris* (1–6)
5. repleta group:
   - *D. aldrichi* (1, 2)
   - *D. anceps* (1)
   - *D. bifurca* (1, 2)
   - *D. californica* (1)
   - *D. canapalpa* (1)
   - †*D. fulvimacula* (1, 2)
   - *D. hamatofila* (1, 2)
   - *D. hexastigma* (1)
   - **D. hydei* (1–6)
   - *D. hydeoides* (1)
   - *D. icteroscuta* (1)
   - *D. leonis* (1, 2)
   - *D. linearepleta* (1, 2)
   - *D. longicornis* (1, 2)
   - *D. melanopalpa* (1)
   - *D. meridiana* (1)
   - *D. m. rioensis* (1)
   - †*D. mercatorum* (1, 2, 6)
   - *D. nigricruria* (1, 2)
   - *D. nigrohydei* (1, 2)
   - *D. racemova* (1, 2)
   - **D. repleta* (1–6)
   - *D. ritae* (1, 2)
   - *D. spenceri* (1)
   - *D. subviridis* (1)
6. cardini group:
   - **D. cardini* (1, 2)
   - **D. cardinoides* (2)
   - **D. neocardini* (1, 2)
   - *D. similis* (2)
7. immigrans group:
   - **D. immigrans* (1–6)
8. macroptera group:
   - *D. alafumosa* (1)
   - *D. aurea* (2)
   - *D. macroptera* (1)
   - *D. magnabadia* (1)
   - *D. submacroptera* (1, 2)
9. rubrifrons group:
   - *D. nubiluna* (1)
   - *D. rubidifrons* (1)
   - *D. rubrifrons* (1)
   - *D. spadicifrons* (1)
   - *D. uninubes* (1, 2)
10. annulimana group:
    - *D. gibberosa* (1, 2)
11. guaraní group:
    - *D. subbadia* (2)
12. pallidipennis group:
    - †*D. p. centralis* (2)

Unclassified species:
- *D. bipunctata* (1)
- *D. canalinea* (1, 2)
- **D. castanea* (1, 2)
- *D. crassa* (2)
- *D. fragilis* (1)
- *D. fulvalineata* (1, 2)
- *D. gigas* (1, 2)
- *D. macropolia* (1)
- *D. parachrogaster* (1)
- *D. pollinospadix* (1)
- *D. pondorosa* (1)
- *D. setapex* (1)
- *D. triangula* (1)

Total forms = 89

* South America also.
† Another subspecies in South America.

localization in the narrow 19° to 22° zone and its immediate vicinity. Figures 20 to 22 and 31 to 33 show this accumulation of species having only a few members with ranges extending any distance beyond this area. A few of the species which extend northward into the plateau or the southwest are Mexican or tropical forms, such as *parachrogaster, tranquilla, nebulosa,* and *cardini.* More often these are forms with a wide Mexican and southwestern distribution, such as *fulvalineata, macroptera, rubrifrons, victoria,* and members of the melanica, funebris, and quinaria groups, together with a few western species, such as *pseudoobscura.* The species *carbonaria* is found also in the mesquite association. The more widespread species, such as *rubrifrons* and *macroptera,* with ranges extending up to the Rockies, have species groups within this narrow prolific zone. Other groups are more nearly restricted to this area (Table 19).

As a reasonable deduction, we have said that forms replace closely related forms. In the east this replacement is often by subspecies; but in the west, and particularly in Mexico, the mixed and broken types of distribution, as well perhaps as lack of adequate records, makes recognition of such replacements very difficult. Of course two species, even in different subgenera, may replace each other, as Dobzhansky and Epling (1944) showed for *pseudoobscura* and *occidentalis,* but this type of competition is difficult to recognize. In the case of *aldrichi, mulleri,* and *meridiana,* where all types of larvae have been taken from the same cactus, there is obvious evidence of competition, but this does not seem to prevent their coexistence.

Epling (1944) has made some bold estimates of the age of *pseudoobscura,* even including certain inversion sequences. He states that the gene arrangements are unique and that phylogeny is certain, and that explanations of distribution must be in accord with the phylogeny. Furthermore, he states that so far as was known at that time, the gene arrangements have equal selective values.

The last statement is no longer tenable, in the light of further information on the different selective values of the several gene arrangements under different conditions, which we have already discussed. There is little doubt that part of Epling's discussion of the distribution due to the present major selective values of arrangements (including the values of the heterozygotes) is based on fact. However, as Dobzhansky (1947a, b, c; 1948a, b) has shown, the same gene sequence captured at different localities has very different selective

values. Furthermore, there does not seem to be any differential selective value between the several arrangements at 16°C., so that any argument on distribution due to differential selective values of arrangements can only be valid back through the time when general temperature was the prevailing one. Stebbins (1945), in supporting Epling's theory that not only *Drosophila pseudoobscura* but also the modern gene arrangements and their distribution was determined in the Miocene, makes the following statement concerning the Pleistocene:

> More important is the fact that the scanty evidence available indicates that in this entire region the climate of the last pluvial period was cooler as well as moister than at present. Meinzer (cf. Epling, 1944, page 163) suggests a Pleistocene pluvial climate in southeastern Arizona similar to that now found in southern Oregon, while Hubbs (1945) states that "during late Glacial time the whole western region was . . . cold (not warm) and moist. . . ."

Such a climatic condition would make selection of the gene content of the several arrangements and its control of migration very different in effect from the modern conditions. The fact that *pseudoobscura* can live with certain plant associations now occurring in the west does not imply continued coexistence or an origin related in time. If certain periods of the Pleistocene were cold and moist, *pseudoobscura* might have been completely displaced to southern Mexico by *athabasca,* which is a species apparently better adapted to the cold, for it displaces *pseudoobscura* to the north in Canada and in the high Rockies. Drosophila can invade a large area as such or evolve to suit an area in a relatively short time. For example, all the territory now occupied by *lacicola,* as a migrant from elsewhere or a local evolution, had to be reforested and invaded by that species since the last Pleistocene glaciation.

Simpson (1945) has shown that it is not practical to prove with kinds of evidence available that any particular species of Drosophila, or even a genus itself, is actually bradytelic (having a very slow rate of evolution, Simpson, 1944). Our present knowledge of the species of this genus, which is somewhat better than that used by Stebbins and Simpson, does not suggest any convincing evidence to us on this point. Zimmerman (1948) has brought out the potential rapidity of evolution under suitable circumstances in considering the remarkable endemic insect fauna of Hawaii, which includes many bizarre species

of Drosophila. He gives evidence to prove that the endemic flora and fauna of Hawaii have all evolved since the late Pleiocene.

Mayr (1945) pointed out the fact that the distributions of the several gene sequences are recent, in the sense that they have different selective values under different conditions. The present distribution, then, must reflect the adaptive values of the gene arrangements to local conditions. It has been proved that the same gene arrangement will have different selective values (gene content) at different localities, therefore it is not possible to give a selective value to a particular arrangement, but only to a local race of an arrangement. Furthermore, the selection is complicated by the greater fitness of many inversion heterozygotes, as shown by Dobzhansky and Levene (1948).

Simpson (1945) has shown that there is no direct fossil evidence of Drosophila evolution. He has warned against using present zoogeographical distribution to infer the age of species. We are therefore unwilling to attempt to decide the time of origin of this genus and its members.

4 CHROMOSOME EVOLUTION IN THE GENUS DROSOPHILA

INTRODUCTION

The first studies of any note on the chromosomes of Drosophila were made by Stevens (1907, 1908), who called attention to the fact that, in Diptera, the chromosomes are associated in pairs in metaphase figures. This was found to be true even in cells somewhat removed from the sphere of the maturation process, such as in spermatogonia, ovarian follicle cells, and in some embryonic cells. The observations of Stevens were confirmed and greatly extended for Drosophila in a series of studies by Metz (1914, 1916a, 1916b), who followed the plan of representing the metaphase chromosome configurations of different species by means of diagrams and assigning to a given "type" species which had similar metaphase patterns.

There are several types of changes that occur in the genetic system (genome) during the course of evolution. These include: (1) gene mutations; (2) changes in relative gene frequency (aneuploidy); (3) changes in absolute gene frequency (polyploidy); and (4) changes in gene association through reorganization of a chromosome or chromosomes. Several of these changes lead to changes in the configuration of the chromosome set, the karyotype.

There are no known polyploid species of Drosophila, so polyploidy has not contributed appreciably to the karyotype evolution in this genus. Gene mutations are frequent, but mutations that change the visible chromosome complement have rarely been demonstrated. This is a possible explanation of some of the observed changes. Single genes, or at least single bands on the salivary gland chromosomes, are known which contribute a large and disproportionate part to the ordinary metaphase chromosomes. Muller, Raffel, Gershenson, and Prokofyeva-Belgovskaya (1937) showed that there

existed two separate single salivary gland chromosome bands which contributed a relatively large part of the so-called inert region of the X chromosome of *Drosophila melanogaster*. One called block A was not necessary to the viability and fertility of females that lacked it, although its absence visibly shortened the chromosome. The addition of the other, called block B, to the dot chromosome by translocation about doubled the size of this chromosome. Hinton (1942) showed that there was a single band of the salivary gland chromosome of this type on each side of the centromere of the second chromosome of *melanogaster*. These also contributed a similar disproportionate part to the metaphase chromosome. Certain of the chromosome changes discussed below must be due to shifts and others to mutation or deletion of these types of single-band structures in the salivary gland chromosomes.

Both aneuploidy and especially changes in gene association have contributed to the modification of the chromosome pattern in some of the species studied. We shall limit our discussion to changes in the genus Drosophila, as White (1945) has reviewed chromosome evolution in animals in general.

Sturtevant (1921c) suggested that the inversion of segments of the chromosome might explain the effect of several crossover inhibitors (C factors) that had been found in *melanogaster*. In later publications (Sturtevant, 1926, 1931) he proved the correctness of this explanation. Furthermore, Sturtevant and Plunkett (1926) proved that two related species, *melanogaster* and *simulans,* differed by a major inversion in the third chromosome. This was the first analysis of karyotype differences between related species in the genus and was made by demonstrating the different linkage associations of allelic genes in the two species.

Bridges (1917) reported a case of deficiency of several genes, including *forked* and *Bar,* in the X chromosome of *melanogaster*. The presence of duplications in *melanogaster* material was shown by Bridges (1919). In addition to these spontaneous aneuploid types, Bridges (1923) discovered the first case of spontaneous translocation in *melanogaster*. This translocation was a complex deletion and insertion, involving at least three breaks in chromosomes 2 and 3. A number of additional spontaneous translocations have been found in *melanogaster* since then.

These cases proved that chromosomal rearrangements did occur

in Drosophila and that differences in gene associations did exist between species. The genus is in fact uniquely suited for the study of karyotype evolution, in part because so many of the species breed well in the laboratory. Certain fundamental genetic discoveries were necessary before a real analysis of the chromosomes of the genus could be made.

The analysis of karyotypes in Drosophila has been greatly facilitated by two discoveries made in this laboratory. The first of these was Muller's (1927) discovery that X rays could be employed as a means of inducing mutations and chromosomal rearrangements at a rate far exceeding the occurrence of such changes in natural populations. The use of this method has made it possible to study in great detail various kinds of chromosomal changes, such as inversions, translocations, duplications, and deficiencies. Painter and Muller (1929) demonstrated cytological abnormalities induced by X rays in ordinary metaphase chromosome preparations. The second advance was made by Painter's (1933) development of the salivary gland technique, and especially by his demonstration that the bands of these giant chromosomes precisely follow the sequence of known mutant genes as shown on the genetic maps of Drosophila. These two technical methods have been of the greatest assistance in studying chromosomal rearrangements and their application to speciation in Drosophila.

It has become a common practice in recent years to study the chromosomes of Drosophila by means of acetocarmine smear preparations of the larval ganglia. The giant nerve cells of these organs often give satisfactory division figures. The advantage of this method is threefold: first, it is a very rapid way of obtaining preparations; second, certain structural details, such as the secondary constrictions, are often more clearly revealed than with any other type of preparation; third, the location and extent of heterochromatic segments are hereby brought out in prophase stages. However, this method of preparing slides has certain disadvantages. Unless care is taken in making such preparations, the normal paired arrangement of the chromosomes, and even the morphology of the individual chromosome, may be distorted, making it difficult to recognize the homologues. This is the main reason for our decision to use semidiagrammatic drawings for illustrating the metaphase configurations.

The occurrence of a sufficient number of hybrids is necessary for

an intensive analysis of karyotype evolution. Heterozygotes for comparison between individuals, races, subspecies, and species all add to the knowledge of the nature and degree of divergence. In 1934 only two cases of species hybrids were known in Drosophila. Eight years later Patterson (1942c) listed thirty-one known cases. There are 101 cases listed in Chapter 9, Table 80, a gain of ninety-nine cases in eighteen years; and we know of several other cases which have not been completed for publication. These hybrids provide most favorable material for the study of the details of chromosome evolution.

A number of investigators have studied the effect on reproductive efficiency of the several types of chromosomal rearrangements. Such information is necessary to evaluate the selective advantage or disadvantage enjoyed by such rearrangements, both heterozygous and homozygous. The simpler and more frequent types of rearrangements are diagrammed in Figure 37. Their general effects as demonstrated are discussed below.

Cytological determination of differences between strains or species due to *paracentric inversions* (A) can be easily detected only in the heterozygote. They do not change the metaphase chromosome configuration when heterozygous or homozygous. If both strains are available, the difference in organization can be detected in the homozygotes only by a laborious comparison of the banding of the salivary gland chromosomes.

Homozygous *pericentric inversions* (B) can be identified in the salivary gland chromosomes and even in the metaphase chromosomes, provided they involve detectably unequal segments on the two sides of the centromere. Both types of inversions can readily be analyzed if heterozygous in the salivary gland nuclei.

Figure 37 C, D, and E illustrate several possible types of mutual translocations. Differences between strains or species such as C illustrates can be detected in the heterozygote by examining the salivary gland chromosomes. Differences involving major unequal segments, such as D and E, can be detected in both the heterozygote and homozygote in metaphase and salivary gland chromosomes. If the two chromosome types are available, the particular chromosome or chromosomes involved, as well as the limits of the changes, can always be determined with the salivary gland chromosomes, but only part of the time for the ordinary metaphase chromosomes.

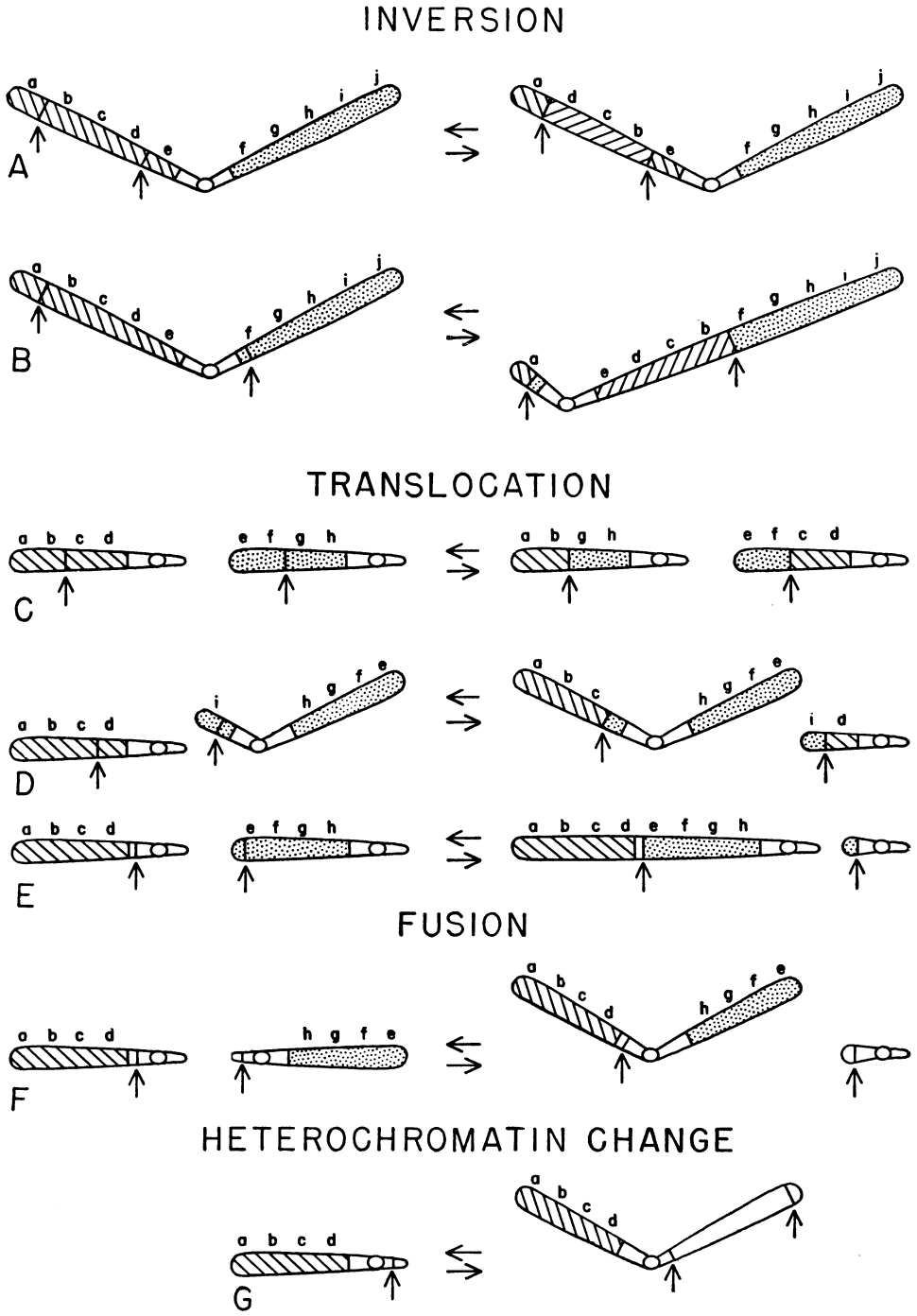

Fig. 37 Diagram illustrating chromosome changes.

The next two types of rearrangements can be observed more readily in the metaphase or by linkage tests for fusions than in the salivary gland chromosome configurations. F is a special type of translocation called a fusion. If the small fragment with a centromere contains no necessary genes, it may be lost by nondisjunction or retained as a supernumerary chromosome, as in *putrida* I and *putrida* II (Figure 42 B and C). G represents the addition or loss of heterochromatin. Such changes have occurred several times in the evolution of this genus. The available information on the effects of these several types of rearrangements is summarized below.

INVERSIONS. Sturtevant and Beadle (1936) have presented the basic genetic evidence and interpretation of the effects of inversions in Drosophila. Since crossing over does not occur in Drosophila males, the effects of inversions will be different from that found in organisms with crossing over in both sexes. (1) Inversions do not cause nondisjunction, so all sperm carry the normal haploid chromosome complement. (2) Homozygous inversions do not affect disjunction or the total amount of crossing over in females, although the distribution of crossovers may be shifted. (3) Simple *paracentric* inversions heterozygous have several effects. (a) Short inversions reduce crossing over inside and outside the limits of the inversion, particularly near the points of rearrangement. (b) Heterozygous long inversions do not materially reduce crossing over. (c) The meiotic divisions are so oriented that the dicentric chromatids (bridges) and the acentric ones (fragments) formed by single exchange within the inversion are eliminated during these divisions by passing into the polar body nuclei, while the female pronucleus receives one of the noncrossover chromatids.

In case of double crossing over, two- and three-strand double exchanges produce gametes which are either noncrossovers or doubles, owing to the orienting effect of such bridges. Four-strand double exchange causes the formation of two bridges in the first division, which so orients the maturation division that the female pronucleus produced in this case contains no chromatid from that tetrad. These eggs produce inviable zygotes, or patroclinous males for X chromosome inversions. The amount of crossing over outside the limits of heterozygous inversions may be reduced, but leads to no abnormal effect. However, the rare three-strand double exchanges, with one exchange within the inversion and the other between the inversion and the

centromere, produce a bridge in the second meiotic division, and so one-half the eggs in such cases form inviable zygotes. (4) A simple heterozygous *pericentric* inversion produces aneuploid gametes when single- or three- or four-strand exchanges occur within the limits of the inversion, but two-strand double exchanges give normal recombination. (5) A transposition of the centromere without a change in gene order through a three-break rearrangement (a centromere shift) produces a situation similar to a *paracentric* inversion and is not at the great selective disadvantage of a *pericentric* inversion.

To summarize, inversions do not increase the rate of nondisjunction. When chromatid exchanges occur within the limits of a heterozygous *paracentric* inversion, or a transposition of the centromere, they produce so few aneuploid gametes that they are at only a slight selective disadvantage; *pericentric* inversions produce relatively many aneuploid gametes, and so are at a selective disadvantage. Homozygous inversions have no selective advantage or disadvantage *per se*.

TRANSLOCATIONS. Brown (1940) has made the most complete and pertinent analysis of the effect of translocations on crossing over and disjunction in Drosophila. The main points are: (1) Disjunction is normal in both males and females homozygous for translocations. Crossover values may be altered by changes in lengths of chromosome arms and by changes in centromere position. Reduction in length of a chromosome arm does not reduce crossing over, unless a part of the arm is moved closer to the centromere. (2) The males heterozygous for translocations produce abnormal gametes as a result of nondisjunction, which reduces by about one-half in *melanogaster* the functional zygotes produced from mating these males to normal females. This was true for the several types of translocations involving segments of the same or different lengths. As crossing over does not occur with any appreciable frequency in the male of Drosophila, this result is generally applicable throughout the genus.

In females heterozygous for a translocation, both crossing over and disjunction are affected. Normal crossing over and normal disjunction are interfered with to a greater extent if the breaks are in the chromosome arms than if they are adjacent to the centromeres. In the latter case, crossing over may be normal in the gametes recovered when the heterozygous female is crossed to a normal male. The rate of nondisjunction decreases in the most favorable cases to give a minimum of 30 per cent aneuploid gametes. Although chias-

mata help orient disjunction, the mechanism is not perfect in Drosophila, but varies with the circumstances of the configurations (see Brown, 1940).

In general, all translocations if heterozygous reduce the number of normal gametes about 40 to 50 per cent in the male. In the female, the reduction in number of functional gametes varies in the known cases from 30 to 50 per cent. Therefore, heterozygous translocations are at a decided selective disadvantage in Drosophila. In fact, Wright (1941) has shown that the selective disadvantage expected in these cases is of such a magnitude that translocations would be retained very rarely and only under exceptional circumstances.

FUSIONS. In *melanogaster,* X-4 fusions have been studied by Stone (1934), Painter and Stone (1935), and Stone and Griffen (1940). Crossing over is not usually affected, although it was reduced slightly in the region of the fusion in some cases. Nondisjunction due to fusion has not been studied in *melanogaster.* One of the cases pertinent to the evolution of new species, the X-4 (A-B) fusion of *Drosophila americana americana,* has been tested for its effect on disjunction (Stone, 1949). The study was made possible by the fact that the subspecies *Drosophila americana texana* does not have this fusion.

The *americana/texana* hybrid males and females are viable and fertile. When these hybrids heterozygous for the fusion are tested to a homozygous *texana* stock, the egg hatch is normal—around 95 per cent for crosses with both male and female hybrids. As this egg hatch is normal, the fusion is at no selective disadvantage in the heterozygous and suffers no disadvantage in the homozygous condition.

HETEROCHROMATIN. We have very little information on the loss or addition of heterochromatin. As mentioned above, Muller, Raffel, Gershenson, and Prokofyeva-Belgovskaya (1937) obtained viable and fertile females which had lost an easily detectable portion of their heterochromatin, block A. At least one extra Y (heterochromatin) may be added to either sex in *melanogaster* without adverse effect.

Many important studies have been made on the chromosomes of Drosophila, using the techniques described above. Here we are concerned with the results of those investigations which have contributed to the analysis of the metaphase patterns of the different species. Among such investigations should be mentioned the following: Metz (1916a, b) on various Drosophila; Sturtevant and

Dobzhansky (1936) on the affinis subgroup, and various other studies from the Pasadena Laboratory on *pseudoobscura* and related forms; Kikkawa and Peng (1938) on the Japanese species; Sturtevant (1942) on members of the saltans group and other forms, and Sturtevant and Novitski (1941b) on chromosome elements; Buzzati-Traverso (1941) on members of the obscura subgroup; Patterson, Stone, and Griffen (1940b, 1942) on members of the virilis group; Dobzhansky and Pavan (1943a) and Pavan and da Cunha (1947) on various species from South America. The results from the Texas laboratory have been summarized in a series of papers by Wharton (1942, 1943, 1944), who examined the chromosomes of the larval ganglia and the salivary gland nuclei of a large number of different forms and analyzed their metaphase configurations. More recently Ward (1949) has studied a number of additional forms. Other references will be found in the text.

There are a number of records found in the literature on the cytology of Drosophila in which a difference of opinion exists regarding the exact composition of the metaphase configuration for a given species. Some of these differences have been cleared up in subsequent studies, but several still remain. There are three possible explanations to account for these differences. The disagreements may be due to observational errors or to differences in interpretation of the divisional figure by different observers. In some cases the confusion is due to the fact that two different forms were involved, one of which was incorrectly identified. A third possibility is that the chromosome configuration may vary in different geographical strains of the same species. A number of such cases have been reported in the literature. Thus Miller (1939), working on different strains of *algonquin,* describes a metaphase configuration due to a pericentric inversion which differs from the one reported by Sturtevant and Dobzhansky (1936a) for the same species. Kikkawa (1936b) has described two "races" of *montium* with different configurations. Wharton (1943) reported two strains each of *putrida* and *micromelanica* which had different metaphase patterns. Carson and Stalker (1947) found that in *robusta* a pericentric inversion, which had transformed an autosomal V into a J, was common among specimens in the northern part of the range of this species. Finally, Ward (1949) has studied several additional cases of such variations.

ANALYSIS OF CHROMOSOME CONFIGURATIONS

Figures 38 through 46 display 121 diagrams of metaphase configurations of 215 different forms of Drosophila. Many of these illustrations have been redrawn from previously published figures, or else they have been constructed from descriptions of chromosome sets. The figures represent the male set in all cases in which the sex chromosomes have been identified, and unless otherwise indicated the X chromosome is on the left and the Y on the right at the bottom of each figure. We shall refer in the text to the haploid complement, and not to the illustrated diploid number of chromosomes. The different species are presented in the same general systematic order as was followed in Chapter 2. We have attempted to analyze each configuration in terms of modifications of the basic Drosophila chromosome complement of five rods and a dot. Where information on both the metaphase and salivary chromosomes was available, a satisfactory analysis was not too difficult. Final analyses of cases where the salivary gland chromosomes are unknown must await further study.

The summary of our analyses is given in Tables 20 to 27. These show both the metaphase and salivary gland chromosome configurations and the number of the figure best illustrating the metaphase pattern. In case a species has a pattern with no counterpart in its own group, the appropriate figure (marked with a star) from another group is given. Whenever possible, we have given as our authority the author who has analyzed the two configurations from the same strain of a species, and in other cases we have given the author followed. We have also shown under appropriate headings the fusions, pericentric inversions, and additions of heterochromatin, which represent the simplest explanation for the changes from the basic chromosome complement.

Hirtodrosophila

The chromosomes of only three of the known species of this subgenus have been worked out. These are *orbospiracula, longala,* and *duncani.* There are six chromosomes in *orbospiracula*—four autosomal rods of about equal length, one longer rod with a proximal constriction representing the X chromosome, and a dot. The salivary

gland preparations show five long euchromatic strands and a short strand representing the dot (Wharton, 1943). In the male the X chromosome does not have a synaptic mate, as the metaphase divisional figures of the larval brain do not show a Y chromosome (Figure 38 A). The male of this species therefore belongs to the "XO type," of which only four cases have been reported for the genus Drosophila. It is possible that the Y chromosome material is represented in the heterochromatic regions of one or more of the rod-shaped chromosomes.

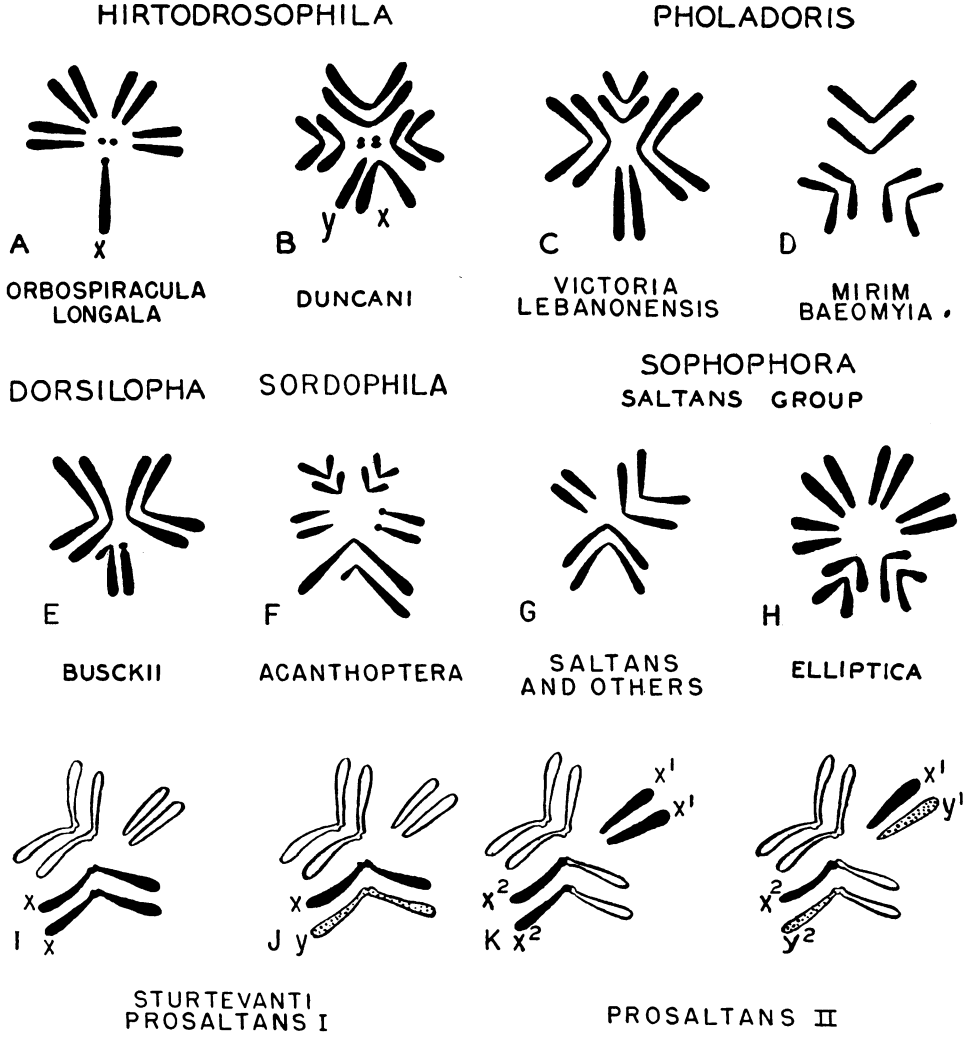

Fig. 38 Metaphase configurations of members of the Hirtodrosophila, Pholadoris, Dorsilopha, and Sordophila subgenera, and the saltans group of the Sophophora.

There are only five chromosomes in *duncani,* instead of six as in *orbospiracula;* but in the female all chromosomes are V's, including three autosomes, the X, and a microchromosome (Figure 38 B). In the male the Y chromosome appears as a rod. The salivary gland nuclei have eight long strands and a dot. According to Wharton (1943), this condition in *duncani* has been brought about by pericentric inversions in the X and two of the larger autosomes, and perhaps also in the microchromosome, which has extra heterochromatin. The other V-shaped autosomes must have arisen through a fusion of two rods (Table 20).

Pholadoris

Six species have been analyzed in this subgenus. These consist of *victoria* and *lebanonensis,* which are closely related and have the same configurations, consisting of two large V's and a small V and a rod (Figure 38 C). The Japanese *coracina* differs from these two in having a dot in place of a small V (Figure 39 C). New World species, *mirim* and *baeomyia,* are alike and have three V's (Figure 38 D). The European *nitens* has four V-shaped chromosomes (Figure 39 G). In *victoria* and *lebanonensis,* the chromosome configuration is the result of one autosomal pericentric inversion, one fusion between two rod-shaped autosomes, and one fusion of an autosome with the dot, which has added heterochromatin (Table 20).[1]

Dorsilopha

The type and only known species belonging to this subgenus is the cosmopolitan *busckii.* It has but three metaphase chromosomes, con-

[1] In this and several other tables throughout the book it was necessary to use certain abbreviations in listing the authors, as follows: de B., de Barros; B. *et al.,* Burla, Cordeiro, Dobzhansky, Malogolowkin, Pavan; Buzz., Buzzati-Traverso; C. & S., Carson and Stalker; D. or Dob., Dobzhansky; D. & P., Dobzhansky and Pavan; Emm., Emmens; Frol., Frolowa; F. & A., Frolowa and Astaurow; G., Griffen; H., Hsu; Hugh., Hughes; Kik., Kikkawa; K. & P., Kikkawa and Peng; Le Cal., Le Calvez; Mag., Magalhaes; M. or Main., Mainland; Mill., Miller; Pain., Painter; P. or Pat., Patterson; P. & G., Patterson and Griffen; P. & M., Patterson and Mainland; P. and S., Patterson and Stone; P. S. G., Patterson, Stone, and Griffen; Pav., Pavan; P. & C., Pavan and da Cunha; S., Sheng; Sok., Sokolov; Stalk., Stalker; S. G. P., Stone, Griffen, Patterson; Stvt., Sturtevant; S. & N., Sturtevant and Novitski; T., Tan; T.H.S., Tan, Hsu, and Sheng; Wh., Wharton; Wheel., Wheeler.

TABLE 20

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-A, X-D, A-A, A-D				Peri. X A D			Heter. X A D		
Hirtodrosophila														
duncani	2V, 2v, 1Dv	8A, 1D	38 B	Wh., '43			1		1	2				1
longala	5R, 1D	5A, 1D	38 A	Ward (un.)										
orbospiracula	5R, 1D	5A, 1D	38 A	Wh., '43										
Pholadoris														
victoria	1R, 2V, 1v	6A, 1D	38 C	Wh., '43			1	1		1				1
lebanonensis	1R, 2V, 1v	6A, 1D	38 C	Ward, '49			1	1		1				1
coracina	1R, 2V, 1D		39 C*	K. P., '38										
mirim	3V	6A	38 D	D. P., '43a										
baeomyia	3V	6A	38 D	Ward (un.)										
nitens	4V		39 G*	Buzz., '43										
Dorsilopha														
busckii	1J, 2V	5A, 1D	38 E	Wh., 43		1	2							
Sordophila														
acanthoptera	2R, 1V, 2v	7A, 1D	38 F	Ward, '49	1					2				1
Sophophora														
saltans	1R, 2V	5A, 1D	38 G	Wh., '43	1		1	1						
sturtevanti	1R, 2V	5A, 1D	38 I	Wh., '43	1		1	1						
prosaltans I	1R, 2V	5A, 1D	38 I	Wh., '43	1		1	1						
prosaltans II	1R, 2V	5A	38 K	D. P., '43b	1		1	1						
emarginata	1R, 2V	5A	38 G	Ward (un.)	1		1	1						
cordata	1R, 2V		38 G	Stvt., '42										
rectangularis	1R, 2V		38 G	Stvt., '42										
earlei	1R, 2V		38 G	Metz, '16b										
elliptica	4R, 1v, 1J		38 H	Stvt., '42										
neosaltans	1R, 2V		38 G	P. M., '50										
neoelliptica	1R, 2V		38 G	P. M., '50										
nebulosa	1R, 2V, 1D	5A, 1D	39 B	Wh., '43	1		1							
willistoni	1R, 2V	5A	39 A	B. *et al.*, '49	1		1	1						
equinoxialis	1R, 2V	5A	39 A	B. *et al.*, '49						1				
paulistorum	1R, 2V	5A	39 A	B. *et al.*, '49						1				
tropicalis	1R, 2V	5A	39 A	B. *et al.*, '49										
capricorni	1R, 2V		39 A	D. P., '43a										
fumipennis	1R, 2V		39 A	D. P., '43a										
sucinea	1R, 2V		39 A	P. M., '44										
bocainensis	1R, 2V		39 A	P. C., '47										
melanogaster	1R, 2V, 1D	5A, 1D	39 C	Pain., '33			2							
simulans	1R, 2V, 1D	5A, 1D	39 C	Patau, '35			2							
rufa	1R, 2V, 1D	5A, 1D	39 D	Ward, '49			2							

sisting of two large autosomal V's and a rodlike X. Wharton found that the X was occasionally bent near its proximal end and may appear J-shaped (Figure 38 E). She also found that the salivary gland nuclei have five long euchromatic strands and a dot. Hence the two autosomal V's must represent fusion of rods, and the dotlike element is probably united with the X to form the J-shaped figure (Table 20). It is interesting to note that a Russian strain of this species has a satellite at the proximal end of the X and a J-shaped Y chromosome (M. I. Sirotina; D.I.S. 11, page 49), but this satellite was not demonstrable in the South African *busckii,* although the number of strands seen in the salivary gland nuclei justifies its presence (G. Eloff; D.I.S. 13, page 71). These determinations do not agree with the reports of Metz (1916a) and Kikkawa and Peng (1938), who state that *busckii* has four chromosomes, with the dot distinct in metaphase figures.

Sordophila

This subgenus has but a single known species, *acanthoptera,* which has one large V, two small V's, and two rods, one of which has a proximal constriction (Figure 38 F). The salivary gland nuclei have seven long strands and a dot. The cytological check shows that this pattern is the result of an X-A fusion, two autosomal pericentric inversions, and the addition of heterochromatin to the dot (Table 20).

Sophophora

saltans group

From the standpoint of their metaphase configurations, the ten species of the saltans group would appear to consist of two types. The first type has two large V's and a rod (Figure 38 G), as determined by Metz (1916b) for *earlei;* by Sturtevant (1942) for *cordata, emarginata, rectangularis, sturtevanti* (*biopaca*), and *prosaltans* (*sellata*); and by Pavan and Magalhaes (1950) for *neoelliptica* and *neosaltans.* A slight variation of this type is found in *saltans,* which Wharton (1943), in a strain supplied by the Wooster Laboratory, reported had one V, one J, and one rod-shaped chromosome. The second type is represented by *elliptica* (Sturtevant, 1942), which has six chromosomes—four rods, one small V, and a small J (Figure 38 H). Since

the salivary gland nuclei of the three species examined by Wharton (*saltans, sturtevanti, prosaltans*) each had five long strands and a dot, it is evident that the latter chromosome, which does not show in metaphase figures, must have become fused to one of the other chromosomes, apparently to the end of the rod which sometimes appears J-shaped (Sturtevant, 1942).

It is not possible to determine by inspection of metaphase figures which chromosomes represent the sex chromosomes, as the metaphase plates seem to be the same in both sexes. However, Dobzhansky and Pavan (1943b) have shown that in *sturtevanti* one of the pairs of V's represents the sex chromosomes (Figure 38 I, J). They also stated that *prosaltans* II could have arisen from *prosaltans* I by a mutual translocation of the sex chromosomes and the rod-shaped autosome (Figure 38 K, L). This analysis would further indicate the presence of X-A and A-A fusions (Table 20). The metaphase configuration of *elliptica* is entirely different from those of the other seven species and must represent another line of evolutionary divergence.

willistoni group

The chromosomes of eight members of this group have been examined and their metaphase configurations determined. These determinations have been made by Dobzhansky and Pavan (1943a, 1949) for *willistoni, paulistorum, capricorni,* and *fumipennis,* by Patterson and Mainland (1944) for *sucinea,* by Dobzhansky (1946b) for *equinoxialis,* and by Pavan and da Cunha (1947) for *bocainensis.* According to the findings of these investigators, these seven species have three chromosomes, consisting of two V's and a rod (Figure 39A). There has been some difference of opinion about the presence of a dot in the metaphase plates of *willistoni.* Metz (1916b), using a strain of *pallida* (synonym of *willistoni* Sturtevant), first reported the presence of a free dot in metaphase figures. Wharton (1943) also reported a free dot in metaphase figures. However, Lancefield and Metz (1921) state that the chromosomes of this species correspond to those of *melanogaster,* with the possible exception of the absence of the dot. Dobzhansky and Pavan (1943a) and Dobzhansky (1946b) state definitely that the South American strains of *willistoni* do not show a dot in metaphase figures. Two possible explanations may be suggested to account for this apparent difference in observation. Either two species are involved, or else the dot is fused in some strains and is free in

others. Sturtevant and Novitski (1941b) have pointed out that neither cytological nor genetic evidence gives a clue as to the location of the dot in *willistoni*. They suggest that the most likely place to look for it

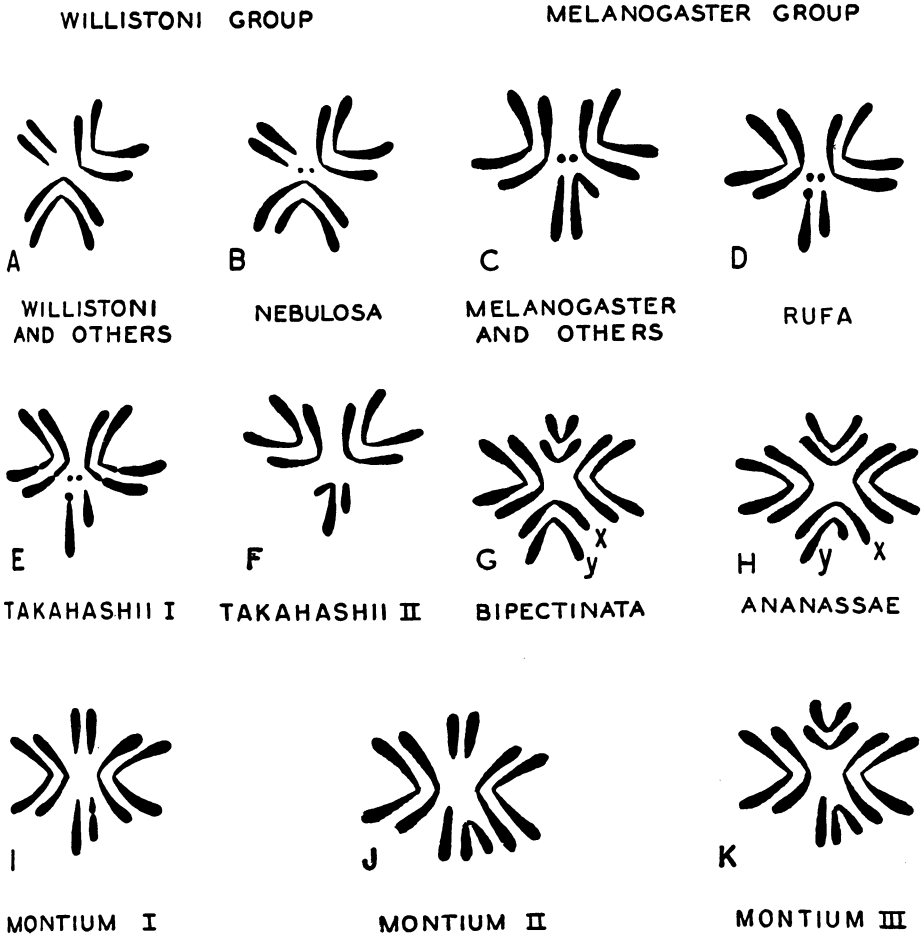

Fig. 39 Metaphase configurations of members of the willistoni and melanogaster groups.

would appear to be as a short arm on the apparently rod-shaped chromosome. Finally Sturtevant (1942) states that, in both the saltans and willistoni groups, the dot is attached to a rod to give a J, the short arm of which is often overlooked.

The eighth species, *nebulosa,* undoubtedly shows a dot in metaphase figures in some strains (Figure 39 B), as determined by Metz (1916b, synonym *limbata*) on a strain from Cuba and by Wharton

(1943) on a strain from Texas. In *nebulosa* and *willistoni,* Wharton found that the salivary gland nuclei contained five long euchromatic strands and a dot. Pavan (1946a) and Ward (1949) found strains of *nebulosa* which had no free dot chromosome. Hence, the dot is probably included in the rod-shaped autosome.

Lancefield and Metz (1921), using a nondisjunction strain of *willistoni,* showed that one of the pairs of V-shaped elements represented the sex chromosomes. This seems to be generally true in the group, as judged by the fact that *nebulosa* also has a V-shaped sex chromosome (Pavan, 1946a; Ward, 1949). In addition to this X-A fusion, there is an A-A fusion as well as an A-D fusion in most forms (Table 20).

melanogaster group

The chromosomes of twelve of the species listed under this group in Chapter 2 have been studied, and, according to the descriptions of the different investigators, the metaphase configurations are of four types. The first is the well-known type of *melanogaster* and *simulans,* both of which have two autosomal V's, a rod-shaped X, and a dot. The Y chromosome is J-shaped in *melanogaster* and is either a small rod or a J in *simulans* (Figure 39 C). Kikkawa and Peng (1938) list six additional species as having this same type of configuration (Figure 39 C, D, E). These are *auraria, ficusphila, lutea, rufa, suzukii,* and *takahashii.* However, according to Sturtevant (1942), the dot element of *takahashii* is attached to the X (Figure 39 F). In all of the species of this group in which the X chromosome has been identified, it has been found to be rod-shaped, but the Y chromosomes may vary from a small rod to a J (Kikkawa and Peng, 1938). More recently, Tan, Hsu, and Sheng (1949) have added another species, *pulchrella,* to this group. It has the typical melanogaster-type pattern. These authors and Ward (1949) found that the dot was present as such in the Chinese strain.

The second type includes *D. montium,* which has three races that differ from each other in the morphology of one chromosome (Kikkawa, 1936b; Tan and Hsu, 1944; Ward, 1949). These races are really three different chromosome-type strains. In one strain (I) there are two V's and two rods, one of which is the X chromosome. Ward found the Y chromosome to be a small rod (Figure 39 I). The second strain (II) differs only in that the Y is a small V (Figure 39 J). In

the other strain (III) there are also four chromosomes, but the autosomal rod has been transformed into a small V, while the other chromosomes are similar to the corresponding ones of the second strain (Figure 39 K). The salivary gland nuclei show six strands branching off from the chromocenter, but the shortest strand is also attached to this body at its distal end and is largely heterochromatic, according to Kikkawa (1936b), who worked on strains II (B) and III (A).

The third type includes *bipectinata* and *ananassae,* each of which has four V-shaped chromosomes, two large, one medium, and one small. The medium-sized V is the X chromosome in both species. The Y chromosome is also a V in *bipectinata,* but is J-shaped in *ananassae* (Figure 39 G and H). As first shown by Metz (1916b, as *caribbea* Sturtevant), the small V in *ananassae* represents a dot chromosome. A part of the X has been translocated to the dot, which is largely heterochromatic (Kaufmann, 1937; Kikkawa, 1936a, 1937, 1938). This chromosome in *bipectinata* and the three strains of *montium* probably arose from a common ancestor. The remainder of the X chromosome in both *ananassae* and *bipectinata* has acquired a median centromere and is V-shaped. The salivary gland nuclei have six long strands and a dot in both species (Wharton, 1943). Both Kaufmann (1937) and Kikkawa (1938) have shown that in *ananassae,* in addition to the six long strands, the two arms of the 4 chromosome are represented by heterochromatic masses embedded in the chromocenter.

In the light of our knowledge concerning the nature of the salivary gland chromosomes in several of these species, the following analysis of the metaphase chromosome configurations in the melanogaster group may be offered. It will be observed that two large V's are common to all nine diagrams shown in Figure 39 C to K. It is reasonable to assume that, in each case, two rods have fused to form a V, which is known to be true for most of the species. The dotlike chromosome is seen in metaphase of the several species represented in diagrams C to E, but is absent as such in the rest of the series. In one strain (F) it is attached to the X, and in others (G to K) it has received material from the X by translocation, as judged by *ananassae*. The X chromosome is rod-shaped in six of the diagrams C, D, E, I, J, K), J-shaped in one (F), and V-shaped in two (G, H), owing to pericentric inversions. The Y chromosome is rod-shaped in

several cases (D, E, F, I), J-shaped in two (C, H), and a small V in three (G, J, K).

obscura group

The members of this group reported here may be divided into three subgroups, as follows: (a) obscura subgroup consisting of six species from Europe; (b) pseudoobscura subgroup consisting of four species from North America; (c) affinis subgroup of eight species from North America, plus one European species (*helvetica*) tentatively assigned to this subgroup.

The metaphase chromosome configurations of the six European forms are illustrated in Figure 40 A to F. The first five diagrams were constructed from the account of Buzzati-Traverso (1941), while the sixth was taken from the report of Frolowa and Astaurow (1929). We have included *obscura,* although Buzzati-Traverso states that its taxonomic status is somewhat uncertain. Until a more detailed study has been made of the chromosomes of these species, including salivary gland chromosomes, it will be difficult to give accurate analyses of the different configurations. It is possible, however, to indicate some of the probable changes which have taken place in the subgroup.

It will be observed that all six species have the dotlike element present in metaphase figures, although it varies in size from a mere speck in *ambigua* to what amounts to a short rod in *bifasciata.* The simplest pattern is found in *subobscura,* which has the basic Drosophila number of five rods and a dot (Figure 40 A). The next species in order would be *obscuroides,* which also has six chromosomes, but two of these are V's and one is a J (Figure 40 B). One arm of the X is slightly longer than the other, and one of the autosomes is an equal-armed V, while another is distinctly J-shaped. In each of the four remaining species there are only five chromosomes, of which the four largest are either V's or J's. The X chromosome is a rod in *subobscura,* V-shaped in *bifasciata, ambigua,* and *obscura,* and slightly J-shaped in *obscuroides* and *tristis.* The Y chromosome is a rod in *subobscura, obscuroides,* and *obscura,* a J with one short arm in *bifasciata* and *ambigua,* and distinctly J-shaped in *tristis.* Buzzati-Traverso (personal communication) reports that the salivary gland chromosome configuration shows eight arms and a dot in *ambigua, bifasciata,* and *tristis.* This must mean that one fusion and three pericentric inversions are present in each of these species.

The chromosomes of three species belonging to the pseudoobscura subgroup have been extensively studied. Metz (1916a, 1916b) appears to have been the first to report on the chromosome number of

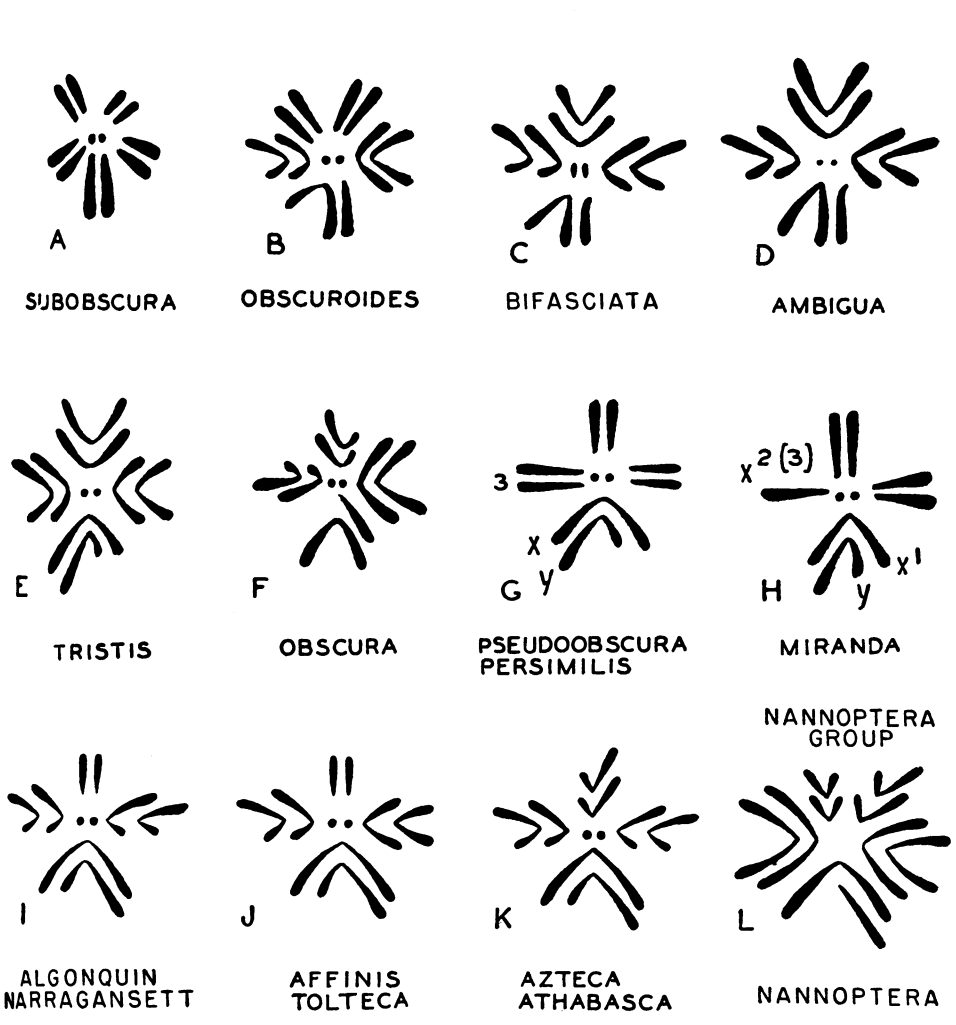

Fig. 40 Metaphase configurations of members of the obscura and nannoptera groups.

what is now known as *D. pseudoobscura* of North America (at that time supposed to be the same as *D. obscura* Fallén of Europe). He reported the female as having five chromosomes: a V-shaped X, three rods, and a dot. The Y chromosome was said to be rod-shaped and about half the length of the X. We now know that the metaphase

configurations of the females of all three members of this subgroup are essentially the same as reported by Metz for *pseudoobscura.* Lancefield (1922), on the basis of genetic evidence, suggested that the X chromosome of *pseudoobscura* is compound, and this is now known to be true for all three species. According to Sturtevant and Novitski (1941b), the left-hand arm, or XL, is equivalent to their element A and XR to element D. It must therefore represent a fusion. The spermatogenesis of *pseudoobscura* has been studied by Metz (1926), Koller and Townson (1933), Koller (1934), Dobzhansky (1934), and Darlington (1934); and that of *miranda* by Dobzhansky (1935b), MacKnight and Cooper (1944), and Cooper (1946). Dobzhansky (1935a) found that the Y chromosome is variable in *pseudoobscura* (and *persimilis*) and may have a median, submedian, or subterminal centromere (Figure 40 G). In *miranda* an original 3 chromosome has become a sex element, and one 3 chromosome has been incorporated in the Y and has lost many of its genes. The free X^2 (3) chromosome and the original X^1 chromosome are now both homologous with the Y chromosome (Figure 40 H). This relation will be more fully discussed in a later chapter.

The chromosomes of six members of the affinis subgroup have been investigated. Those of *algonquin, affinis, azteca,* and *athabasca* have been reported on by Sturtevant and Dobzhansky (1936a), and the three diagrams shown in Figure 40 I to K are based on their descriptions and accompanying illustrations of metaphase plates of the giant nerve cells of the larval ganglia. According to their account, the metaphase configuration of *algonquin* has a V-shaped X, two J-shaped autosomes, a rod-shaped autosome, and a dot (Figure 40 I). However, Miller (1939), using a different standard stock, gives a somewhat different account, in that he designates one of the autosomes as a V instead of a J and indicates that the centromere of the rod is not quite terminal. He points out that the change from a J to a V is due to an inversion across the centromere of this chromosome. Ward (1949) has shown that the chromosome pattern of *narragansett* is like that of *algonquin.* In *affinis,* one of the autosomes is distinctly V-shaped (Figure 40 J). We have examined the chromosomes of *tolteca* and find that its metaphase configuration, as seen in the division figures of the giant nerve cells of the larval ganglia, is practically the same as in *affinis.* The only differences noted were that the short arm of the Y is longer and the short arm of the autosomal J somewhat

shorter than in *affinis*. The metaphase configurations of *azteca* and *athabasca* appear to be identical, each with three J-shaped autosomes (Figure 40 K). In these six species of the affinis subgroup, the Y is J-shaped and the X is a V. The salivary gland nuclei all have seven long strands and a dot, and, judging from this condition, the X chromosome must represent a fusion of two rods. The rod of *azteca* and *athabasca* has accumulated heterochromatin beyond the centromere, making it J-shaped. The other V- and J-shaped chromosomes must have resulted from pericentric inversions.

nannoptera group

The metaphase configuration of the single known species of this group has three large V's and two smaller unequal-armed V's, one of which is distinctly J-shaped. The salivary gland nuclei show six arms and a dot, and that there is an X-A fusion. In addition, there is one pericentric inversion, and heterochromatin has been added to two autosomes and the dot (Figure 40 L).

bromeliae group

The three species included in this group are *bromeliae, florae,* and *bromelioides* (Table 21). The salivary gland chromosomes are not known for any of the three. The metaphase configurations of the first two are similar to that of *melanogaster* (Figure 39 C*) according to Metz (1916b), but the metaphase configuration of *bromelioides* (Pavan and da Cunha, 1947) resembles that of *victoria* (Figure 38 C*).

Drosophila

A majority of the known species of the complex subgenus Drosophila has now been divided into a series of twenty-two species groups, which vary in size from a single species to one with over fifty known forms. These will be presented in the same order as was followed in Chapter 2.

quinaria group

Both the metaphase and salivary gland chromosomes of ten species of this group have been worked out by Wharton (1943). The metaphase configurations of these species are illustrated in the seven dia-

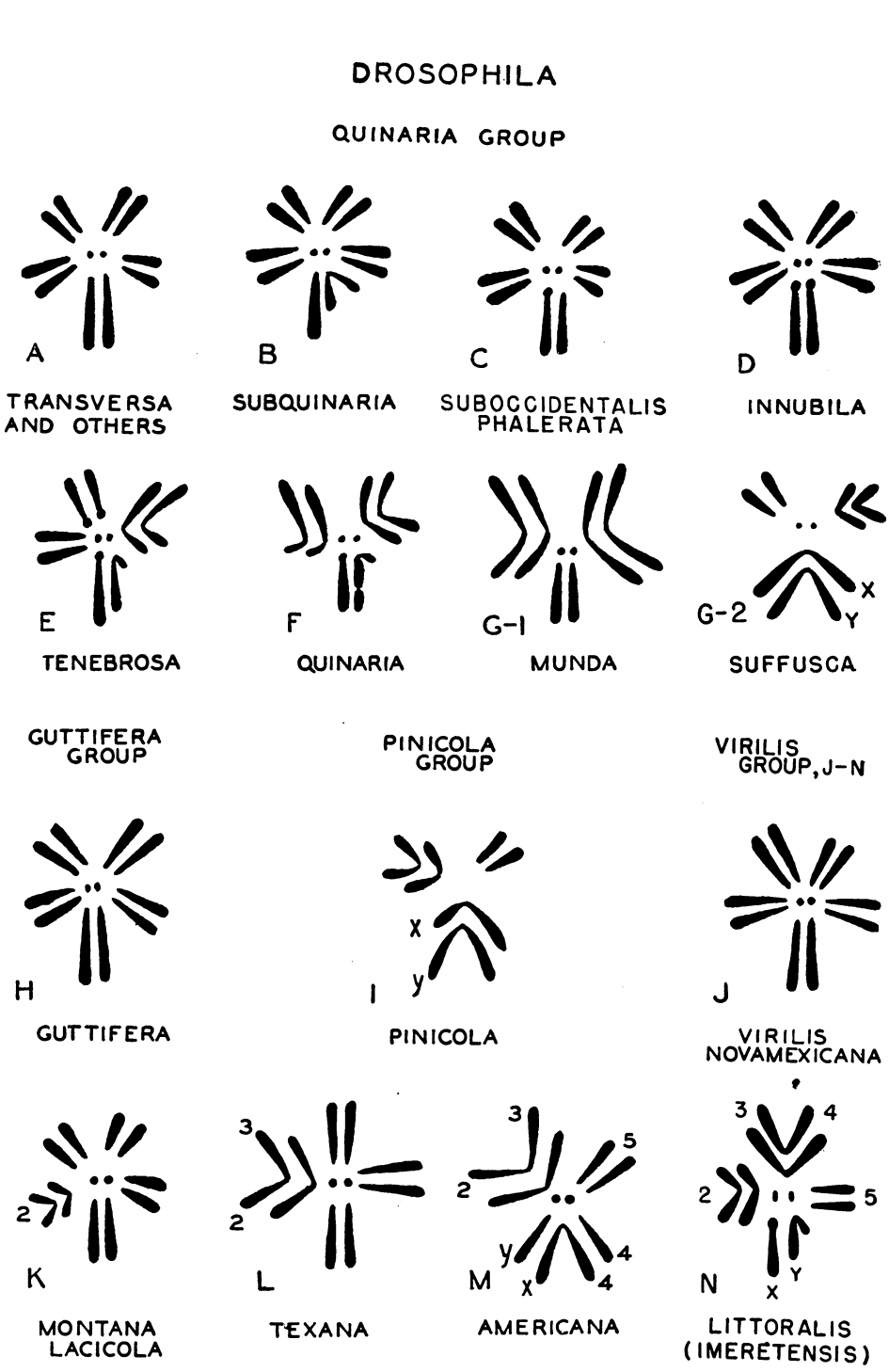

Fig. 41 Metaphase configurations of members of the quinaria, guttifera, pinicola, and virilis groups.

grams shown in Figure 41, A to G. Four of the species (*tranversa, palustris, subpalustris,* and *occidentalis*) appear to have identical metaphase patterns, and to this list should be added *mutandis* (Tan, Hsu, and Sheng, 1949). Each species has five rods and a dot (Figure

TABLE 21

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-A, X-D, A-A, A-D	Peri. X A D	Heter. X A D
auraria	1R, 2V, 1D		39 C	K. & P., '38			
ficusphila	1R, 2V, 1D		39 C	K. & P., '38			
lutea	1R, 2V, 1D		39 C	K. & P., '38			
pulchrella	1R, 2V, 1D		39 C	T.H.S., '49			
suzukii	1R, 2V, 1D		39 C	K. & P., '38			
takahashii I	1R, 2V, 1D	5A, 1D	39 E	Ward, '49	2		
takahashii II	1R, 2V		39 F	Stvt., '42	1 2		
montium I	2R, 2V	5A, 1D	39 I	Ward, '49	2		1
montium II (B)	2R, 2V	5A, 1D	39 J	Kik., '36	2		1
montium III (A)	1R, 2V, 1v	5A, 1D	39 K	Kik., '36	2	1	1
bipectinata	2V, 2v	6A, 1D	39 G	Kik., '38	2	1 1	1
ananassae	2V, 2v	6A, 1D	39 H	Kik., '35	2	1 1	1
subobscura	5R, 1D	5A, 1D	40 A	Emm., '37			
obscuroides	2R, 2V, 1J, 1D		40 B	Buzz., '41			
bifasciata	2V, 2J, 1D	8A, 1D	40 C	Buzz., '41	1	3	
ambigua	2V, 2J, 1D	8A, 1D	40 D	Buzz., '41	1	3	
tristis	3V, 1J, 1D	8A, 1D	40 E	Buzz., '41	1	3	
obscura	2V, 2J, 1D		40 F	F. & A., '29			
pseudo-obscura	3R, 1V, 1D	5A, 1D	40 G	Tan, '35	1		
persimilis	3R, 1V, 1D	5A, 1D	40 G	Tan, '35	1		
miranda	3R, 1V, 1D	5A, 1D	40 H	Dobz., '35	1		
affinis	1R, 2V, 1J, 1D	7A, 1D	40 J	S. & N., '41b	1	2	
algonquin	1R, 1V, 2J, 1D	7A, 1D	40 I	Mill., '39	1	2	
tolteca	1R, 2V, 1J, 1D	7A, 1D	40 J	Ward, '49	1	2	
azteca	1V, 3J, 1D	7A, 1D	40 K	S. & N., '41b	1	2	1
athabasca	1V, 3J, 1D	7A, 1D	40 K	Mill., '39	1	2	1
narragansett	1R, 1V, 2J, 1D	7A, 1D	40 I	Ward, '49	1	2	
nannoptera	3V, 1J, 1v	6A, 1D	40 L	Ward, '49	1	1	2 1
bromeliae	1R, 2V, 1D		39 C*	Metz, '16b			
florae	1R, 2V, 1D		39 C*	Metz, '16b			
bromelioides	1R, 3V		38 C*	P. & C., '47			

41 A). The longest rod represents the X. In the first four species, as well as in the other seven members studied, the salivary gland nuclei have five long strands and a dot. Very close to this series of species are four other forms, which have essentially the same metaphase pattern but differ in showing slight variations in the morphology of the sex

chromosomes. In *subquinaria,* the Y chromosome is V-shaped instead of a rod (Figure 41 B). In *suboccidentalis* and *phalerata* (Ward, unpublished), the X chromosome has a distinct knob (Figure 41 C), and in *innubila* both sex chromosomes have knobs (Figure 41 D).

The metaphase configurations in the next three species have undergone greater morphological changes than any of those just described. Thus, in *tenebrosa,* there are five chromosomes instead of six, a change resulting from a fusion of two rods to produce a J-shaped autosome. In addition, one of the rod-shaped autosomes and the X each has a knob and the Y is J-shaped (Figure 41 E). Both *quinaria* and *munda* have but four chromosomes in metaphase. Those of *quinaria* are a rod-shaped X with a proximal constriction, a V-shaped autosome, a J-shaped autosome, and a dot. The Y chromosome is slightly J-shaped, with a constriction near the middle of the long arm (Figure 41 F). The metaphase configuration of *munda* is somewhat different, in that both autosomes are V's and sex chromosomes are rods without any apparent constrictions (Figure 41 G-1). The species *suffusca* is similar except that the X represents a fusion (Figure 41 G-2).

guttifera group

This is also a monotypic group, with *guttifera* as the single known species. It is very closely related to the quinaria group, and its metaphase pattern is very similar to the transversa type, which has five rods and a dot (Figure 41 H). As might be expected, the salivary gland nuclei show five long strands and a dot (Wharton, 1943).

pinicola group

This is also a small group, with *pinicola* as the single described species. According to Sturtevant (1942), it has but three chromosomes: a J-shaped X; a V-shaped Y, of which each arm is about as long as the long arm of the X; a rod-shaped autosome; and a V-shaped autosome, with the rod and the two arms of the V about equal in length to the short arm of the X (Figure 41 I). Sturtevant states that its closest relative in the subgenus apparently is *virilis,* and that it is nearest to the genus Scaptomyza and to the obscura species group of the subgenus Sophophora. In spite of its peculiar chromosome pattern, which is difficult to interpret, Sturtevant regards

this species as a very primitive form. An undescribed member of this group from the northwestern part of United States has the primitive chromosome combination like *virilis.*

virilis group

The ten known members of this group show five different types of metaphase configurations. In *virilis* (Metz, 1916a; Heitz, 1934) and *novamexicana* (Patterson, Stone, and Griffen, 1942) there are five rods and a dot (Figure 41 J). This configuration is modified in *montana* (Stone, Griffen, and Patterson, 1942) and *lacicola* (Patterson, 1944) by a pericentric inversion in chromosome 2, changing this element into a small V (Figure 41 K). Another modification is found in *texana* (Patterson, Stone, and Griffen, 1940a; Stone and Patterson, 1947) in which chromosomes 2 and 3 have become fused to form a large V (Figure 41 L). Finally, *americana* (Hughes, 1939a, b) has a metaphase configuration which is still more complex. In this species the male has a V-shaped autosome which has the same composition as its homologue in *texana,* a rod-shaped autosome, a dot, a V chromosome comprised of a fused X and chromosome 4, a free rod-shaped 4 chromosome, and a rod-shaped Y (Figure 41 M). This arrangement forms a complex sex-determining mechanism which will be discussed in a later chapter. The salivary gland preparations show five long strands and a dot in *virilis, novamexicana, texana,* and *americana;* but in *montana* and *lacicola* there is an additional long strand, owing to the pericentric inversion in chromosome 2.

The two Palaearctic species have very similar metaphase configurations (Figure 41 N). As determined by Ward, the chromosome complement of *littoralis* consists of two pairs of rods, a pair of large V's, a pair of J's, and a pair of dots. The rod-shaped X has a subterminal centromere, and the Y is J-shaped. He also determined that the salivary gland nuclei have six long strands and a dot. Such a configuration must have resulted from a fusion of two rods and a pericentric inversion. According to Sokolov's description of the chromosomes of *imeretensis,* its chromosomal complement is very similar to, if not identical with, that of *littoralis.*

As indicated in Chapter 2, Patterson has added two new North American members to this group, *D. flavomontana and D. borealis;* their chromosomes are like those of *montana* (Figure 41 K).

testacea group

The two species of this group are the fungus-feeders *testacea* and *putrida,* and Wharton (1943) and Ward (1949) have analyzed the metaphase chromosomes of both species. In *testacea,* there are four chromosomes: a rod-shaped autosome, a V-shaped autosome, a dot, and a rod-shaped X (Figure 42 A). Two strains of *putrida* were examined by Wharton, one from Texas and one from Florida. The Texas strain has a metaphase pattern like that of *melanogaster,* with two large autosomal V's, a rod-shaped X, and a large dot (Figure 42 B). The Florida strain, which was discovered by Dr. A. B. Griffen, has much the same type of configuration, except that, in addition to the regular dot, an extra "double" dot is present (Figure 42 C). The salivary gland chromosomes of both strains of *putrida* were found to consist of five long strands and a dotlike element. There are two possible ways of explaining the origin of the metaphase figure seen in the Florida strain. The first would be to assume that it represents the original condition from which the Texas type arose by loss of the extra dotlike element. In this case this small element would represent the residual "free" centromere from one of the fusions. The second explanation would be that the Texas strain represents the original condition and that the Florida strain was derived from it, thus representing an increase of the number of centromeres from four to five. Such an increase has been accomplished experimentally in *melanogaster* (Stone and Griffen, 1940) and has occurred in *trispina* (see below). Wharton points out that, if the free centromere carries any genes, they must be few in number, since only one dot was found in the salivary gland nuclei of the Florida strain.

The salivary gland chromosome configuration of *testacea* shows that it has undergone a more complex series of rearrangements than has that of *putrida.* The configuration consists of four long strands and a dot, but one of the strands is double-length, apparently composed of two complete chromosomes. There are two possible explanations of this case: (1) that one autosomal V underwent a major pericentric inversion to give the double-length rod; (2) that two independent autosomal rods were involved in a translocation which transferred all of the necessary gene material of one chromosome onto the free tip of the second.

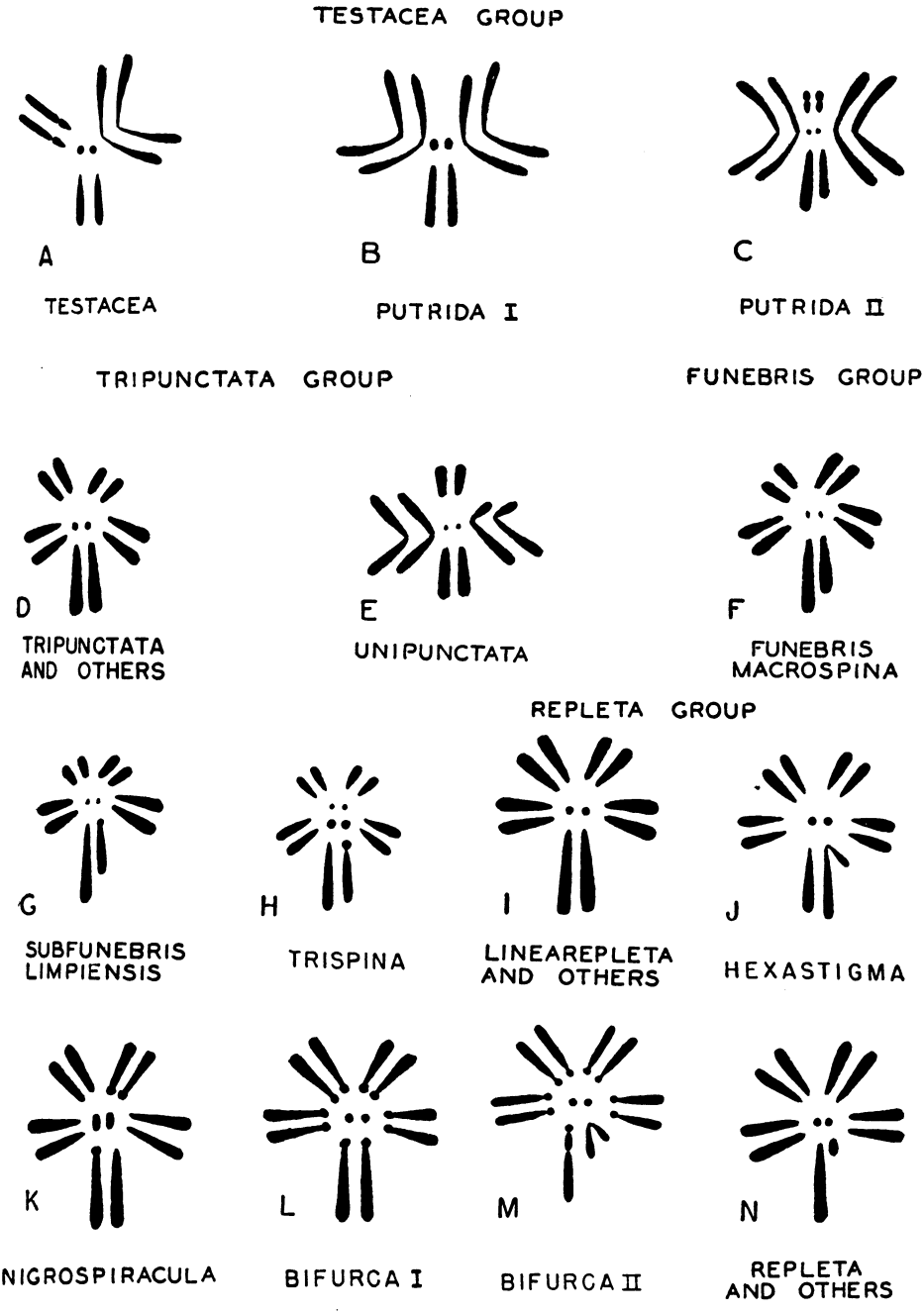

Fig. 42 Metaphase configurations of members of the testacea, tripunctata, funebris, and repleta groups.

tripunctata group

Of the six species investigated which belong to this group, the metaphase configuration for *tripunctata* was determined by Metz and Moses (1923), and those of *crocina* and *unipunctata* by Wharton. The configurations of *tripunctata* and *crocina* are very similar; each has a long rod-shaped X, four relatively short rods, and a dot (Figure 42 D). Except for a few minor differences in the length of some of the chromosomes, the metaphase configurations of *mediopunctata, mediosignata,* and *mediostriata* are similar to those of *tripunctata* and *crocina.* In *unipunctata,* there are but five chromosomes: a short rod, a large V, a J, a dot, and a rod-shaped X (Figure 42 E). Wharton found that *tripunctata, crocina,* and *unipunctata* each had five long strands and a dot in the salivary gland nuclei. This would indicate that *unipunctata* could have evolved from a form with a configuration like *tripunctata,* by a fusion of two rods to produce the large V and a shift or addition of heterochromatin (Table 22).

funebris group

The metaphase configurations of *funebris, subfunebris, macrospina,* and its subspecies *limpiensis* have been determined, the first species by Metz (1916b) and the other three forms by Wharton (1943). *D. funebris* and *macrospina* have very similar metaphase patterns. Each species has six chromosomes: one long rod which is the X chromosome, four short rods of different lengths, and a dot. The Y chromosome is also a rod, but is shorter than the X (Figure 42 F). The only detectable difference between the configurations of the first two forms and those of *subfunebris* and *limpiensis* is found in the morphology of the Y chromosome. This element has a proximal constriction and is shorter in *subfunebris* and *limpiensis* than in the other two forms (Figure 42 G). This difference in the character of the Y chromosome may indicate a closer relationship between *subfunebris* and *limpiensis* than between *subfunebris* and *macrospina,* for in addition it hybridizes more readily with the former subspecies than with the latter. Wharton found that the salivary gland nuclei of all had five long strands and a dot.

Recently we have collected a most interesting member of this group, *Drosophila trispina.* It is the only known species of the genus which has an increase in chromosome number above the basic six

TABLE 22

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-A, X-D, A-A, A-D	Peri. A D	Heter. A
Drosophila							
transversa	5R, 1D	5A, 1D	41 A	Wh., '43			
mutandis	5R, 1D		41 A	T.H.S., '49			
palustris	5R, 1D	5A, 1D	41 A	Wh., '43			
subpalustris	5R, 1D	5A, 1D	41 A	Wh., '43			
occidentalis	5R, 1D	5A, 1D	41 A	Wh., '43			
subquinaria	5R, 1D	5A, 1D	41 B	Wh., '43			
subocciden-talis	5R, 1D	5A, 1D	41 C	Wh., '43			
phalerata	5R, 1D	5A, 1D	41 C	Ward (un.)			
innubila	5R, 1D	5A, 1D	41 D	Wh., '43			
tenebrosa	3R, 1J, 1D	5A, 1D	41 E	Wh., '43	1		
quinaria	1R, 1V, 1J, 1D	5A, 1D	41 F	Wh., '43	2		
munda	1R, 2V, 1D	5A, 1D	41 G-1	Wh., '43	2		
suffusca	1R, 2V, 1D	5A, 1D	41 G-2	Ward (un.)	1 1		
guttifera	5R, 1D	5A, 1D	41 H	Wh., '43			
pinicola	1R, 1J, 1V		41 I	Stvt., '42			
virilis	5R, 1D	5A, 1D	41 J	Heitz, '34			
novamexicana	5R, 1D	5A, 1D	41 J	P.S.G., '42			
montana	4R, 1v, 1D	6A, 1D	41 K	S.G.P., '42		1	
lacicola	4R, 1v, 1D	6A, 1D	41 K	Pat., '44		1	
texana	3R, 1V, 1D	5A, 1D	41 L	P.S. G., '40	1		
americana	1R, 2V, 1D	5A, 1D	41 M	Hugh., '39b	1 1		
littoralis	2R, 1J, 1V, 1D	6A, 1D	41 N	Ward (un.)	1	1	
imeretensis	2R, 1J, 1V, 1D		41 N	Sok., '48			
testacea	2R, 1V, 1D	4A, 1D	42 A	Ward, '49	2	1	
putrida I	1R, 2V, 1D	5A, 1D	42 B	Wh., '43	2		
putrida II	1R, 2V, 2D	5A, 1D	42 C	Wh., '43	2		
tripunctata	5R, 1D	5A, 1D	42 D	Wh., '43			
crocina	5R, 1D	5A, 1D	42 D	P. & M., '44			
mediosignata	5R, 1D		41 J*	D. & P., '43a			
mediostriata	5R, 1D		41 J*	D. & P., '43a			
mediopunc-tata	5R, 1D		41 J*	D. & P., '43a			
unipunctata	2R, 1V, 1J, 1D	5A, 1D	42 E	Wh., '43	1		1
funebris	5R, 1D	5A, 1D	42 F	Wh., '43			
macrospina	5R, 1D	5A, 1D	42 F	Wh., '43			
subfunebris	5R, 1D	5A, 1D	42 G	Wh., '43			
limpiensis	5R, 1D	5A, 1D	42 G	Wh., '43			
trispina	5R, 2D	5A	42 H	Ward, '49			

elements, it having seven pairs of chromosomes (Figure 42 H). The additional chromosome is heterochromatic, for it has not been detected in the salivary gland nuclei. Ward (1949) demonstrated its segregation in hybrid crosses with *limpiensis,* thus proving its chromosomal character (Table 22).

repleta group

A majority of the members of this large species group may be arranged under four subgroups, as indicated in Chapter 2, but in considering the morphology of their chromosomes we have grouped the several species under different types which follow the increasing complexity of their metaphase configurations. The twenty diagrams representing forty-two different forms are shown in Figures 42 I to N and 43 A to N. In thirty-one of the forms the dot, or its equivalent, is seen to be present; but it is absent as a dot in the remaining eleven. Unless otherwise stated in the text, these diagrams are based on the studies of Wharton (1943, 1944). It may be said at the outset that the salivary gland nuclei of the thirty-four forms checked all showed one short and five long euchromatic strands, irrespective of the complexity of their metaphase configurations (Tables 23 and 24).

Twenty-three forms may be included in the first general type of metaphase configuration. In all of these there are five rods and a dot, with the longest rod representing the X chromosome (Table 23). The typical members of this type are *linearepleta, ritae, fulvimacula, flavorepleta, brevicarinata, racemova, nigricruria,* and *ramsdeni* (Metz, 1916b). In each of these species, the long rod-shaped X and Y chromosomes are of equal length and the four rod-shaped autosomes are also of about equal length (Figure 42 I). Very close to this group of species is *nigrospiracula,* in which the metaphase configuration differs from the others in having constrictions at the proximal ends of the X and one autosome (Figure 42 K). A more extreme type of this variation is found in *bifurca I,* in which all five rod-shaped chromosomes show proximal constrictions (Figure 42 L). In *bifurca II* (Figure 42 M), the Y chromosome is modified to form a small V. Wharton (1943) found another configuration in a strain of *bifurca* different from I by a pericentric inversion of heterochromatic material of the X chromosome. There are twelve species with metaphase configurations similar to the linearepleta type, but differing chiefly in the shape of the Y chromosome. In *repleta* and *brunneipalpa* the Y chro-

TABLE 23

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-Y	Fusions A-A	Hetero. X, A D
linearepleta	5R, 1D	5A, 1D	42 I	Wh., '43			
brevicarinata	5R, 1D	5A, 1D	42 I	Wh., '43			
fulvimacula	5R, 1D	5A, 1D	42 I	Ward, '49			
flavorepleta	5R, 1D	5A, 1D	42 I	Ward (un.)			
nigricruria	5R, 1D	5A, 1D	42 I	Wh., '43			
racemova	5R, 1D	5A, 1D	42 I	P. & M., '44			
ramsdeni	5R, 1D		42 I	Metz, '16b			
ritae	5R, 1D	5A, 1D	42 I	Wh., '43			
hexastigma	5R, 1D	5A, 1D	42 J	P. & M., '44			
nigrospiracula	5R, 1D	5A, 1D	42 K	Wh., '43			
bifurca I	5R, 1D	5A, 1D	42 L	Wh., '43			
bifurca II	5R, 1D	5A, 1D	42 M	Ward, '49			
repleta	5R, 1D	5A, 1D	42 N	Wh., '43			
aldrichi	5R, 1D	5A, 1D	42 N	Wh., '43			
arizonensis	5R, 1D	5A, 1D	42 N	Wh., '43			
brunneipalpa	5R, 1D	5A, 1D	42 N	D. & P., '43a			
buzzatii	5R, 1D	5A, 1D	42 N	Wh., '43			
longicornis	5R, 1D	5A, 1D	42 N	Wh., '43			
meridiana	5R, 1D	5A, 1D	42 N	Wh., '43			
mojavensis	5R, 1D	5A, 1D	42 N	Wh., '43			
mulleri	5R, 1D	5A, 1D	42 N	Wh., '43			
hamatofila	5R, 1D	5A, 1D	43 A	Wh., '43			
peninsularis	5R, 1D	5A, 1D	43 A	Wh., '43			
hydei	4R, 1V, 1D	5A, 1D	43 B	Kikk., '35	1		1
rioensis	3R, 1V, 1D	5A, 1D	43 C	Wh., '43		1	
betari	3R, 1V, 1D		43 C	D. & P., '43a			
pararepleta	3R, 1V, 1D	5A, 1D	43 C	Wh., '44		1	
paranaensis	3R, 1V, 1D	5A, 1D	43 C	deB., '50		1	
mercatorum	3R, 1V, 1Dv	5A, 1D	43 D	Ward, '49		1	
onca	1R, 1V, 1J, 1D		43 E	D. & P., '43a			
fascioloides	3V, 1D		43 F	D. & P., '43a			

mosome is about one-tenth the length of the X (Figure 42 N). In six other species, all members of the mulleri subgroup (*aldrichi, arizonensis, buzzatii, meridiana, mojavensis, mulleri*), it is slightly less than a third of the length of the X; and in *longicornis* it is nearly half as long as the X. Other variations occur in *hamatofila* and *peninsularis,* in which the Y chromosome is a small V (Figure 43 A); and in *hexastigma,* which has a J-shaped Y that is slightly longer than the X (Figure 42 J). A different metaphase configuration occurs in *hydei,* which has a V-shaped X and a Y that is slightly J-shaped (Figure 43 B). Since one arm of the X is entirely heterochromatic, Wharton (1943) has suggested that this sex chromosome complex may be the result of an X-Y fusion.

In the second general type of metaphase configuration in the repleta group, two rods have fused to form an autosomal V. This type is found in *paranaensis* and in *rioensis,* which is a subspecies of

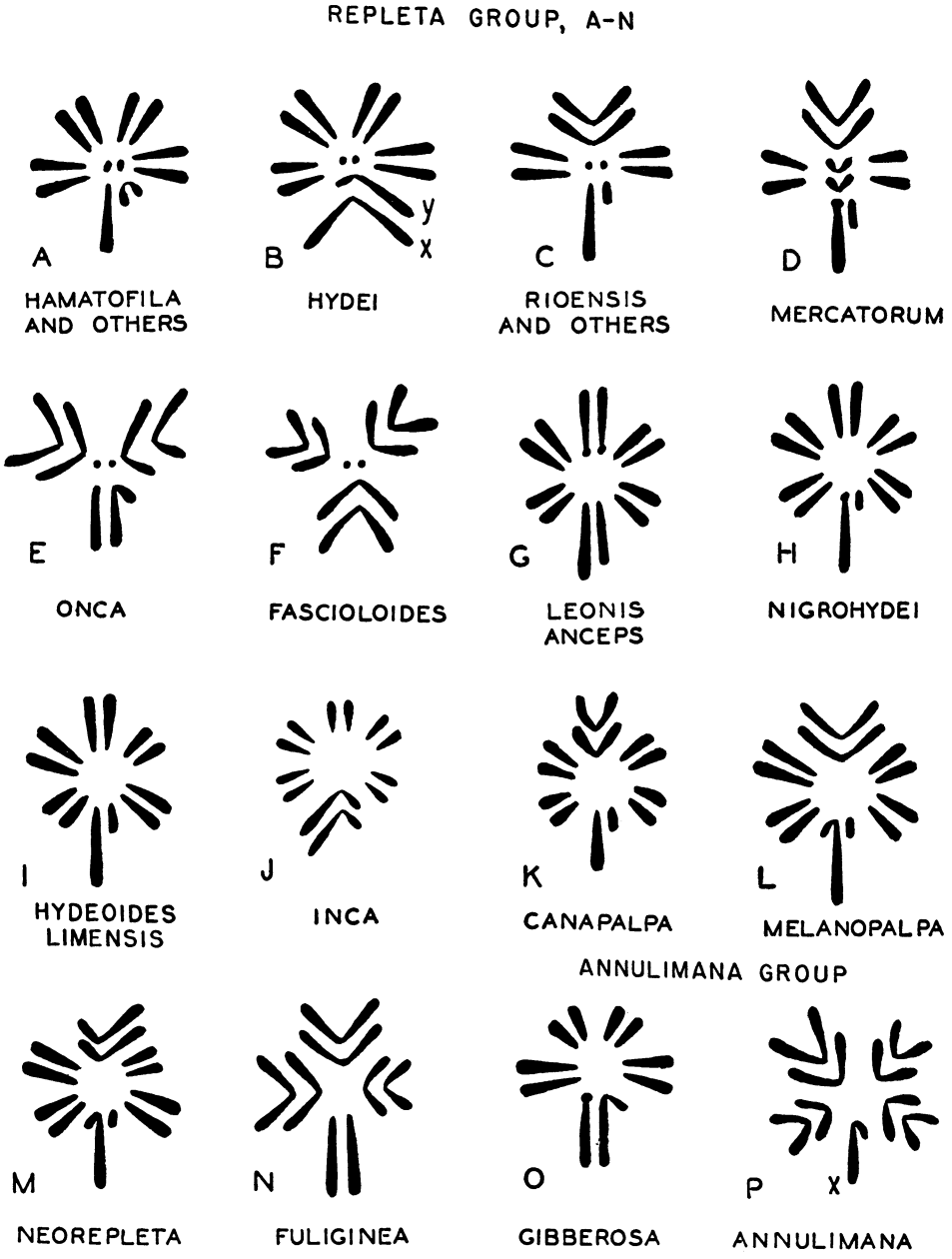

Fig. 43 Metaphase configurations of members of the repleta and annulimana groups.

meridiana (Figure 43 C); and, according to the account of Dobzhansky and Pavan (1943a), *betari* and *pararepleta* belong here. We also include *mercatorum* in this type, although, as Wharton (1944) has shown, the addition of heterochromatin has changed the dot into a small V (Figure 43 D). This would imply that *mercatorum* evolved from a form like *pararepleta.* As a matter of fact, on the basis of morphological similarity and cross-fertility tests, these two forms are ranked as a pair of closely related subspecies.

The third type of metaphase configuration includes two species from Brazil, both described by Dobzhansky and Pavan (1943a). In *onca,* there are four chromosomes: a V- and a J-shaped autosome, a dot, a rod-shaped X, and a J-shaped Y (Figure 43 E). The second species, *fascioloides,* also has four chromosomes, but three of these are V's and one is a dot (Figure 43 F). The sex chromosomes were not identified. In the absence of a study of the salivary chromosomes of these two species, it is difficult to offer satisfactory analyses of their metaphase configurations. Apparently the V and J autosomes of *onca* and two of the V's of *fascioloides* were produced by fusions of rods.

This brings us to a consideration of the eleven species in which the dotlike element as such is not visible in metaphase figures (Table 24). Six of these species constitute a fourth type of configuration consisting of six rod-shaped chromosomes. In two of the species, *leonis* and *anceps,* one of the autosomal rods has a proximal constriction and the Y chromosome is nearly as long as the X (Figure 43 G). In another species, *nigrohydei,* the X has a proximal constriction and the Y is much shorter than the X (Figure 43 H). In the species *hydeoides* and *limensis,* there are no apparent chromosome constrictions and the Y is also much shorter than the X (Figure 43 I). According to Wharton's observations, the salivary gland nuclei of each of these five species have five long strands and a dot. Obviously fusions or pericentric inversions have not occurred in these forms, and, as Wharton suggests, the best explanation to account for the presence of six rods is that the dotlike element has been modified into a rod by the addition of extra heterochromatin. The metaphase configuration of *novemaristata* resembles that of *hydeoides* and *limensis,* but the salivary gland chromosomes are unknown. Dobzhansky and Pavan (1943a) have described the chromosomes of *inca* as consisting of five rods and a long J-shaped chromosome (Figure 43 J). They did not describe the

TABLE 24

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-A, A-A, A-D	Peri. X A	Heter. X A D
leonis	6R	5A, 1D	43 G	Wh., '43			1
anceps	6R	5A, 1D	43 G	P. & M., '44			1
nigrohydei	6R	5A, 1D	43 H	Wh., '43			1
hydeoides	6R	5A, 1D	43 I	Wh., '43			1
novemaristata	6R		43 I	D. & P., '43a			1
limensis	6R	5A, 1D	43 I	Ward, '49			1
inca	5R, 1J		43 J	D. & P., '43a			
canapalpa	5R, 1V	5A, 1D	43 K	P. & M., '44			1 1
melanopalpa	4R, 1V, 1J	5A, 1D	43 L	Wh., '43			1 1 1
neorepleta	4R, 2J	5A, 1D	43 M	Wh., '43			1 1 1
fuliginea	1R, 3V	5A, 1D	43 N	Wh., '43	2		1
annulimana	4V, 1J	8A, 1D	43 P	D. & P., '43b	1	3	1
gibberosa	5R	5A, 1D	43 O	Wh., '43	1 (X-D)		
arapuan	1R, 2V, 1D		39 C*	P. & C., '47			
ararama	4R, 1J		44 A	P. & C., '47			
arassari	5R, 1D		42 I*	P. & C., '47			
arauna	2R, 4V			P. & Nac., '50			
araicas	4V, 1D			P. & Nac., '50			
robusta	3V, 1D	6A, 1D	44 B	C. & S., '47	1 1	1	
colorata	2R, 1J, 2V, 1v	7A, 1D	44 D	Wh., '43		1 1	1 1
sordidula	2R, 2V, 1D		44 C	K. & P., '38			
cheda	1R, 2V, 1D		39 C*	T.H.S., '49			
pullata	4V, 1D		38 B*	T.H.S., '49			
melanica	2R, 2V, 1D	6A, 1D	44 E	Wh., '43	1	1	
parame-lanica I	2R, 2V, 1D	6A, 1D	44 E	Wh., '43	1	1	
parame-lanica II	3R, 2V	6A, 1D	44 F	Ward, '49	1	1	1
"*melanissima*"	2R, 2V, 1D		44 E	K. & P., '38			
melanura I	2R, 1V, 1v, 1Dv		44 G	Mill., '44			
melanura II	2R, 1V, 1v, 1D	6A, 1D	44 H	Ward, '49	1	1	1
nigrome-lanica I	2R, 2V, 1Dv	6A, 1D	44 I	Wh., '43	1	1	1
nigrome-lanica II	3R, 1V, 1v	6A, 1D	44 J	Ward, '49	1	1	1
afer	1R, 2V, 1J, 1D		40 J*	T.H.S., '49			
microme-lanica I	5R, 1D	5A, 1D	44 K	Wh., '43			
microme-lanica II	2R, 1V, 1v, 1D	6A, 1D	44 L	Wh., '43	1	1	
microme-lanica III	4R, 1v, 1D	6A, 1D	44 M	Ward, '49		1	
microme-lanica IV	4R, 1v, 1r	6A, 1D	44 N	Ward, '49		1	1

salivary gland chromosomes; nor did they identify the sex chromosomes.

The fifth type of metaphase configuration is exhibited in *canapalpa, melanopalpa,* and *neorepleta,* which are three closely related members of the repleta group. Each of these species has six chromosomes, and one autosome is either V- or J-shaped. In *canapalpa* this autosome is a V, and the X and Y chromosomes are rods, the Y being much shorter than the X (Figure 43 K). The metaphase configuration of *melanopalpa* is similar, but the X has a subterminal spindle fiber attachment and is slightly J-shaped (Figure 43 L). The configuration in *neorepleta* is much like that of *melanopalpa,* except that the one autosome is J-shaped rather than a V (Figure 43 M). The salivary gland nuclei in each of these species show five long strands and a dot. In this, as in the preceding type, fusions or pericentric inversions of euchromatic material have not occurred, and one of the chromosomes must represent the dot which has acquired extra heterochromatin.

The metaphase configuration of *fuliginea* may be regarded as representing a sixth type. It has but four chromosomes: two large and one small V, and a long rod which probably represents the sex chromosome (Figure 43 N). The small V represents the dot, which has acquired extra heterochromatin, while the two large V's must have resulted from fusions of rods (Table 24).

robusta group

The chromosomes of five members of this group have been examined (Table 24). Carson and Stalker (1946, 1947), reporting on the metaphase and salivary gland chromosomes of *robusta,* found that the metaphase configuration showed three V's of decreasing sizes and a dot. The largest V is the X chromosome; and the Y is also a V, which is indistinguishable from the X (Figure 44 B). The salivary gland nuclei show six long strands and a dot. They discovered an interesting variation resulting from a pericentric inversion which has transformed the middle-sized V into a J. This variation is common to the northern part of the range of this species. Their determination does not agree with those of Metz (1916b) and Wharton (1943). We now know that Wharton studied an incorrectly identified robusta-like form. This configuration must be the result of X-A and A-A fusions, together with a pericentric inversion of the other autosome.

Kikkawa and Peng (1938) state that the metaphase configuration

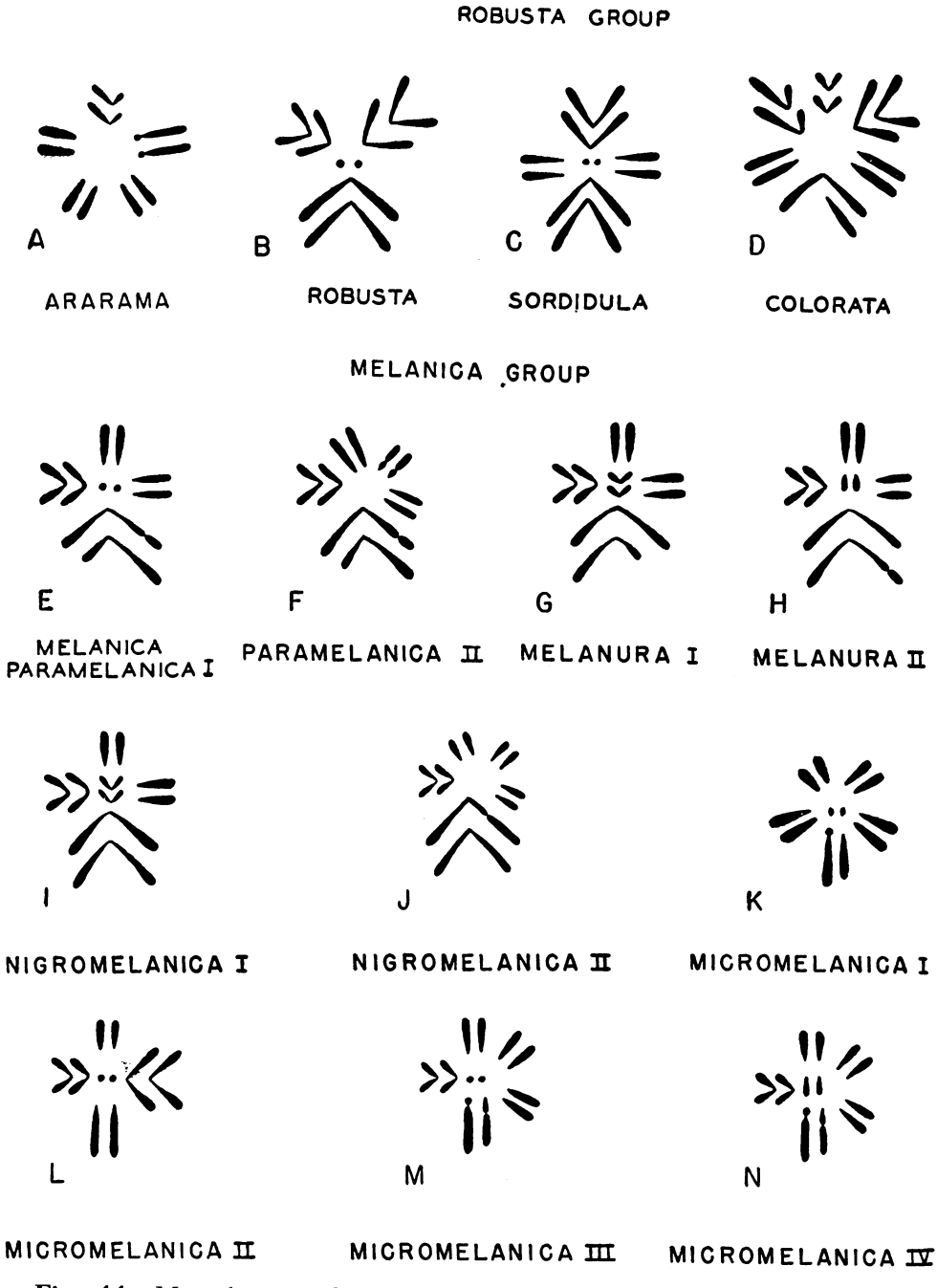

Fig. 44 Metaphase configurations of members of the annulimana, robusta, and melanica groups.

of *sordidula* belongs to their "E-type," which has one large V, one smaller V, two rods, and a dot. They indicate that the X is V-shaped (Figure 44 C). In the absence of any statement concerning the nature of the salivary gland chromosomes, one might infer that the larger V is the product of a fusion of two rods and that the other V resulted from a pericentric inversion or added heterochromatin, as only five chromosomes are present in the metaphase figure. In the stock of *colorata* examined by Wharton, there were six chromosomes: two autosomal rods, one large V, one very small V, a J, and a V-shaped X (Figure 44 D). The Y chromosome is a rod. The salivary gland nuclei have four short and three long strands, in addition to the dot. This configuration must be the result of pericentric inversions of the X and one autosome, together with the addition of heterochromatin to one autosome and a dot. The metaphase patterns of two additional species from China by Tan, Hsu, and Sheng (1949) are known. No figure is given as such, but they are referred to forms in other groups with similar patterns: *cheda* has the familiar melanogaster-type (Figure 39 C*), and *pullata* resembles *duncani* (Figure 38 B*).

melanica group

The metaphase configurations are known for six species, but these have ten different chromosome forms (Table 24). In the subspecies *melanica* (Metz, 1916b; Wharton, 1943) and *paramelanica* I, there are two rods, a large V, a small V, and a dot (Figure 44 E). The Japanese *"melanissima"* has the same chromosome configuration (Kikkawa and Peng, 1938). Ward (1949) has described another configuration for *paramelanica* II. In this form the dot has sufficient added heterochromatin to change it into a small rod (Figure 44 F). There are two known chromosome forms of *melanura;* in I the dot has added heterochromatin to form a small V (Figure 44 G), and in II the Y and the dot have each added heterochromatin, but the latter is not V-shaped (Figure 44 H). There are two chromosome configurations in *nigromelanica.* One resembles *melanura* I, except that the Y is V-shaped rather than J-shaped (Figure 44 I), and the other has added heterochromatin to the dot to form a small rod (Figure 44 J). In each of the seven forms for which the salivary gland chromosomes are known, there are six arms and a dot. Besides the added heterochromatin, these configurations have resulted from an X-A fusion and a pericentric inversion of an autosome (Table 24). The

Chinese species, *D. afer,* is not illustrated with the group. According to Tan, Hsu, and Sheng (1949), the metaphase configuration resembles that of *D. affinis* (Figure 40 J*).

Four chromosome strains of *micromelanica* are known (Table 24). Strain I has the primitive chromosome configuration (Figure 44 K). This is modified in strain III by an autosomal pericentric inversion to give four rods, a small V, and a dot (Figure 44 M). Strain IV differs from III only by the addition of heterochromatin to change the dot into a short rod (Figure 44 N), whereas strain II differs from III by an autosomal fusion (Figure 44 L).

polychaeta group

The only member of this group for which the chromosomes have been analyzed is *polychaeta.* This species has six chromosomes: a long rod-shaped X, a short autosomal rod, two J's, a V, and a dot. The X has a proximal constriction, and the rod-shaped Y is slightly shorter than the X (Figure 45 A). There is one short strand and seven long ones in the salivary gland nuclei (Wharton, 1943). This would indicate that two of the large autosomes had resulted from pericentric inversions and that the third one had acquired heterochromatic material beyond the centromere (Table 25).

carbonaria group

The single member of this group, *D. carbonaria,* has a metaphase chromosome configuration of two rods, a V, two J's, and a dot (Figure 45 B). Ward (1949) found eight arms and a dot in the salivary gland nuclei, thus indicating three pericentric autosomal inversions, with heterochromatin added to make one J large (Table 25).

cardini group

The metaphase configurations of six members of this group have been described (Table 25). These are *cardini, cardinoides, neocardini, polymorpha, prosimilis,* and *similis.* The salivary gland chromosomes of the first three have been checked. *Drosophila cardini* (Ward, 1949) has five long and one short rod, so that heterochromatin has been added to the dot (Figure 45 C). This agrees with Metz's 1916b account, except that the size of the dot was not indicated. Dobzhansky and Pavan (1943a) described the metaphase configuration of *cardinoides,* which has one rod, two V's, and a dot (Figure 45 D).

Wharton (1943) described the same configuration in a strain from Florida as *cardini,* but we now believe this to be *cardinoides,* which we have collected in Mexico. Ward (unpublished) has analyzed this

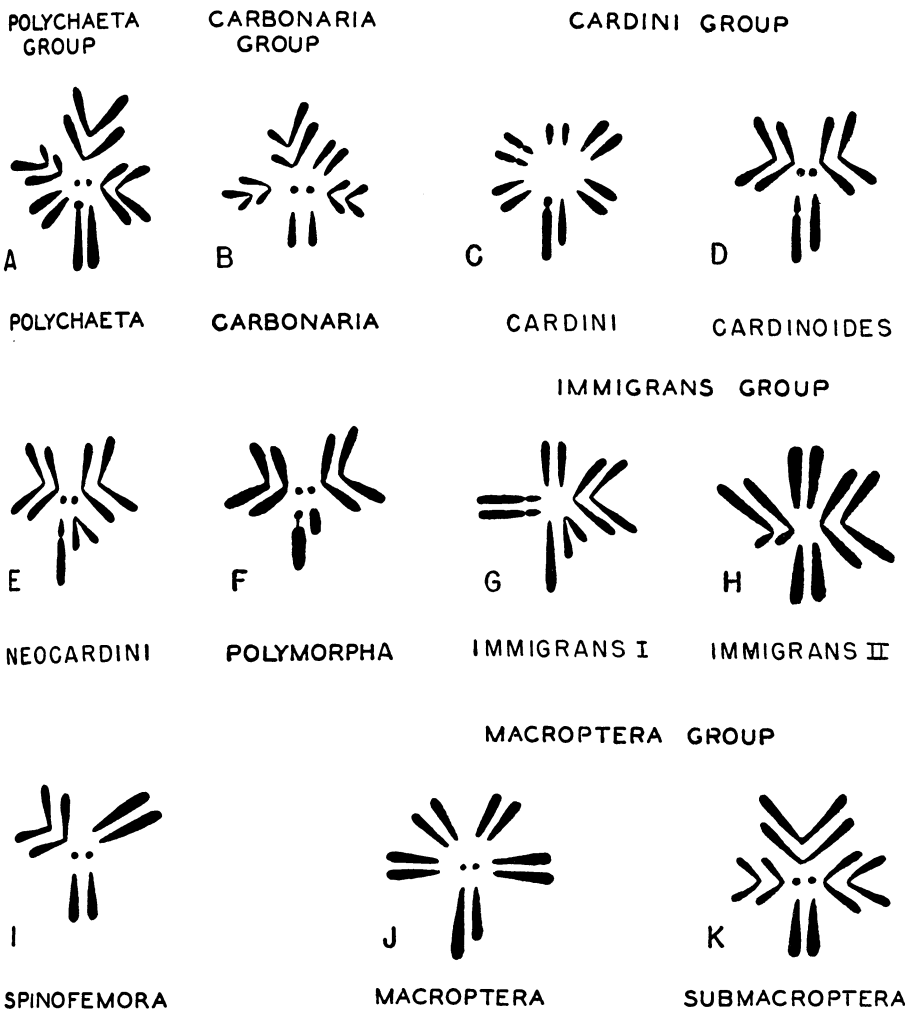

Fig. 45 Metaphase configurations of members of the polychaeta, cardini, immigrans, and macroptera groups.

material from Mexico and finds the same condition as reported by Wharton. This configuration represents two autosomal fusions, as does also *neocardini.* Streisinger (1946) described the metaphase configuration as consisting of a rod, two V's, and a dot. Ward (unpublished) has checked both the metaphase and salivary gland chromosomes of this species (Figure 45 E). Dobzhansky and Pavan (1943a)

gave the metaphase configuration for *polymorpha* which differs from *neocardini* in the shapes of the sex chromosomes (Figure 45 F), and for *prosimilis* which is like *similis*. Sturtevant (1942) states that

TABLE 25

Species	Metaphase	Salivary	Fig.	Authority	Fusions X-A, A-A, A-D	Peri. A	Heter. X A D
polychaeta	2R, 1V, 2J, 1D	7A, 1D	45 A	Wh., '43		2	1
carbonaria	2R, 1V, 2J, 1D	8A, 1D	45 B	Ward, '49		3	1
cardini	5R, 1r	5A, 1D	45 C	Ward, (un)			1
cardinoides	1R, 2V, 1D	5A, 1D	45 D	Ward, (un)	2		
neocardini	1R, 2V, 1D	5A, 1D	45 E	Ward, (un)	2		
polymorpha	1R, 2V, 1D		45 F	D. & P., '43a			
similis	5R, 1D		41 J*	Metz, '16b			
prosimilis	5R, 1D		41 J*	D. & P., '43a			
immigrans I	3R, 1V	4A, 1D	45 G	Le Cal., '48	2	1	1
immigrans II	2R, 1V, 1J	5A, 1D	45 H	Emm., '37	1 1		
spinofemora	2R, 1V, 1D	4A, 1D	45 I	Wh., '43	2	1	
komaii	3R, 1V		45 G	K. & P., '38			
hexastriata	2R, 1V, 1D		45 I	T.H.S., '49			
nixifrons	3R, 1V, 1D		41 L*	T.H.S., '49			
macroptera	5R, 1D	5A, 1D	45 J	Wh., '43			
submacroptera	1R, 3V, 1D	7A, 1D	45 K	Wh., '43	1	2	
guaramunú	5R, 1D	5A, 1D	46 A	King, '47			
griseolineata	5R, 1D	5A, 1D	46 B	King, '47			
guarajá	3R, 1V, 1D	5A, 1D	46 C	King, '47	1		
guarú	4R, 1V, 1D	5A, 1D	46 D	King, '47			1
guaraní	5R, 1V	5A, 1D	46 E	King, '47			1 1
subbadia	5R, IV	5A, 1D	46 F	King, '47			1 1
bizonata	3V, 1D		46 G	K. & P., '38			
heterobristalis	1R, 2V, 1D		39 C*	T.H.S., '49			
meitanensis	1J, 2V, 1D		46 H	T.H.S., '49			
pallidipennis	4R, 1V, 1D	5A, (1D)	46 I	Dobz., '44			1
centralis	4R, 1V, 1D	5A, (1D)	46 I	P. & M., '44			1

similis may belong to the cardini group, and Metz (1916b) gave its metaphase pattern as consisting of five rods and a dot, similar to that of *virilis* (Figure 41 J*).

immigrans group

The metaphase chromosomes are known for the following species of this group: *immigrans* (two strains), *spinofemora, komaii, hexastriata,* and *nixifrons* (Table 25). In strain I of *immigrans* there are

three rods and a V (Figure 45 G). The X is a rod and the Y a small V in the strain examined by Ward (1949). This is the metaphase configuration given by Metz (1916a). The same chromosome type was reported by Le Calvez (1948) from Europe, who gave the salivary gland chromosomes as four and a dot, which agrees with Ward's observations. The interesting feature of this case is the double-length autosomal arm. Strain II was described by Emmens (1937) and by Wharton (1943). The metaphase plate shows two rods, a V and a J (Figure 45 H), while the salivary gland nuclei show five arms and a dot. This configuration is best explained by A-A and A-D fusions. Strain I probably arose from a parent type with two autosomal fusions, by a complete pericentric inversion changing a V into a double-length rod. An alternative explanation for the double-length rod is by total translocation of one rod chromosome minus its centromere onto the tip of another rod. In either case, heterochromatin has been added to the dot. Wharton (1943) described for *spinofemora* a metaphase configuration of two rods, a V, and a dot (Figure 45 I). Here again there are four arms and a dot in the salivary gland nuclei. The explanation is the same as for *immigrans* I except that no heterochromatin has been added to the dot.

The salivary gland chromosomes of the other three species are unknown. The metaphase configuration of *komaii* (Kikkawa and Peng, 1938) resembles that of *immigrans* I, while *hexastriata* (Tan, Hsu, Sheng, 1949) resembles that of *spinofemora.* The metaphase plate of *nixifrons* (Tan, Hsu, and Sheng, 1949) shows a simpler pattern of three rods, one V, and one dot (Figure 41 L*).

macroptera group

The chromosomes of the two species belonging to this group have been described. The first of these is *macroptera,* which has five rods and a dot. The X chromosome is the longest rod, while the rod-shaped Y is shorter than the X (Figure 45 J). The salivary gland nuclei show five long strands and a dot. In *submacroptera,* there are five chromosomes: one large and two small V's, a rod, and a dot. The rod represents the X chromosome, which is of about the same length as the Y (Figure 45 K). The salivary gland nuclei have seven long strands and a very short strand representing the dot. Evidently the metaphase configuration of this species could have been derived from *macroptera,* or a form like it, by the fusion of two rods to produce

the large V; and a pericentric inversion in each of the other two rods would account for the two small V's.

annulimana group

The metaphase chromosome configurations of seven members of this group are known (Table 24). According to the descriptions of Dobzhansky and Pavan (1943a, 1943b), the metaphase configuration of *annulimana* has one very large and three small V's, and a short rod or J which is the X chromosome. There are only nine chromosomes in the male, the X being without a partner which makes the species an XO type (Figure 43 P). A detailed study of the salivary chromosomes of this species reveals that the salivary preparations have one short and eight long strands. To account for this large number of strands in *annulimana,* Dobzhansky and Pavan suggest that the minimum number of changes from the ancestral number of one short and five long strands might be as follows: (a) fusion of two rods to produce the large V; (b) pericentric inversions in two rods with subterminal centromeres to account for two of the small V's; (c) translocation of enough material from one rod onto the dot to form the third small V. We consider that an alternative explanation is more probable. This is through autosomal pericentric inversions to form the three small V's, a fusion of the fourth autosome to the dot with added heterochromatin, and heterochromatin (perhaps the Y) added to the X.

Drosophila gibberosa has five chromosomes: four relatively short autosomal rods, and a longer rod-shaped X with a proximal constriction. The Y chromosome is J-shaped, with a constriction slightly distal to the middle of the long arm (Figure 43 O). The X and Y chromosomes of this species are strikingly similar to those of the distantly related *quinaria* (see Figure 41 F). The salivary gland nuclei have five long strands and a dot. The dotlike element must be fused with one of the other chromosomes.

The salivary gland chromosomes of the five remaining species are unknown. The metaphase configuration of *ararama* has four rods, one having a proximal constriction and a J-shaped element (Figure 44 A). The configurations of the other four are not illustrated as such, but equivalent metaphase configurations are indicated for *arapuan,* melanogaster-type (Figure 39 C*), and *arassari,* linearepleta-type (Figure 42 I*). The metaphase plate of *ariacás* has one large and

three small V's, and a pair of dots; while that of *araúna* has one large and three short V's, a pair of long rods, and a pair of short rods (Pavan and Nacrur, 1950).

bizonata group

The metaphase configurations of the three known members of this group have been reported, but the salivary gland chromosomes are unknown (Table 25). The metaphase plate of *bizonata* (Kikkawa and Peng, 1938) shows three V's and a dot (Figure 46 G), while *meitanensis* (Tan, Hsu, and Sheng, 1949) differs in that the sex chromosomes are J-shaped instead of V's (Figure 46 H). Tan, Hsu, and Sheng report the chromosomes of *heterobristalis* resemble those of *melanogaster* (Figure 39 C*).

guaraní group

The chromosome configurations of members of this group have been described by King (1947a). The six species of this group are very similar morphologically, but, on the basis of their cytology and certain genetic tests, they can be divided into two subgroups. King places in the first subgroup *guaramunú, griseolineata,* and *guarajá;* and in the second subgroup *guarú, guaraní,* and *subbadia.* He bases his descriptions of the metaphase and prophase stages on the giant nerve cells of the larval brain. Our own diagrams are based on his descriptions and figures.

The metaphase plate of *guaramunú* consists of six chromosomes: four short rods, a long rod, and a dot. The long rod is the X, which has two constrictions near its middle. The Y chromosome is also a long rod. All of the short rods give evidence of subterminal centromeres or at least constrictions, and one of them often shows a distinct satellite (Figure 46 A). The chromosome configuration of *griseolineata* also has six chromosomes: four short rods without constrictions or satellites, a long rod-shaped X, which usually shows a submedian constriction, and a tiny dot. The Y chromosome is a long rod with a definite satellite (Figure 46 B). The third member of this subgroup is *guarajá,* which has three short rods of different lengths, a V-shaped chromosome, and a dot. The X is the shortest rod, and the Y is a rod with a large satellite (Figure 46 C). As King points out, the V chromosome is the result of a fusion of two rods.

In the second subgroup, the chief characteristic of the metaphase

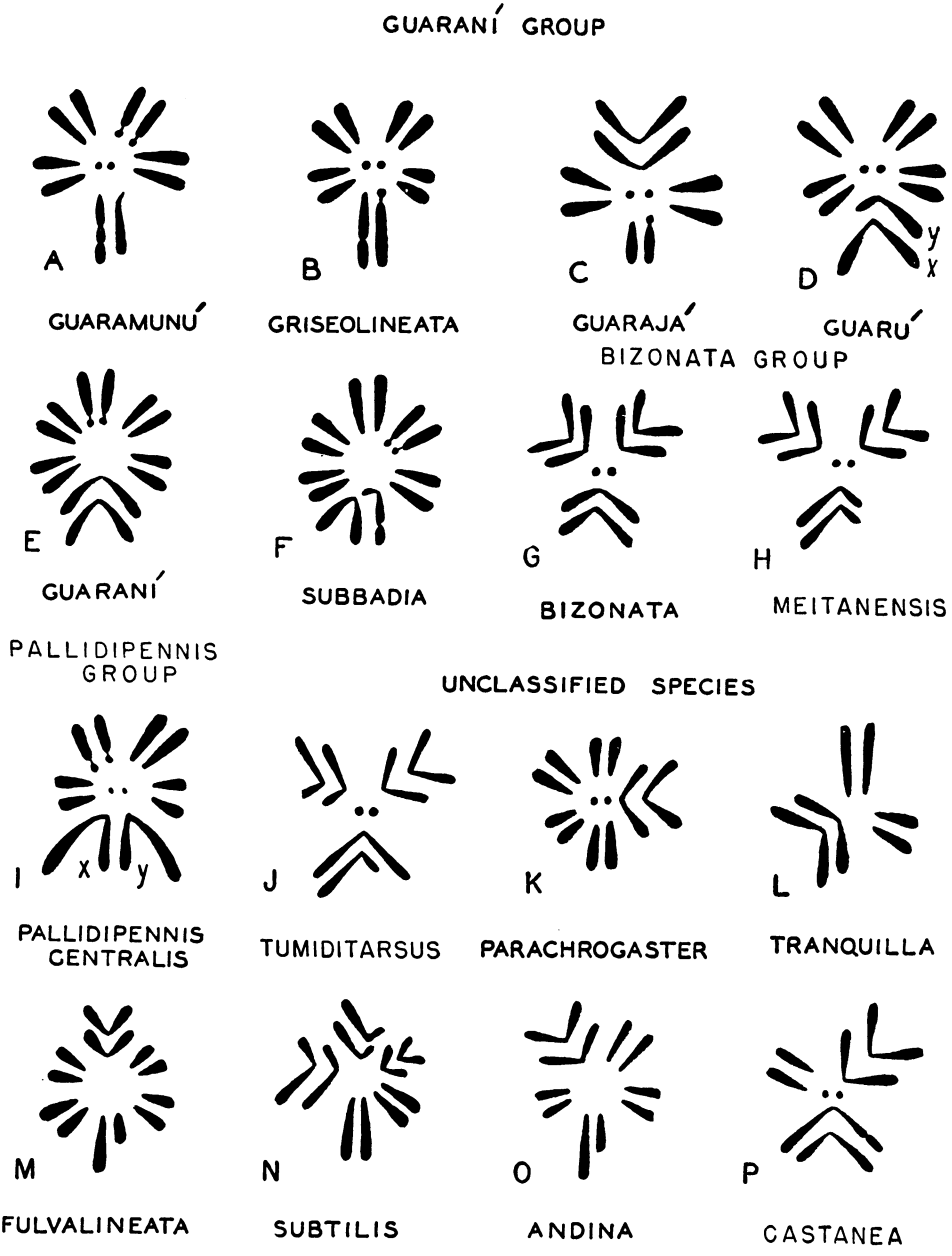

Fig. 46 Metaphase configurations of members of the guaraní, bizonata, and pallidipennis groups, and of seven unclassified species.

chromosome configuration is the presence of a V-shaped X in each of the three species. In *guarú,* there are four short autosomal rods, the V-shaped X, and a dot. The Y chromosome is J-shaped (Figure 46 D). The rods give evidence of subterminal centromeres. The X has large heterochromatic sections, especially in one arm. The metaphase figures of *guaraní* show five short autosomal rods, the V-shaped X, with the Y chromosome also a V (Figure 46 E). All of the rods give evidence of subterminal centromeres, especially clear in one of them. Prophase figures show that the X is euchromatic in one arm only. In prophases, one of the rods appears to be wholly heterochromatic with a distinct satellite at one end, thus suggesting that heterochromatin has been added to the dotlike chromosome. This would explain the configuration of five rods and a V in this species. The metaphase figures of *subbadia* also show five autosomal rods and a V-shaped X. The Y chromosome is a long J with two constrictions, one near the middle and another about halfway between this and the end (Figure 46 F). The sex chromosomes are more heterochromatic than four of the rods. The fifth rod is also heterochromatic, with a satellite probably representing the dot. The salivary gland chromosomes of all six species show five long arms and a dot.

King suggests that the differences in the metaphase configurations of these six species can be explained, with but one exception, as being due to differences in the amount or arrangement of heterochromatin. The exception is seen in *guarajá,* which has a fusion autosomal V.

pallidipennis group

This group includes the two subspecies *pallidipennis* and *centralis,* which are morphologically similar and appear to have identical metaphase chromosome configurations. Each form has six chromosomes: four short rods, one of which has a satellite, a very small dot, and a relatively enormous V which is the X chromosome. The Y chromosome is similar to the X, and the arms of these two elements are unequal (Figure 46 I). In the nuclei of the giant cells of the larval brain, the dotlike pair of chromosomes usually occupies the center of the metaphase plate, with the four pairs of rods forming a semicircle about them. The large V's lie either next to each other on one side of the plate or on opposite sides. They never lie inside each other as such chromosomes of Drosophila usually do as a result of somatic pairing (Dobzhansky, 1944b). In his detailed study of the chromo-

somes of *pallidipennis,* Dobzhansky found that in the salivary gland nuclei the autosomes (except the dot) showed no heterochromatin at the centromeres, but do have some interstitial heterochromatic sections. Most of the heterochromatin is restricted to the X and Y chromosomes, both of which are composed chiefly of this material. A similar detailed study of the chromosomes of *centralis* has not been made, but Patterson and Dobzhansky (1945) found that the homologous elements in the salivary gland preparations of the F_1 hybrid larvae of the two subspecies indicated that synapsis was complete. The only difference between the gene arrangement of the two forms is a fairly long inversion in one of the autosomes (Table 25).

dreyfusi group

The two species thus far included in this group are *D. dreyfusi* and *D. camargoi* (Table 15). Dobzhansky and Pavan (1943a) reported that *dreyfusi* has one J and two V's in metaphase, and five arms and a dot in the salivary gland nuclei. This is interpreted to be the result of an X-D and two A-A fusions (Figure 38 E*).

Unclassified Species

The chromosome configurations of eighteen species unclassified as to species groups but belonging in the subgenus Drosophila are given in Table 26.

The metaphase pattern of *castanea* has one rod, two V's, and a dot. The X chromosome is a V, and the Y is a J (Figure 46 P). As the salivary gland nuclei show five arms and a dot, this pattern must be the result of X-A and A-A fusions.

Drosophila fulvalineata (Wharton, 1943) has six arms and a dot in the salivary gland nuclei, and five rods and a V in the metaphase plate (Figure 46 M). One autosomal rod has undergone a pericentric inversion, while heterochromatin has been added to the dot. As opposed to the formation of V's by fusion of rods in these two species, *parachrogaster* (Wharton, 1943) has added heterochromatin to an autosomal rod to form a V; for the metaphase plate has four rods, one V, and one dot, while the salivary gland configuration is the familiar five arms and a dot (Figure 46 K). *Drosophila tranquilla* (Wharton, 1943) is another species which has a double-length rod composed of two chromosomes. The metaphase plate has two rods

and a V (Figure 46 L), while the salivary gland nuclei show four arms and a dot. The dot must be fused with a rod (assumed to be the X in our interpretation, Table 26), as it does not show in metaphase. The large V is a fusion of two rods. The double-length chromosome came from a fusion of two rods followed by a complete pericentric inversion of one arm, or by a total translocation of one rod minus the centromere to the free tip of a second rod.

The Asiatic species *tumiditarsus* has two V's, one J, and a dot. Hsiang (1949) showed that one V resulted from the fusion of two

TABLE 26

Species	Metaphase	Salivary	Fig	Authority	Fusions X-A, X-D, A-A			Peri. A	Heter. X A D
dreyfusi	2V, 1J	5A, 1D	38 E*	D. P., '43a		1	2		
castanea	1R, 2V, 1D	5A, 1D	46 P	Ward, '49	1		1		
fulvalineata	5R, 1V	6A, 1D	46 M	Wh., '43				1	1
parachro-gaster	4R, 1V, 1D	5A, 1D	46 K	Wh., '43					1
tranquilla	2R, 1V	4A, 1D	46 L	Wh., '43		1	2	1	
tumiditarsus	2V, 1J, 1D	6A, 1D	46 J	Hsiang, '49	1		1	1	1
subtilis	2R, 2J, 1v		46 N	K. P., '38					
andina	4R, 1V		46 O	D. P., '43a					
bandeiran-torum	4R, 1V, 1D		41 K*	D. P., '43a					
calloptera	3R, 1V, 1D		41 L*	Metz, '16b					
canalinea	1R, 1V, 1D		39 C*	P. M., '44					
caponei	3V, 1D		43 F*	P. C., '47					
fumosa	1R, 2V, 1D		39 C*	P. C., '47					
histrio	5R, 1D		41 J*	Frol., '26					
melanospila	4R, 1V, 1D		41 K*	Stvt., '42					
mesophrag-matica	3R, 1V, 1D		41 L*	P. C., '47					
pulla	3R, 1V, 1D		41 L*	P. C., '47					
trivittata	2R, 2V, 1D		44 B*	Frol., '26					
vibrissina	1R, 2V, 1D		39 C*	Frol., '26					

autosomes, the second V from an X autosome fusion, and the J from a pericentric inversion. The dot chromosome has no euchromatic part, but does have the nucleolus organizer. The Y is heterochromatic also, but the X and autosomes have only β-heterochromatin, which does not form any appreciable mass in the salivary gland chromosomes. This case is somewhat analogous to that of *ananassae,* but here the dot chromosome seems even more a specialized α-heterochromatic body.

The salivary gland chromosomes of the remaining thirteen species are not known, and hence an analysis of their configurations is not justified. The metaphase configurations which these species resemble

most closely are given in Table 26. These will not be discussed further, except to state that none of them demands an explanation differing from cases already analyzed.

DISCUSSION

In analyzing the chromosome changes which have occurred in the genus Drosophila with the necessarily incomplete data available, we shall use certain working hypotheses. The evidence to support these assumptions will be developed in the analysis, or they are general statements which we consider most probable:

1. The basic cytological configuration is five rods and a dot, i.e., six separate chromosome elements, as discussed by Muller (1940) and especially by Sturtevant and Novitski (1941b).
2. Only one increase in chromosome number has thus far been found, and therefore increase in chromosome number must be difficult and has seldom been accomplished in the genus.
3. A new species group usually evolves from one species. It is possible that much less frequently two or more species split off together and, by parallel evolution at the species level, form a new species group.
4. A species group which contains members with several chromosome numbers was either descended from a common ancestor with the highest represented number (or a higher one) or from a mixed group which contained a member with the highest number. Gains in chromosome number are relatively rare. Stated in another way, differences in chromosome number within a species group must usually have evolved subsequent to the formation of the group.

Before attempting the general analysis of the chromosome evolution in the genus, the results of a series of genetic studies of the chromosome elements of several species, mostly of the subgenus Sophophora, will be reviewed. The general system of nomenclature suggested by Muller (1940) is followed, using information summarized and discussed at length by Sturtevant (1940) and by Sturtevant and Novitski (1941b). Since the gene system of *Drosophila melanogaster* has been studied more extensively than that of any other species, it is taken as standard. The autosomal fusions present in *melanogaster* introduce complications and possible sources of error, for the shift

of genes across the centromere may have occurred. This would change the gene association from the primitive condition found in forms with five pairs of rods and a pair of dots. Table 27, which is modified from that of Sturtevant and Novitski, gives the homologies between the six basic elements and the gene linkage maps that have been made. The homologies of chromosomes could be tested directly in some species using the salivary gland chromosomes of the hybrids, but most homologies are based on similarity of mutants and their groupings. The similarities of mutant genes at different loci on different chromosomes in the same species make this procedure difficult to evaluate. The method is open to a further objection, in that the suppressor type of mutation might shift the active locus of a gene (Price, 1949b). However, when a number of different mutants from similar groups in two species are considered—and this criterion has always been used—the conclusion that they represent the same element will usually be correct, even though the gene order is not the same.

TABLE 27

Species	Elements						Authority
	A	B	C	D	E	F	
Sophophora							
melanogaster	X	2L	2R	3L	3R	4	Muller, 1940
simulans	X	2L	2R	3L	3R	4	Sturtevant, 1922
ananassae	X(–)	3R	3L	2R	2L	4(+X_T)	Kikkawa, 1938
montium	(X)	3L	3R	2R	2L	(F+X_T)	Stvt. & Nov., 1941b
willistoni	X(A–D)	2(B–C)	2(B–C)	X(A–D)	3	(F)	Stvt. & Nov., 1941b
pseudoobscura	XL	4	3	XR	2	5	Lancefield, 1922
persimilis	XL	4	3	XR	2	5	Tan, 1935
miranda	XL	4	X_2	XR	2	5	Dobz. & Tan, 1936
affinis	XL	4	3	XR	2	5	Sturtevant, 1940
algonquin	XS	C	A	XL	B	D	Miller, 1939
azteca	XS	C	A	XL	B	D	Dobz. & Socolov, 1939
Drosophila							
hydei	X	4	3	5	2	6	Spencer, 1949
virilis	X	4	5	3	2	6	Chino, 1936
americana	X(X–4)	4(X–4)	5	3(2–3)	2(2–3)	6	Hughes, 1939
texana	X	4	5	3(2–3)	2(2–3)	6	Stone & Patt., 1947
novamexicana	X	4	5	3	2	6	P. S. G., 1942
montana	X	4	5	3	2	6	S. P. G., 1942
lacicola	X	4	5	3	2	6	Patterson, 1944
littoralis	X	4(3–4)	5	3(3–4)	2	6	Hsu, 1951

In addition to the information on homologies, which can be obtained from critical parallel mutations in two species, the gene homologies of forms which hybridize can be extended to all these species

when homologies of the elements of one of them are known. In this connection it is interesting to note that Hughes (1939b) established it for the X–4 (A–B) and 2–3 (D–E) fusions in *americana,* and that Stone and Patterson (1947) established the 2–3 (D–E) fusion of *texana.* In neither case have there been pericentric inversions to destroy the homologies in the A, B, D, or E elements. The pericentric inversion in chromosome 2 (E) of *montana* and *lacicola* does not disrupt the homologies, for this element was not fused with any other chromosome. The situation in *robusta* does show that pericentric inversions do destroy chromosome homologies. Carson and Stalker (1947) demonstrated the presence of two pericentric inversions in *robusta,* one in chromosome 2, the larger autosome, which presumably represents the fusion of two separate elements, and another in chromosome 3, the smaller autosome, which presumably arose by a pericentric inversion. The pericentric inversion of chromosome 3 would not affect the chromosome elements, as it consists of only one element, so that this case is comparable to the pericentric found in chromosome B (element E) of *algonquin,* which is also a single element. The pericentric inversion in 2 of *robusta* is widespread in certain localities, and thus the stability of the chromosome elements would depend in this species on the strain used in gene tests for homologies.

The study by Wharton (1943) has been the most extensive and useful source of information for our analysis of chromosome evolution in the genus. She demonstrated the presence of a large number of pericentric inversions. The evidence is of course particularly clear when six, seven, or eight long strands, in addition to the dot, are found in the salivary gland nuclei of forms with six chromosomes. In the vast majority of these cases, pericentric inversions have occurred. The evidence that these cases are but seldom the result of translocations will be developed in this analysis. From a knowledge of this type of information, Wharton wrote as follows:

> It may also be concluded that in the course of the divergence and evolution of Drosophila species some widespread alterations have changed the original six chromosome elements in species of the genus. It is futile to assume the integrity of chromosome elements in the light of the evidence presented by fusions, translocations and pericentric inversions, which have been shown to occur throughout the genus. Homology in terms of whole chromosome elements has doubtless been disrupted by

such events. There must be, in some instances, particularly in closely related species or species groups, some, or even extensive homologies, but this is not an indication of stable and inviolable linkage relationships. It simply means that at the particular moment when the Drosophila population is sampled genetically, these species present this evidence of common origin. It is not an assurance that in their future evolution they will retain such relationships in their homologies. In the study of chromosome homologies, therefore, as in every phase of the study of evolution, we may expect discontinuity because of the extinction of some species, thus precluding a step-by-step analysis of the changes which have altered the homology of chromosome elements.

Obviously, those cases where Wharton could demonstrate pericentric inversions most readily in her material are not the ones that affect the chromosome elements. The large number demonstrated by her and the still larger number presented in our material, analyzed in the same way, prove that pericentric inversions could be expected to be present in a number of cases where separate elements are fused, and such cases would disrupt the chromosome elements.

Spencer (1949), referring to Wharton's findings, states: "The writer considers that, on the basis of the data, she has overstated the case. Actually most of the cases which she has interpreted as pericentric inversions might well involve only one of the six chromosome elements, and still leave these as continuous structures with median or sub-median centromeres."[1] He is overlooking the implications of her data and stating that, because pericentric inversions can be demonstrated in one kind of material by her type of analysis, they cannot be inferred in other types of material where pericentric inversions would affect chromosome elements. Such pericentric inversions of fused chromosome elements are at no more selective disadvantage than the simple pericentric inversions of rods demonstrated by Wharton and in this material.

Unfortunately, the problem of establishing chromosome homologies based on similarities of mutants in two species that cannot be crossed is most difficult. At present it is complicated by the fact that *melanogaster* has been used as the base species. This form has two autosomal fusions, which we can only hope have not undergone reor-

[1] Spencer, W. P., "Gene Homologies and the Mutants of *Drosophila hydei*," page 25. *Genetics, Paleontology and Evolution.* Princeton University Press, Princeton, N. J. 1949.

ganization. To prove the permanent reality of these elements, the proponents would need to establish a simple six-chromosome standard, either *virilis* or *hydei,* or preferably both together. Then the forms with fusions in different species groups could be compared with the standard forms. Spencer (1949) in fact suggested that there exists a small translocation difference between *hydei* and *melanogaster,* and that *hydei* and *virilis* were alike. If this is so, it would automatically make it most probable that *melanogaster* was a changed and derived form. The tremendous labor necessary for such analyses makes them slow, and at present we cannot draw many final conclusions.

In those cases where hybrids can be produced between species, the homologies can be established. Double-length rods, which clearly illustrate loss of homology, are known to occur in *testacea, immigrans* I, *spinofemora,* and *tranquilla.*

TRANSLOCATION

Only in proven cases have we resorted to translocation (other than fusions) to explain changes in the chromosomes and their configurations. The evidence that translocations have played a minor role in such changes follows from the information we have on known cases.

Dobzhansky and Tan (1936) indicated that several small translocations had occurred in the evolution of *miranda,* as judged from *pseudoobscura/miranda* hybrids. MacKnight (1939) has questioned the presence of translocations in this case, and Sturtevant and Novitski (1941b) agree with him, although neither presented evidence to refute Dobzhansky and Tan's conclusions.

A second case is the very interesting one involving the X and 4 chromosomes (A and F elements) in *ananassae.* As Kaufmann (1937) and especially Kikkawa (1938) have shown, the nucleolus organizer and the locus of *bobbed* have been translocated from the X to the 4 chromosome. This creates an unusual situation, for the Y chromosome retains this portion; i.e., it is now in part homologous to chromosome 4, so that these chromosome regions are present three times in the male. This represents a case of fixation of a translocation with hyperploidy in one sex. The chromosome configuration present in *ananassae* is probably also present in the closely related *bipectinata* and in *montium.* These represent the only proven

cases of fixation of translocations as the replacement of some previous condition in the genus.

In addition to the spontaneous translocations in laboratory strains of *melanogaster,* already mentioned, there are two other cases of translocation in wild populations. Dobzhansky and Dreyfus (1943) have reported the presence of a heterozygous mutual translocation in a strain of *ananassae* in Brazil. This translocation was recovered from two of seven females captured in Mogi das Cruzes. In this case the exchange involved breaks in the euchromatin of chromosomes 2L and 3L. The translocation was lost and has not again been recovered from wild populations. It therefore probably represents the detection of a translocation before its elimination from the population as a result of the production of aneuploid gametes. Ward has found a case of spontaneous translocation between the X and 4 chromosomes of *melanica* in a laboratory stock.

Dobzhansky and Pavan (1943b) and Cavalcanti (1948) have demonstrated an exchange between the fusion (X–A) sex chromosome and an autosome to give a complex sex chromosome system in the Bertioga strain of *prosaltans,* as compared with other strains from South America and Mexico (Fig. 38, I, J, K, L). The information available on such a transfer would indicate that it should be at some selective disadvantage, but this situation was not investigated. It is not known how widespread this odd arrangement is in *prosaltans,* but it was obtained only once.

Brown (1940) has proved the relations between crossing over and disjunction in *melanogaster* and reviewed the pertinent literature. Wright (1941) has shown that there is very little chance for the spread and fixation of translocations. The two proven cases of fixation of translocations involve very small segments. In fact, the one in *ananassae* is a segment of such a nature that it was fixed as a hyperploid segment in the male.

The X–4 (A–F) translocations and fusions in *melanogaster* give some idea of the kinds and relative frequencies of such changes. Stone (1934) analyzed fifteen such cases. Number 1 was obtained in a separate experiment where translocations and fusions could be detected. The remaining fourteen were detected in a series of similar experiments (Patterson *et al.,* 1934). Six of these were translocations and eight were fusions. One of these cases (number 10) is of special interest, for it was a total translocation of all the

long euchromatic arm of the 4 chromosome to the tip of the X beyond any known genes. The remaining portion of the 4 was so small that its fate could not be followed satisfactorily. This gives a ratio in X-ray-induced translocations of one total translocation to six ordinary translocations (one of them quite complex) to eight fusions. If this is anything like the relative ratio of spontaneous translocations, which seems probable, we can get some idea of the expected versus the actual frequency of these found in populations.

Table 28 was constructed to show an estimate of the number of the several kinds of rearrangements in Drosophila. This is based on the simplest assumptions capable of explaining the changes in chromosomes observed and on the assumption that any particular change occurred only once in a species group. For example, in the repleta group, the largest number of fusions in any one species is two; hence

TABLE 28

Subgenera	Species Groups	Fusions Y-A	X-A	X-D	A-A	A-D	Pericentric X	A	D	Added Heterochromatin X	A	D
Hirtodrosophila					1		1	2				1
Pholadoris			1		1	1		1				1
Dorsilopha				1	2							
Sordophila			1					2				1
Sophophora	*saltans*		1		1	1					2	1
	willistoni		1		1	1		1				
	melanogaster			1	2		1		1			1
	obscura	1	1					3			1	1
	nannoptera		1					1			2	1
	unassigned				2							1
Drosophila	*quinaria*		1		2							
	guttifera											
	pinicola		1		1	1						
	virilis		1		2			1				
	testacea				2			1				
	tripunctata				1						1	
	funebris											
	repleta				2					1	1	1
	annulimana					1		3		1		1
	robusta		1		1		1	1			1	1
	melanica		1		1			1				1
	polychaeta							2			1	
	carbonaria							3			1	
	cardini				2							1
	immigrans				2	1		1				1
	macroptera				1			2				
	guarani				1					1		1
	bizonata				2					1		
	pallidipennis									1		
	unassigned		1	1	2			1			1	1
	Totals	1	12	3	32	6	3	26	1	5	11	16

these are assumed to be all of the fusions that have occurred. Obviously this is a minimum estimate, and in several cases it is very probably too low. This, with other information, gives a ratio of three total translocations (*spinofemora, testacea, tranquilla*) to two ordinary translocations (*miranda, ananassae*) to about fifty fusions. When it is remembered that there is another and more probable explanation of double-length rods, namely, total pericentric inversions of fusions, this ratio obviously departs very markedly from expectation if all types have had equal chance of fixation.

In the analysis of the metaphase and salivary gland chromosome patterns, certain possible configurations are missing. If we consider only six centromere forms which have no fusions, we find a number of cases of pericentric inversions. There are no forms where a large part of the euchromatic arm is translocated to another chromosome to leave a medium-sized fragment and make a half chromosome, either as a J or an overlength rod or dot. The frequency of induced mutual translocations is about that of fusions, judging from the X–4 cases in *melanogaster,* but these forms are seldom found in natural populations. When all of these data are considered, it seems probable that the majority of the visible structural reorganizations of the metaphase chromosomes in the genus have been the result of fusion and pericentric inversions or added heterochromatin, and that only special types of translocations are fixed.

FUSIONS

Fusions have been referred to repeatedly, but it is well to explore further certain pertinent cases, as well as to consider the net effect of these rearrangements. Sturtevant (1940) and Sturtevant and Novitski (1941b) have reviewed most of the information on chromosome homologies which is presented in Table 27. The evidence on homologies is not as complete as we would like, except between members of the virilis group and between those of the pseudoobscura subgroup. Here homologies can be established by analyzing the salivary gland chromosome synapses.

An examination of the table shows that the analyzed members of the two American subgroups of the obscura complex share the X–A (A–D) fusion, so we can presume a single ancestral origin for this fusion. As the willistoni group is closely related to the obscura group and also has the same X–A fusion, it is probable that this

change arose early in the Sophophora. The X–A fusion, which is characteristic of the saltans group but has not been identified, may be the same one.

It seems more probable that the B–C fusion of *willistoni,* which Sturtevant and Novitski point out is poorly identified, is of independent origin from the B–C fusion of the melanogaster group. The relationships in the Sophophora show why the common origin is not probable (Figure 34).

When we correlate the information in Tables 20 through 28 with the phylogeny, it seems highly improbable that any other fusions are common to two or more groups. One evidence against this assumption is the fact that almost all species groups have some six centromere forms.

The importance of fusions, with a few total translocations which also combine two complete chromosomes (minus one centromere) into one, in the evolution of the genus can best be demonstrated by the frequencies of species with different chromosome numbers. This information is summarized in Tables 20 through 26, on the basis that any reduction represents one of these total combinations of two chromosomes. There are 215 analyzed forms which are shown as 7, 6, 5, 4, or 3 chromosome forms (Figure 47).

The frequencies of all types in the genus, as well as of the two large subgenera, are presented in the form of histograms. The difference between the total for the genus and the Sophophora plus the Drosophila is due to the few members belonging to other subgenera (Table 20). The one gain in chromosome number (0.5 per cent of the total) found in *trispina* shows how infrequently this type of change is retained when compared to the 86 (40.0 per cent) that are unchanged, the 52 (24.2 per cent) with one fusion, the 51 (23.7 per cent) with two fusions, and even the 25 (11.6 per cent) with three fusions. All reductions in chromosome number are counted as fusions in the calculations.

There are 147 forms where both metaphase and salivary gland chromosomes are known. The analysis of these cases shows the same relationships. Here there is one (0.7 per cent) with a gain in number of chromosomes, 70 (47.6 per cent) with no fusions, 35 (23.8 per cent) with one, 27 (18.4 per cent) with two, and 14 (9.5 per cent) with three. The fact that reduction in chromosome number has occurred so often in this genus is remarkable, especially when it is

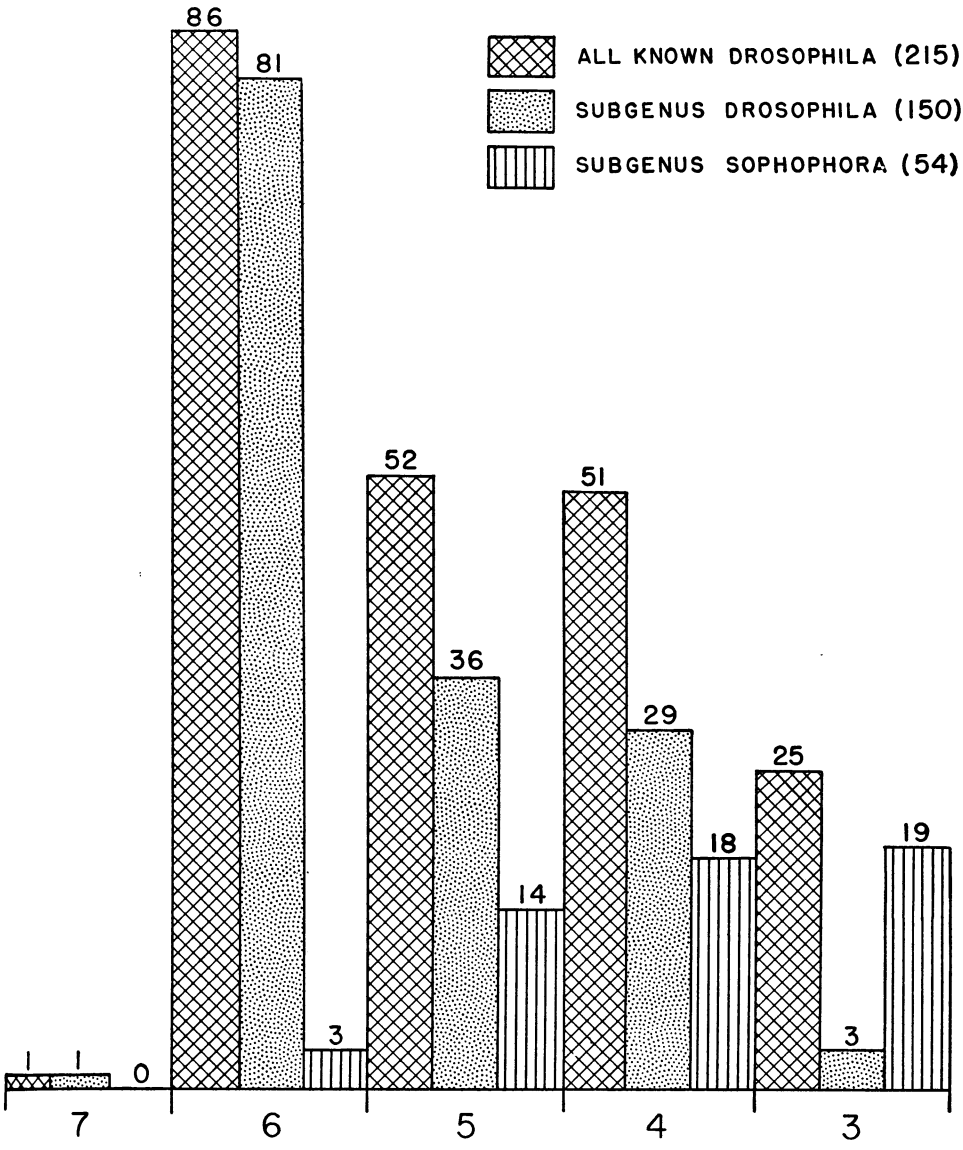

Fig. 47 Histogram showing chromosome number in Drosophila and its two large subgenera.

remembered that probably only one of these fusions is common to several species groups in the Sophophora.

Table 28 was made up to show the minimum numbers of rearrangements necessary if these occurred independently in the species groups. Only one fusion is common to several groups; therefore some fifty separate fusions are known to have occurred. This is the largest class of changes known, other than pericentric inversions. This is an average of one fusion per four species. Actually, the frequency is higher in most groups, for it is low in the large repleta group.

One type of fusion requires special consideration. This includes fusions involving either the X or Y chromosomes and an autosome. The changes in genic balance will be discussed later, but the problem of disjunction with a complex sex chromosome is of considerable interest. This question was investigated by genetic methods in *americana* (Stone, 1949), as previously referred to. It has been investigated by cytological methods in *miranda* by Dobzhansky (1935b, 1937a), Koller (1939), MacKnight and Cooper (1944), and Cooper (1946). Dobzhansky and Koller were unable to find evidence of regular pairing of the X_1 and X_2 (3) with the Y. MacKnight and Cooper (1944), and especially Cooper (1946), presented convincing evidence that there is regular pairing without chiasmata together with a regular oriented disjunction, so that the Y goes to one pole and the X_1 and X_2 to the other at the first meiotic division. It has been shown by MacKnight (1939) that the compound Y–3 element has undergone numerous rearrangements with the loss of some loci. Nevertheless, the presence of the original Y–X_1 homology, together with the Y–X_2 is sufficient to cause this regular association and disjunction. Hence, almost all sperm are normal, i.e., they have either the Y, 2, 4, 5 or X_1, X_2, 2, 4, 5 chromosome complement.

HETEROCHROMATIN

The initial studies of the cytological differences between euchromatin and heterochromatin were made by Heitz, who later reviewed the information and gave the relations between heterochromatin in ordinary cells and in the salivary gland nuclei (Heitz, 1935). The general characteristics of heterochromatin which differentiate it from euchromatin, or the ordinary gene chromosome, are usually based on differentially deeper staining and heteropycnosis. The heterochro-

matin and euchromatin are not single separate substances, but integrate in part, as shown by Dobzhansky (1944b) in *pallidipennis,* where the amount and position of the differential staining is quite variable. We do not mean to imply that the two types of chromatin are fundamentally and completely different, for example, that the euchromatin is the genic part. It is more probable that they share properties in common and yet have some unique functions.

We will not discuss here the relation between heterochromatin and position effect, for such genetic studies do not relate to changes in the physical karyotype such as we are discussing. A general review of the situation in other animal forms is given by White (1945).

Heitz (1935) showed that, in some forms, there are four types of heterochromatin, as follows: (1) α heterochromatin, which often includes the Y chromosome and certain blocks near the centromeres. These often fuse and are not often well organized in the salivary gland nuclei. (2) β heterochromatin, which retains chromomere structures that stain deeply and are usually longer than euchromatic chromomeres. (3) Intercalary heterochromatin, which has been identified by its differential staining and other properties. (4) Heterochromatin associated with the nucleolus, including, at least in *ananassae,* the nucleolus organizer.

Goldschmidt (1949d) has discussed certain features of "heterochromatic heredity." He includes the above-mentioned types plus the heterochromatin of supernumerary chromosomes as still another, and discusses one type of genetic system, the *podoptera* effect, which he has shown to be influenced by heterochromatin such as the Y chromosome. He believes that heterochromatin has genic action and undergoes genetic mutation just as does euchromatin. Its action is usually concerned with the process of growth, and especially with early differentiation.

Goldschmidt points out that the group of mutations he calls homoeotic mutations causes large departures from the normal, and that the *podoptera* effects produce changes which are of such magnitude that they could be considered morphological changes at a class level. Since he believes that such large changes by single mutation are particularly important in evolution, he asks the following question: "Should heterochromatic mutation be considered a major factor in evolution?" Unfortunately, the information at our disposal from the observable heterochromatin changes in the karyotype evolution does not answer

the question. We shall discuss a few representative cases from the available literature.

Heitz (1935) pointed out one of the essential differences in heterochromatin distribution when comparing *virilis,* where every major chromosome has a large heterochromatic region adjacent to the centromere, to *funebris,* where almost all of the heterochromatin is restricted to the X and Y chromosomes. The effect of this difference in distribution probably accounts for the differences in metaphase pattern (cf. Figures 41 J and 42 F).

Bauer (1936) demonstrated that there was a number of strands (chromonema) in the heterochromatin and euchromatin in the salivary gland nuclei of several different species. The heterochromomeres are much larger, and in some places the heterochromatin is only *loosely* associated, as contrasted to the very tight pairing of euchromatin. The clumping of the centromere required of the chromosomes, owing to mutual attraction of this heterochromatic material, also adds to the difficulty of following individual chromosomes in the chromocenter region.

The translocation of the *bobbed* locus and nucleolar organizer from the X to the 4 chromosome in *ananassae* (Kikkawa, 1938) changed the chromosome configuration so that the dot became a small V (or rod in *montium*). This is a transposition of the nucleolar organizer plus other heterochromatin, as Kaufmann (1937) has demonstrated. In this same article, Kaufmann figured XO males as well as triplo-4 individuals. Kikkawa (1938) also studied triplo-4 and haplo-4 individuals. There was a reduction in the viability and fertility in the haplo-4 females, which were *Minute,* just as were haplo-4 flies of *melanogaster.*

Wharton (1942, 1943) demonstrated the marked difference in heterochromatin between closely related species in the repleta group, even though the salivary gland chromosomes differ but little. Only one major inversion shows in the salivary gland nuclei of hybrids between *repleta,* with no heterochromatic arms as such in metaphase, and *melanopalpa,* with three heterochromatic arms. Such marked differences may have developed in several ways. First, there may have been a translocation of heterochromatic material from the Y or some other sources followed by the segregation to fix the hyperploid condition much as in the male of *ananassae.* Second, there may have been a pericentric inversion, or a transposition across the centromere of the

single-band type, which produces a block of heterochromatin. This type has been demonstrated in the X chromosome of *melanogaster* by Muller, Raffel, Gershenson, and Prokofyeva-Belgovskaya (1937); and in the 2 chromosome by Hinton (1942), where large blocks of heterochromatin, as seen in the ordinary prophase and metaphase, are due to single bands on the salivary gland chromosomes. It might also have originated by a mutation of an existing gene which gave it the new power of forming one of these blocks. The only proof for this possibility is that such genes exist.

We cannot determine which of these or other alternatives explain this type of phenomenon. Whatever the changes were that added the heterochromatin to *melanopalpa,* as compared with *repleta,* it did not reduce the size of the other parts of the chromosome in doing so, as Wharton (1942) has shown. Therefore the material which enlarged the chromosomes of *melanopalpa* must have been added, or else the phenomenon observed by Ward (1949) must effect the apparent size. Ward noted that the condition of double-length rods (e.g., in *testacea, immigrans,* and *spinofemora*) showed in prophase, but that by metaphase and anaphase the double-length rod had shortened to appear the same size as the other chromosomes. Obviously, this must be an adaptation to reduce the difficulty of moving such long elements in the spindle system. It may be, then, that differences in coiling and shortening conceal the place of origin of heterochromatin when it has been shifted in such cases.

Dobzhansky (1944b) published a detailed study of the chromosomes of *pallidipennis.* This species, like *funebris,* has almost all of the heterochromatin on the heteropycnotic X and Y, which are comparatively enormous, being larger than all of the other chromosomes combined. Dobzhansky showed that, in the salivary gland nuclei, heterochromatin was attached to the X and that the autosome broke free of this chromocentral mass easily, as a result of pressure on the cover slip. In addition, there were a few short interstitial heterochromatic sections in the euchromatin and the dot chromosome was heterochromatic.

One very interesting and suggestive feature of this study was the "heterochromatization" of the euchromatic material in certain cells. In some cells, the euchromatic part of the chromosome next to the heterochromatin of the X was distinct and synapsed normally outside of the chromocenter. In other cells, this region appeared heterochro-

matic and could not be differentiated in the chromocenter. This is an excellent illustration of the lack of ease in separation between these types of chromatin, at least in some areas of the chromosome.

Pavan (1946b) demonstrated the presence of two types of heterochromatin in *nebulosa* by the use of translocations. These two types stain alike in mitotic prophases of the neuroblasts. The X and Y chromosomes have one type, so that a few chromomeres of the salivary gland chromosome make large heteropycnotic regions in the ordinary prophase. The 2 chromosome possesses the second type of heterochromatin, for a large mass of this material is present in the usual prophase of neuroblasts. Therefore, there are two types of heterochromatin in *nebulosa.* One type, found in the 2 chromosome, expands as does the euchromatic material in the formation of the giant chromosomes of the salivary gland nuclei. The second type, found in the X and Y chromosomes of this species, does not expand as does the euchromatin of the X chromosome in this peculiar enlargement in the very actively secreting salivary cells.

Cavalcanti (1948) studied the chromosomes of several strains of *prosaltans.* The matter of particular interest here is that he found the same type of distribution of heterochromatin in this species as Pavan reported for *nebulosa.* This strengthens our deduction, although the evidence is not conclusive, that the X–A fusion in saltans group is the same as that in the willistoni group.

Hsiang (1949) has shown that *tumiditarsus* has these two types of heterochromatin. In this species, the X and the major autosomes have only the nonexpanding type, while the dot is a large mass of heterochromatin, which includes the nucleolar organizer.

Ward (1949) has found this difference very well illustrated by contrasting two species. In *carbonaria,* an expanding mass of heterochromatin is present adjacent to the centromere in each of the major chromosomes, although no heterochromatic arm as such is present. In contrast to this condition, *nannoptera* has two large heterchromatic arms, but in the salivary gland nuclei even in the male there is no chromocenter and no obvious heterochromatic bands.

There are several XO forms among the species tested. Wharton (1944) used an XO strain of *mercatorum* and followed the effect in inheritance in crosses to *pararepleta.* Ward (1949) has found that some other strains of *mercatorum* have a small Y. Wharton found no

Y in *orbospiracula,* and Ward discovered a similar condition in *longala* (Figure 38 A). Dobzhansky and Pavan (1943b) described *annulimana* as an XO form. Wharton decided that the Y material of *mercatorum* might be incorporated in the dot, which is enlarged to form a small V at metaphase. The location of the Y in the other forms is uncertain. Either the necessary Y heterochromatin—and the Y is usually necessary for fertility in the male—has been translocated to another chromosome or chromosomes in these cases, or other genes have taken over the necessary functions.

The number of cases where extra heterochromatic arms are present in this material can be determined from Tables 20 to 26 by comparing the number of chromosome arms in the metaphase with that found in the salivary gland nuclei. On the basis that similar changes are of single origin within the species group, we have summarized in Table 28 the number of times heterochromatic arms have been added.

The peculiarities of the heterochromatin, its capacity for fixation of aneuploid conditions, its relation to position effect, and its relation to growth and development all combine to indicate that it deserves a further intensive and extensive study.

PERICENTRIC INVERSIONS

We have assumed that, when the number of strands seen in the salivary gland nuclei increased above five long arms and the dot, that the increase was due to a pericentric inversion of euchromatic material for each additional arm. Certainly the evidence on mutual translocations indicates that this is almost always correct. Tables 20 to 26 indicate these pericentric inversions in the several species, and Table 28 summarizes the minimum number of this type of transformation in each species group.

This generally applicable method is subject to the possible errors already mentioned, so that gene homologies have been used as a check whenever feasible to separate this type of change from possible translocation. Table 27 gives the gene homologies established between the members of the obscura group which is pertinent here. This information makes it very probable, as the homologies are almost certainly justified here, that two J- or V-shaped autosomes in the affinis subgroup of the obscura complex differ from the corresponding rod-

shaped autosomes in the pseudoobscura subgroup by pericentric inversions or centromere shifts.

The first case of pericentric inversion demonstrated by direct cytological observation in a natural population is the one reported by Miller (1939) in the B chromosome (element E) of *algonquin.* This pericentric inversion (B3, Miller) exists homozygous or heterozygous with a sequence which differs from it by two overlapping inversions (the Standard, Miller). The difference is sufficient to reduce the crossing over to such an extent that probably few aneuploid gametes are formed. This was not measured directly, but the wide coexistence of these two gene sequences prove that the heterozygote is not at a too serious selective disadvantage.

A second case of cytologically detected pericentric inversion was described by Wharton (1943). This consisted of a small simple pericentric inversion in the X chromosome of *D. bifurca.* The inversion was small, involving the heterochromatic region near the centromere. Crossing over is infrequent near the centromere in Drosophila, and so this small inversion was probably at little selective disadvantage even when heterzygous.

Carson and Stalker (1947) describe two simple pericentric inversions in *Drosophila robusta.* One is rare, but the other is common in certain regions where it is often heterozygous with the standard sequence. The actual effect of these cases on disjunction and the production of aneuploid gametes is unknown. Certainly they afford evidence that pericentric inversions can persist in certain natural populations.

Finally, there is the case of fixation of a pericentric inversion in the virilis group, which has been demonstrated cytologically. Stone, Griffen, and Patterson (1942) have shown that the 2 chromosome (element E) of *montana* differs from *virilis, texana, americana,* and *novamexicana* by a pericentric inversion. Patterson (1944) has shown that *lacicola* has this same pericentric inversion.

Burla *et al.* (1949) reorganized the taxonomy of the sibling species in the willistoni group. They established the chromosome pattern of both metaphase and salivary gland chromosomes. One case showing the effect of pericentric inversions on gene homologies has been found in these four sibling species. Burla *et al.* report that there is a pericentric inversion of the 2 chromosome of *paulistorum* and *equi-*

noxialis, as compared with *tropicalis* and *willistoni*. Since no hybrids are produced, the presence of this inversion was determined from the characteristic banding configurations. These physical homologies can be recognized, as there is comparatively little reorganization in this chromosome.

In order to test more fully the negative selective effect of heterozygous pericentric inversion, Alexander (1952b) tested two in the 2 chromosome of *melanogaster*. These are *Plum*^{2} and *Glazed*. The breaks of the pericentric in *Plum*^{2} are very near *purple,* between this gene and the centromere in the *purple-curved* region and, at the *brown* locus, between *plexus* and *speck*. The breaks are in the 40F and 59E sectors of Bridges' map of the salivary gland chromosome. In effect, it is an inversion of nearly all the right arm of the V-shaped chromosome, changing it to a very short-armed J or rod. The euchromatic portion of the left arm is not changed up to the break, just to the left of the centromere. *Glazed* is broken far out in the two arms of the 2 chromosome at 27E and 51D, between *dumpy* and *black* on the left and *purple* and *curved* on the right. The left end region is only about half as long as the right end region, while about three-fifths of the midregion of the chromosome is inverted. The effect of these inversions on crossing over was tested with the marker stock *aristaless* (0.0), *dumpy* (13.0), *black* (48.5), *purple* (54.5), *curved* (75.5), *plexus* (100.5), and *speck* (107.0). The two pericentric inversions caused very different modifications of crossing over. *Plum*^{2} heterozygous gave 52.9 per cent recombination in the left arm from *aristaless* to *purple* as compared with a standard of 54.5 per cent, while no crossovers were recovered from *Glazed* in these regions. In the right arm there was 2.1 per cent crossing over with *Plum*^{2} in the regions between *purple* and *speck,* all due to double crossing over. *Glazed* had no crossovers in the *purple-curved* region, 2.8 per cent (standard 25.0 per cent) in the *curved-plexus,* and 1.8 (standard 6.5 per cent) in the *plexus-speck* region. In each case this crossing over was due to single exchange outside the inversion. An egg hatch test was run with each inversion, to determine the frequency of the production of aneuploid gametes. The control consisted of the inversion heterozygous in a male crossed to normal females, while the test heterozygous females were crossed to normal males. The results were as follows:

Control: $Pm^2/+$ ♂ × + ♀ = 4,651 hatched from 4,913 eggs = 94.7%
Test: $Pm^2/+$ ♀ × + ♂ = 4,774 hatched from 5,628 eggs = 84.8%
Control: *Gla*/+ ♂ × + ♀ = 3,770 hatched from 4,050 eggs = 93.1%
Test: *Gla*/+ ♀ × + ♂ = 4,299 hatched from 4,975 eggs = 86.4%

If we use these controls, *Plum*^{2} produced only 9.9 per cent aneuploid gametes (16.2 per cent if we assume 100 per cent hatch), while *Glazed* females heterozygous produced only 6.7 per cent aneuploids (or 13.6 per cent on the basis of 100 per cent hatch expected).

These are both major inversions and together test the two types of pericentrics, symmetrical and asymmetrical. If the inversions had been shorter, they would have reduced the amount of exchange within the inversion even further, and so produced less or no aneuploid gametes. Obviously, shorter pericentrics that had fewer crossovers within the inversion, and so produced very few aneuploid gametes, could be retained in a population.

The argument that pericentric inversions will be eliminated rapidly from a population owing to the production of aneuploid gametes from crossing over within the limits of the heterozygous inversion is undoubtedly sound and is reflected in the data given showing the egg mortality. There seem to be three situations where pericentrics may be retained. The first Miller (1939) suggested from the case in *algonquin,* namely, that the pericentric is shielded from selection by being heterozygous with a paracentric inversion that prevented crossing over. The inversions found by Carson and Stalker (1947) in *robusta* are not so shielded and must fall in the second class, which consists of cases where the inversion is short enough that it does not cross over to an appreciable degree when the inversion is heterozygous. The third type is a shielding effect which would result if the pericentric were superimposed on a rare paracentric. In some cases the complex of inversions would prevent crossing over. In others, which involved suitable long inversions, an elimination of the abnormal chromatids formed by crossing over would occur at the first maturation division, since the complex would act as a paracentric. Thus few, if any, aneuploid gametes would be formed. The frequency of changes in chromosome configuration from rod to small J or V in this genus suggests that these factors have been effective in a number of cases.

INCREASE IN CHROMOSOME NUMBER

Sturtevant and Tan (1937) postulate that an increase in chromosome number could be accomplished by use of the relatively inert Y chromosome, and Dubinin (1934) has used this method experimentally. Stone and Griffen (1940) showed that an extra or "free" centromere can be made available for future use by certain types of translocations and fusions. Apparently neither the ever-present Y nor the supernumerary chromosomes provided by other rearrangements have been utilized to any extent to increase chromosome number by the analyzed members of this genus.

In the 215 forms analyzed (at least 198 separate species), there was only one where the chromosome number was increased above the basic six. This was *trispina,* where the chromatin nature of the extra element was proved by its segregation in hybrids with *limpiensis.* There is another case of a "free" centromere (other than the Y) where the chromosome seems to be heterochromatic, *putrida* II. This case suggests that such an extra chromosome, when compared to *putrida* I, arose as the alternate heterochromatic chromosome when two rods fused to form one of the autosomal V's (Figure 37). The large size of this extra chromosome suggests that an appreciable part of the heterchromatin of one rod was retained by the supernumerary in the fusion. The diagrams in Figure 37 where heterochromatin is left unmarked show such changes. The lack of this supernumerary chromosome in *putrida* I indicates a possible evolutionary heterochromatin diminution which might result in forms with a reduced number of chromosomes and the minimum of heterochromatin.

The supernumerary chromosome in *trispina* must have arisen in the same way, but in this case the compound chromosome was lost in the segregation from the original fusion or translocation, and the supernumerary was fixed in the population.

These supernumeraries, no matter which of these ways they arose, are available for further modification of the chromosome complex. If present with V's, they afford an opportunity to go from a V to two rods. They would be available, together with the Y, for translocation to establish additional short chromosomes. The absence of such translocation forms in the genus again emphasizes the difficulty of fixation of translocations except the special whole armed type such as fusion.

SIBLING SPECIES AND CHROMOSOME RACES

There are represented in our data a number of cases of twin species or races. These are forms which are phenotypically very close together, even indistinguishable, but which differ in some fundamental characteristic or characteristics. The first pair of twin species to be established as such were *D. pseudoobscura* (Frolowa) and *D. persimilis* (Dobzhansky and Epling). In this case the chromosomes are alike except for paracentric inversions, which will be discussed later. The members of the affinis subgroup of the obscura complex are so similar that Sturtevant and Dobzhansky (1936a) state: "The eight forms concerned here are so similar in appearance that we have been unable to devise satisfactory methods of distinguishing pinned females." Living females are more easily separated in some cases and the males can often be separated by using the sex combs. Dobzhansky (1946b) showed that *D. equinoxialis* Dobzhansky resembles *D. willistoni* Sturtevant, so that it is nearly impossible to separate the two species. Burla *et al.* (1949) have added two additional sibling species to the willistoni group, *tropicalis* and *paulistorum.* The metaphase chromosomes are alike (Figure 39 A), but differences can easily be seen in the chromosomes of the nuclei of the salivary glands. The external morphology is so similar that positive identification is more readily made from the disk pattern of these large chromosomes (Dobzhansky *et al.,* 1950).

There are recorded in Tables 20 to 26 numerous cases of sibling forms which have different chromosomal configurations. These are often chromosome races, but may even represent separate species in a number of cases that have not been tested genetically. This situation had developed so far that Ward (1949) made a special study of a number of strains of different species to look for variability in chromosome pattern. The ten cases in the six species groups where the twin forms are designated I, II, and so on are obvious in Tables 20 to 26 and the corresponding figures. Some of these are shifts of heterochromatin, but others are more drastic changes.

There are several cases to be mentioned in addition to those so listed. Wharton (1943) reported a small pericentric inversion in a strain of *bifurca* that changed the rod-shaped X into a J.

King reports that members within one of the two subgroups of the *guaraní* complex are very difficult to tell apart in several cases. In

some of these the chromosomes differ. These species can be differentiated genetically in all cases.

There are a number of cases in which the species or subspecies resemble each other very markedly, but which have chromosome differences. These are *cardini* and *cardinoides, occidentalis* and *subocciden-talis, texana* and *americana, meridiana* and *rioensis, mercatorum* and *pararepleta,* and *heterobristalis* and *meitanensis.*

Finally, there are groups where different investigators have reported different chromosome configurations for several species. We believe that many of these will prove to be such twin forms. Among these are *robusta,* which has three V's and a dot, according to Carson and Stalker (1947). However, Metz (1916b) and Wharton (1943) state that it has a rod, two V's, and a dot. Sturtevant (1942) gives a somewhat different description of the chromosomes of *victoria* from southern California from those of the strain used here (Wharton, 1943). Metz (1916b) and Wharton (1943) describe both *willistoni* and *nebulosa* as having a dot. Dobzhansky and Pavan (1943a) failed to find a dot in the strain they identified as *willistoni,* and Pavan (1946) reports no dot in *nebulosa.* Finally, Metz (1916b) and Kikkawa and Peng (1938) report that *busckii* has a dot, while Wharton (1943) failed to find one in the strain she examined. Some of these may be cases of oversight or misidentification of chromosomes, but the variability of the chromosome pattern definitely proved suggests that at least some of these will prove to be different chromosome forms.

GENERAL OBSERVATIONS

The limitations of variation in chromosome number in this group reflect the differential effects of selection on the several types of rearrangements. The *trispina* configuration with seven pairs of centromeres indicates that an even higher increase in number is theoretically possible. On the other hand, a fusion of the two rods in *tranquilla* could form a species with only two chromosomes, even if it involved the X chromosome, for X-A fusions have been rebalanced genetically and established in several species.

The use of prophase, metaphase, and salivary gland chromosomes has allowed us to follow the reorganization and reduction of chromosome numbers. The demonstration of the addition (or subtraction)

of heterochromatin showed us the limitations of metaphase configurations for determining gene content.

This analysis of chromosome evolution is not as complete or thorough as we could wish it to be. The main patterns seem clear. In this material there are few, if any, translocations, except special cases such as that of heterochromatic material (*ananassae, tumiditarsus*) and fusions whch are fairly common. Paracentric inversions will be shown to be very frequent in Chapter 5, and pericentric inversions have occurred a number of times. Although some of the cases we have classed as pericentric inversions might be due to centromere shifts, this seems improbable, as no other three-break rearrangement has been found. The several mechanisms which protect pericentric inversions are probably sufficient to explain the fixation of the known and inferred cases. The addition, subtraction, and shift of heterochromatin has accomplished some extensive changes in metaphase chromosome pattern without a correspondingly great shift of genes in the system. It would seem that these data and those in Chapter 5 have established the main patterns of chromosome evolution in the genus Drosophila.

5 SALIVARY GLAND CHROMOSOMES

The gigantic chromosomes found in the nuclei of the salivary glands are very important and useful in studying phylogeny and divergence. For one thing, these chromosomes can be said to show the effect of most cytological divergence and phylogeny, particularly of closely related forms. Almost all the types of rearrangements illustrated in Figure 37 can be detected if both salivary gland nuclei and ordinary ganglionic or gonadial metaphase chromosomes can be examined, usually with very considerable certainty in forms that hybridize.

Kostoff (1930) was the first investigator to show the structure of the chromosomes in the nuclei of the salivary glands of *D. melanogaster.* He showed a photograph of these chromosomes and stated:

> The linear arrangement of the genes necessitates the assumption that the chromosomes are made up of chemically different components. The discoid structure of the chromosomes shown in this photograph, indicates the existence of such chemical differences in the varying capacity to absorb haematoxylin. These discs may represent the actual packets in which inherited characters are passed from generation to generation.[1]

Kostoff did not present experimental evidence to support his hypothesis. Painter (1933; 1934a, b; 1935) first demonstrated and used these chromosomes to map and identify the location of the gene, often to very narrow limits. He used chromosomal abnormalities, such as deletions, translocations and inversions, in this study. For our analysis, the fact that these differences in gene sequence were so readily localized and obvious in this material made this tool of great importance in studying both intraspecific and interspecific differences in gene order. The salivary gland chromosomes have since been used

[1] Kostoff, Dontcho. "Discoid Structure of the Spireme," pages 323–324. *Journal of Heredity.* American Genetics Association, Washington, D. C. 1930.

extensively by Painter and others to study both induced and natural differences between chromosomes in Drosophila in general. We shall review some of the work on differences within and between species.

Gene mutations occur and reoccur in a population. If gene differences between a pair or even several pairs of alleles exist in populations under test, it is often impossible to demonstrate them. This is not so of chromosomal rearrangements of a magnitude more than one or two bands on the salivary gland chromosomes, for these can be detected whenever heterozygotes can be obtained, i.e., always within a species and often between members of a species group.

Furthermore, any particular gene arrangement has only one origin, for, unlike mutations, the same rearrangements probably occur twice in a species so seldom as to have no effect on the results of analysis based on unitary origin. This single origin can be contrasted with the occurrence of a particular mutant allele in a population, for many mutations may recur a number of times before being fixed in a population.

Dobzhansky (in Dobzhansky and Epling, 1944) has discussed the evidence against the repeated origin of a particular gene arrangement. The facts are: (1) Rearrangements are very infrequent in control material; for example, no translocation was found in ten thousand different sets of salivary gland chromosomes examined by him. (2) There are many thousands of places where the breakage could occur along the chromosomes to produce rearrangements. Among the rearrangements studied only two or three have one breakage point in common, and these are not otherwise alike. In the complex sequence phylogenies of paracentric inversions, as discussed below, no case exists where the same rearrangement had to occur twice to account for the observed gene sequences. In fact, the retention of even paracentric inversions, which are certainly the most frequent type of change in gene sequence, are uncommon. Some fifty to a hundred would probably account for the most extreme cases of reorganization known within a species group where crosses are still possible, for example, in the variable pseudoobscura subgroup. In fact, in some groups, distinct species have little if any difference in gene order. The fact that extensive reorganization has occurred, at least within the chromosome elements, can be inferred from the difference in sequence between apparently homologous genes, as reviewed by Sturtevant and Novitski (1941b) and Spencer (1949).

The detailed salivary gland chromosome analysis is possible because of the distinctive banded nature of these chromosomes. The salivary gland chromosomes are in effect a bundle of very closely synapsed, uncoiled, extended and enlarged chromonemata (Koltzoff, 1934). The variation in pattern of bands and unbanded areas reflects the variation in size and distribution of the chromomeres along the chromonema. Examination of unstained salivary gland chromosomes with a phase microscope shows that the banded structure is characteristic of differential light adsorption and reflection, and not an artifact of staining. Presumably the chromosomes are in the main nucleoproteins, nucleic acids and proteins with other less common particular constituents, but the quantitative evidence on these points is as yet unsatisfactory (Commoner, 1949; Danielli, 1949).

Blumel and Kirby (1948) have published an introductory study showing some of the main amino acid constituents of these salivary gland chromosomes. This type of investigation will require a great deal of further detailed work. A general discussion of the salivary gland chromosomes and chromosome structure as such is not within the scope of this study.

In the analysis of intraspecific and interspecific differences, the intimate pairing of homologous chromosomes allows for the detection of even minute reorganizations. The difficulty lies in the fact that homologous regions of chromosomes synapse less intimately in some species crosses. This is well illustrated by comparing Figs. 48 and 49, taken by Dr. A. B. Griffen. Figure 48 is a photograph of the salivary gland chromosomes of a *virilis* female. The homologous chromosomes are completely paired, except for a short region in the X chromosome, which allows us to see that the arms which seem to be single elements are formed by the somatic synapsis of the two homologues. Unfortunately the dot chromosome is not well shown in either photograph, but the other chromosomes are identified. Figure 49 is a *virilis/americana* female hybrid. The inversions in chromosomes 2, 4, and 5 show well, but the complex inversion in the X is unpaired. Here the less intimate union in somatic synapsis of homologous chromosomes is apparent for regions where the banding of the two chromosomes is obviously identical although they often lie somewhat apart. This difference is retained in backcross hybrids, sometimes with only one or a part of a chromosome from one ancestral species.

The differences between species in chromosome organization are

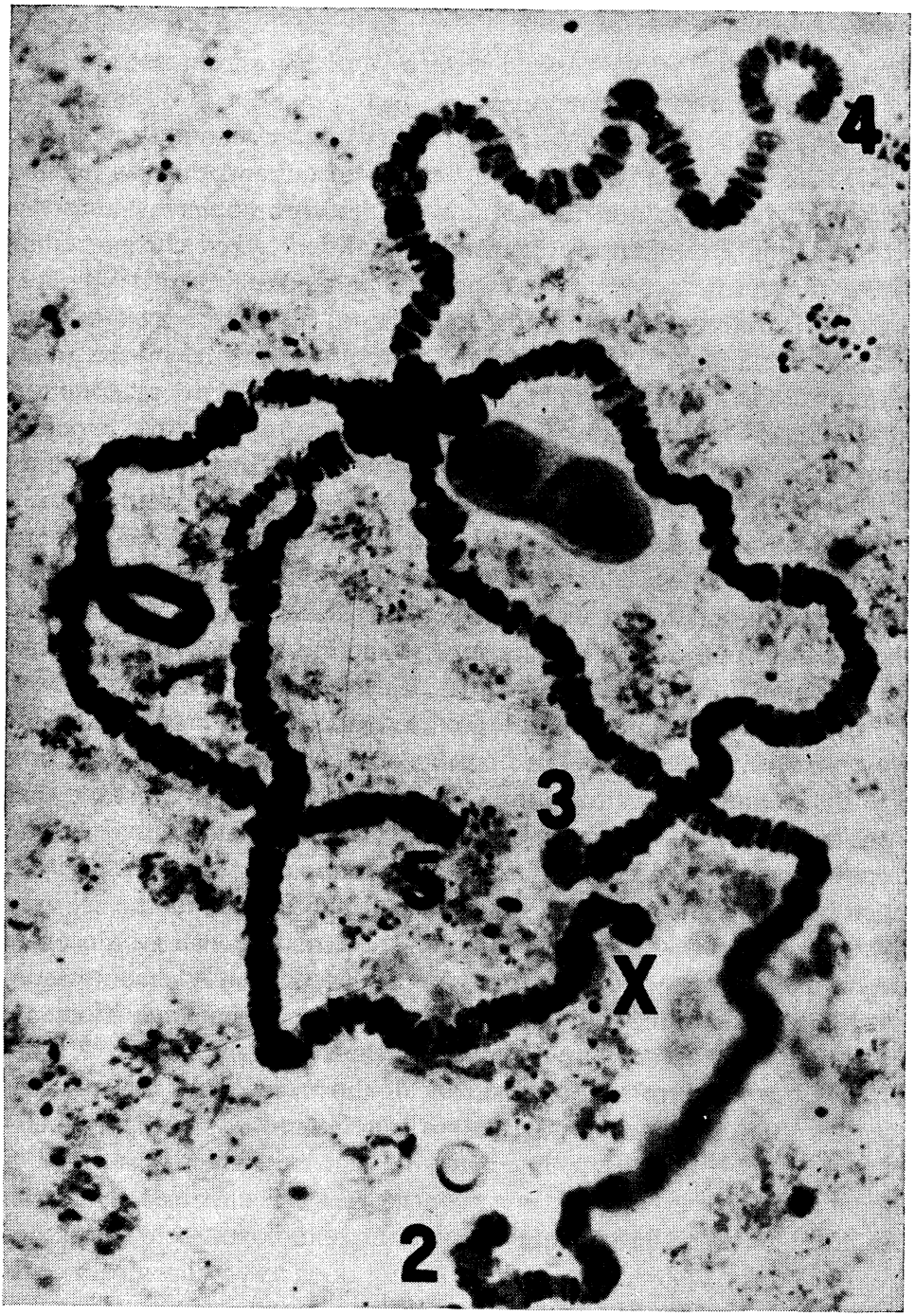

Fig. 48 Salivary gland chromosomes of *D. virilis* female. (Photograph by A. B. Griffen.)

Fig. 49 Salivary gland chromosomes of *virilis/americana* hybrid female. (Photograph by A. B. Griffen.)

often reflected in the differences in staining technique needed to obtain good slides (Warters, 1944). This difference in staining is a property of the chromosome itself. Fujii (1942) called attention to the fact that crossing over could be detected cytologically in the 6 chromosome of *virilis*. The 6 chromosome of a New Orleans strain stains much more lightly than the ordinary 6 chromosome in other strains. If crossing over between this lightly staining and a normal staining 6 chromosome is followed by gene markers, the recombinations show the effect of the parent type 6 chromosome in the differential staining capacity in the recombined chromosome, at least to some extent.

In *victoria,* Wharton (1943) found that the two lobes of the salivary gland did not develop equally, so that this species, and the closely related *lebanonensis* (Ward, 1949), have asymmetrical salivary glands. In the well-developed section of the gland, the chromosomes develop and stain well, while they are unusable in the poorly developed portion of the gland. The differences in properties of the chromosomes reflected in their pairing and staining are sometimes extensive, even within a species group.

The type of rearrangement most useful in the study of phylogeny in Drosophila is the paracentric inversion. It is the most common aberration and suffers little selective disadvantage, even when heterozygous. Sturtevant (1926, 1931) first demonstrated the presence of paracentric inversions in *Drosophila melanogaster*. Sturtevant and Plunkett (1926) demonstrated that *melanogaster* and *simulans* differed by a major paracentric inversion in chromosome 3 R (element E). These were demonstrated by genetic means, and the inversions were not delimited cytologically at that time.

McClintock (1933) had shown that, in *Zea mays,* inversion heterozygotes can be detected both by prophase pairing configurations and by characteristic bridges and fragments in meiosis. Drosophila material is not suitable for this type of study of meiosis, but the development of the salivary gland chromosome technique made even more exacting comparisons possible. In order to facilitate comparisons, the several groups will be considered separately.

The Subgenus **Sophophora**

Sturtevant (1926, 1931) first demonstrated inversions genetically with linkage studies in *melanogaster*. This same method was used by Sturtevant and Plunkett (1926) and Sturtevant (1929), who proved

the existence of inversion differences between the closely related *melanogaster* and *simulans*. These genetic methods are slow and laborious, so that it was not until the method of analysis with the salivary gland chromosomes had been demonstrated by Painter (1934a) that gene sequence differences between strains or species could be demonstrated easily and efficiently.

Some of the American representatives of the obscura group have been most thoroughly investigated for cytological differences, both within and between species. Before taking up particular cases, it is necessary to present the method of studying chromosome phylogeny as developed by Sturtevant and Dobzhansky (1936), Dobzhansky and Sturtevant (1938), and Dobzhansky (1944c). The basis for chromosome phylogeny lies in the fact that a particular rearrangement, a pericentric or, more usually, a paracentric inversion, occurs only once. Therefore any strain that has this arrangement, or any modification of it, must be directly descended from this single original animal. As spontaneous rearrangements are very rare events, the evidence on the single occurrence of any one rearrangement is shown best in experimental material.

Painter (1934a, 1934b, 1935) studied a series of translocations and inversions in *melanogaster*. No two rearrangements involved breaks at the same place in the euchromatic regions of the salivary gland chromosomes, although breaks in certain heterochromatic regions next to the centromeres may have been involved in several different rearrangements. This is difficult to ascertain in *melanogaster*. Painter made no special study of this problem, but Bauer, Demerec and Kaufmann (1938), Bauer (1939), and Kaufmann (1940b) have made extensive and careful studies on the distribution of breaks. These authors concluded that the frequency of breakage was proportional to chromosome length and the distribution was at random along the chromosome, except in the heterochromatin. Baker (1949) found a generally similar situation in *virilis*. Kaufmann (1940b) has shown that some regions have a somewhat greater tendency to break than others, but this does not increase significantly for our purposes the probability that even a two-break rearrangement has occurred twice in a species. The problem of multiple breaks followed by rearrangement can best be developed by studying the relation between two-break rearrangements and four-break rearrangements. This is particularly applicable, for in natural populations many gene sequences differ from each other by a two-break rearrangement; many

others differ by rearrangements involving four breaks, with new attachments of genes. Single-break arrangements do not survive as such in Drosophila. These relations can best be illustrated by giving the possible changes in gene association.

Let the gene system along a chromosome be designated by letters. We will consider a chromosome system with four possible breakage points and the rearrangements that could result if two breaks occurred, if two breaks with rearrangement were followed by the other two breaks with rearrangement, and finally the possibilities if all four breaks were present and all the possible rearrangements occurred. The results are as follows:

Chromosome showing four possible breakage points:

A	BC	DE	FG	H
	1	2	3	4

Arrangements from any two breaks in regions indicated:

(1) A. CB. DE FG H (1, 2)
(2) A. ED CB. FG H (1, 3)
(3) A. GF ED CB. H (1, 4)
(4) A BC. ED. FG H (2, 3)
(5) A BC. GF ED. H (2, 4)
(6) A BC DE. GF. H (3, 4)

Arrangements from two-break rearrangement followed by other possible two-break rearrangement:

(7) A. CB. DE. GF. H (1, 2 then 3, 4; or 3, 4 then 1, 2)
(8) A. GF. DE. CB. H (1, 4 then 2, 3; or 2, 3 then 1, 4)
(9) A. ED. GF. BC. H (1, 3 then 2, 4)
(10) A. FG. CB. ED. H (2, 4 then 1, 3)

In addition to the four arrangements, (7), (8), (9), (10), there are twenty-one additional detectable four-break rearrangements possible if all four breaks were present at once and the broken ends rejoined at random. These are:

(11) A. CB. ED. GF. H
(12) A. CB. FG. DE. H
(13) A. CB. FG. ED. H
(14) A. CB. GF. DE. H
(15) A. DE. BC. GF. H
(16) A. DE. CB. GF. H
(17) A. DE. GF. BC. H
(18) A. DE. GF. CB. H
(19) A. ED. BC. GF. H
(20) A. ED. FG. BC. H
(21) A. ED. FG. CB. H
(22) A. ED. GF. CB. H
(23) A. FG. BC. ED. H
(24) A. FG. CB. DE. H
(25) A. FG. DE. BC. H
(26) A. FG. DE. CB. H
(27) A. FG. ED. BC. H
(28) A. GF. BC. ED. H
(29) A. GF. CB. DE. H
(30) A. GF. CB. ED. H
(31) A. GF. DE. BC. H

Figure 50 shows the synapsis of some of the possible combinations, a normal chromosome with the sequence change given, as well as two heterozygous inversions synapsed.

The occurrence of overlapping inversion differences between two strains indicates that at least four breaks have happened, either two at a time or all four at one time. Dobzhansky (1944c), reviewing the material known to date, states that overlapping inversion differences, numbers (9) and (10), are present between strains. In addition, there are independent (7) and included (8) inversion differences. However, there are no four-break rearrangements such as (11) through (31). In addition, Dobzhansky (1944c) stated that there are no sure cases of three-break rearrangements known in Drosophila species. From this information it is possible to infer that the inversions usually occur as two-break rearrangements and that complex differences are in fact the sum of a series of such steps. As the same inversions do not occur with detectable frequency, we can have $+ \rightarrow$ (2), and $+ \rightarrow$ (5) then have heterozygotes $+/2$, $+/5$, and $2/5$. In this case we would not get (9) or (10), for these involve sequential superimposition of one inversion on the other rather than on normal. Consequently, if complex differences are found between strains, the intermediate steps may be predicated as existing or having existed in the past. As these methods of analysis were developed using the pseudoobscura subgroup, it will be considered next as it is the most extensively tested group.

obscura group

The obscura group has an Old World complex of species and a New World complex. We prefer to separate the New World complex into the pseudoobscura subgroup and the affinis subgroup, as these are natural divisions, judged from the chromosomes, cross fertilities, and taxonomic types.

(a) pseudoobscura subgroup. The pseudoobscura subgroup has four known members, and hybrids have been obtained between *pseudoobscura* (*pseudoobscura,* race A), *persimilis* (*pseudoobscura,* race B), and *miranda.*

Tan (1935) and Koller (1936) studied the chromosomal rearrangements in *pseudoobscura, persimilis,* and their hybrids. For a long time these two species were treated as two races and will here be treated together. The differences that have been demonstrated between their chromosomes have all been paracentric inversions. The

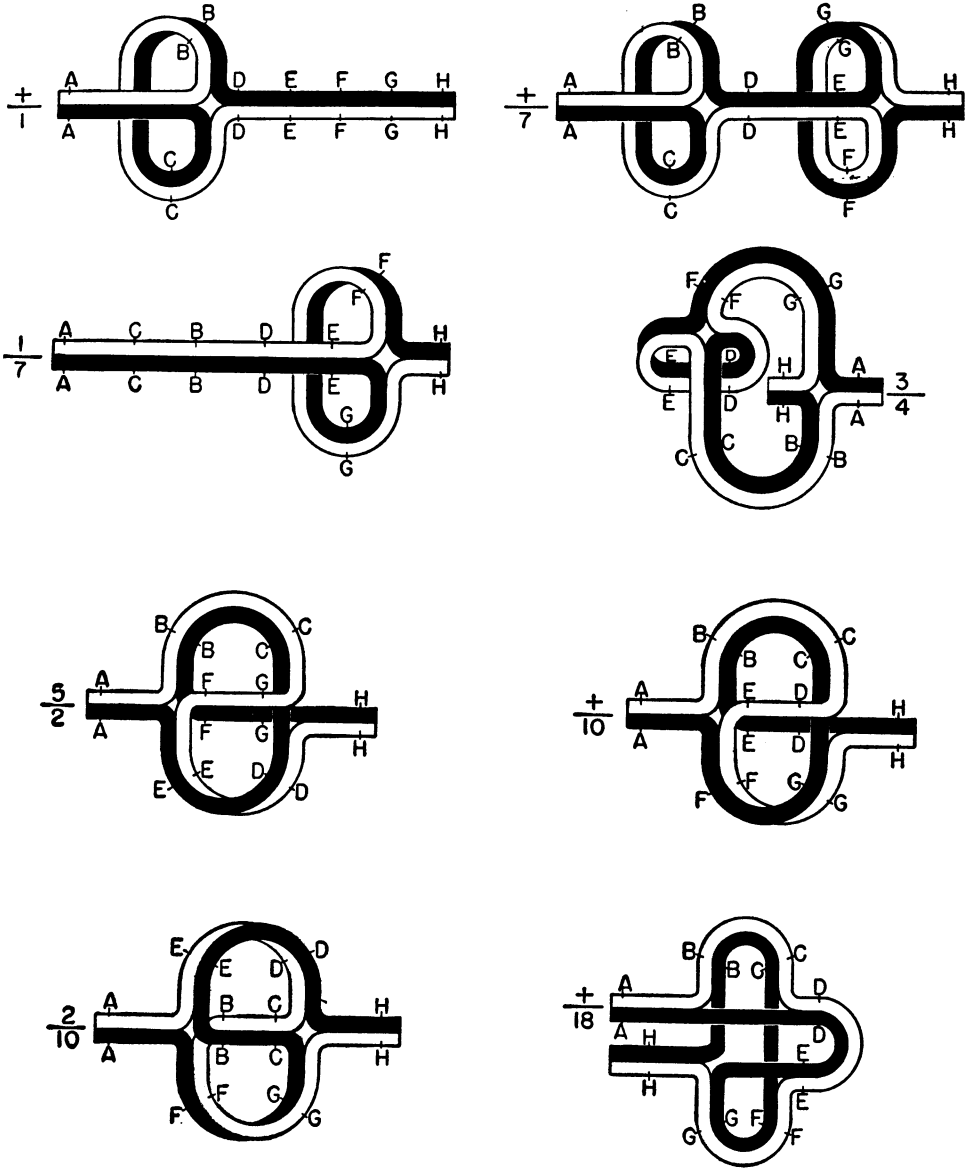

Fig. 50 Inversion configurations. (Modified from Dobzhansky, 1944c.)

genetic situation has been investigated further by Sturtevant and Dobzhansky (1936b), Dobzhansky and Sturtevant (1938), and Dobzhansky (1944c, which includes a bibliography of the work on this problem). Dobzhansky summarizes the evidence to that date, and only a few new arrangements have been added. The 3 chromosome (element C) of the sibling species *pseudoobscura* and *persimilis* has a large number of gene arrangements. Their analysis has produced a very convincing chromosome phylogeny, for with it as yet unknown chromosome arrangements have been predicted which were later found. Figure 51 gives the phylogeny after Dobzhansky (1944c).

In addition to Figure 51 showing the phylogeny, Figure 52 shows the gene arrangements in *pseudoobscura* and Figure 53 shows those in *persimilis*. The Standard arrangement is common to both strains and will be used as the basic gene pattern. It is assumed that this was the original pattern, although either Hypothetical or Santa Cruz might have been the basic pattern (see Dobzhansky, 1944c). In theory, any one of the gene patterns might have been the original pattern, with the others derived from it, following the steps indicated by the lines in Figure 51. Actually, this is made very improbable from the distributional and sequence change data. All gene sequences are based on cytologically detectable differences. The corrected map of the 3 chromosome of *pseudoobscura* and *persimilis,* Standard sequence, was published by Dobzhansky and Sturtevant (1938). The changes in sequence can readily be followed from Figures 51, 52, and 53. In these cases each sequence in Figure 51 differs from the sequence to which it is joined by an arrow by one inversion, whereas those connected through one intermediate sequence differ by two inversions, and so on. Figure 50 shows the types of inversion synapsis expected in several of these cases. Figure 54 shows Dobzhansky and Sturtevant's (1938) map and certain of the inversion differences illustrating simple and cumulative changes in sequence. Dobzhansky (1944c), after some introductory comments, goes on to describe the relationships illustrated in Figures 51, 52, and 53, as follows:

> Some of the gene arrangements in the scheme are peripheral, that is, related by a single inversion to only one or two other gene arrangements. Others, namely, Standard, Tree Line, Santa Cruz and Klamath, occupy central points in the scheme; each of them is related simply to four or more arrangements. Standard, Tree Line, Santa Cruz, and Klamath are the heads of "families" or "phylads" of gene arrangements, and it seems

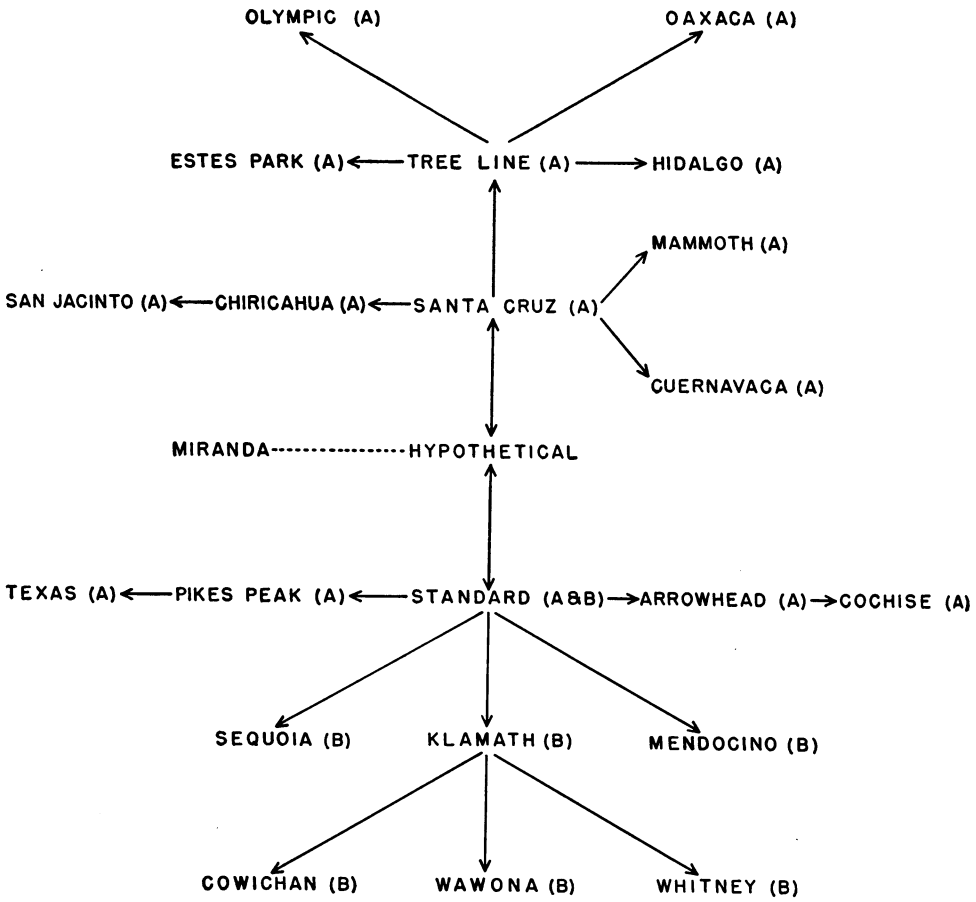

Fig. 51 The phylogeny of gene arrangements in the third chromosome of *Drosophila pseudoobscura* (A) and *Drosophila persimilis*. (From Dobzhansky.)

reasonable to suppose that the gene arrangement phylogenetically ancestral to all others is most likely to be found among them. Standard is not only the head of a large phylad, but also the only arrangement which is known to occur in both *D. pseudoobscura* and *D. persimilis*. It would therefore be reasonable to assume that it existed in the common ancestor of these two species. An alternative, the Hypothetical, has not yet been found in nature, but it occupies perhaps the most central position of all. Furthermore, it is remarkable in another respect. As shown by Dobzhansky and Sturtevant (1938) and by observations made since the publication of that paper, the gene arrangement of the third chromosome in *D. miranda* is more like that of Hypothetical than it is like any other arrangement in *D. pseudoobscura* or *D. persimilis*. Hypothetical or a closely similar arrangement might therefore have existed in the phyletic

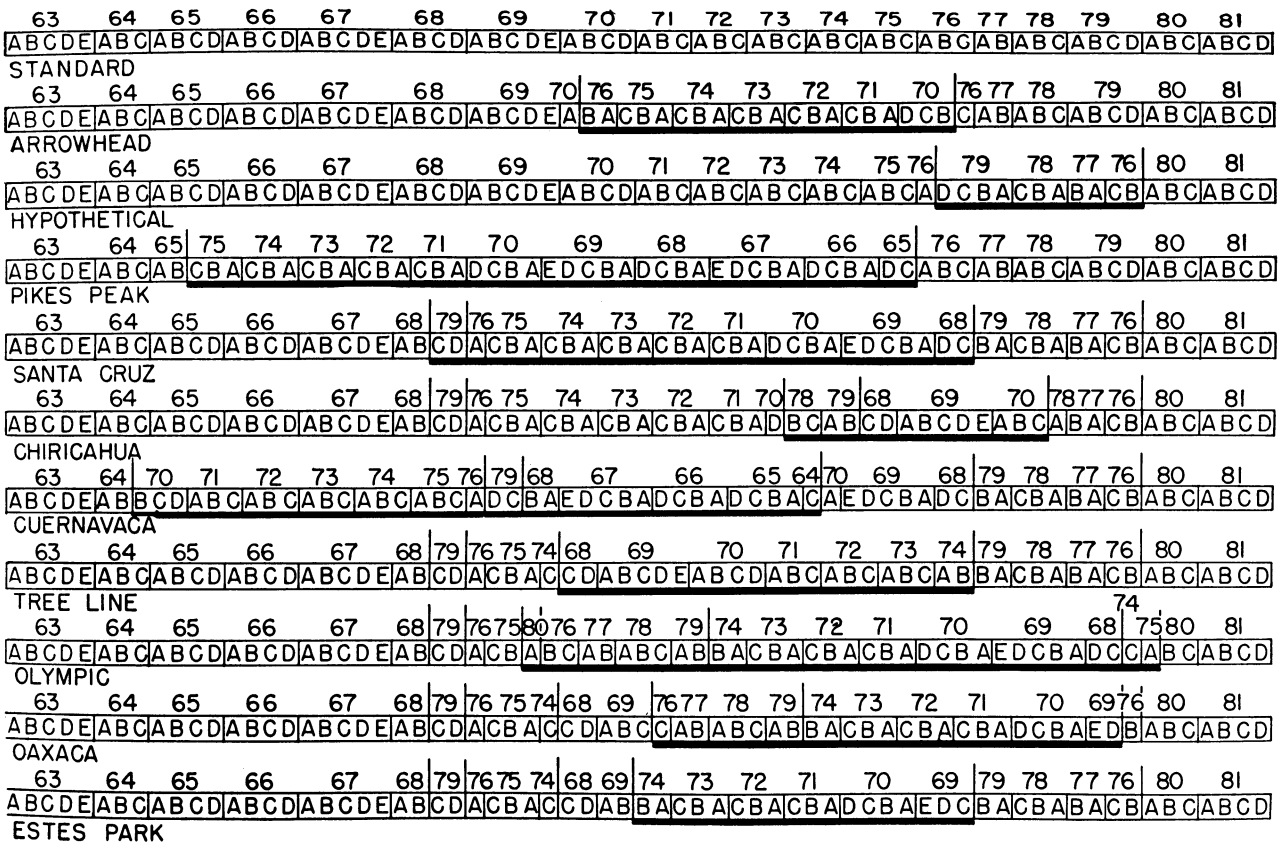

Fig. 52 The principal gene arrangements in the third chromosome of *Drosophila pseudoobscura.* (From Dobzhansky and Epling.)

stock which gave rise to *D. miranda* on the one hand and to *D. pseudoobscura* and *D. persimilis* on the other. Finally, a third arrangement, Santa Cruz, occupies a position similar to that of Standard, if Hypothetical be considered the most central arrangement. That Santa Cruz is of great antiquity is suggested by its present geographic distribution and the distributions of its derivatives (see below). Hypothetical, Standard, and Santa Cruz therefore seem to be the ancestral arrangements. This assumption is symbolized in [Figure 51] by the double-headed arrows which connect these arrangements, while other arrows are single-headed. If one of these arrangements be accepted as the starting point, the remainder of the scheme becomes fixed.

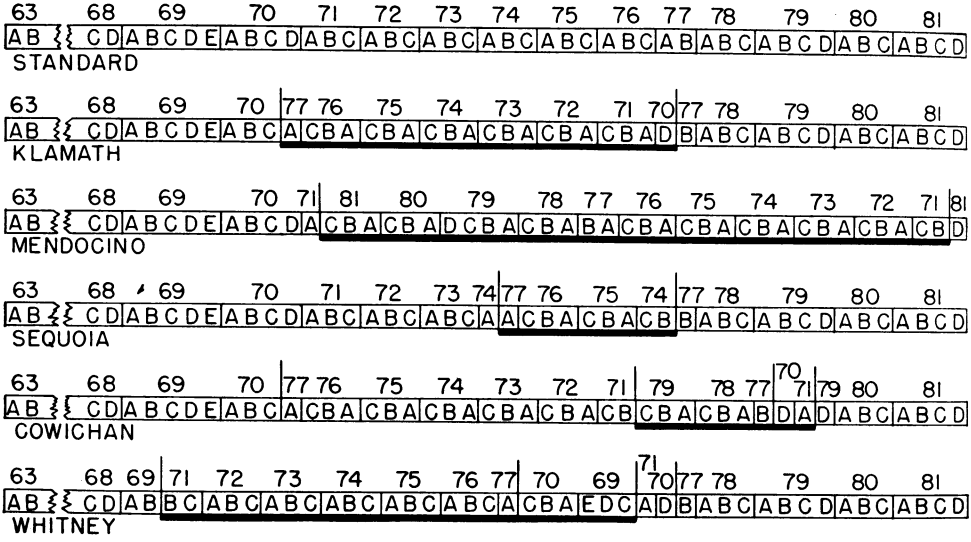

Fig. 53 The gene arrangements in the third chromosome of *Drosophila persimilis*. (From Dobzhansky and Epling.)

He goes on to point out that the distribution of the thirty-eight well-established breaks in the 3 chromosome is not quite at random, but there is no obvious explanation for this departure presented. Helfer (1941) had already studied the distribution of X-ray-induced breaks of the chromosomes of *pseudoobscura*. The distribution is not the same as those of the breaks found in natural populations. Also, Helfer found no difference between the rates of breakage in the chromosomes of *pseudoobscura* to account for the fact that the 3 chromosome has many more rearrangements in wild populations than any of the other chromosomes. The explanation for these discrepan-

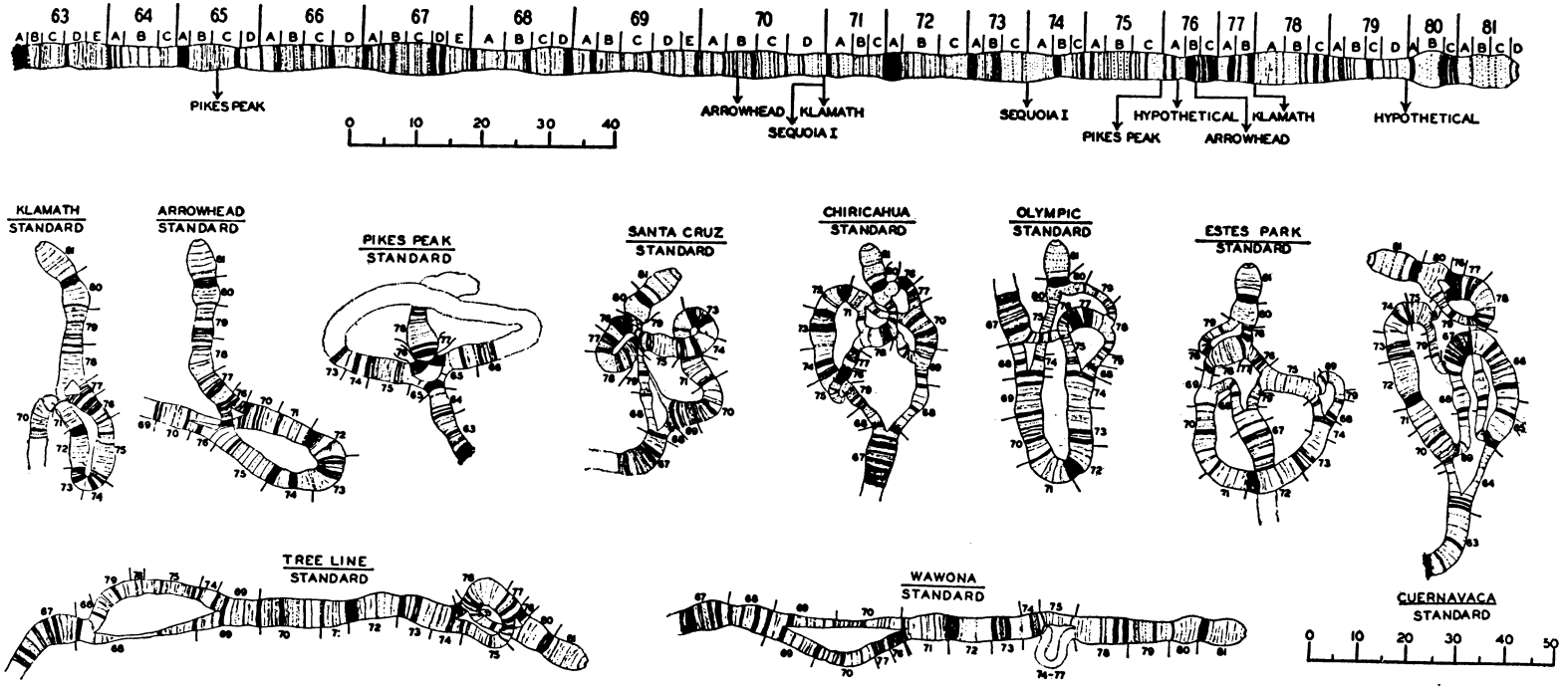

Fig. 54 Standard map and inversions of chromosome 3 of *Drosophila pseudoobscura.* (From Dobzhansky and Sturtevant.)

cies in distribution of breakage points leading to these arrangements is not known.

The chromosomes other than the third have but few arrangements. The 4 chromosome (element B) is alike in *pseudoobscura* and *persimilis,* with one simple inversion known in the former. Tan (1935) showed that the 2 chromosome (element E) in these two species differed by one inversion, and two other inversions have been found in each of the two species (see Dobzhansky, 1944c). No rearrangement has been reported for the small 5 (element F) chromosome in natural populations.

The X chromosome in the two species is a large V with XL element A and XR element D. The XL arm in the two species differs by one inverted segment. The XR has a sequence in common, the standard for both species. In *pseudoobscura,* this is a normal genotype; in *persimilis,* this is the "sex ratio" genotype, and normal strains differ from it by a simple inversion. The "sex ratio" sequence in *pseudoobscura* differs from the standard sequence by three independent inversions. The distal one is interesting of itself, as it appears to be a terminal inversion. That is to say, all the detectable darkly-staining bands at the normal end of the chromosome have been inverted, which is as close to a cytological proof of terminal inversion as it has been possible to arrive at. These additional rearrangements are too few to add very much to an understanding of the phylogeny and migrations of these species.

The cytological relationships between *pseudoobscura, persimilis,* and *miranda* have been studied by Dobzhansky and Tan (1936) and MacKnight (1939). The first point of note is that, although chromosome 3 of *pseudoobscura* and *persimilis* is exceedingly variable within the species and the other chromosomes are relatively stable, the difference between *pseudoobscura* (or *persimilis*) and *miranda* involves all chromosomes. The number of breaks involved in rearrangements suggested as a minimum difference between *pseudoobscura* and *miranda* by Dobzhansky and Tan (1936) are XL = 6, XR = 4, 2 = 12, 3 = 11, 4 = 15, and 5 = 1. In fact, the 4 chromosome has more differences than 3, even though it is shorter in the salivary glands. Most of the differences here are due to inversions, either major or perhaps small rearrangements, but a few translocations were reported. In fact, the differences were so extensive in some cases as to preclude a decision on homology between parts of the chromo-

somes. Dobzhansky and Tan (1936) estimate that, if most differences are to be accounted for by changes in gene association, there are over one hundred points of breakage leading to rearrangements in the gene order. This is an exceptionally large amount of difference in gene order between species which produce hybrids and is the most extensive difference analyzed. This extensive difference may account in part for the infrequent and less intimate somatic pairing in hybrids with *miranda* than between *pseudoobscura* and *persimilis.*

The distribution of the several sequences of genes in the 3 chromosome of *pseudoobscura* and *persimilis* and their relations to each other as shown in the phylogeny (Figure 51) have been studied. Figures 55, 56, 57, and 58 show the distributions of some of the central groups in the phylogeny. No single sequence or phylad of closely related sequences are universally distributed throughout the range of these species. Some are very widespread; others, usually the peripheral ones in the phylogeny, may be very local. There is some grouping in distribution and considerable seasonal fluctuation in relative frequencies of the several gene sequences. There have been found from one to seven arrangements present in a population. The seasonal fluctuations of the relative frequency of arrangements are not necessarily alike in different localities, even those close together.

Dobzhansky (1944c) decided that the populations of different gene arrangements were in the expected combinations, homozygous and heterozygous, that would be expected from the Hardy-Weinberg equilibrium $(a + b + c -)^2$, and that this panmixia was at least the rule (Hardy, 1908; Weinberg, 1908). His more recent investigations do not bear this out (see Chapter 6).

(b) affinis subgroup. Miller (1939) studied the chromosome variation in *algonquin* and mentioned examining the salivary gland chromosomes in hybrids between *algonquin* and *athabasca.* In these hybrids there were some similiarities in gene order, but the extensive differences have not been analyzed. In the species *algonquin,* Miller found no inversions or other cytological differences in the XL (element D) or the dot (element F). There was one paracentric inversion in XS (element A), two in the chromosome called A (element C) where the second inversion overlapped the first, and the single and double inversions both exist, and two in Miller's C chromosome (element B). Miller's B chromosome (element E) showed several gene arrangements. There were two simple paracentric inversions as

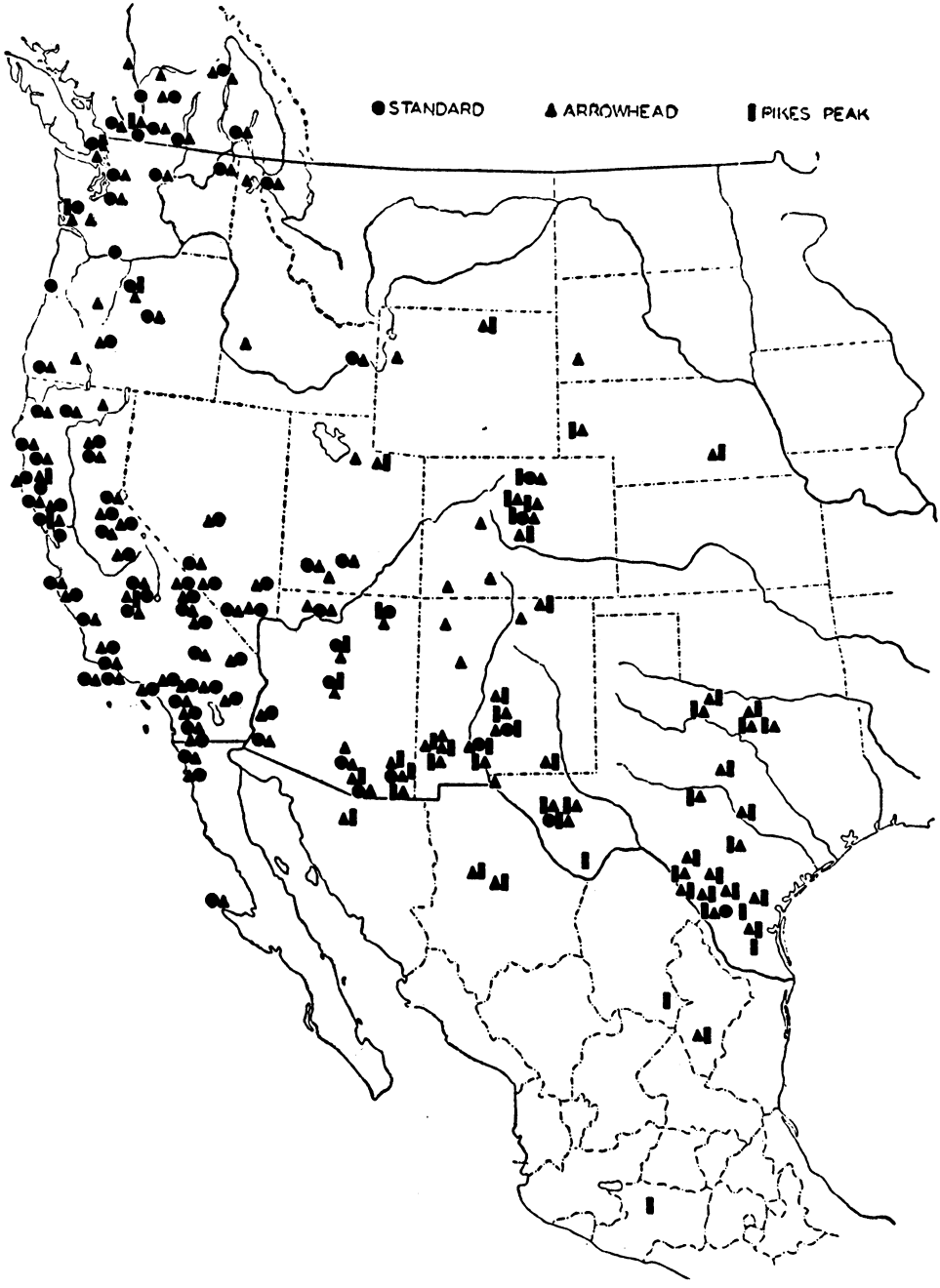

Fig. 55 Geographic distribution of the standard phylad of gene arrangements in the third chromosome of *Drosophila pseudoobscura*. (From Dobzhansky and Epling.)

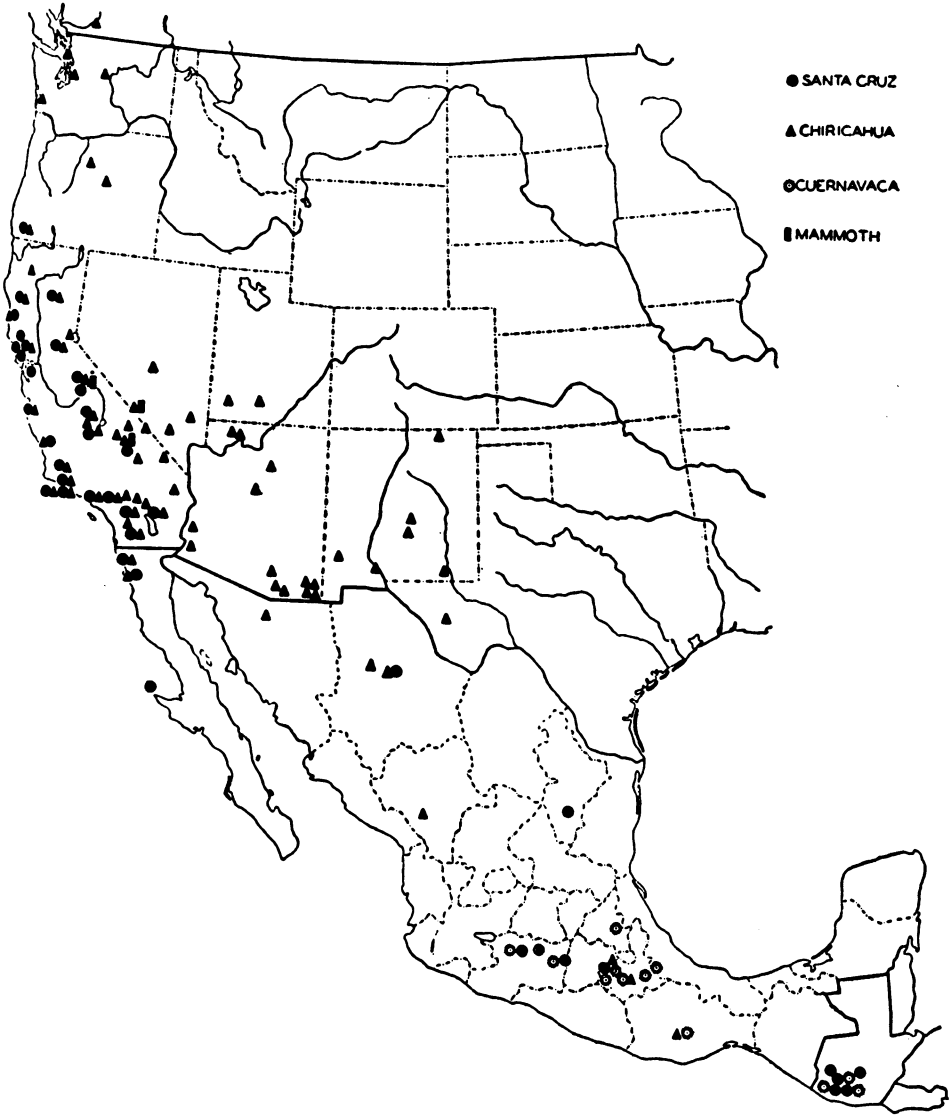

Fig. 56 Geographic distribution of the Santa Cruz phylad of gene arrangements in the third chromosome of *Drosophila pseudoobscura*. (From Dobzhansky and Epling.)

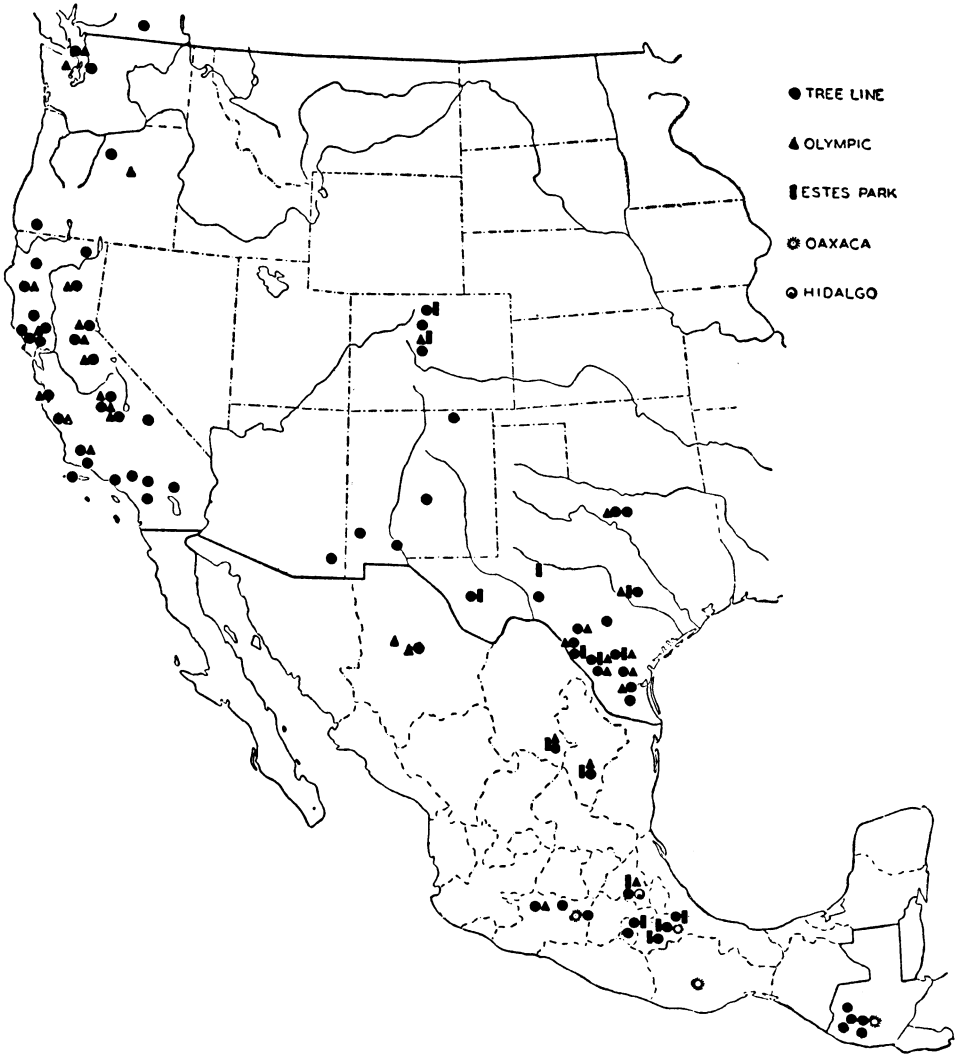

Fig. 57 Geographic distribution of the Tree Line phylad of gene arrangements in the third chromosome of *Drosophila pseudoobscura.* (From Dobzhansky and Epling.)

compared to the Standard sequence. In addition, there was a complex inversion sequence called B_3 which differed from Standard by a pericentric inversion, (Miller's Hypothetical sequence) followed by a paracentric inversion. In the diagrams of chromosome rearrangements, this would be $+ \rightarrow (5) \rightarrow (10)$, with the centromere between D and E. If the original order of genes was the Standard used here, there would have been a time when a large pericentric inversion was heterozygous, with the resulting reduction in reproductive efficiency

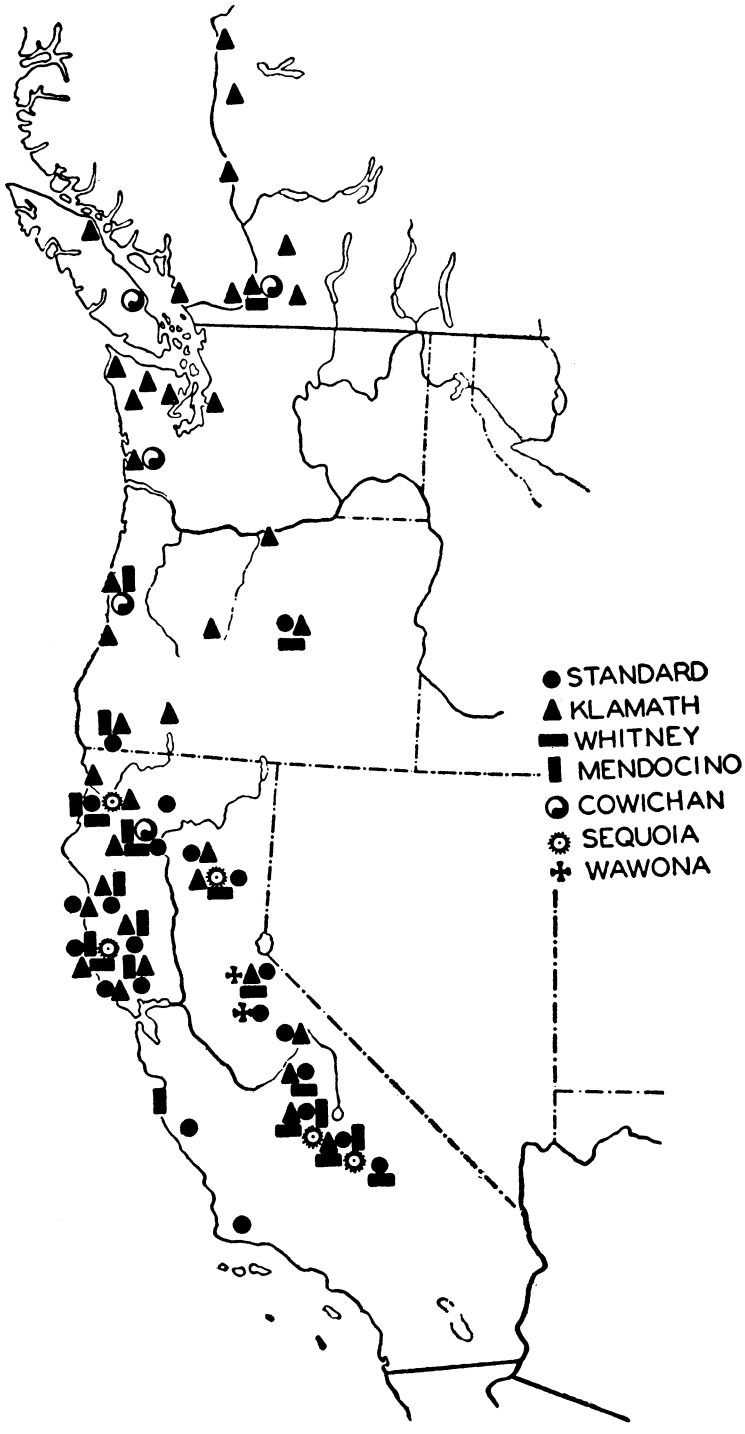

Fig. 58 Geographic distribution of the gene arrangements in the third chromosome of *Drosophila persimilis*. (From Dobzhansky and Epling.)

due to the production of aneuploid gametes. However, the B_3 sequence may have been the original. In this case the changes would be (10) → (5) → +. It could give rise to the paracentric inversion, which would not reduce the reproductive efficiency. The pericentric inversion imposed on this to give Standard would then not give aneuploid gametes with B_3, but would with this intermediate sequence. This last sequence has not been found, which lends some credence to the hypothesis that B_3 was the original sequence. An alternative hypothesis offered by Miller is that the intermediate sequence is the original one. Whatever the phylogeny may have been, this is the first proven case of pericentric inversion in a population. The net rearrangements are such that the reproductive efficiency of the species is not impaired by the presence of these differences in gene sequence.

Dobzhansky and Socolov (1939) reported on the major chromosomal rearrangements in *azteca.* The dot (element F) and the short arm of their C chromosome (element B) were homozygous in the strains they examined. Sturtevant and Novitski (1941b) point out that Dobzhansky and Socolov did not include the long arm of their C chromosome in their analysis; we presume that there were no rearrangements in this arm in their material. The XS (element A) has one very short inversion, whereas the XL (element D) has five different independent paracentric inversions as compared with Standard.

Their A chromosome (element C) is homologous to the variable 3 chromosome of *pseudoobscura* and *persimilis.* As compared with their Alpha, the standard sequence, there have been six single inversions, some of them superimposed on others to form a branching phylogeny. One of these intermediate arrangements, Zeta, was only predicted by Dobzhansky and Socolov, but was found and reported by Dobzhansky (1941). One arm of their B chromosome (element E) has an inversion in the proximal portion, as compared with their standard sequence, which is independent of the inversions in the distal section. In this latter section, there are three inversions in addition to normal to form a branched sequence of changes, so that there is one simple and two different overlapping inversion sequences compared with their standard. No inversions are reported in the other arm of the B chromosome.

Dobzhansky and Socolov suggest that there is a better spread of rearrangements between the chromosomes of *azteca* than in the sibling

species *pseudoobscura* and *persimilis*. Bauer and Dobzhansky (1937) report hybrids between *azteca* and *athabasca*. The difference in gene sequences in these two species is very great, for the most part due to inversions, as far as they could tell. Bauer and Dobzhansky suggest that some translocations may be present, but pairing is so incomplete that the analysis was necessarily unsatisfactory. The affinis subgroup is characterized by the presence of numerous inversions both within and between species.

Novitski (1946) analyzed the gene arrangements in a number of strains of *athabasca*. The chromosome variation in this form resembles that of *pseudoobscura*. The details of the variability are best shown in Table 29, taken from Novitski, which shows the number of rearrangements known for *algonquin, athabasca, azteca,* and *pseudoobscura* in the several homologous chromosome elements.

TABLE 29

Comparison of the number of different sequences found in the chromosomes of *D. athabasca* and the homologous chromosomes of closely related species (from Novitski)

Species	Elements				
	A	B	C	D	E
D. athabasca	2	4	17	2	2
D. algonquin	3	3	4	1	4
D. azteca	2	1	6	3	6
D. pseudoobscura	2	2	22	3	6

Thus in nineteen strains of *athabasca,* Novitski found nearly as many gene arrangements in element C as have been found in the homologous element in all the studies of *pseudoobscura* and *persimilis*.

Novitski suggests an ingenious mechanism for the multiplication of inversions in one chromosome. Here the presence of one inversion heterozygous increases the tendency for a second to occur related to the first. This would lead to an accumulation of inversions in certain regions of a chromosome. He points out that there are many more distal than proximal inversions in both *pseudoobscura* and *athabasca* to support this idea. In view of the distribution of inversions in other forms, for example, *nebulosa,* this does not seem to be the only reason for the differential accumulation of inversions.

Novitski was able to construct a good phylogeny of inversions in element C. Using this and other inversions, he concluded that some sequence changes were old and common to both the eastern and western strains he studied, but that other sequences indicated local and separate evolution. None of these changes lead to any cross-sterility or other symptoms of serious separation and divergence. Table 29 shows that even the members of the obscura complex are not uniform in variability of gene sequence.

EUROPEAN SPECIES. Some species of the related obscura complex of Europe have been analyzed by Dubinin, Sokolov, and Tiniakov (1937). These are identified only as *Drosophila obscura, Drosophila obscura* 2, and *Drosophila obscura* 3. They write:

In *Drosophila obscura* one inversion was found in the left arm of chromosome II and a small inversion in the right arm of chromosome II. . . . In *Drosophila obscura* 2 six different inversions and one intrachromosomal displacement were found. In *Drosophila obscura* 3 the following eight mutations were met, four inversions, two rearrangements and two complex aberrations.

At another place they write:

The investigation of *Drosophila obscura* 2 and *Drosophila obscura* 3 present pictures of intrachromosomal variability which differ from those of *Drosophila melanogaster* or *Drosophila funebris.* Firstly, not only inversions but also other rearrangements have been found here (intrachromosomal displacements). Secondly, populations of these species are exceptionally richly permeated with mutations. Individuals were found which carried simultaneously eight chromosomal mutations (4 inversions, 2 rearrangements and 2 complex aberrations) which were connected with 20 breaks and corresponding exchanges of chromatin. This great number of rearrangements was found in populations which simultaneously carried many inversions in the same chromosomes. The variability of the chromosome structure of these species is so great that the karyotype of many individuals, collected in nature, recall the type in intraspecific hybrids obtained from combining chromosomal elements, which were separated by a long period of evolution.

Sokolov and Dubinin (1941, in *Drosophila Information Service* 15) reported further on the species called *Drosophila obscura* 3. This species has five rods and a dot in metaphase. They report that

chromosome 5 is always heterozygous for complex rearrangement "B," except for a few very rare homozygotes which they attribute to crossing over. This species is not common, but they obtained like results for three consecutive years on strains collected from several localities. The other major autosomes also proved to be heterozygous for rearrangements. In the stocks collected at Sochi, they reported chromosome 2 with 66 per cent heterozygous for inversion "A," chromosome 3 with 45 per cent heterozygous for inversion "B," chromosome 4 with 91 per cent heterozygous for complex rearrangement "A," and chromosome 5 with 100 per cent heterozygous for complex rearrangement "B." Sokolov and Dubinin believe that *obscura* 3 is a permanent heterozygote for the inversions in chromosome 5 and that, just as in *Oenothera,* homozygotes for this chromosome can occur only after crossing over has eliminated some lethal conditions.

Philip, Rendel, Spurway, and Haldane (1944) have reported briefly on *Drosophila subobscura.* They point out that *obscura* 3 mentioned above may be *subobscura,* for strains fertile with English strains of *subobscura* occur on the Continent. However, this was not proved. The obscura complex of the Continent is not well worked out, and Philip *et al.* state that *subobscura* in England has other rare relatives, presumably in the obscura complex. They further state that almost all larvae from wild parents or laboratory stocks show inversion (presumably paracentric) of from one to five for the long arms in the salivary gland nuclei. In some cases the alternate gene orders seem equally viable, in others the heterozygote is apparently at a selective advantage. They point out that, after fifteen generations, several of the gene arrangements remain heterozygous—a condition expected without selection in only one in thirty-seven thousand of such stocks. This indicates the fallacy of assuming homozygosity of a stock solely because the strain has been closely inbred. Some arrangements differ by simple inversions, others by complex or compound inversions. No map or detailed analysis is included with this preliminary report.

All of these observations indicate that the obscura complex is prone to retain rearrangements in its populations, as well as to change gene arrangements drastically in evolving new species.

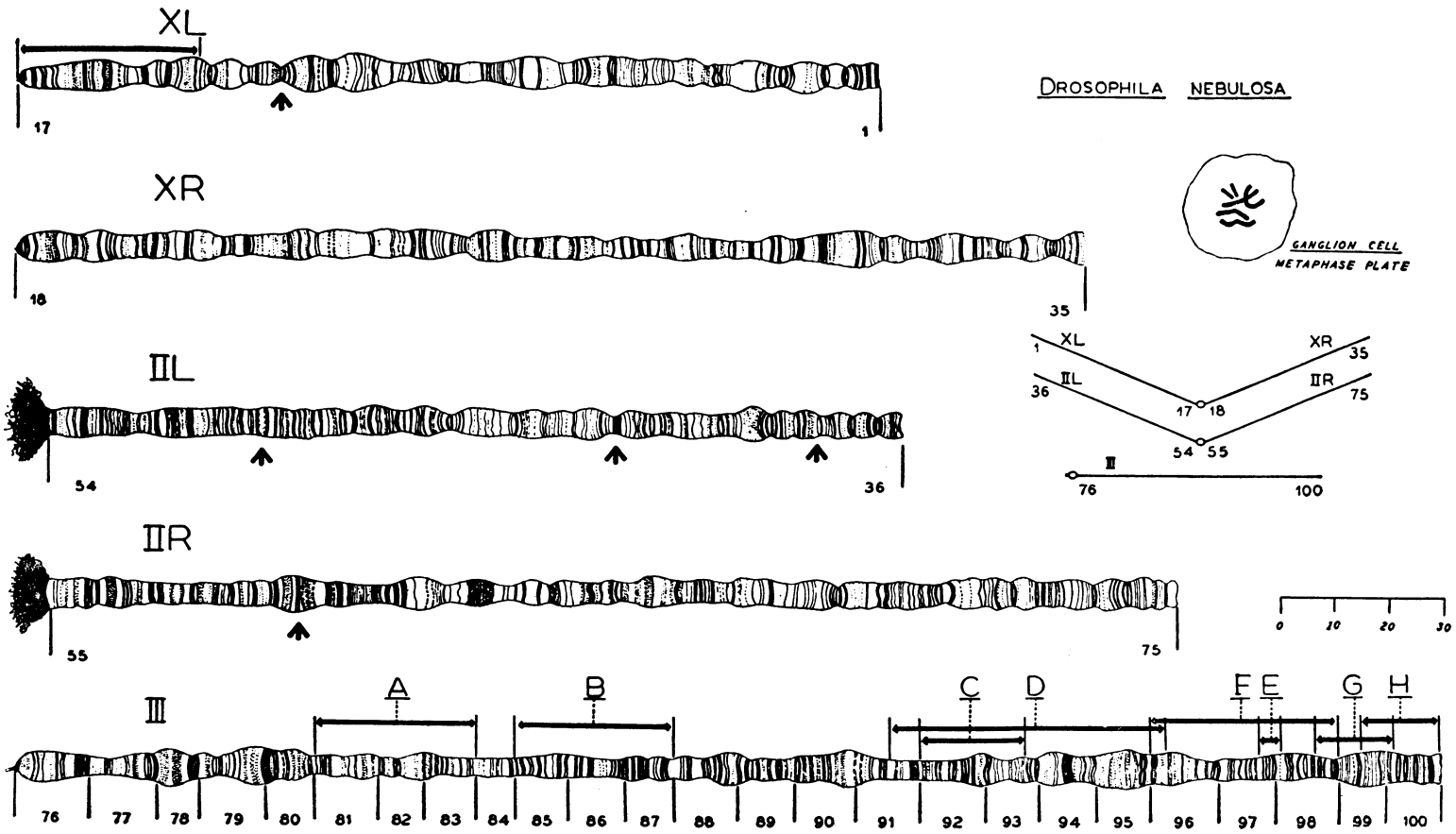

Fig. 59 Salivary gland chromosomes of *Drosophila nebulosa,* with inversions indicated. (From Pavan.)

saltans group

Dobzhansky and Pavan (1943b) gave some preliminary data on the chromosomes of *prosaltans*. In this paper they mention two independent inversions in one arm of the V-shaped autosome, as well as the peculiar X-chromosome situation in the Bertioga strain, as compared with *prosaltans* I, which has a regular X-A fusion V-shaped sex chromosome with both strands haploid in the male. Cavalcanti (1948) worked out the cytology of *prosaltans* in some detail. He showed that the Bertioga strain was aberrant as far as the sex chromosome mechanism is concerned, whereas the other strains of *prosaltans* which he examined are similar to *sturtevanti*. The peculiar sex chromosome complex of the Bertioga strain seems to represent an exchange of an X chromosome arm for chromosome 3, so that XL is now a free rod and XR is fused with 3.

Dobzhansky and Pavan (1943b) indicate that 3 is homozygous and XR is hemizygous in the male, as is XL, judged by the staining in the salivary gland chromosomes. This would imply—and they indicate that this is their belief from their diagram (our Figure 38 I,J,K,L)—that there had been a like transfer with the Y, making both X and Y chromosomes complex. The case needs more study but no further strains have been found, even at Bertioga.

Cavalcanti (1948) reports no inversions in XL, one large submedian inversion in XR in some strains as compared with his standard map. He found that 2R contained numerous heterochromatic regions and considers the inversions reported by Dobzhansky and Pavan to be artifacts, resulting from some nonspecific heterochromatic pairing. Chromosome 2L shows several inversions from the standard A sequence, to give rise to the B sequence by a single inversion and also to the C sequence by a single inversion. The D sequence came from C by an additional inversion. The B and C are broadly overlapping, for both are long and nearly terminal, but C comes to within one band of the end of the chromosome. Thus A and D differ by two overlapping inversions, while B and D have a complex difference resulting from three overlapping inversions.

The 3 chromosome has an interesting strain difference, for all Brazilian strains tested have one sequence, the standard, while all Mexican and Guatemalan strains have a sequence which differs from the standard by two independent inversions. The 3 chromosome also

often fails to pair in the proximal and distal regions in a variable manner. No difference in banding was detected, although one chromosome seems more extended in this region than the other. This failure to pair resembles the situation existing in numerous species hybrids and again suggests that it is locally determined.

willistoni group

Pavan (1946a) studied the gene arrangements in *Drosophila nebulosa,* using one strain from Del Rio, Texas, and seven strains from Brazil, as listed below. Figure 59 shows his chromosome maps of this species, with the inversions marked by black lines with double arrows. Two important facts are indicated with these inversions. In the first place, the XL inversion extends to the heterochromatin in the centromere region. As mentioned previously, this is the compact one-band type heterochromatin. Inversions involving the diffuse type heterochromatin such as that in the centromere region of the 2 chromosome have seldom been found in natural populations, although they are common in X-ray-induced rearrangements. They may have a negative selective value either because of position effect or because inversions so close to the centromere disrupt normal disjunction to some extent, but neither factor has been established.

In the second place, all other inversions are restricted to the rod-shaped 3 chromosome, presumably element E, judging from its length. The following is a list of the combinations of inversions found in the strains studied; in each case the standard sequence is indicated by lower-case letters and the inverted sequence by capital letters, corresponding to the inversions indicated on the map:

(1) a b c d e f g h — (Standard) Del Rio, and Belém
(2) a b c d e f g H — Del Rio and Belém
(3) a b c d e f G h — Prata
(4) a b c D e f g H — Belém
(5) a b C d e f g H — Ilhabella
(6) a b c D e f g h — Belém
(7) a b C d e f g h — Ilhabella
(8) a b C d E f G h — Ilhabella
(9) A B C d e f g H — Iporanga
(10) A b C d e f g H — Ilhabella and Bertioga
(11) A b C d e f G h — Prata, São Jose dos Campos, and Santos
(12) A b C d e F g h — Santos
(13) A B C d e f g h — Bertioga

(14) A b C d e f g h — Bertioga, Ilhabella, São Jose dos Campos, Prata, and Iporanga
(15) A b c D e f g h — Belém
(16) A b c d e f g H — Iporanga and Belém
(17) a b C d e f G h — Ilhabella and Prata

One interesting point is that all inversions are small. Another is that all the inversions are independent and represent changes from the standard sequence; that is, although the breakage points of the several inversions overlap on the map, all actual inversions present together in a stock are independent. Despite this fact, which in some cases even helps analysis, Pavan could make a very convincing phylogeny of this chromosome. He points out that some of the combinations of rearrangements may be due to crossing over, but that some represent a sequence in occurrence in the same strain. This represents the most remarkable case of restriction of rearrangements to one chromosome thus far recorded.

A series of studies have been published on the willistoni sibling species, *willistoni, tropicalis, paulistorum,* and *equinoxialis.* Burla *et al.* (1949) compared the chromosomes of this group. They pointed out that these four siblings, which are entirely cross-sterile, are so similar in external morphology that they can best be identified by crosses to known forms or by the pattern of banding and other structures of their salivary gland chromosomes. The authors give drawings of XR from each of these species to show the general similarity of certain regions, as well as the fact that characteristic landmarks are recognizable in all four species. There are characteristic repeats common to and recognizable in the XR of all four siblings. The rod-shaped 3 chromosome has been so thoroughly reorganized by rearrangements, presumably paracentric inversions, that it is not sufficiently alike to recognize similarities. Their drawings of this latter chromosome from three of the species show the reorganization to be extensive. Another important point is that there exists a pericentric inversion difference between *willistoni* and *tropicalis* on one hand, and *paulistorum* and *equinoxialis* on the other. The V-shaped 2 chromosome has exchanged parts of each arm, so that it is 2L distal-2L proximal-centromere-2R proximal-2R distal in two species and 2R distal-2L proximal-centromere-2R proximal-2L distal in the other two. The authors point out that there has been a considerable amount

of intrachromosomal reorganization of the chromosomes in the four siblings.

Dobzhansky (1950) gives a detailed map of the chromosomes drawn from the salivary gland nuclei of *willistoni*. He points out that there exist certain characteristic "weak points" recognizable in the homologous chromosomes of all these siblings. The organization of the heterochromatin is similar to that given by Pavan for *nebulosa* in these four siblings, in the other members of the willistoni group, and in *sturtevanti*. The heterochromatin in the X is in one or a very few bands, while there is a large mass of diffuse heterochromatin in 2. This distribution of heterochromatin is constant in the saltans and willistoni species groups.

Dobzhansky *et al.* (1950) described the chromosomal polymorphism in the willistoni siblings. They examined the progeny from females fertilized by one or more males in nature. A summary of the number of inversions found, indicating the number of females whose progeny were examined, is given below:

		Inversions					
Species	♀♀ tested	X L	X R	2 L	2 R	3	Total
willistoni	2,500	9	5	8	5	13	40
paulistorum	800	6	3	5	5	15	34
tropicalis	74			2		2	4
equinoxialis	79		1		1	2	4

As the number of progenies of different females is increased, the number of inversions is increased, but with a diminishing return, so that nearly as many inversions were found in *paulistorum* with eight hundred tests as in *willistoni* with twenty-five hundred tests. On the other hand, the mean number of heterozygous inversions per individual is much higher in *willistoni* than in its siblings, varying from 0.8 to between 9 and 10 as compared with 0.6 to 1.8 for *paulistorum,* 0.14 for *tropicalis,* and 0.11 for *equinoxialis.* The species *willistoni* is by far the most widespread, extending north as far as southern Florida and south through the tropics to southern Brazil. The second species, *paulistorum,* is more restricted, but extends north to Trinidad and Costa Rica and south to São Paulo, Brazil. *Drosophila tropicalis* has a still smaller distribution, being found in Brazil and Bolivia, while *equinoxialis* is restricted to the Amazon rain forests. In the twenty-one regions from which samples were available to test, *willistoni* was the most frequent species in eighteen and *paulistorum* in

three. *Drosophila willistoni* is the most abundant and most polymorphic with respect to gene arrangement of any species in Brazil (da Cunha *et al.,* 1950). Only paracentric inversions were found, usually short ones, as were those found by Warters in *macrospina* and by Pavan in *nebulosa.* Some of these inversions occurred in heterozygous combinations in the population in excess of the frequency expected from the Hardy-Weinberg ratio, and so must have possessed a selective advantage when heterozygous. The populations from different geographical regions were often cytologically different races, sometimes homozygous for certain gene arrangements differing from those of other regions, at other times having different frequencies of certain gene arrangements.

The chromosomal polymorphism is greatest where the Drosophila population is largest, and the most plentiful species are usually the most polymorphic. For *willistoni,* these are usually the regions where there are more ecological niches, such as types of fruit. In regions with fewer available environments, the polymorphism is less. An attempt is made by da Cunha *et al.* (1950) to show that the chromosome polymorphism is adaptive to take advantage of the multiplicity of available environments. Although this relationship may exist, they failed completely to present suitable evidence, for no case of proven advantage for a particular selected environment of a particular gene order is given.

melanogaster group

In the history of genetics during the first half of the twentieth century, *Drosophila melanogaster* has proved to be one of the most favorable organisms for genetic study. It was used by Morgan and his students and associates to establish many basic genetic principles. Muller used it to demonstrate the effectiveness of X rays in the production of mutations and chromosomal abnormalities. Further, Painter used *melanogaster* to demonstrate the analysis of chromosomal abnormalities and the localization of the genes on particular regions of the salivary gland chromosomes, sometimes to a single band. The properties of chromosomal abnormalities, including their effect in meiosis, have been established by the use of this organism. We cannot hope to cover all of these facts and their importance in this volume, and shall here refer only to some studies of chromosomal variation in natural populations.

Bridges spent a great deal of time and effort trying to establish as accurately as possible both the cytological and genetic picture of this organism. Bridges and Brehme (1944) published a summary of information on both the spontaneous and induced mutations and chromosomal rearrangements of this organism, together with references to the original and other source material where these were analyzed. Any one interested in an over-all picture of the genetic divergence in a species is referred to this volume with its references.

Sturtevant (1926, 1931) had been able, by genetic tests, to demonstrate paracentric inversions (called C factors) in strains of *melanogaster* from a number of localities. The limitations of the technique did not allow exact cytological localization of these rearrangements at that time. Dubinin, Sokolov, and Tiniakov (1937) studied Russian material and Warters (1944) American strains, using the salivary gland check for heterozygous inversions. Dubinin, Sokolov, and Tiniakov checked 34,515 chromosomes in *melanogaster* from a number of localities. They found one X-chromosome deletion and seven inversions, one in 2L (element B), two in 2R (element C), and four in 3R (element E). They showed that the 2L and the long 2R inversion are equivalent to the C2L Cy C2R found by L. Ward in Michigan. In all, twenty localities yielded 525 of these seven inversions in the population of 34,515 chromosomes examined.

Warters (1944) examined from two to seven strains from thirty-five different localities in the United States, and from four in Mexico and one in Hawaii. No rearrangement was found in the X (element A), nor the 4 chromosome (element F). The seven inversions were: one in 2L, one in 2R, two in 3L, and three in 3R. Several of these rearrangements are widespread. Warters' 2L was the same inversion as the 2L Cy found by Ward in Michigan and the 2L found in Transcaucasus by Dubinin, Sokolov, and Tiniakov. The 3RA sequence found by Warters was equivalent to the Russian workers C3R-K2. Other rearrangements were very restricted in their distribution; for example, Warters' 3L-B was found only in Denver, Colorado.

Ives (1947) reported on variability of the 2 chromosome in *melanogaster*. He found the In (2L)t inversion as well as the In (2R)NS inversion, which had been described by Bridges (Bridges and Brehme, 1944), present in a population from South Amherst, Massachusetts. He points out that Warters (1944) 2L-A inversions may have been the In (2L)t rather than In (2L)Cy, as Warters

thought. It is also probable that the common 3 chromosome inversions Warters described as 3L-A and 3R-B are the Payne inversion In (3L)P, In (3R)P (see Bridges and Brehme, 1944). He points out that when Warters' and his material are compared with that of Dubinin, Sokolov, and Tiniakov, it shows some consistent differences in both chromosomes 2 (elements B and C) and 3 (elements D and E).

Sturtevant and Plunkett (1926) first demonstrated the presence of an inversion difference between *melanogaster* and *simulans* by extensive linkage studies. The salivary gland technique was used by Pätau (1935), Kerkis (1936, 1937), Horton (1939), and Slizynski (1941) to investigate the visible cytological differences between these two species. The large inversion described by Sturtevant and Plunkett (1926) and Sturtevant (1929) was conspicuous in these preparations. In addition, there are a number of minor rearrangements. Horton (1939) described at least five of these as inversions from two to ten bands long on the salivary gland chromosome. Additional regions synapsed well only occasionally, and the differences in several chromosome ends were not analyzed. Therefore it seems that there are, in addition to the one major inversion, a number of minor rearrangements of the chromatin of a type that could be detected only in such chromosomes as those found in the salivary gland nuclei. Horton also described the difference between the fourth chromosomes as being due to an inversion, and Slizynski worked this out in detail. The hybrids between these two species are sterile. However, the only cytological differences are the one major rearrangement and these several minor rearrangements. The detection of these very minor rearrangements gives a good measure of the potential accuracy of the study of chromosomal differences in the salivary gland nuclei.

Kaufmann (1936), Kikkawa (1935, 1936a, 1938), and Dobzhansky and Dreyfus (1943) have studied the gene arrangements in *ananassae*. In all, six inversions have been described, in addition to the heterozygous translocation described by Dobzhansky and Dreyfus in Brazilian material. Four of these inversions were common to both the Asiatic material available to Kikkawa and to the material from the United States and from Brazil available to Kaufmann and to Dobzhansky and Dreyfus. One inversion was present only in the Asiatic material, and another only in the Brazilian material. Thus certain rearrangements must have occurred at some considerable time

ago and been widely disseminated in the range, or rather in the two separate nonconnected ranges of the species.

There is among these six inversions one which all investigators have described as terminal. A further peculiarity of *ananassae,* described by Kikkawa and noted also by Dobzhansky and Dreyfus, is the presence (or absence) of extra bands at the free ends of the 2 chromosome (elements D and E) and the free end of 3L (element C). It is not known if these differences represent duplications or deficiencies, but as both strain types are fully viable and fertile homozygous these unexplained differences do not seem to have selective effects.

Osima (1940), using Japanese material, and Freire-Maia (1947), using material collected in South America, checked *montium* both genetically and cytologically, but found no cytological abnormalities.

The melanogaster group so far as analyzed has not shown the chromosomal variability of the obscura group. The melanogaster group presents a picture of species with standard gene arrangements, but with a few widespread and presumably old rearrangements, together with a few local rearrangements.

Subgenus **Drosophila**

Only the virilis group in the subgenus Drosophila has been analyzed as extensively as the pseudoobscura subgroup of the Sophophora. No other group so far has proved to be as amenable to a study of chromosome phylogeny, but this may be lack of adequate analysis. Some of these species groups show very few rearrangements. Others that show more rearrangements have not been studied with an adequate sample of the whole population.

quinaria group

Members of the quinaria group do not make easily analyzed salivary gland preparations and have not been studied in detail. Blumel (1949) reports that *munda* shows one very small inversion, and that there is at least one inversion in each of the species *innubila, transversa, quinaria, subquinaria, suboccidentalis,* and *tenebrosa,* while *palustris* has two different inversions. In addition to the variability within species, there may be differences between species. However, some larvae of *innubila/palustris* hybrids show no rearrangements.

The salivary gland chromosomes of hybrid larvae between *subocci-dentalis* and *munda* show that extensive changes have occurred, while the *transversa/innubila* hybrid larvae show only a few cases of very incomplete synapsis, owing to the major changes present between them.

virilis group

The virilis group is considered in detail in Chapter 10. As the inversions and fusions present are used extensively in determining relationships, we defer a discussion of this material.

funebris group

Dubinin, Sokolov, and Tiniakov (1937) analyzed 5,517 chromosomes in *funebris* and found five different inversions. Only 207 chromosomes were inverted in the seven populations sampled. They found regular geographic distributions of the several inversions. In a subsequent series of papers by Dubinin and Tiniakov, the selective value of different gene arrangements has been investigated but this will be discussed in Chapter 6.

Warters (1944), in her investigation of chromosome variability in a number of Drosophila species, studied several members of this group. She found no rearrangements present in the single strain available of *Drosophila subfunebris,* a species so far found only in southern California. A study of 460 larvae from crosses of different strains of the subspecies *macrospina limpiensis* to the Limpia Canyon, Texas, strain revealed no rearrangements. There were several strains from Texas, New Mexico, Arizona, Utah, and Sonora, Mexico, included in this test, so no variability was found in this southwestern subspecies.

However, the subspecies *macrospina macrospina,* found throughout the South, and *macrospina ohioensis,* found in Ohio and Michigan, show variation in gene order when tested with a standard strain from Georgetown, Texas.

In *macrospina,* Warters found no inversions in the X, 3, or 6 chromosomes. These chromosomes lacked inversions in *ohioensis,* and in addition none was found in 4. In *macrospina,* there was a small inversion in chromosome 4 heterozygous, with the Standard sequence in a few stocks. A similar inversion in chromosome 5 was present fairly frequently, but always heterozygous, so it appeared to act as a

lethal when homozygous. This chromosome 5 inversion was present in some *ohioensis* strains, but those from Wooster and Cokeley were homozygous for a different small inversion.

Chromosome 2 is the most variable in both these subspecies. There are five inversions in *macrospina* and an additional inversion, F, in some strains of *ohioensis,* in all cases representing paracentric inversions. The *macrospina* series are: A, a short distal inversion; B, a very small inversion that falls within the limits of A; E, a medium inversion near the centromere; and C and D, very short inversions which fall within the limits of E, fairly close together. Table 30 shows the associations and distribution of these inversions. The most interesting facts are that all these rearrangements are of independent origin from the standard. Also, they occur together in all types of association, so that their recombinations must be the result of crossing over. Too many combinations are present to suggest the necessary repeated occurrence of these rearrangements if their combinations were not due to crossing over. The potential for crossing over does not seem to be interfered with too much by these rearrangements.

Only one strain of the new species, *Drosophila trispina,* was available for test by Ward (1949). It was homozygous and differed from *limpiensis* by only one autosomal inversion. The species *subfunebris* differs from *limpiensis* by several rearrangements which have not been analyzed, although they appear to be paracentric inversions.

repleta group

The repleta group is a large complex of forms that has several functional subdivisions in which hybrids occur. A few representative forms have been checked, but no very extensive analysis has been made.

Members of the melanopalpa subgroup differ among themselves by six inversions, four in 2 chromosome and one each in 3 and 5. These inversions are indicated on Wharton's (1942) map of the *repleta* chromosomes (Figure 67). The *hydei* inversion on 2 chromosome is also indicated. The genetic evidence given in Chapter 9 shows that *repleta* is most nearly the modern representative of the ancestral form. In these species, *limensis* (L) differs from *repleta* by a short proximal inversion in 2 chromosome and a very short inversion in the middle of 3 chromosome. *Drosophila canapalpa* (C) differs by a short proximal inversion in 5 and by a much longer inver-

TABLE 30

Arrangements of inversions in chromosome 2 of subspecies *macrospina* and *ohioensis* (from Warters)

D. m. macrospina		A	AB	C	D	CD	E	ED	AC	AD	ACD	AE	ABE	ADE	ABDE	BE	BCDE	CDE	F	Standard
Texas, Aldrich farm	57.2				*															*
Aldrich farm	118.5			*	*	*														*
Aldrich farm	140.7			*	*	*														
Aldrich farm	527.6a	*								*	*									*
Aldrich farm	577.6								*		*									*
Onion Creek	114.4b				*		*													*
Austin	239.5						*													*
Slaughter Creek	727.7a						*													*
Belton	312.5				*															
Bell County	740.8a				*															*
Rusk	748.4b				*															*
Ft. Worth	613.8b				*															
Ft. Worth	735.7			*																*
Smithville	774.6a	*			*					*										*
Alvin	778.3b				*						*									*
Houston	790.3c									*	*									*
San Gabriel Pk.	823.2f				*															*
Mason	861.9b							*												*
Del Rio	874.9a				*															*
Del Rio	874.9b				*					*										*
Beaumont	881.8										*									
Oklahoma, Wichita Mts. ...	988.3				*			*						*						*
Tulsa	996.3a														*					
Tulsa	996.3b				*		*						*	*	*					
Tulsa	996.3f						*								*					
Tulsa	996.3g												*			*				

A = terminal; B = included terminal; C = upper median; D = lower median; E = basal; F = distal.

TABLE 30 (*Cont.*)

D. m. macrospina	A	AB	C	D	CD	E	ED	AC	AD	ACD	AE	ABE	ADE	ABDE	BE	BCDE	CDE	F	Standard
Missouri, Springfield 1046.5										*				*					
Arkansas, Petit Jean 1291.10										*				*					
Louisiana, New Orleans 1112.6b											*								*
New Orleans 1112.6c					*					*									*
New Orleans 1112.6d					*		*									*			
Mississippi, Columbus 854.4						*													*
Columbus 1268.9										*			*						
Florida, L. McKethan 1148.8	*			*	*	*			*										
Tennessee, G. Smoky 1272.15					*			*									*		
Chimney Camp 1273.13b											*		*						
Shelby Forest 1279.12a		*												*					
Shelby Forest 1279.12b				*									*						*
Shelby Forest 1279.12c											*								*
Shelby Forest 1279.12f								*	*					*					
Shelby Forest 1280.12a										*									
Shelby Forest 1280.12b							*						*						
Shelby Forest 1280.12c							*						*						
	3	1	3	17	6	7	5	3	6	10	3	2	7	7	1	1	1	0	23
D. m. ohioensis																			
Ohio, Overton, Sp. 1				*	*													*	
Licking, Sp. 2	*																		*
Wooster, Sp. 3a														*					
Wooster, Sp. 3b														*					
Cokeley, 1027.2														*					
Piqua, 1035.3							*			*									
West Chester		*					*			*	*		*						*
	1	1	0	1	1	0	2	0	0	2	1	0	1	3	0	0	0	1	2

sion in 2, which is present only part of the time. The right-hand breakage point of this latter inversion is very close to the left-hand break of the *limensis* inversion. The species *neorepleta* and one of the *melanopalpa* strains (W) studied by Wharton have an included inversion in 2, while the second strain of *melanopalpa* (M) tested by Ward has these inversions and the same small inversion in 5 as the one in *canapalpa*. Despite the genetic complexity of the differences between these forms, their chromosomes show little variation. Even so, the synapsis between *repleta* and *limensis* chromosomes in the salivary glands of the hybrids is sometimes incomplete.

Warters' (1944) check of *hydei* showed only one inversion in the 2 chromosome (the longest autosome), using Wharton's (1942) map of *repleta,* for the chromosomes of *hydei* are so similar that ready identification of the individual regions of the chromosomes is possible. This arrangement is very widespread, perhaps coexistent with the normal sequence, for it is included in material from South Africa and Hawaii. Wharton (1944) also reported only one inversion between the standard and a Brazilian strain.

The two species in the hydei subgroup, *hydeoides* and *nigrohydei,* show no rearrangements in the hybrids between them. Warters (1944) found no rearrangements in the few stocks of *bifurca* available for testing, although Wharton (1943) had found a small pericentric inversion in the X chromosome of another stock.

Patterson and Crow (1940) and Crow (1942) make no mention of variation in gene order within species in the mulleri subgroup. When *mulleri/aldrichi* hybrids were examined, no major rearrangements were detected, although synapsis was poor. However, *mulleri/mojavensis* hybrids showed some inversions as well as poor pairing in some chromosome regions. The *mojavensis/arizonensis* hybrids showed several inversion differences, but pairing was good. This subgroup has some internal reorganization of the chromosomes, although all members have the characteristic chromosome complex of five long rods and a dot. Warters checked 251 larvae from twenty-one strains of *meridiana,* but found no rearrangements.

Drosophila mercatorum mercatorum, from the United States and Mexico, and *Drosophila mercatorum pararepleta,* from South America, are a pair of cross-fertile subspecies whose metaphase chromosomes differ. However, the hybrid larvae show close synapsis of

homologous chromosomes and only one complex autosomal inversion (Wharton, 1944).

The South American species *pararepleta* and *paranaensis* have been investigated by de Barros (1949c) and by Dreyfus and de Barros (1948, 1949). In the former species, *pararepleta,* de Barros discovered a spontaneous repeat duplication of about one-fourth of the X chromosome. This consisted of some sixty-three bands and was inserted reversed in place, so that it usually synapsed with the homologous region. As this was found in only one of 167 larvae examined from a strain collected in Monjolinho-Anapolis, Goyaz, Brazil, its genetic effect could not be tested. The author assumed that it resulted from an abnormal exchange between sister chromatids.

Dreyfus and de Barros describe cytological differences between *pararepleta* and *paranaensis.* They show that there are several differences between the chromosomes, so that they do not synapse well at the tips of the 2 and 3 chromosomes, and that differences exist at the bases of the 3 and 4 chromosomes. There may be two inversions present in the 2 chromosome between strains of *paranaensis.* There are two other inversions between the 2 chromosome of *pararepleta* and that of normal *paranaensis.* These are simple independent inversions; but when all four inversions are present, a complex configuration results. Dreyfus and de Barros (1948) illustrate a translocation of the tip of chromosome 2 and 3 of *pararepleta,* which was detectable in backcross of hybrid females to *paranaensis* males. It consists of a few bands at the ends of these chromosomes, which may synapse end to end. They believe it is due to a translocation, rather than nonspecific pairing of heterochromatic material. This small *mercatorum* complex presents several interesting genetic and cytological problems.

In general, it may be said that the repleta group shows few rearrangements within or between species when compared with such forms as those in the obscura group.

robusta group

Drosophila robusta has been studied extensively by Carson and Stalker (1947, 1949) and Stalker and Carson (1948, 1949). In comparison to their standard sequence, they describe six inversions in the X, three in each arm; five inversions in the second, one a pericentric; and two inversions in the third, one of these a pericentric. These represent a series of two-break inversions. The positions of

the breaks in these rearrangements were such that some combinations would be overlapping, others included. Carson and Stalker found that some rearrangements were rare, local, and recent; others were widespread; and still others formed north-south clines, with shift in frequencies.

One of the pericentric inversions found was rare, the other widespread, so that it may make up as much as 30 per cent of the chromosomes in some localities. The effect on reproductive efficiency has not been determined, but it is able to persist in the populations. Carson and Stalker also report that two of the inversions involve the heterochromatic regions near the centromere. The interpretation of the distribution of rearrangements will be considered in Chapter 6.

melanica group

A preliminary investigation of this group was made by Griffen (1942). The forms studied were *Drosophila melanica melanica, Drosophila melanica paramelanica,* and *Drosophila nigromelanica.* There are a number of inversions present, both within and between species. In the subspecies *paramelanica,* when different strains are compared with the standard, inversions were found in the longest autosome in several stocks and a third inversion in another strain. In another stock, three small inversions were present in the X, two proximal overlapping in the longest autosome and two small inversions in another autosome.

The subspecies *melanica* differed from standard *paramelanica* by a long inversion in the X and a more complex rearrangement in the longest autosome.

Some strains from the southwest differed from the usual *melanica* by two small distal inversions in the longest autosome. In comparing some other strains from Texas and Oklahoma, a series of overlapping inversions was found in one autosome. As *nigromelanica* does not synapse satisfactorily with *melanica,* it is not possible to decide what differences exist. Two inversions were found in different stocks of this species.

Despite the lack of adequate analysis, the presence of a number of rearrangements in the X makes this species group of considerable interest cytologically. Ward (1952), who made a thorough study of *melanica,* found a translocation between the X and an autosome with both breaks well out in the euchromatic arms in a stock of *melanica*

from Oklahoma. This is another case of spontaneous translocation, but it was not characteristic of the stock; it may have occurred in the laboratory.

Sturtevant and Novitski (1941a) reported their preliminary observations on the cytological differences between Arizona and Texas strains of *micromelanica.* The autosomes are rods in both, but the salivary gland chromosomes showed one inversion in one autosome and three in a second, one independent and two overlapping. This group seems to be cytologically quite variable.

guaraní group

King (1947a) published some information on the *guaraní* species group, although he pointed out that the salivary gland chromosomes of this group are not especially suitable for analysis. In *guarú,* there are a number of inversions in both the X and autosomes. The X has several inversions; both the A and B chromosomes have complex inversions and another different rearrangement, while C has at least two separate inversions. No inversions are recorded in D or E, although several additional unidentified rearrangements were seen. In crosses of this species to *subbadia,* several additional rearrangements are found, although the chromosomes pair well. The complex triple hybrid (*guarú* × *subbadia*) × *guaraní,* shows still further rearrangements, including some very small inversions or other rearrangements which interfere locally with pairing. This group shows a sufficient variability to be regarded as resembling the variable forms, such as the pseudoobscura subgroup.

DISCUSSION

There are a number of kinds of information that can be obtained from a study of the salivary gland chromosomes. The organization of the chromosomes, as seen in this magnified version, with the differences in heterochromatin and heterochromatization, has already been mentioned. Another interesting and important factor is one on duplication. Bridges (1935) showed that there were several short duplications called repeats in the chromosomes of *melanogaster.* These usually involved only a few bands of the salivary gland chromosomes and might lie in place or in another part of the chromosome, leading to pairing between different regions of the chromosomes in

some cases. These probably are duplications in the strict sense like *Bar,* which was shown to be a duplication in place by Sturtevant (1925), Bridges (1936), and Muller, Prokofyeva, and Kossikov (1936). This type of repeat duplication has been found in other species besides *melanogaster.* They have been reported in other Diptera, for example, by Metz and Lawrence (1937) in *Sciara ocellaris.* This represents one of the important mechanisms for increase in gene number, especially in forms such as Drosophila, which do not give rise to polyploid species. Nevertheless, these duplication types have not yet been demonstrated as differences between related species. One important exception is that found by Warters (1944), who reported a repeat duplication fixed in some strains of *melanogaster* from western North America.

A second important factor related to rearrangements is position effect. The *Bar* case mentioned above was demonstrated by Sturtevant to be the result of position effect, or gene association effect, and remains the classical case in *Drosophila melanogaster.* This form has been studied extensively for this effect, and it has been shown that there are numerous cases where change in association of genes is related to a change in their function. This effect has been demonstrated in several species of Drosophila, but its general applicability to animal and plant forms is unknown.

In the present case, there is no evidence that position effect has played any role as such in the evolution of Drosophila. There are a number of inversion differences within and between species. These may have position effects, but none has been demonstrated.

The selective value of the inversions in *pseudoobscura* seems to be due, in the main, to gene mutations. The several inversion differences between *pseudoobscura* and *persimilis* do not seem responsible as such for the differences between these forms. We are left with no conclusive evidence for or against position effect as an agent in evolution.

There are several ways that the salivary gland chromosomes are unique in showing the history and relationships within and between species. As has been pointed out, many mutations recur in a population, and in most cases it is not possible to differentiate between a recurrent and a nonrecurrent mutant. Inversions and other chromosomal rearrangements are nonrecurrent, for all practical purposes. There is no evidence that the same rearrangement has occurred even

twice spontaneously. Thus rearrangements present either within a population or between species tell of differences which have come into being and, in some cases, give good phylogenies. In the best cases, these give the sequence of rearrangements in the populations. The phylogenies in *pseudoobscura* and *persimilis,* in *athabasca* and *nebulosa,* are good and have allowed the investigators working with these forms to establish certain relations between age and distribution of rearrangements. Dobzhansky (1944c) gave a summary of the situation in *pseudoobscura* and *persimilis,* which have been most thoroughly analyzed.

The species *persimilis* is restricted to the west coast, where it occurs from California to Canada (Figure 58). As the differences in salivary gland chromosomes are the easiest way to tell these two species apart, the distribution was in large measure determined by their use. The species *pseudoobscura* has a much wider distribution, from Guatemala to Canada. No one sequence of genes is universally distributed throughout this range. The chromosome races in *pseudoobscura* are divided by Dobzhansky into phylads, based on the three gene sequences that form centers of chromosome variation, Standard, Santa Cruz, and Tree Line (Figure 51). The distributions of these phylads are shown in Figures 55, 56 and 57. The Standard phylad has a denser distribution in the northern and eastern part of the range, and has a much more consistent distribution in the middle portion of the range, but does not extend to lower Mexico and Guatemala. Both Tree Line and Santa Cruz extend to the southern limits, but the latter is not present in the eastern section, while the former is very sparse in the intermontane section, especially in Arizona, Nevada, and Utah. Neither is present in the northeastern limits of the range. Epling (1944) tried to use the distribution of certain gene sequences to establish an extensive history of these species and the gene arrangements, but there are too many unknown factors, particularly in the absence of satisfactory paleontological evidence. Even these phylogenies give only very limited information on the history of the species.

The most important use of the inversions within a species is that of cytological markers, which in certain cases can be used to study the genetic rather than the cytological facts (see Chapter 6).

The evidence from rearrangements studied within a species points to the fact that all of the more complex rearrangements are in fact the sum of a series of two-break rearrangements. This is true in

macrospina (Warters, 1944); in *nebulosa* (Pavan, 1946a), *pseudoobscura,* and all others known to Dobzhansky (1944c); in *athabasca,* so far as can be told (Novitski, 1946); and in *miranda* (Koller, 1939). These facts make simple centromere shift without pericentric inversion, which must be at least a three-break rearrangement, less probable as such in Drosophila. It still remains to be proved whether the changes in the position of the centromere that have occurred between species are pericentric inversions, such as occur in *algonquin* (Miller, 1939) and *robusta* (Carson and Stalker, 1947), or centromere shifts, which have not been proved anywhere in Drosophila.

There have been some suggestions at one time or another that the genes are not distributed at random in the chromosomes. These rearrangements, both within and between species, insure changes in gross distribution of genes as well as changes due to mutation. The net result of both processes would be a random distribution of genes in the chromosomes if not opposed by some form of selection, and none is apparent.

The inversion differences impose restrictions on exchange of genes. In fact, only double (or quadruple) exchange leads to recombination of genes within the limits of two gene sequences that differ by an inversion. As Dubinin, Sokolov, and Tiniakov (1937) suggest, this restriction of free recombination allows the accumulation of several mutant genes at once, so that different combinations can be tested from a heterozygous population. These lead to detectable differences in selective value of different gene arrangements.

Certain cytological peculiarities show up both within and between species. One such peculiarity is the so-called terminal inversion, such as that in *ananassae* and other forms. The question of their terminal position is in doubt because of several facts. Ordinarily, broken ends of chromosomes do not become free ends and, conversely, free ends do not attach themselves to other chromosomes or parts of chromosomes. The evidence for the terminal nature of these inversions consists of the fact that there is no obviously stained band terminal to the limits of these inversions. In view of the fact that the salivary gland chromosome is banded because of alternation of deeply staining and unstained sections, it seems odd that the lack of a deeply staining band beyond the limits of an inversion should make it terminal. There is no *a priori* reason why the telomere should be large or stain heavily. At any rate, most inversions are not terminal.

Another factor of interest, which undoubtedly is connected with the divergent evolution between species, is the failure of the usual intimate somatic synapsis in the case of some species hybrids. This poor pairing occurs in the absence of marked cytological differences in gene order, for example, in *mulleri/aldrichi* hybrids. It is the property of the chromosomes, and not of the cytoplasm in any simple sense; for it holds true in backcross hybrids for different chromosomes in the virilis species group. It can occur between strains within a species, as found by Cavalcanti (1948) in *prosaltans,* where it is limited to a certain region of the chromosome. In such cases, the tendency to pair intimately is certainly a local phenomenon in the chromosome.

There is both direct evidence from crossing and indirect evidence that the gene sequence within a chromosome element is both generally and even locally quite different in the different species. Paracentric inversions will change the association of genes within an arm of a chromosome. In cases like *spinofemora, immigrans, tranquilla,* and *testacea,* these paracentric inversions will obviously destroy the two separate elements that went into the double-length rod. Only the very rarely fixed translocations can change homologies, unless a fusion has occurred between two elements. After fusion has occurred, of course the pericentric inversions, such as that in chromosome 2 of *robusta* and between the willistoni siblings, will change the homologies of the chromosome elements. In the absence of crossing between species, some evidence can be obtained on the retention of individuality by the chromosome elements by mutation parallels. Other evidence may be obtained if the chromosomes in the salivary gland nuclei are similar, as they are between *repleta* and *hydei.* Even if the chromosomes are quite different in appearance, Sturtevant (1942) does not believe that this proves lack of homologous elements, but only lack of a similar sequence of genes. In case no fusion is present, this is probably almost always true; but after fusion has occurred, only hybrids give good proof of homology.

The species *pseudoobscura* and *persimilis* share one gene arrangement in chromosome 3 and have many more different arrangements. These do not seem to be responsible for the factors which separate these forms. The pseudoobscura subgroup differs from the affinis subgroup in that the known members of the latter have two pericentric inversions, changing the rods into J-shaped chromosomes. It is not

known whether these differences, or the many other changes in gene sequences in these groups, are responsible for any of the fundamental isolation between them. There is a lack of conclusive proof that paracentric inversions affect the separation or isolation of two forms. It may be that large pericentric inversions and translocations do contribute if these differences are fixed in semi-isolated populations, for then there would be selective advantage to select isolating factors into a population as such.

The funebris group studied by Warters (1944) is an excellent example of the lack of correlation between chromosome variability and gene divergence. There are a number of inversion sequences within the subspecies *macrospina* and *ohioensis* when compared with the standard *macrospina*. There is no detectable genic isolation between these two subspecies. The subspecies *limpiensis* has no known rearrangements and has the standard *macrospina* sequence; yet there is genic isolation between *limpiensis* and *macrospina* or *ohioensis*. The separate species *trispina* differs from *limpiensis* by only one autosomal inversion; and the cytological differences between *subfunebris* and *limpiensis* are not much, if any, greater than the differences between strains of *macrospina*.

There are extremes in the amount of variation in gene order which may be represented in the genus. In the obscura complex, there are a great many gene orders present within a species. In fact, Dobzhansky and Epling (1948) showed that several of the inversions in the third chromosome of *pseudoobscura* were extensive or complex enough to reduce crossing over markedly. Some gene orders could hardly exchange genes. The situation in *willistoni* particularly and in *paulistorum* to a marked degree shows the same extensive variation in gene order. At the other end of the series, the repleta group shows comparatively few rearrangements. Only *hydei, canapalpa,* and *paranaensis* have been found heterozygous for one (or two) inversions, and the melanopalpa subgroup of five species differs by only half a dozen rearrangements.

These inversions and fusions are very remarkable tracers, in many ways more remarkable than the more spectacular isotope tracers of chemistry. Any chromosome arrangement that is characteristic of one or several species, but which differs from other gene orders, demonstrates that all individuals of even a tremendous species or species group descended from the one ancestor that first possessed it.

6 GENE VARIATION, SELECTION, AND GENIC BALANCE

INTRODUCTION

Variation in an animal or plant species may be due either to variations in the environment or to variations in the inherited (genetic) system of the organism. Variation due to environmental causes, that is, responses and adaptations other than induced genetic changes, are not inherited; but it must be emphasized that the types of adaptive response made to environmental stimuli are limited by the genotype of the organism. We are concerned here with the inherited variation within the genus Drosophila, and our problem is to show that this variation can explain its evolution.

The problem of genetic diversity might be approached by determining the number of genes in the several species of Drosophila. This has been estimated in *melanogaster* by a number of workers using different methods. For example, Muller (1947) estimates that there are well over a thousand, perhaps as many as ten thousand, genes in this species. Komai and Takaku (1949) estimate that there are between eight and twenty-five thousand genes in *virilis,* but consider the number to be closer to the first figure. If we had an accurate determination of the number of gene loci in the several species, the number of gene differences within the species, and the number of gene differences between the species, we might be able to assign an amount of genetic difference that constitutes the necessary minimum between species. This is obviously impossible, for we do not know any of these variables within wide limits, and especially which genes are important and which are incidental in species differences. It is impossible to get the actual historical sequence and say that at one time there was species A which accumulated through time many genetic changes to become a different species B. As an additional

limitation, there is no good paleontological evidence on the evolution of the genus Drosophila. We must therefore measure our genetic evolution from the relationships and comparisons between members of the genus as it now exists.

The extensive genetic diversity found in the genus as represented by *melanogaster* was ably demonstrated by Morgan and his students and co-workers, Sturtevant, Bridges, and Muller. We will not attempt to review their basic work. Morgan, Sturtevant, Muller, and Bridges (1915), and Morgan, Bridges, and Sturtevant (1925) have reviewed the earlier work. Muller (1939) has published a comprehensive bibliography of Drosophila literature through the year 1938. Those interested in the details of the investigations will find these references very complete for that period. Bridges and Brehme (1944) have summarized most of the known gene mutations and chromosomal abnormalities for that species. This very extensive list of genetic changes, both spontaneous and induced, gives a comprehensive picture of the tremendously varied types that have been found. This variability must be known and appreciated to understand the unlimited potentiality for change that is apparent in the species which has been tested most extensively.

The presence of the great array of genetic variabilities in *melanogaster* and the occurrence of gene mutations in homogeneous material early raised the issue of the induction of mutations. It has been claimed repeatedly that the environment induces adaptive mutations which increase the fitness of the organism to survive in that particular environment. All attempts to produce inherited changes or increase the rate of mutation by environmental agents failed until Muller (1927) discovered that X rays induced mutations in Drosophila. Further experiments since then have shown that short-wave ionizing radiations in general will induce mutations and chromosomal rearrangements. Stadler and Sprague (1936) proved that ultraviolet radiation will also cause mutations; but Stadler (1941) has shown that, although it is responsible for very short deletions, ultraviolet seldom induces gross structural rearrangements. In fact, Muller (1928), Goldschmidt (1929), and others have demonstrated that higher temperature or temperature shock increases the mutation rate to some degree. Auerbach and Robson (1944, 1946) and Auerbach (1949) showed that chemical agents induce mutation, especially mustard gas and allied compounds. Since that time a wide range of

agents, all of them very toxic or lethal to the organisms, except in very low concentrations, have been found to have some mutagenic properties. Some of them, such as short-wave radition and the mustards, are very effective. The general ineffectiveness of many previously tested agents shows that the chromosomes are buffered in the cell system against effects by any except a few active agents, which are of very low incident in the usual environment of living forms.

Although these environmental agents are able to induce mutation, there is no evidence that they act to induce selected mutations which adapt the organism. If this argument were true, agents which are known to induce mutations should produce adaptive changes. Both X rays and the mustards cause serious burns in living tissue; yet these agents produce all types of mutations, most often detrimental, as well as chromosomal rearrangements. They seldom produce protective mutations which reduce their deleterious effects. None has been so produced, although genetic differences controlling responses to radiation are known. Variations have been essentially at random, although the mutation rates of different genes are not the same.

The presence of all types of mutations occurring at random with all sorts of effects is the necessary condition for evolution if it is to occur through the natural selection of mutations and combinations of genes. Even the effects of nonrandom sampling in small populations depend on the available array of genetic variables. In all the data on spontaneous and induced mutations, the only undeniable case of directed mutation is that in *Diplococcus pneumococcus,* worked out by Avery, MacLeod, and McCarty (1944). This is an exception to the general situation, for mutations in bacteria are not otherwise directed (Lewis 1934, and others). It must be stressed that, at best, this is a case of directed mutation, but not a Lamarkian adaption in a simple sense. The only case of cytoplasmic inheritance in Drosophila is the CO_2 sensitivity factor (L'Heritier and Teissier, 1937), so we do not consider cytoplasmic inheritance of serious importance in the evolution of this genus.

The criticism that the mutations found in laboratory stocks of Drosophila are the result of artificial conditions is invalidated by the study of mutations present in wild strains, as carried out by Dubinin, Dobzhansky, Sturtevant, Spencer, and others. If we are to depend on the available mutations to provide the raw material of evolution, then

we must answer the following questions: Are there enough mutations available in these wild populations to account for the changes that have occurred, if we assume that all species of Drosophila had a common ancestor? Are these mutations of the right kinds to account for the evolution in this genus?

The tremendous genetic variability uncovered in every species studied answers the first question in the affirmative. The answer to the second question must be general. As pointed out before, it is impossible to obtain a parent species and its different descendants, except perhaps in certain polyploids, which do not concern us in the analysis of Drosophila. It is furthermore impossible to determine which of the mutant alleles fixed in the origin of a new species are of primary importance and which are incidental. It is to be understood that any organism is a balanced genetic system, and that the replacement of one gene by its allele may be necessary for the establishment of a subsequent mutation at another locus. We therefore must fall back on the proof that all types of genetic changes occur.

The adaptive changes in a population depend on the occurrence of mutations with a different activity from their original alleles. Muller (1932, 1947, 1950) reviewed the evidence on different types of mutations and certain aspects of their adaptive value. Types of mutations are known which reduce the effect of the gene, in some cases apparently to no activity; others increase the activity of the gene; others change it to a new activity; and still others have an antagonistic action with that of the normal allele. All these effects are measured in terms of the end product in the organism and represent changes in the heterocatalytic function of the gene. The loss of a gene would be equivalent to a cessation of gene activity.

As the species in the genus Drosophila are sexual forms, with normal crossing over in the female but little or none in the male, the various mutations can occur in numerous combinations. Such recombinations test the several types of mutations which have different selective values and effects in the various combinations. The formation of a new species may be characterized as the formation of a new and different harmonious combination of genes. In some cases, the new species is established to fit a different environmental niche from that of the parent species. In that event the two species can live in the same area.

MUTATIONS

The mutations that are important in species differentiation may not be directly correlated with the visible morphological differences between species. It has been argued that the morphologically visible differences between species are not adaptive. The validity of this argument is highly improbable, for there is no known genetic mechanism that would fix numerous selectively indifferent mutations except unilateral mutation pressure or chance fixation in small populations. Neither is probable on a scale sufficient to account for the many visible differences between the known species. There is a large body of genetic data showing the existence of different selective values for different combinations of genes, but very little, if any, proof for the existence of many really selectively indifferent mutations.

The classes of mutations effecting the phenotypic changes which make related species differ should be found among the spontaneous mutations within a species. It has been said on several occasions that the mutations studied in the laboratory have nothing to do with and bear no resemblance to the factors responsible for the difference between species. For example, Goldschmidt (1940) quotes a part of the following from East (1936) who wrote:

> The situation is so peculiar that taxonomists have little interest in the characters with which geneticists deal, maintaining that they are wholly unnatural material for evolutionary processes. Professor C. T. Brues has examined the published descriptions of mutational effects in Drosophila, at my request, and finds that only a limited few characteristics of similar type have ever survived in nature, and these often in distant genera, thus indicating that the germinal causes are not identical. It is worthy of note, however, that the characters resembling the Drosophila mutations that have survived elsewhere in the insects are invariably retrogressive simplifications.
>
> The tendency of the Morgan school, in the face of this situation, is to remain silent. When the subject is mentioned, the reader is led to infer that only a very meager percentage of chance mutations should be expected to be of evolutionary importance.[1]

Such statements by Brues and East cannot be ignored, as they deserve, for they were utilized by Goldschmidt and others to question

[1] Goldschmidt, R. B. *The Material Basis of Evolution,* page 10. Yale University Press. New Haven, Connecticut. Copyright 1940 by Yale University Press.

the genetic basis of evolution. These statements could be made only by persons unfamiliar with the mutations and allelic systems, and with the phenotypic differences between related species of Drosophila. Such opinions indicate a failure to understand evolution in terms of genetic systems, as already developed by Fisher (1930) and especially by Wright (1931). For example, the mutations in Drosophila are rejected on the basis that only a small percentage could be immediately used in evolution because of their reduced selective value. Yet the only alternative to evolution by selection among random mutations, with the majority of mutations detrimental at the time and place of their occurrence, is directed mutation to fit the needs of the organism, possible only under supernatural guidance, although this is seldom the name applied to such a concept.

Goldschmidt goes on to quote East as follows:

> I suggest that constructive mutations are numerous, but have ordinarily remained unnoticed simply because destructive mutations are more easily described, catalogued and scored, and therefore have been more convenient in genetic research. There is evidence of a varied nature, nevertheless, in support of the idea that constructive mutations occur with remarkably high frequency.[1]

To quote further from East (1936) directly:

> I have presented elsewhere a study of a large series of species hybrids in the genus Nicotiana where normal genoms developed under natural selections are pitted against other and different genoms developed under natural selection. There the effect of innumerable gene differences of the constructive type may be examined; and where the effects concern essential characteristics, dominance is practically absent. Development of the alien genoms together gives a harmonious result in which the normal activity of each is traceable in the resultant pattern of every organ.

This is a remarkable argument to the effect that, as there are many different useful genes in each species, such mutations must have been frequent. East could give no evidence on how long it took these species to acquire and fix these useful alleles. No evidence on the high frequency of occurrence of truely beneficial mutations is available. There is very convincing evidence that different genes of positive selective value exist in numerous kinds of populations. East's

[1] Goldschmidt, R. B. *The Material Basis of Evolution,* page 10. Yale University Press. New Haven, Connecticut. Copyright 1940 by Yale University Press.

work quoted above demonstrated this very well. Unselected Drosophila populations demonstrated such factors in *hydei* and *virilis* through heterosis tests (Stone, 1942). This is made especially obvious in *pseudoobscura* by the recent work of Dobzhansky.

Allelic differences and mutations which change the characters used in Drosophila systematics are indeed plentiful in all extensive mutation studies of any species. Some of the phenotypic characters of the adult which are used to differentiate between the species of Drosophila are: arista branches and pattern; eye color; bristle size and distribution, including relative sizes; number of rows of acrostichal hairs; relative and absolute sizes of parts; presence and absence of sex combs in the male; wing size (relative) and relative proportions, as reflected in the several wing vein indices; body color; pattern of body colors; anatomy of certain internal organs, such as the parts of the reproductive systems; color of testes, and so on. Table 31 lists a few of the mutations that affect some of these characters. No attempt is made to be exhaustive, but only to give a few examples. The mutations listed in this table are taken from Bridges and Brehme (1944). A very similar series of mutants could have been made up from the lists of Chino (1936, 1937, 1941) for *virilis* or those of Spencer (1949, unpublished) for *hydei.*

Certain mutants are included which are more extreme than any incorporated in Drosophila species (e.g., white eye is a more extreme change than any recorded as characteristic of a species). This was done to illustrate certain relations between the known mutations and development. The development of an organism under the control of the genotype is an integrated series of changes and specializations, coordinated both in time and space. The coordinated activity of all the genes in the genotype, called genic balance in certain contexts, must initiate, integrate, and limit the development of the parts to make a functional whole. The sequential series of actions of genes and the time of action of some of the genes in developmental processes has been determined. In such a system the genes that act on secondary control systems, such as hormone systems, may have many effects, whereas those that work late in development on specific systems may be limited in effect. Several allelic series illustrating quantitative and qualitative differences in gene action are included in Table 31. If we take any series of mutations affecting any one of the types of variables listed, such as body shape, eye color, bristle pattern,

TABLE 31

Types of mutations in *Drosophila melanogaster*

Mutant	Characteristics	Remarks
1. *achaete*	Hairs and bristles missing	Varies with alleles
2. *scute*	Various bristles missing	Varies with alleles
3. *spineless*	Bristles reduced	Alleles differ
4. *slight*	Small fly, fine bristles	
5. *Minutes*	Bristles fine and thin	Homozygous lethal
6. *polychaetoid*	Some extra bristles	Varies
7. *Hirsute*	Extra bristles	Homozygous lethal
8. *aristaless*	Arista reduced	Varies with alleles
9. *stubarista*	Abnormal, irregular branches	Sometimes extra branches
10. *thread*	No branches to arista	
11. *cross-veinless*	Cross veins absent	Alleles
12. *veinlet*	Veins L, 3, 4, 5, with terminal gaps	Cancelled by plexus
13. *plexus*	Extra wing veins	
14. *shifted*	Veins L, 3, & 4 closer, or fused	Varies with alleles
15. *giant*	Flies about 1.7 times normal	Varies, longer development
16. *giantoid*	Body larger, head especially	
17. *ascutex*	Furrough-less, scutellum inflated	
18. *chubby*	Short thick fly	
19. *chunky*	Short thick fly with short wings	
20. *narrow abdomen*	Abdomen narrow in both sexes	Female sterile
21. *expanded*	Wings and body large	Eyes small
22. *shrunken*	Small body	Sums with *abbreviated*
23. *abbreviated*	Bristles reduced	See *shrunken*
24. *rotund*	Wings short, tarsi three-jointed	Sex combs absent, sterile
25. *ancon*	Wings and legs short	Overlaps normal
26. *four-jointed*	Short, legs four-jointed	
27. *proboscipedia*	Oral lobes like tarsus or arista	Homozygous starve
28. *eyeless*	Eyes small, variable	Alleles vary
29. *Lobe*	Eye small, changed shape	Various alleles
30. *small eye*	Eyes small, round, high	
31. *scarbrous*	Eyes large, bulging	Supernumerary acrostichal rows
32. *broad*	Wings short and wide	
33. *dumpy*	Wings short, truncated, etc.	Varies with alleles
34. *dachsous*	Wings, abdomen and legs shorter	
35. *lancelate*	Wings narrow, eyes small	
36. *short wing*	Wings short and broad	
37. *square wing*	Blunt but normal length	
38. *vestigial*	From nick to all of wing gone	Varies with alleles
39. *apterous*	Wings and balancers reduced	Thorax small
40. *tetraltera*	Wings halterelike	Variable, overlaps normal
41. *bithorax*	Metathorax like mesothorax	Balancers winglike
42. *tetraptera*	Halteres winglike	Variable to normal
43. *lemon*	Yellowish body, bristles black	Sterile
44. *straw*	Body color and bristles pale	Varies with alleles
45. *yellow*	Body color and bristles yellow	Varies with alleles
46. *green body*	Body dark greenish tinge	Overlaps normal
47. *Blackoid*	Black body	
48. *ebony*	Black body, intense pattern	Varies with alleles
49. *sable*	Dark body, trident sharp	
50. *spot*	Dark spot below eye	
51. *stripe*	Heavy stripe on thorax	
52. *trefoil*	Scutellum, trident, and head dark	
53. *Velvet*	Dilutes eye color	Hairs on eyes conspicuous
54. *white*	Eyes white	Large allelic series
55. *bordeau*	Dark wine eye color	Overlaps normal
56. *cardinal*	Yellowish vermilion eyes	Varies with alleles
57. *cinnibar*	Scarlet eye color	Varies with alleles
58. *scarlet*	Eyes bright scarlet	Some alleles near normal
59. *vermilion*	Eyes bright scarlet	Some alleles near normal
60. *brown*	Eyes clear brown	Many alleles, some dominant

TABLE 31 (*Cont.*)

Mutant	Characteristics	Remarks
61. *carnation*	Eyes dark ruby	Very dark in old flies
62. *sherry*	Eyes sherry color	Crossfertile, sterile *inter se*
63. *dark eye*	Eyes soft, dark, dull chocolate	Darker than normal
64. *Henna*	Eyes dull dark sepia	Varies with alleles
65. *sepia*	Eyes reddish, sepia to black	Darker than normal
66. *comb gap*	Gap in L, 4, sex combs large	Female sterile
67. *sex combless*	Sex combs absent	Abnormal, sterile
68. *degenerate spermatheca*	Abnormal epithelial cells	Pigmented, fertility good
69. *mutability factor*	Increases mutation rate	Viable and fertile
70. *supressor of sable*	Suppresses sable	Also suppresses vermilion

arista, wing shape, and so forth, we find that some among those known have slight effects; some have more extensive effects, increasing or decreasing the growth of the organ system; some have effects on various organ systems; some are so drastic as to be lethal. Tests of numerous allelic and multiple factor systems giving rise to final characters, such as eye color and body shape, show that the organism can be changed in numerous small or large steps, usually in several possible directions—more, less, or different for colors and patterns; larger, smaller, or redesigned for shape.

This demonstrated genetic variability lends credence to the assumption that all other variable systems (and we have mutations modifying all types of taxonomic characters) can be changed by small viable and fertile steps by other alleles or mutations. Let us review some of the differences between closely related species and compare them with mutational changes such as those recorded in Table 31.

In comparing Drosophila species, it is obvious that the characteristics of the living forms are those which have evolved, even though these may not show in pinned specimens. In lieu of the living forms for comparison, a great deal of information may be obtained from the descriptions and colored illustrations of a number of Drosophila species found in the publications by Patterson (1943) and by Patterson and Mainland (1944, Mexico). Many differences in shape, proportion, and color are apparent in the illustrations, which were made from living material. The descriptions do not contrast characters, such as chunky versus slender body, large versus small head (or eyes), and the like. The body color and pattern and eye color differences are also illustrated in the descriptions and the paintings.

Table 31 shows a number of mutants affecting the several morphological features of *melanogaster,* but of course any particular differences between two related species resembling the mutants given for *melanogaster* may involve either homologous or quite different loci. A check of the types of phenotypic changes will show many separate divergent and parallel changes in the several species groups.

The simplest mutational differences would be expected between closely related species, although phenotypic differences as great as those characterizing other separate species are found on occasion even between geographical strains. The numbers used below correspond to mutations in Table 31. The anatomy of the internal reproductive systems is markedly different between species. One difference often found is in sclerotization of the spermatheca (68, *degenerate spermatheca*). The species *equinoxialis* and *willistoni* differ in the shape of the spermatheca, although they are otherwise indistinguishable (Spieth, 1949). Such differences are present both within and between species; for example, Patterson and Wheeler (1947) describe an inherited difference in the growth pattern of the spermathecae between two strains of *peninsularis.*

Many body color differences exist between closely related species. One interesting color which is very useful in identification of two dark members of the repleta group, *bifurca* and *subviridis,* is a decided greenish color of the abdomen (46, *green body*). The color patterns may differ within and between species (see 43 to 51). There are three abdominal color phases in *polymorpha* that occur throughout the population in Brazil. These are due to a single pair of alleles, which give an intermediate heterozygote (da Cunha, 1949). Both alleles have sufficient selective value, so that both the heterozygote and homozygotes are retained. This is a demonstration that a very marked difference in color pattern, one in fact which caused differences greater than between many related species, is due to a single pair of alleles. The fact that both have selective value in a population shows how easily such differences between species can arise. One of the *straw* alleles causes light spots in the pigmented pattern as well as reduces the total pigment. Another case of polymorphism in abdominal color pattern is known in *montium* where an autosomal pair of factors determine color (Freire-Maia, 1949). Here the dark allele is dominant, and apparently the heterozygote has some selective advantage; for the alleles come to an equilibrium in artificial popu-

lations, roughly similar to that found in the natural populations in Brazil.

The very similar species, *melanogaster* and *simulans,* can be separated most easily by the male genitalia; but *simulans* is of somewhat different shape, corresponding roughly to the mutants *chubby* (18) and *chunky* (19) of *melanogaster.*

In the mulleri subgroup, the subspecies *rioensis* differs from *meridiana* in color markings and in the fact that the eye is sepia (darker, duller) rather than deep red. In the group that gives hybrids, *mulleri* (the standard color) and *mojavenis* have the same eye color; *aldrichi* has a brighter, more orange eye, although not quite as clear a color; *arizonensis* has a still brighter, more orange eye; and *buzzatii* has eyes that are a darker, duller red. The species *longicornis* and *hamatofila* both cross occasionally to *mulleri.* Here the eye colors are the same. The body colors in this group, as well as the pigment pattern, are quite different. *D. mulleri, longicornis, aldrichi,* and *arizonensis* are grayish brown, while *hamatofila* often has a reddish tinge and *mojavensis* is a still lighter yellow. There is considerably less difference in ordinary phenotypic characters between *mulleri* and *aldrichi,* which give sterile hybrids, than between *mojavensis* and *arizonensis,* which give many fertile hybrids and are actually much more closely related.

In the melanica species group, several of the differences in phenotype are of the kinds illustrated by the *melanogaster* mutants. For example, *micromelanica* and *melanissima* are usually smaller than *melanica, paramelanica,* or *nigromelanica.* Furthermore, *melanissima* has wings longer than the body, as contrasted with the other four. The species *micromelanica* has the lightest eyes (cherry red); those of *paramelanica* are of a duller, less orange color; and *melanica* has still duller eyes. The eyes of *nigromelanica* are red without the orange component, and *melanissima* has a much darker, duller red of the same kind. In body colors the eastern *melanica, paramelanica,* and *micromelanica* are a dark brownish, *nigromelanica* is much darker, and *melanissima* is shining black. There is an interesting additional variation in the color of the western *melanica,* which is a light yellowish brown, a typical lighter, "desert-adapted" form.

The question of the gene types needed to explain the phenotypic changes is answered by the evidence of their presence in *melanogaster* and their frequent occurrence in wild populations. This will be developed in the next section. One comment must be made about the

nature of these phenotypic differences, such as eye color, which are often considered to be of superficial nature. The normal alleles of the *vermilion* and *cinnabar* mutants are concerned in the production of the *v*+ hormone, kynurenine, and the *cn*+ hormone, which is 3-hydroxykynurenine. These substances are tied in with tryptophane and nicotinic acid in Neurospora (Haskins and Mitchell, 1949) and are linked with the production of body color pigment also in *melanogaster,* through the mutant *sable.* A gene acting as a *sable* suppressor, *su*^{2}*-s,* prevents the formation of an added dark body color pigment by the mutant *sable,* and also acts as a suppressor of the *vermilion* eye color. In this gene combination, the normal eye color pigment is present because kynurenine is formed (Beadle and Ephrussi, 1936b). In analyzing these cases biochemically, Green (1949) showed that nonprotein tryptophane accumulated in the *vermilion* mutant in *melanogaster* (and also in *virilis*). The *su*^{2}*-s* gene with *sable* in *melanogaster* caused the oxidation of about half the nonprotein tryptophane to kynurenine. Kikkawa (1950) has shown that the synthesis of tryptophane in insects is probably similar to the cycle in mammals and differs from that in *Neurospora,* but this does not modify these conclusions. In view of this type of biochemical interrelation, the assumption that genes which affect external characters or pigmentation are necessarily superficial and do not have selective importance is obviously false.

Some of the mutations listed in Table 31 occurred in wild populations, others in laboratory stocks; while some were the result of X-ray treatments. Muller (1947), who has studied a great number of both spontaneous and X-ray-induced mutations, states that the types of mutations recovered are the same. Both represent random changes in all directions in gene function. The types of mutants found in natural populations are illustrated below.

MUTATIONS IN WILD POPULATIONS

Spencer (1947a) has reviewed the work on spontaneous mutations found in wild populations of Drosophila. He states:

> Most biologists know that many mutations have been discovered in flies of the genus Drosophila. Few of them have any idea of the order of magnitude of the number of these mutations. The latest editions of several modern texts on General Biology state this number as somewhere between

500 to 1000. Actually the number of natural and induced mutations found in this genus would be much closer to 100,000 and probably in excess of this figure. There are individual investigators who have discovered hundreds of natural visible mutants in Drosophila, and others who have certainly found well over 1000 natural and induced lethals. Even fewer biologists realize that, at certain seasons, within a few hundred yards of almost any biology laboratory in the country there occur wild or semi-wild populations of Drosophila carrying many more than the 1000 mutants reported in the textbooks. These and other Drosophila populations provide a relatively untapped source of genetic variability, ready to be extracted and fashioned into the tools of the laboratory geneticist. Actually, certain kinds of mutants can be secured more easily from wild populations than by radiation or other methods of induction. However, the main interest in the genetic variability of populations relates to its evolutionary significance.[1]

Those interested in an extensive analysis of the mutations found in wild populations should consult the article by Spencer (1947a) and the details in the articles listed in this bibliography. Basically, the mutations in wild populations must show the necessary visible and physiological variability to account for the differences between related species. In addition, mutations, especially lethals, may be used to study population structure and cycles. We give below some illustrations of each use of mutations in several of the species groups.

Drosophila melanogaster

There have been extensive studies of mutants in wild populations of this species, so that much information on genetic variability is available. H. A. and N. W. Timofeeff-Ressovsky (1927) reported the genetic variability found in a Berlin population. Here seventy-eight females produced ten mutant types, including some weakly dominant mutants and a sex-linked lethal. A number of these were recovered several times (up to twelve), so that the populations included several closely related flies; for, as Spencer points out, the descendants of one female showed five mutants, two had three each, and twelve had two each.

Dubinin and his collaborators (Dubinin *et al.*, 1934, 1936, 1937) have analyzed a number of strains of *melanogaster* from Russia both

[1] Spencer, W. P. *Advances in Genetics,* I, pages 359–360. Academic Press, New York, N. Y., 1947. Copyright 1947 by Academic Press Inc.

for visible mutations and for lethals. They followed the lead of Tschetwerikoff (1926), who demonstrated the extensive genetic variability in natural populations. Table 32 shows the visible variation found in several widely separated localities in the U.S.S.R. through several years (Dubinin *et al.,* 1937). This is a record of the visible variation, part of which is genetic and part noninherited phenocopies. Gelendzhik is in the Novorssiisk region, on the eastern shore of the Black Sea. Derbert is on the western shore of the Caspian Sea, and Tashkent lies in Central Asia. Dubinin *et al.* (1937) wrote:

> The vast literature of systematics describes a great number of aberrations in all groups of the animal kingdom, especially in vertebrata, insects and land molluscs. However, up to the present there exist no genetic tests analyzing this aberrant variability in detail. Our findings show that aberrant polymorphism is widely spread in *D. fasciata* (*melanogaster*). Studies on 129,582 individuals in nature, taken from geographically remote localities, revealed 2800 aberrant forms (over 2 per cent). Genetic tests of these variant forms have largely contributed to the existing data on the nature of aberrant polymorphism, and have shown that the occurrence of aberrations in nature is caused by recessive and dominant genes, as well as non-hereditary variability.

In addition to the visible mutations, these Russian workers reported a number of phenocopies. Goldschmidt (1940), who has worked extensively on this type of abnormality, points out that the phenotypic effect of almost any genetic factor can be simulated by a noninherited phenotypic variation, a phenocopy, by the use of the correct inducing agent during development, such as heat shocks, X rays, or chemical treatments. Goldschmidt points out that the total range of phenocopies covers most of the range of gene-induced variability. These phenocopies are rare, except under the influence of powerful (sublethal) agents in the laboratory, which suggest the presence of equally powerful agents in natural environments. A number of the visible abnormalities were tested to determine whether they were genetic changes or phenocopies. Certain variants have figures after them in parentheses, indicating that some (not necessarily all) of these types were tested: (1), phenocopies only; (2), genetic changes only; and (3), both genetic changes and phenocopies among those tested. Among the genetic types, some were dominant, some recessive, and others were represented by some dominant and some recessive genes.

Dubinin *et al.* (1936) reported the tests for mutations in all chro-

TABLE 32

Visible variant types found in wild populations of *melanogaster* in U.S.S.R.
(from Dubinin *et al.*)

Locality	Gelendzhik			Durbent		Tashkent
Year	1933	1934	1935	1934	1935	1934
Number tested	10,000	14,765	6,960	3,971	7,088	5,888
VARIANTS						
trident	2,096	1,026	1,001	662	563	256
extra bristles (2)	252	94	15	10	24	51
small bristles (1)	102	13	...	1	...	...
bristles, mosaic (3)	101	33	4	1	5	3
bristle comb (2)	19	...	...	...	...	...
wavy bristles (1)	55	...	...	...	2	...
reduced bristles (3)	11	...	...	...	...	...
small eye (1)	37	...	5	...	...	...
rough eye (3)	41	1	...	1	...	...
dark eye (3)	16	...	5	1	1	...
mottled eye (3)	5	1	...	3	...	1
sepia eye	2	...	...	...	...	...
garnet, abnormal wing (2)	1	...	...	...	...	...
dark body (3)	3	7	1	1	...	...
yellow body (1)	1	...	...	...	...	...
dachs legs	1	...	...	...	...	...
plexus 2 (2)	5	10	...	23	15	6
analis incompletus	16	...	...	...	...	3
crossveinless	2	...	2	...	1	1
turned up wing (1)	14	12	...	...	5	4
divergent wings (3)	...	1	...	...	...	...
extra cross veins	...	...	1	1	...	2
extra-analis (2)	...	8	...	22	...	1
extramedia	...	...	1	...	...	...
light eye	...	1	...	...	2	2
notched wings	...	2	...	...	...	...
truncate wings	...	1	...	...	...	...
comma on thorax	...	1	...	...	...	...
tumor on abdomen	...	5	...	...	...	...
absent head bristles	...	...	...	...	...	1
Total changes (trident excluded)	684	190	34	64	55	75

mosomes in samples for 1933, 1934, and 1935 from Gelendzhik. The results are given in Table 33. This analysis gives some idea of the tremendous store of genetic variability present in a population. In these populations, no sex-linked mutation was found. Indeed, sex-linked mutants were very rare; but two or three were found in all the tests, including a *yellow* mutant at Buinaksk. The large number of gross visible and lethal mutations is indicated in these studies. To

this, Dubinin *et al.* (1937) add the information that slight visible variants which may cumulate to give appreciable effects are much more numerous than variants with large effects. They go on to point out that some of the genes are obviously detrimental and do not occur in the expected Hardy-Weinberg frequencies (see below), while others occur in about the expected frequency of heterozygotes and homozygotes in the wild populations. Furthermore, the mutant *ebony* gives heterosis with its normal allele, so that the heterozygote is more frequent than expected. A laboratory test crossing heterozygotes for the *ebony* mutant recovered in the Gelendzhik population gave 1,529 normal, 3,960 heterozygotes, and 1,467 *ebony* homozygotes, which is a marked departure from the expected 1:2:1 ratio. Dubinin *et al.* point out the effect of natural selection in eliminating some genes (homozygotes missing), and especially as the agent which reduced the frequency of recovered X-chromosome mutants to such a very negligible number. They account for the variation in frequency of mutants present from year to year in part as the result of gene drift through small winter populations.

Hardy (1908) and Weinberg (1908) independently developed the relation between allele frequencies in a population. If in a sexual form with a large panmictic population the frequency of allele *A* is q and *a* is (1 — q), the expansion $[qA + (1 - q)a]^2$ will give the frequency of zygote combinations, provided there is no modification by selection between alleles, migration, or mutation.

Dubinin (1946) summarized the work in U.S.S.R. on lethal mutations in *melanogaster*. He reported both on the lethals present in the populations and on the rate of lethal mutations of selected wild chromosomes. The mutation rates for the second chromosomes tested by him in three populations were $0.33 \pm 0.07\%$, $0.44 \pm 0.08\%$, and $0.45 \pm 0.10\%$. He points out that some of the data indicate different rates of mutation in different populations. Referring to the different mutation rates encountered in these data and in those reported by other workers (see below), he again states that the direction and rates of mutation of all genes are an adaptive character of the species, which is subject to evolutionary change (Dubinin *et al.*, 1936). This is undoubtedly true in a general way, but we have no data on cases where such an adaptive change in mutation rate has been utilized by a species. Such data would be very difficult to obtain and evaluate.

Despite the fact that allele tests showed that some lethals occurred more than once, Dubinin points out that in these tests, as well as in previous ones, the actual frequency of lethals in the chromosomes is less than the expected frequency, provided the lethals are not selected against in the heterozygote and the population breeds completely at random. There is no information that permits us to determine whether either inbreeding or selection against the heterozygote are operating, although he considers that some of the frequencies of lethals are determined by gene drift in small populations. There is an increase to a concentration of about 39 per cent in November, although there is no convincing argument as to why this increase should happen. However, the elimination rate calculated for November of 0.248 per cent was approaching elimination equilibrium with the mutation rate.

The genetic structure of populations of *melanogaster* from several sections of the United States have been studied by Ives (1945, see bibliography in this paper). Ives tested for lethals in the second chromosome, using as markers the two *Curly* inversions, which prevent crossing over. The tested chromosome was obtained heterozygous with *Curly* and inbred. As *Curly* is lethal homozygous, we expect 66.7 per cent *Curly* to 33.3 per cent normal if no lethal is present. The tests were run through two generations of over two hundred flies each. If no normal flies appeared, this strain was classed as lethal; if from 0 to 17 per cent were normal, it was classed as semilethal; and if 17 to 30 per cent were normal, it was classed as a deleterious mutant. The visible mutations in the second chromosome would be detected. Most of the second chromosomes tested were deleterious or semilethal, but a few had normal viability. Visible mutations in the other chromosomes might be detected during the inbreeding. Ives lists a few of the genes which had previously known alleles: *net, dumpy, black, purple, cinnabar, Lobe,* and *brown* on the second chromosome; *scarlet, pink, cardinal,* and *claret* on the third chromosome; *shaven* on the small fourth chromosome. There were numerous other mutations, many of them previously unknown or undescribed. This list may be compared with the mutants listed in Table 31. In addition, the mutant *cardinal* was found at one collecting area in Massachusetts in 1931, 1932, 1934, and 1938. These records indicate the survival and continuity of the *melanogaster* population carrying the *cardinal* gene in that area.

The amount of genetic variability of the *melanogaster* populations

TABLE 33

Mutations in Gelendzhik populations of *melanogaster* (from Dubinin *et al.*)

Mutation	Year 1933 Tested 1,754		Year 1934 Tested 1,232		Year 1935 Tested 1,594	
	Number	Autosomes	Number	Autosomes	Number	Autosomes
extra bristles	102	2, 3	234	2, 3	224	2, 3
comma	57	3	172	3	108	3
divergent wings	1	3	2	3	...	...
cinnabar	3	3	5	3	...	...
dark eyes	7	3	8	3	...	...
sepia	2	3	2	3	...	...
bristles absent	2	3	3	3	...	...
divergent wings	19	2	12	2	3	2
cross-veinless	13	2	3	2	16	2
cut wings	2	2	...	...	...	...
extra bristles	3	2	...	...	10	...
dachs-legged	3	2	...	...	...	...
small bristles	1	2	...	...	3	3
drooping wings	3	2	2	2	...	...
mottled eye	1	2	...	...	...	...
dwarfs	2	2	...	...	...	...
small wings	2	2	...	...	...	...
reduced bristles	1	2	...	...	...	...
mottled + spot	1	2	...	...	...	...
rough eye	1	2	2	2	...	...
analis incomplete	...	...	6	2	1	2
deformed wings	...	...	2	2	6	2
abnormal wing, thorax	...	...	5	2	...	...
brown eyes	...	...	1	2	1	2
bent bristles	...	...	2	2	...	...
extra vein, small	...	...	9	2	4	2
dark eyes	...	...	...	...	1	3
brown eyes, small wings	...	...	...	...	10	2
rough mottled	...	...	...	...	10	2
balloon wing	...	...	...	...	6	2
reduced eyes	...	...	...	...	1	2
abnormal wings	...	...	...	...	2	3
upturned wings	...	...	...	...	1	2
extra vein	...	...	...	...	3	2
lethals	70	2	78	2	70	2

is very great. Using the criteria given above, only 12 per cent of the chromosomes collected in Massachusetts in 1938 seemed to have normal viability, while 32 per cent were lethal, 13 per cent semi-lethal, and 43 per cent deleterious. Some 22 per cent of these showed visible effects. A Florida population collected in 1940 had even fewer

normal chromosomes, with proportionally more lethals and semi-lethals. There is an astonishingly low number of normal chromosomes present in the populations tested. Tables 34 and 35 show the frequency of lethal chromosomes and the number and allelism of the lethals. It should be noted that some chromosomes had two or more lethals present. Ives showed that the inbred nonlethal stocks were less viable, as compared with *Curly,* than crossbred heterozygotes from different areas. Heterosis was not very pronounced in the crosses between stocks from Massachusetts and Ohio. This implies that there existed in general as many (or as few) genes in the *Curly* inversions leading to heterosis as in the normal chromosomes from the wild populations.

TABLE 34

Results of lethal tests on 1,202 second chromosomes of *D. melanogaster* from six wild populations from the United States and from the Maine 42 stock after 3½ years of laboratory culture (from Spencer after Ives)

Collection	Chromosomes Tested	Number of Lethals	Percentage of Lethals
Massachusetts 38	151	68	45.0%
Massachusetts 41	108	64	59.3
Florida 40	227	152	67.0
Florida 42	110	68	61.8
Ohio	177	88	49.7
New Mexico	203	126	62.1
Maine 38	115	59	51.3
Maine 42	111	38	34.2
Totals	1,202	663	55.2%

There was a large number of lethals present in each population, and an appreciable part of these occurred more than once in the samples. The tests were made by crossing two lethals heterozygous for *Curly* together and also by locating the lethals with other gene markers. Comparing the percentage of allelic lethals in his control series with the percentage from wild populations, Ives concluded that they were similar, so that probably most lethal mutations in these natural populations were of independent origin. He calculated that the average proportion of identical lethal chromosomes in the several samples tested was 0.00202. He estimated that there were about 495 ($\pm$ 106) genes mutating to lethal alleles. This estimate compares

favorably with that of 295 gene loci in chromosome 3 in *pseudoobscura* as calculated by Wright, Dobzhansky, and Hovanitz (1942), in view of the fact that the third chromosome of *pseudoobscura* is homologous to 2R of *melanogaster,* or roughly half that chromosome. These estimates of the number of loci which produce lethal mutations are much lower than the frequency of genes, if one assumes that each band on the salivary gland chromosome is a gene (see, for example, Bridges and Bridges, 1939).

TABLE 35

Frequency of different lethal genes determined by cross tests within a sample of lethals from each population and within two samples of new lethals arising in the laboratory; not quite all of the cross tests were made for the Lab 2 and the Florida 40 samples (from Spencer and Ives)

Origin	le Chrom's	le Genes	Frequency of Appearance 1	2	3	4	5	6	7	13
Lab 1	27	27	27	0	0	0	0	0	0	0
Lab 2	(75)	68	61	7	0	0	0	0	0	0
Massachusetts 38	49	46	43	2	1	0	0	0	0	0
Florida 40	(50)	46	42	4	0	0	0	0	0	0
Florida 42	47	41	35	6	0	0	0	0	0	0
Ohio	48	44	42	1	0	1	0	0	0	0
New Mexico	48	19	7	3	4	3	0	1	1	0
Maine 38	41	10	2	1	1	1	1	3	1	0
Maine 42	34	17	13	1	0	1	1	0	0	1

In discussing the natural populations of *melanogaster* in the United States, Ives (1945) stated:

The two major conclusions are, first, that American *Drosophila melanogaster* breeds in comparatively large populations; and second, that these large populations are continuous from year to year not only in tropical and subtropical areas but also in the temperate zones of the northern United States. These conclusions are based on three lines of evidence: the low proportion of identical lethal genes found among lethal chromosomes in Massachusetts and Florida collections; the high proportion of lethal chromosomes in these collections; and the continuous presence of the infrequent mutation, *cardinal* eye color, in collections from one Massachusetts area over a period of eight years. The first of these lines of evidence indicates a large breeding population continuous for at least several generations, since the proportion of identical lethal genes among wild lethal genes was no greater than that among a sample of lethals which

arose in the laboratory independently of each other. The second, considered in conjunction with the first, suggests an accumulation of lethal genes over a long period of time. The third indicates a population continuous for at least eight years in the area in which the collections were made.

He goes on to point out that there is a marked difference in the populations analyzed from the U.S.S.R. and those he studied, perhaps reflecting a large fluctuation in numbers in the U.S.S.R. population through the seasonal cycles.

Ives gave the results of tests for the rate of lethal mutations in stocks from different localities. These varied from 0.51 ± 0.13% to 7.16 ± 1.38% lethal mutations for the stocks tested. This type of variation, including high rate lines, had been reported by Demerec (1937), Neel (1942), and Mampell (1943). He points out that the variation he reports, even from stocks which came from the same area, makes an estimation of the actual rate of mutation for the population of *melanogaster* very difficult, so that we can only roughly approximate the average mutation rate.

Ives presents data on recovery of zygotes carrying a lethal from individuals heterozygous for a lethal when cultured under normal temperature conditions and when the germ cells were treated with a temperature shock in the pupal stage. At normal temperature, the controls gave rise to 49.6 ± 2.6% (186 +/+; 183 +/lethal) lethal zygotes, whereas both males and females treated with temperature shocks produce offspring only 35.4 ± 2.7% of which carry a lethal (204 +/+; 112 +/lethal). This indicates that temperature shock (and perhaps other adverse environmental agents) select against germ cells with a lethal. If this type of selection were to prove a general rule, adverse environmental agents would be very useful in improving a population (see Dubinin *et al.* below). More experimental evidence is needed on this point.

Drosophila pseudoobscura

Drosophila pseudoobscura and its sibling species, *D. persimilis,* have been more intensively studied than any other species in the subgenus Sophophora except *melanogaster.* The chromosomes have been studied genetically as well as cytologically by the use of inversion markers. Sturtevant (1937) reported on lethals in *pseudoobscura*

from wild populations. He found that about 20 per cent of the third chromosomes carried lethals and that the second chromosomes had about the same percentage of lethals as the third. Dobzhansky and collaborators published a series of articles developing the information on lethals and other mutations in *pseudoobscura*. Dobzhansky and Queal (1938) found that, in California populations, 11.9 per cent of the third chromosomes carry recessive lethals, 3.1 per cent semilethals, and 3.5 per cent visible mutations. They point out that 39 per cent of the third chromosomes carry genes which reduce viability and 2 per cent carry genes which increase viability. Dobzhansky (1939) extended this study to Mexican populations of this species. In the stocks he tested from Mexico and Guatemala, about 30 per cent of the third chromosomes contain lethals or semilethals and, in addition, roughly 40 per cent carry deleterious factors. He goes on to point out that the individuals homozygous for any of these third chromosomes are usually at a selective disadvantage, as compared with the heterozygotes. In his opinion, the difference between these populations and those reported by Sturtevant (1937) and Dobzhansky and Queal (1938) from the Death Valley region was due to differences in breeding size of the populations.

Dobzhansky and Wright (1941) and Wright, Dobzhansky, and Hovanitz (1942) studied the origin and allelism of lethals. Table 36

TABLE 36

Frequency of allelism of lethals (including semilethals) according to whether flies were collected at same or different stations of same locality, and whether collected simultaneously or at different times (San Jacinto only) (from Wright, Dobzhansky, and Hovanitz)

	Same Station				Different Stations			
	Number Tested	Same Locus	%	SE	Number Tested	Same Locus	%	SE
Simultaneous	594	15	2.53	0.64	691	9	1.30	0.43
Different times	1,474	29	1.97	0.36	1,593	11	0.69	0.21
Totals	2,068	44	2.13	0.32	2,284	20	0.88	0.20

shows the origin and cross tests for allelism of lethals from the same locality, and Table 37 shows the tests for allelism between lethals from different localities. Lethal mutation rates for certain strains from

the Death Valley region were tested both by an occurrence test and an accumulation test. The rates were 0.251 ± 0.056% for the direct test and 0.313 ± 0.038% for the accumulation tests. This lack of a significant difference is taken to mean that, under the usual laboratory conditions, there is no selection against heterozygous lethals. The lethal mutation rates in the Death Valley region, Mexico, and Guatemala strains are shown in Table 38. The frequency of lethals in the populations as tested were 15.29 ± 0.83% for Death Valley, 28.1 ± 3.3% for Mexico, and 34.2 ± 5.2% for Guatemala.

TABLE 37

Frequency of allelism of lethals, including semilethals, according to locality and whether collected at same or different stations of same locality (same or different locality of region in the case of Death Valley) (from Wright, Dobzhansky, and Hovanitz)

	Same Station				Different Stations			
	Number Tested	Same Locus	%	SE	Number Tested	Same Locus	%	SE
San Jacinto								
Keen	978	24	2.45	0.49	1,369	11	0.80	0.24
Pinon	681	13	1.91	0.52	524	7	1.34	0.50
Andreas	409	7	1.71	0.64	391	2	0.51	0.36
Totals	2,068	44	2.13	0.32	2,284	20	0.88	0.20
Death Valley, 1937	772	24	3.11	0.63				
Wildrose, 1939	183	1	0.55		230	2	0.87	0.67

TABLE 38

Data on rate of occurrence of lethal mutations in the third chromosome of *Drosophila pseudoobscura* (from Wright, Dobzhansky, and Hovanitz)

Source of Stock	Kind of Experiment	Chromosomes Tested	Lethal Mutations Number	%	SE	Averages %	SE
Death Valley	Direct	3,580	9	0.25	0.08	0.297	0.047
Death Valley	Cumulative	9,892	31	0.31	0.06		
Mexico	Cumulative	4,177	15	0.36	0.09	0.325	0.065
Guatemala	Cumulative	3,522	10	0.28	0.09		
Totals		21,171	65			0.307	0.038

In tests of pairs of lethals found in the same locality, 3.11 ± 0.042% were allelic; but only 0.407 ± 0.061% were allelic in tests of pairs of lethals from different localities. Dobzhansky and Wright consider at least nine properties of populations and their general relations that might account for these results. From calculations of the probability of elimination of lethals (and semilethals) through homozygosity, they find, "the observed mutation rate is more than four times as great as necessary to account for the elimination of lethals on the assumption that these are recessive [s (selection against the heterozygote) $= 0$] and that there is random mating within localities [F (the inbreeding coefficient) $= 0$]." They were unable to determine whether the discrepancy between expected and realized frequencies of lethals was due to selection against the heterozygote or inbreeding, and calculated that, if s is the same for all loci, the value $s + F = 0.013$ will account for the elimination of lethals.

They conclude that the breeding structure of the populations determines the distributions of the lethals, that there was a minimum of 289 loci giving lethal mutations in the 3 chromosome of *pseudoobscura,* and that the effective size of population in a locality (ten to twenty traps along a quarter-mile to a one-mile range) was less than twenty-five hundred in the Death Valley region.

Wright, Dobzhansky, and Hovanitz (1942) studied several localities on Mount San Jacinto, in California. Here 13.85 per cent of the chromosomes contained lethals or semilethals, with no significant differences between stations or localities, or for collections made at different seasons of the year. They compared allelism between lethals from different localities (0.413%), different stations in the same locality (0.88 ± 0.20%), and lethals from the same station (2.13 ± 0.32%). The percentage of allelism was slightly, but not significantly, higher between flies from the same collections and those from the same station collected as much as six months apart. The estimate of the number of loci giving lethals is 285. The authors could not determine which of the possible alternatives explained the fact that lethals were much less frequent than expected if recessive in a random breeding population with the observed mutation rates. They conclude that the joint selection against the heterozygote (s) and inbreeding coefficient (F) could be expressed as $(s + F) = 0.0177$ for stations, 0.0194 for localities, and 0.021 for San Jacinto as a whole. In the stations, 7 per cent brother-sister matings, if other matings were

random, would reduce expected lethal frequencies to those observed. They estimated the effective breeding population (N) to be 2 or 3×10^4 for the largest locality (Keen), having an area of about two square miles, if the migration index is only about 1 per cent.

Unfortunately, a complete measure and analysis of factors effecting changes in real populations is not yet possible. An alternative explanation for their results may be that the inbreeding is more pronounced in the early part of the season in the spring, owing to the low mobility of flies at the lower temperatures. This would tend to induce inbreeding in the populations at their lower value, where this would be very effective in reducing the number of lethals by eliminating them as they become homozygous.

Dobzhansky and Wright (1943, 1947) have published studies on the dispersion rates of flies of a laboratory strain of *pseudoobscura,* marked by the bright eye-color mutant *orange* to distinguish them from the wild flies, which were released at a certain point and then collected along a north-south line at twenty-meter intervals for six hundred to fifteen hundred meters from the point of release. The experiments performed at Mather, California, in 1945 and 1946 give some measure of the migration index (m) for further analysis of population dynamics. Similar earlier studies were considered by Dobzhansky and Epling (1944) and have been mentioned previously. In these experiments they again showed that the activity and average distance traveled was correlated with temperature, so that there was little movement below 50° F., but values of two hundred meters per day were probable at 78° F.

The authors estimate that the density of the wild population of *pseudoobscura* was only 0.4 fly per hundred square meters in midsummer, which is only one-tenth to one-twentieth of that found at the corresponding season on Mount San Jacinto. In order to express in another way the spread of a population, the authors state:

> To help a nonmathematical reader visualize the observed rate of diffusion of the gene *orange,* the following very crude figures can be mentioned. We take the figure 0.72 kilometers to represent the standard deviation (in one direction) of the distribution of the progeny of *orange* flies about ten months after their release. This is probably an overestimate for ten months, but may be fairly close as an estimate of the standard deviation one year after the release. Now, if the progeny of the flies one year after the release is normally distributed, then half of this progeny will

be found within a circle with a radius of about 0.85 of a kilometer from the origin. About 95 per cent of the progeny will be found within a circle with a radius of 1.76 kilometers, and about 99 per cent of the progeny within a circle with a radius of 2.2 kilometers.

This general estimate of activity was based on a check for the presence and frequency of *orange* heterozygous in the population in 1946, as carried over by breeding with the wild population from the flies released in 1945.

Dobzhansky, Holz, and Spassky (1942) studied the genetic variability of the second and fourth chromosomes of *pseudoobscura*. They desired to test the chromosomes, not only for major changes such as lethals and sterility factors, but also for mutants affecting viability and developmental rate. They were able to show that a number of the chromosomes affected developmental rates from tests of the effect of 274 different second chromosomes homozygous and 293 fourth chromosomes. Their results show:

Development rate	Second Chromosome	Fourth Chromosome
Normal	31.22 ± 0.42%	27.84 ± 0.41%
Slow	30.55 ± 0.50%	25.96 ± 0.86%
Very slow	19.48 ± 1.80%	21.00 ± 2.30%
Fast		27.00 ± 2.26%

Their tests for sterility show that a number of chromosomes carried factors causing sterility in one or both sexes, which were not tested separately. Of 275 second chromosomes tested, 13.5 ± 2.1% were sterile; and of 297 fourth chromosomes tested, 8.1 ± 1.6% were sterile. Some of these effects on fertility were associated with other detectable abnormalities. Table 39 summarizes these tests, which show that a very large majority of flies in these populations depend on heterozygosity for normal functional and survival value.

Dobzhansky and Spassky (1944) made some extensive tests on the survival value of certain of the second (twenty-six tested) and fourth (twenty-two tested) chromosomes under various environmental conditions. The forty-eight chromosomes chosen produced some mild departures from the normal condition in the previous experiments (Dobzhansky, Holz, and Spassky, 1942). These were studied for effects on viability, development rate, and structure of the homozygotes under different conditions of temperature and population density, and different genic balance or modifier systems. In the tests, the homozygous

chromosomes were compared with a heterozygote of the tested and the marker chromosome used in all tests of the particular chromosome. The viability of some homozygotes varied only slightly with temperature, population density, or culture conditions; others were

TABLE 39

Percentage frequencies of chromosomes containing different types of genetic variants (from Dobzhansky, Holz, and Spassky)

	Second Chromosome	Third Chromosome	Fourth Chromosome
Lethals and semilethals	21.3 ± 1.8	13.9 ± 1.0	25.5 ± 2.2
Minus modifiers of viability	21.1 ± 2.3	30.5	40.7 ± 2.6
Plus modifiers of viability	1.3	0.4	0.5
Minus modifiers of developmental rate	54.0 ± 3.0	?	31.7 ± 2.7
Plus modifiers of developmental rate	0.4	?	3.4
Sterility factors	13.5 ± 2.1	?	8.1 ± 1.6
Visible mutants (minimum estimate)	4.2	3.1	1.9

drastically affected by some or all of these variables. Some of the homozygotes were superior in viability to their heterozygous siblings under some conditions; none was superior under all conditions. As an example, one genotype was lethal at 25.5° C., semilethal at 21.0° C., and nearly normal at 16.5° C. Mutants changing the development rates were more stable, but one was modified from normal much more drastically under some conditions than under others. The several tested genetic modifier systems were variable in their effects, so that none was universally favorable. Dobzhansky and Spassky generalize their results by stating: "Natural populations contain a tremendous variety of genotypes with different reaction norms."

Dobzhansky (1946 a) elaborated on certain genetic interrelations as tested with chromosomes drawn from wild populations. He chose three second chromosomes as follows: (1) A, having viability nearly normal at 16.5° C., semilethal at 21.0° C., lethal at 25.5° C.; development rate slow; survives better in crowded conditions. (2) B, having viability normal or above at the lower temperatures and nearly normal at 25.5° C.; development rate normal; not affected by culture conditions. (3) C, having viability normal or above at all temperatures tested; development rate slightly slower than normal; not affected by culture conditions. He tested a number of the recombinations from heterozygous females, A/B, A/C, B/C, crossed to marked balancer

stock, and compared the properties of the homozygous recombinant or noncrossover with the heterozygote which is considered normal. A great amount of variability was present in these crossover recombinants. He states that:

> The viability of the homozygotes for some of the chromosomes is equal to or even slightly superior to that of the respective heterozygotes; other chromosomes act as recessive deleterious modifiers, still others as semilethals, and finally some act as complete lethals. Homozygotes for some chromosomes are relatively more viable then heterozygotes in crowded cultures, others are favored in cultures with low population densities, and still others are not sensitive to population density variations. Many of the chromosomes produce varying degrees of retardation of the development of the homo- as compared to the heterozygotes.
>
> Particularly interesting is the appearance of synthetic lethal and semilethal chromosomes, which arise through crossing over between chromosomes lacking these properties. One chromosome has a dominant effect of the development rate of its carriers; no such effects were present in the ancestral chromosomes. At least two chromosomes have synthetic effects on the visible morphology of the flies.

Dobzhansky considers that, as the heterozygotes are usually superior to homozygous gene combinations, inversions which achieve a superior gene combination are selected for and retained because of the heterosis effect. This is perhaps an important type of heterosis polymorphism for the third chromosome of *pseudoobscura* (see below). It is by no means true of the second chromosome (the one used by Dobzhansky in this study) or the fourth chromosome in *pseudoobscura.* It is not important in *melanogaster* or in *hydei,* which have even larger populations than *pseudoobscura.* The places where such polymorphic inversion heterozygotes might be important in the genus may be inferred from the information on chromosome variability in Chapter 5.

other Sophophora

The genetics of the European *Drosophila subobscura* has been investigated by several investigators. In the stocks tested, the homozygotes were at considerable selective disadvantage. As Spurway (1948) points out in her report of the interesting mutation, *grandchildless,* ordinarily not more than one autosome could be homozygous without serious reduction in fertility. Females homozygous

for this mutation produce sterile offspring when mated to any type of male. The offspring have very rudimentary gonads, and most of the males have no testes. These males resemble the hybrid males from *persimilis* females crossed to *pseudoobscura* males. This single mutation produces as drastic effect as that of the multiple-gene differences in *pseudoobscura/persimilis* hybrids.

Kikkawa (1938) reported on a number of mutants in *ananassae* and established the linkage relations of many of them. The most important contribution to the general problem of evolution in this genus was the demonstration of the A-F translocation and its establishment in the evolution of this species. Sturtevant studied the mutations present in wild populations of *affinis* and mapped the chromosomes (see homologies below). In addition to these last species, certain others in the subgenus Sophophora have been studied enough to show a similar pattern of mutations.

Subgenus **Drosophila**

Drosophila virilis

In the subgenus Drosophila only two species have been studied extensively, *virilis* by Chino and others and *hydei* by Spencer. Some other species have been sampled for mutations as well, but not intensively. Chino (1936, 1937, 1941) has reported on the mutants and linkage in *virilis* from Japan and other parts of Asia where *virilis* has an extensive population. Previously Metz, Moses, and Mason (1923) had published on the mutants found in *virilis,* but the population in the United States is not a large one. Chino has indicated with his description of mutants which of them originated from irradiation, from laboratory stocks, or from wild populations. Both dominant and recessive mutants have been detected from all types of material. There exists extensive genetic variability in the *virilis* populations. Patterson, Stone, and Griffin (1942) tested a small number of wild populations from the United States and showed that they contained a large number of mutations, including lethals. These tests, together with those reported by Metz, Moses, and Mason, indicate that the genetic variability in the United States populations seems similar to that reported by Chino for Asia. Thanks chiefly to Chino and his collaborators, the mutations and linkages are better known in this species than in other members of the subgenus (see Table 42).

Drosophila immigrans

Spencer (1947 a), in his review of the methods of analyzing populations of Drosophila, points out that there are present species-specific mutants which are widely spread throughout their populations. He calls attention to two noted by Sturtevant, a *light body* color in *repleta,* and *net,* a mutant in *immigrans* which causes extra veins. Spencer (1947 b) compared the populations of *immigrans* collected near New Wilmington, Pennsylvania, in 1944 and in 1946 at places about one-quarter mile apart. Table 40 shows the results, and it is clear that a number of genes were still retained in the local populations during the two years.

TABLE 40

Mutations in *Drosophila immigrans* (from Spencer)

1944	1945
Eye Color:	Eye Color:
brick 8	brick 3
dubonnet 2	dubonnet 4
Wing Veins:	Wing Veins:
broken 1	broken x-vein 1
cross-veinless 1	plexus 1
short-5 1	short-5 1
	slant 1
	net 1
	ragged 1
Bristles:	Bristles:
double 1	pin 1
minute 1	ragged-tiny 1
small 1	small bristle 1
stubble 19	spineless 1
tiny 1	stubble 11
two-bristle 1	two-bristle 1
Wing Shape:	Wing Shape:
	curled 6
	cut-echinus 1
	spread wing 1
Body Shape:	Body Shape:
	fat 1
Phenotypic Complexes:	Phenotypic Complexes:
purple-net-short 1	
purplish-thin-singed 1	
sepia-spineless 1	
Total 40	38

Drosophila hydei

Spencer (1947 a, b, 1949) has made a study of the many mutations present in *hydei*. The comparative genetics are given below. Spencer has shown the distribution of mutations in the several chromosomes of this species (Table 41). A small percentage of the flies give sex-linked mutants, including *vermilion* and *bobbed*. The latter is one of the species-specific mutants of *hydei* (Spencer, 1944, 1945). The term "species-specific mutation" refers to particular mutant alleles which occur in exceptionally high frequencies in this species, as compared with their frequency in others. There exists a series of isoalleles which may produce the normal phenotype if homozygous or in some combinations, but give differing grades of *bobbed* in other combinations, depending on the alleles involved. All of these alleles must be checked in heterozygous or homozygous females, since the sex-linked *bobbed* has a strong dominant normal allele in the Y.

Alexander (1949) also found different *bobbed* alleles widespread in the *hydei* population, as well as two other species-specific mutants,

TABLE 41

Distribution of mutations in *Drosophila hydei* (from Spencer)

Male	Mutant	F_1	F_2-1	F_2-2	F_2-3	F_2-4	F_2-5	F_2-6	F_2-7	Linkage Group II	III	IV	V	VI
9	abnormal				+			+			+			
11	gray	+									+			
11	squat				+	+					+			
18	rough				+	+						+		
21	taxi		+					+		+				
23	gray	+									+			
23	tiny					+				+				
27	rose		+	+	+	+	+		+				+	
28	gray	+									+			
44	nicked		+	+			+		+	+				
49	nicked		+			+			+					
54	tiny				+					+				
56	gray	+									+			
56	orangelike				+						+			
64	rose		+	+	+	+			+				+	
71	facet			+	+		+					+		
97	facet				+							+		
100	javelin	+												+
101	gray	+									+			
111	grooveless								+					+

striped thorax and *pale* thorax pattern, resulting from reduction in size of the pigment spots. She also found a *mottled* which is a species-specific mutant in *Drosophila macrospina limpiensis.* The phenotypic expression of the *mottled* factor overlapped normal and was affected both by culture conditions and modifiers. Other species-specific genes are known, such as *trident* in *melanogaster* (Dubinin *et al.,* 1937). These species-specific mutants are important, for they show that certain mutants can attain appreciable frequencies in a population, and so might replace the normal allele.

The *bobbed* iso-alleles show that a number of alleles of different activities and interactions can be characteristically present even in wild populations. Stern (1926) described two iso-alleles of the *ebony* locus in *melanogaster,* and Stern and Schaeffer (1943) showed that several different iso-alleles existed for the *cubitus interruptus* locus. Timoféeff-Ressovsky (1932) proved that there were two different normal alleles of *white* from differences in their mutation frequency and pattern, while Muller (1935) showed that these genes differed in dominance, as measured by ability to produce pigment in diploids and triploids. From all these related tests, we find that a somewhat heterogeneous population of normal alleles exists, as well as numerous recessive mutants. Haldane (1930, 1939) has discussed the evolutionary importance of the system of multiple normal alleles.

GENE HOMOLOGIES

A very important aspect of the genetic problem is that of gene homologies in the several different species which have been studied. The mutants and linkage relations in *pseudoobscura* and a description of homologies with the chromosomes of *melanogaster* were discussed by Crew and Lamy (1935), Donald (1936), and Sturtevant and Tan (1937). The concept of gene homologies was developed further by Muller (1940) and by Sturtevant (1940). This material and other evidence was critically reviewed by Sturtevant and Novitski (1941), and additions were made by Spencer (1949b). To make the homologies as definite as possible, loci considered homologous, and therefore identical in origin and presumably in function, have usually possessed some peculiarity in mutational properties or a similar series of alleles have existed at the two loci. Mutants where mimic loci occur in several chromosomes are ignored, except as supporting evidence.

The weaknesses of the establishment of homologous loci in two species that do not cross are several. Many nonhomologous loci may be present in two chromosomes suspected of being homologues. The nature of evidence limited by mutation similarities does not allow the detection of lack of homologies, unless there are several unmistakable parallels to different loci in mixed sets of chromosomes of the species being compared. The existence of mimic mutations at different loci on nonhomologous or homologous chromosomes introduces another source of error.

As an extreme example, *vermilion* and *cardinal* in *virilis* are both concerned with the production of kynurinine, which is necessary for the production of brown pigment (Price, 1949b). As far as known, their activities are the same, although *vermilion* is on the X and *cardinal* on the fourth chromosome. Another source of error is suppressor mutations, which in effect may move the reaction control from one locus to another, even in different chromosomes. The cytological shifts between elements by pericentric inversions or centromere shift are a further source of possible mistakes. Translocations seem so rare in Drosophila material that, when the elements are separate, they should ordinarily remain homologous except for mutation divergence as the species evolve. The divergence of species would be a further source of difficulty, for the different normal alleles at the same locus might give similar mutant alleles which would simulate complete homology. Despite these limitations, the concept of homologies is important in that it shows some of the limits of gene divergence and similarities between species that cannot be crossed. The best examples of gene homologies for the X chromosome of several species are illustrated in Table 42. These were taken from Sturtevant and Novitski (1941) and from Spencer (1949). In each case the known linkage map loci of the genes are given. These are taken from the following sources: *melanogaster,* Bridges and Brehme (1944); *simulans,* Sturtevant (1929); *pseudoobscura,* Sturtevant and Tan (1937); *affinis,* Sturtevant (1940); *virilis,* Chino (1941); *ananassae,* Kikkawa (1938); *hydei,* Spencer (1949). These linkage data show the effect of the extensive rearrangements within a chromosome arm, presumably for the most part through paracentric inversion, as judged by the evidence presented in Chapter 5.

Crossing over is one of the important types of recombination of genes. In this group we find that crossing over is low in *melanogaster,*

simulans, pseudoobscura, and *affinis* of the subgenus Sophophora, and high in *virilis* and *hydei* in the subgenus Drosophila. The species *ananassae* is in the melanogaster group; and the X chromosome of this

TABLE 42

Gene homology in the X chromosome, element A (data from Sturtevant and Novitski, and Spencer)

Mutant	Species						
	melano-gaster	*simulans*	*ana-nassae*	*pseudo-obscura*	*affinis*	*virilis*	*hydei*
yellow	0.0	0.0	96.2	59.3	12.0	2.9	38.8
scute	0.0+	...	96.0	59.0	27.0	3.8	15.6
white	1.5	4.1	16.7	65.3	2.0	105.0	19.0
facet	3.0±	7.1	...	...	...	...	...
Notch	3.0±	...	15.5	63.3	...	102.9	22.3
echinus	5.5	...	...	45.9	...	8.7	...
ruby	7.5	9.7	...	...	...	83.5	115.7
cross-veinless	13.7	14.0	...	...	...	25.0	...
vesiculated	16.3	16.4	...	...	...	27.0	...
cut	20.0	+	117.6	21.9	17.0	160.5	77.3
singed	21.0	21.1	112.7	73.1	...	50.0	31.4
lozenge	27.7	+	...	5.4	...	127.0	...
vermilion	33.0	+	44.0	69.1	22.0	25.5	7.4
miniature	36.1	...	45.5	70.9	+	78.0	59.6
dusky	36.2	36.2	46.2	70.9	...	78.1	55.8
garnet	44.4	42.3	...	...	...	136.0	32.5
rudimentary	54.5	53.3	...	...	...	122.6	...
forked	56.7	56.0	41.5	66.5	...	89.0	...
Beadex	59.4	...	+	0.0	...	94.5	...
bobbed	66.0	66.7	on F	73.1	40.0	170.5	116.0

species has more than double the amount of crossing over found in the other tested species of the Sophophora, for the gene *abnormal,* which is not included in Table 42, lies at locus 163.6 (Kikkawa, 1938). It is not clear whether the genes controlling the amount of crossing over are species-specific, or if there is a general difference between the Sophophora and Drosophila. The situation in *ananassae* is unique among the species included, in that the X is a V-shaped chromosome, owing to a pericentric inversion. The large amount of crossing over may be due to this condition. The low amount of crossing over in the autosomes of *ananassae,* as contrasted with that in the autosomes of *virilis,* would support this hypothesis. In any case the amount of crossing over is under genetic control, as is the reduplica-

tion of the genes, as shown by the *sex ratio* abnormality discussed below.

It is obvious from Table 42 that a great many gene homologies exist in the gene systems of the equivalent chromosomes of these species. As *melanogaster* and *simulans* cross, allelism of genes assumed to be homologous can be tested directly, thus affording an independent test of the ability of the investigator to evaluate identity. The marked success in this case makes much more convincing the establishment of homologies based on mutational similarities in species which cannot be crossed. Even here different normal alleles cannot be detected.

Spencer (1949) presents evidence for a possible translocation difference involving elements C and F when *melanogaster* is compared with *hydei* and *virilis*. Such a case is most difficult to establish by mutant homologies because of mimic and modifier mutations. If it be true, the survival of such a translocation may be due to the fact that the small F element is more flexible in that it survives in aneuploid conditions, both hypoploid and hyperploid, much more readily than do the larger elements. The evidence on genetic divergence, or lack of it, presented by such analyses of similarities is important in our understanding of genetic divergence in evolution. It is unfortunate that it gives us information on loci that have similar mutant alleles, but no measure of the number of loci that are different or that have related normal alleles with different functions.

NATURAL SELECTION

Gene differences which have selective advantages under some of the several conditions that exist for natural populations have recently been demonstrated by Dobzhansky and several coworkers. Dobzhansky (1943) showed that different gene arrangements in a particular locality often changed markedly in relative frequency through a seasonal cycle, and that this cycle is repeated through several years. This type of cycle must be due to differences in selective value of each arrangement and its heterozygotes with other gene arrangements as the seasons changed. Dobzhansky demonstrated that the same gene arrangement had different properties at different localities. This indicated that different gene complexes have accumulated in different gene sequences, and even in the same sequences at different localities; consequently position effect as such does not explain the selective effects demonstrated in this material.

L'Héritier and Teissier (1933) and L'Héritier (1937) devised population cages which allowed fresh food to be added as needed to keep the population at a relatively constant level. Such cages were used by Wright and Dobzhansky (1946) to test the selective value of several gene arrangements in the third chromosome of *pseudoobscura*. The Standard, Chiricahua, and Arrowhead gene sequences were tested from flies collected at Piñon Flats, Mount San Jacinto, California. The results lead to the conclusions that the heterozygotes have selective advantage over the homozygotes, but that the selective advantages of the different combinations are not the same when tested at 25.0° C. On the other hand, at 16.5° C. no difference in selective advantage was demonstrated. Wright and Dobzhansky state that the selective values at 25.0° C., indicated by the method of least squares, are: .43 for ST/ST, .05 for AR/AR, .21 for CH/CH, 1.30 for ST/AR, 1.00 for ST/CH, and .71 for AR/CH. At this temperature equilibrium is indicated at 53 per cent Standard, 34 per cent Arrowhead, and 13 per cent Chiricahua.

Dobzhansky (1947 a) demonstrated that an equilibrium population of 70 per cent Standard and 30 per cent Chiricahua would be attained if a mixed population were placed in a cage and allowed to adjust above 20.0° C. The initial mixture could have Standard in excess or below the equilibrium 70 per cent. In such a population cage samples of eggs which were removed and allowed to develop under optimum conditions showed by a check of the salivary gland chromosomes that the population in the cage was essentially panmictic, as the several homozygous and heterozygous combinations occurred in the frequencies indicated by the Hardy-Weinberg formula. However, if adult males were taken from a crowded population cage, a significant excess was heterozygous, indicating that the homozygous classes had lower survival values under these conditions. Dobzhansky concluded that there was a differential mortality between the egg and the adult stages.

Dobzhansky (1947 b) reported the directional and seasonal changes in *pseudoobscura* populations from three localities about fifteen miles apart on Mount San Jacinto, California. These are: Keen Camp, at approximately forty-four hundred feet elevation on the northwestern shoulder of the mountain, in the ponderosa pine belt; Piñon Flats, at about thirty-nine hundred feet elevation on the desert slope, with a very different vegetation dominated by the

piñon pine; and Andreas Canyon, at eight hundred feet elevation on the edge of the desert at the foot of the mountain, with the palm, *Washingtonia filifera,* the most prominent plant. There were seasonal cycles influenced by natural selection in Andreas Canyon and Piñon Flats. Although Dobzhansky studied the seasonal variation for the years 1939 to 1946, he found no long-range trend. However, there was much less seasonal fluctation at Keen Camp and a definite trend with the Standard gene arrangement increasing at the expense of Arrowhead and perhaps Chiricahua. This is shown in Table 43. These are good examples of the changes in populations under the influence

TABLE 43

Percentage frequencies of the gene arrangements in different samples of Keen Camp: ST = Standard, AR = Arrowhead, CH = Chiricahua, TL = Tree Line (from Dobzhansky)

Month and Year	ST	AR	CH	TL	Number of Flies
April, 1939	32.5%	30.0%	32.5%	5.0%	40
May, 1939	32.6	28.5	36.2	2.7	298
June, 1939	24.8	30.8	41.2	3.2	718
July, 1939	28.6	29.3	37.3	4.8	566
August, 1939	29.5	30.5	37.6	2.4	210
September–October, 1939	26.0	35.7	35.1	3.2	154
Total, 1939	27.8%	30.4%	38.3%	3.5%	1,986
April, 1940	30.6%	22.8%	42.7%	3.9%	464
May, 1940	26.8	22.1	47.1	4.0	526
June, 1940	31.9	20.7	43.4	4.0	728
July, 1940	34.5	24.8	37.4	3.3	452
August, 1940	37.6	26.4	31.5	4.5	178
September, 1940	41.2	17.6	32.4	8.8	34
Total, 1940	31.5%	22.6%	41.9%	4.0%	2,382
May, 1941	29.0%	29.4%	37.9%	3.6%	248
June, 1941	38.3	17.6	39.3	4.8	290
July, 1941	35.4	27.2	33.5	3.8	158
September, 1941	38.2	25.0	32.4	4.4	68
Total, 1941	34.7%	24.1%	37.1%	4.1%	764
April, 1942	45.1%	17.6%	29.4%	7.8%	102
May, 1942	45.1	14.7	33.3	6.9	102
June, 1942	28.2	15.4	46.4	10.0	110
July, 1942	26.0	18.0	52.0	4.0	100
Total, 1942	36.0%	16.4%	40.3%	7.2%	414
April, 1945	41.0%	22.2%	29.2%	7.6%	288
April, 1946	52.0	15.5	23.7	8.8	400
June, 1946	48.0	15.0	32.5	4.5	400

of natural selection. The difference shown in the several localities might be due to differences either in selective forces or in genotypes available for selection.

Dobzhansky (1947 c) published additional data on the differential mortality during larval development of the homozygotes, ST/ST and CH/CH, as contrasted with their heterozygote, ST/CH, in crowded population cages. He also showed that the Standard and Arrowhead gene sequences from Piñon Flats established a different equilibrium from the same gene sequences from Mather. The Piñon Flats strains came to equilibrium with about 67 per cent Standard sequence and 33 per cent Arrowhead, while the Mather strains came to equilibrium frequencies with about 55 per cent Standard and 45 per cent Arrowhead. Again, cages tested at 16.5° C. did not show a differential effect in survival.

Dobzhansky (1948 a) reported on *pseudoobscura* and *persimilis* collected at ten different localities which represented a great range of altitudes and life zones of the Sierra Nevada in the Yosemite region. At the four lower locations, *pseudoobscura* was numerous and *persimilis* was not present in large numbers, while *pseudoobscura* did not occur with any frequency at altitudes above five thousand feet whereas *persimilis* was present. Dobzhansky draws several conclusions from the data. The Standard gene arrangement was most frequent at low elevations and decreased in relative frequency as one progressed to higher localities, while Arrowhead was more frequent at higher and decreased at lower altitudes. In certain populations, this gradient of selective value was indicated by an increase of Standard and decrease of Arrowhead as summer progressed, with a reversal during the winter. The population of *pseudoobscura* did not show seasonal changes at Jacksonville, the lowest station, but did at Lost Claim, Mather, and Aspen Valley. *Drosophila persimilis* did not show any consistent seasonal fluctuations. However, the Whitney arrangement was less frequent at lower than at higher levels.

Dobzhansky and Levene (1948) reexamined the available information on gene frequencies in wild populations of *pseudoobscura* and showed that there was an excess of the several heterozygous over the homozygous gene arrangements which could be accounted for only by the process of natural selection. Dobzhansky had demonstrated that there was a differential mortality in larval stages which reduced the number of homozygous adults. This differential mortality could

be tested for flies under natural conditions. The chromosomal constitution of the wild population had been sampled in two ways. First, the eggs from females fertilized in nature were allowed to develop under optimum conditions, so that no larval selection took place. Second, adult males collected from natural populations were crossed to known tester stock females, and the gene arrangements present in the males were determined. The flies from the first type of test showed that the populations were panmictic, as the several combinations occurred as predicted from the Hardy-Weinberg formula. The males in the second test proved to be heterozygous much too frequently, showing that natural selection had differentially reduced the number of homozygous individuals below that expected. These tests indicate that natural selection was affecting the gene frequencies in natural populations.

Dobzhansky (1948 b) published data which establish in some detail the fact that the same gene arrangements from different populations have different selective values. A series of the stocks having the different gene arrangements to be tested were collected in 1945 at Piñon Flats, Keen Camp, and Mather. These were tested in suitable combinations in population cages. In this series of experiments, Dobzhansky isolated a number of approximately homozygous strains of a particular gene sequence, mixed six to sixteen of these strains with a like set of mixed genotypes with another gene order. This procedure insured a heterozygosity most of the time for at least the more detrimental genes present in the original mixture. The cages were kept both in the light and in the dark.

In some experiments, Dobzhansky found again that there was no appreciable selective difference at 16.0° C. The comparative adaptive advantage of the homozygotes, where the heterozygote is considered to have unitary adaptive value, is shown in Table 44. Here the data are taken from previous publications, mixed old and new data, or only new data. One new situation developed. If only the Standard and the Tree Line sequences from Mather are mixed and allowed to compete in a population, the Standard replaces Tree Line, as it has a higher adaptive value than the heterozygote, while the homozygous Tree Line is semilethal. However, the Mather strains of Arrowhead and Tree Line give a very superior heterozygote, thus explaining the retention of Tree Line as a small fraction of that population. In all other cases tested, the heterozygotes exhibit sufficient heterosis to

keep any gene sequence present even if the homozygous form has an inferior selective value. Thus even in Mather we have Tree Line retained as part of the natural equilibrium population. It is the difference in selective values of the several heterozygous and homozygous gene sequences that cause these populations in some localities with particular genotypes to go through the seasonal cycles.

TABLE 44

Comparison of adaptive values (W) between chromosome pairs from different populations (from Dobzhansky)

Gene Arrangements	Piñon Old and New	Keen Camp New	Mather New
ST/ST	0.77 0.85	0.91	0.78
ST/CH	1.00 1.00	1.00	1.00
CH/CH	0.39 0.58	0.42	0.28
ST/ST	0.81	0.79	0.64
ST/AR	1.00	1.00	1.00
AR/AR	0.50	0.58	0.575
AR/AR	0.86	0.54	0.48
AR/CH	1.00	1.00	1.00
CH/CH	0.48	0.60	0.40
ST/ST			1.12
ST/TR			1.00
TR/TR			0.33
AR/AR			0.69
AR/TR			1.00
TR/TR			0.12

Heuts (1947, 1948) used some of these strains of *pseudoobscura* collected at Piñon Flats in 1946 to test the effect of certain environmental conditions as selective agents. In this test, about ten different strains of a particular gene sequence were mixed to reduce the incident of genes fixed by inbreeding, which might lower viability or fertility. He compared flies homozygous for the Arrowhead and for the Chiricahua sequence. The viability as hatchability of pupae was tested under different conditions of humidity by selecting young (light-colored) pupae and placing them in the several different humidities to hatch. Table 45 shows the differences, with Chiricahua superior at 100 per cent humidity and Arrowhead at 0 per cent humidity, while Standard survives better in the relatively high humidity. In testing the

survival of adults without food, the pupae were allowed to hatch at from 70 to 75 per cent humidity or at 100 per cent humidity. In the first experiment, Standard and Arrowhead adults lived longer at 100

TABLE 45

Relation between humidity and survival in *pseudoobscura* (from Heuts)

Percentage Humidity	Geno-type	First Experiment Pupae	Flies	%	Second Experiment Pupae	Flies	%	Totals Pupae	Flies	%
100	ST/ST	300	248	82.6	400	340	85.0	700	588	84.0
100	CH/CH	300	276	92.0	400	363	90.7	700	639	91.0
100	AR/AR	300	220	73.3	400	300	75.0	700	520	74.5
92	ST/ST	300	278	92.6	300	284	94.6	600	562	93.6
92	CH/CH	300	233	77.6	300	258	86.0	600	491	81.8
92	AR/AR	300	256	82.0	300	252	84.0	600	508	84.6
76	ST/ST	500	419	83.2	300	262	87.3	800	681	85.1
76	CH/CH	500	376	75.2	300	239	79.2	800	615	76.9
76	AR/AR	300	265	88.3	300	250	83.3	600	511	85.1
56	ST/ST	...	...	...	300	242	80.6	300	242	80.6
56	AR/AR	...	...	...	200	168	84.0	200	168	84.0
0	ST/ST	300	122	40.8	700	468	66.9	1,000	590	59.0
0	CH/CH	300	94	31.3	700	446	63.7	1,000	540	54.0
0	AR/AR	300	215	71.0	700	526	75.1	1,000	741	74.1

per cent humidity and at 76 per cent humidity, and Arrowhead at 0 per cent humidity. In the second experiment, Chiricahua lived slightly longer at 100 per cent and 76 per cent humidity, but Arrowhead insignificantly longer at 0 per cent humidity. The 0 per cent humidity reduced the survival time by about one half in each case.

Heuts also tested the survival of flies homozygous for Standard and Chiricahua and their heterozygote. The survival differed as follows:

Chromosome type	Survival in Days: At 28° to 30° C. Average longevity in days	At 0° to 4° C. Average longevity in weeks
ST/ST	26.39 ± 0.25	15.71 ± 0.14
ST/CH	23.45 ± 0.28	17.24 ± 0.18
CH/CH	21.63 ± 0.14	14.05 ± 0.16

At the higher temperature Standard was superior to the heterozygote, which in turn was superior to Chiricahua. At 0° to 4° C. the heterozygote is superior to both homozygotes, and Standard is again better than Chiricahua. The experiments on humidity are in agreement with the distributional data, for Arrowhead is the usual gene arrangement found in the drier areas of Arizona, Utah, and New Mexico.

Wallace (1948) reported a number of experiments to determine the adaptive value of the *sex ratio* character in *pseudoobscura.* This is a very interesting factor or factors located in the D element of the compound X chromosome. Most or all the offspring of a male which carries it are females, without any egg mortality such as would result from a lethal. Sturtevant and Dobzhansky (1936 b) reported that, in the *sex ratio* male of *pseudoobscura,* the Y chromosome is not ordinarily included on the spindle. The X chromosome appears as a four-partite structure at the first metaphase and undergoes two equatorial divisions. Presumably the X chromosome reduplicates itself an extra time, as compared to the autosomes, thus indicating genetic control of specific chromosome reduplication. This or an equivalent factor is present also in *persimilis, athabasca,* and *azteca.* Gershenson (1928) reported a similar factor in an European member of the obscura group, *Drosophila obscura* 2 in Sturtevant's classification, but the species concerned is not certainly identified. Sturtevant and Novitski (1941 b) also report *sex ratio* in *melanica,* and from this infer that element D is fused to A to make the V-shaped X of that species.

In *pseudoobscura,* the male with *sex ratio* in his X chromosome produces from 6.2 per cent male offspring at 25.0° C. to 1.2 per cent at 16.0° C. (Darlington and Dobzhansky, 1942). Consequently, practically all the offspring receive the *sex ratio* X chromosome from a mutant male, while only half receive the normal X from a male without this factor. If no other effect resulted from this factor, it should rapidly replace its normal allele in a population up to the limits set by sexual reproduction in this species. The distribution data do not show whether *sex ratio* is or is not increasing in frequency in the population. In order to test the selective value of *sex ratio* in both males and females, Wallace set up four population cages, beginning with a thousand females, five hundred normal males, and five hundred *sex ratio* males. In two of the cages, half the females were homozygous and half heterozygous for *sex ratio,* while in the other two half the females were heterozygous and half carried only a normal X chromosome. One of each type of cage was placed at 16.5° C. and the other at 25.0° C. In seven months, *sex ratio* had disappeared from the cages at 25.0° C. (none in two hundred and thirty chromosomes examined). In thirteen months, at 16.5° C., about 92 to 94 per cent of the chromosomes were standard, so that *sex ratio* had decreased markedly in frequency but had not been eliminated. Both males and especially

homozygous females are selected against in larval competition in the population. Females are at considerable disadvantage in longevity tests, especially at 25.0° C., although males are apparently at a slight advantage. It also appears from these results that the females homozygous for *sex ratio* are less fecund than the normal, although the heterozygotes are more fecund than either. The *sex ratio* males fertilized fewer females than normal males at both temperatures tested. When the three types of females were mated by normal males, the egg hatch showed a differential as follows: at 25.0° C., sr/sr = 20.8%, sr/+ =88.9%, +/+ = 53.7%; at 16.5° C., sr/sr = 72.2%, sr/+ = 71.07%, and +/+ = 68.3%. The homozygous *sex ratio* females are at an obvious disadvantage at the higher temperature.

It is not clear whether *sex ratio* is due to one gene or several. The factor or factors lie near the middle of the XR arm (element D) in *pseudoobscura* and *persimilis*. There are three inversion present in the *sex ratio* X of *pseudoobscura*. Wallace showed that the distal inversion is not involved in the effect. The *sex ratio* factor or factors are never separated from the two proximal inversions, which always occur together. The standard sequence in the *pseudoobscura* X and the standard sequence in the *persimilis* X differ by a single inversion. However, the *sex ratio* strain of *persimilis* had the same gene arrangement as the standard sequence in *pseudoobscura*. It is not clear whether the *sex ratio* factor had a single common ancestry in these two species, although this is probable. However, it is very improbable that it is due to a position effect, since the inversions which characterize these strains in the two species have breakage points some little distance apart.

It is clear, that under some experimental conditions, the reproductive efficiency of the *sex ratio* factor in *pseudoobscura* is at a considerable selective disadvantage, particularly at the higher temperature tested. This does not explain the natural distribution of this X chromosome, for the highest frequency is found in several very hot regions, whereas the coolest part of the range of *pseudoobscura* seems to lack this factor.

Dobzhansky (1949) reviewed certain of the earlier natural selection experiments and reported addition tests. He crossed Standard from Mather and Chiricahua from Piñon Flats, and established a population cage from the heterozygotes. He examined the chromosome complexes of 200 F_2 flies from this cage and found 66

ST/ST, 106 ST/CH, and 28 CH/CH, instead of the 1:2:1 ratio expected, and concluded that the gene sequences from different localities had not been coadapted, as the heterozygote is less fit than the Standard homozygote. It is quite probable in large breeding populations such as *pseudoobscura* that genes are selected into the population which cause heterosis. The published data do not prove this, as Standard will replace Tree Line if only those two arrangements, both from Mather, are placed in a population cage.

The experiments by Koopman (1949) are of considerable interest. Mayr and Dobzhansky (1945) had found that sexual isolation is weaker between *pseudoobscura* and *persimilis* at 16.0° C. Koopman introduced *pseudoobscura* and *persimilis,* each marked by suitable genes, into a population cage at 16.0° C. and discarded the hybrids each generation, although the hybrids actually affect the breeding population very little. After a series of generations, the strains of the two species were tested and gave much higher sexual isolation from each other at this temperature. Koopman assumes that this is due to a form of natural selection against flies that hybridized readily, on the premise that they left fewer offspring with their own species than those which were more reluctant to cross mate.

Dubinin and Tiniakov (1945, 1946a, 1946b, 1946c, 1947) have published some very interesting facts on inversions in populations of *funebris.* Unlike *pseudoobscura,* which is never urban, *funebris* occurs in both rural and urban areas. There are several different inversions included in these studies, II-1, II-2, II-3, III-1, and IV-1. The inversions are very common in Moscow and other urban areas, but rare or absent in the country. Dubinin and Tiniakov (1946b) report a seasonal increase in inversions from March to September in Moscow. The most striking change was from 13.48 per cent to 50.84 per cent in one area. As contrasted to this, the frequency in a village a hundred and fifteen kilometers southeast of Moscow, did not vary appreciably from the average of 1.26 per cent.

Dubinin and Tiniakov (1946a) studied the survival during winter hibernation of samples of from one thousand to five thousand flies, placed in boxes in a cellar at temperatures varying from —2° C. to +3° C. Under these conditions selection was very stringent, for only 6.80 per cent survived one month, 2.5 per cent survived 1.5 months, and 0.56 per cent survived 2.5 months. The inversions decreased in frequency over the winter. In order to test this effect, sets of homo-

zygous normal, homozygous II-1, and their heterozygotes were placed under hibernating conditions. Table 46 shows that, under these conditions, the normal homozygote was superior to both the heterozygous and homozygous inversion. They judged that the detrimental effects of the inversion were dominant, as both the heterozygotes and homozygous inversions were reduced in viability.

An even more remarkable finding reported by these authors is that the fecundity of females that survived hibernation is superior to that of the general population and that, since it is inherited by their daughters, this fecundity represents a genetic character. In this case the females surviving hibernation were mated to fresh males, for the sperm stored in their receptacles became nonfunctional during hibernation. Their eggs were counted over a ten-day period; and the egg hatch from a set of control females, which had not come from a hibernating population, was tested at the same time. Those that survived hibernation averaged 55.7 eggs per day, and the control 42.3 eggs per day. The daughters of hibernated females averaged 64.2 eggs per day, and their control 54.6. Females, which must have been at least a hundred and eighty days old when caught, were collected

TABLE 46

Survival of different karyotypes of *funebris* under conditions of hibernation (from Dubinin and Tiniakov)

Days of Hibernation	Types	Initial Number	Number Survived	Percentage Survived
15	+/+	2,000	593	24.6%
30	+/+	3,092	367	11.2
45	+/+	1,092	12	1.1
15	+/I	2,000	285	14.2
30	+/I	2,318	94	4.1
45	+/I	1,483	1	0.1
15	I/I	1,847	270	14.6
30	I/I	3,483	147	4.2
45	I/I	1,471	3	0.2

in the Botanical Gardens and similarly tested. They proved superior to control females. Since these tests were run over a ten-day period and the test animals were always superior to the controls, these conclusions seem valid. This is a good example of the action of natural selection to increase the fitness of the organism by differential mor-

tality, which left only the more vigorous organism to replace the population.

Dubinin and Tiniakov (1946b) reported varying concentrations of inversion heterozygotes in the populations in Moscow, ranging from 16.74 to 88.54 per cent. Again, in the rural areas around Moscow, the inversion frequency was about 1.5 per cent. The frequency of inversions in Ivanovo was 37.23 per cent, but none was found in the rural areas around it. Dubinin and Tiniakov (1946c) reported the effect on the local population at the Biological Station at Kropotovo of the release early in June of approximately one hundred thousand *funebris* homozygous for inversion II–1. The local *funebris* population had only about 0.70 per cent heterozygotes for this inversion, as checked before the experiment. Table 47 shows that the populations had bred to establish the Hardy-Weinberg equilibrium frequency in July. However, selection acted against the inversion homozygote as well as migration. In this table q is the frequency of the inversion. As stated above, in July the population showed an approximate equilibrium population $q\mathrm{I} + (1 - q)\mathrm{N}$. All samples after July indicated an excess of heterzygotes, with a small deficiency of normal and a large deficiency of inversion homozygotes. The deficiency of normal homozygotes indicated that natural selection against the inversion was affecting the frequencies of the genotypes, rather than migration by itself. In this case the heterosis of the heterozygote was not sufficient to retain the inversion in high concentration in this rural locality.

Dubinin and Tiniakov (1947) reported the general distribution of the several inversions studied. There is a definite gradient of concentration of inversions from the rural into the urban areas, and apparently the heavily industrialized cities have an even greater concentration. For example, in the central portion of Moscow, 88.1 per cent of the flies were heterozygous for inversions; in the next zone, "Circle B," 55.5 per cent were heterozygous for inversions; in the third zone, which grades into the rural, the region of the Circuit Railway, only 12.1 per cent of the flies were heterozygous for inversions; in the fourth zone, including suburban villages twenty to two hundred kilometers from Moscow, only 1.8 per cent carried inversions; finally, in the fifth zone, two hundred to five hundred kilometers north of Moscow, no inversions were found. In comparing Moscow with other cities, it is obvious that the smaller places have a lower frequency of inversions. The several frequencies for inversion heterozygotes men-

TABLE 47

Observed and expected numbers of inversion and standard homozygotes and heterozygotes found in different collecting localities in different months (from Dubinin and Tiniakov)

Locality and Month	Standard Homozygotes	Hetero-zygotes	Inversion Homo-zygotes	q	Flies Examined
Biological Station, July	Obs. 29 Exp. 28.5	57 57	28 28.5	49.5	114
Biological Station, Aug.	Obs. 70 Exp. 72.8	52 46	4 7.2	23.8	126
Biological Station, Sept.	Obs. 144 Exp. 150	73 61.5	1 6.4	17.2	218
Biological Station, Oct.	Obs. 187 Exp. 187.4	43 41.3	1 2.2	9.7	231
Near Kropotovo, July	Obs. 70 Exp. 73.8	55 47.6	4 7.6	24.4	129
Near Kropotovo, Aug.	Obs. 80 Exp. 82.8	63 57.3	7 9.9	25.7	150
Near Kropotovo, Sept.	Obs. 10 Exp. 10.8	5 3.9	0 0.3	16.7	15
Far Kropotovo, July	Obs. 52 Exp. 51.8	16 16.8	2 1.4	14.3	70
Far Kropotovo, Aug.	Obs. 88 Exp. 89	26 24.2	1 1.7	12.2	115
Besovo, August	Obs. 37 Exp. 37.3	9 8.2	0 0.5	9.8	46
Besovo, September	Obs. 67 Exp. 68.2	4 2.8	0 0.05	2.8	71
Maloe Kropotovo, Aug.	Obs. 12 Exp. 12.5	5 4.2	0 0.4	14.7	17
Maloe Kropotovo, Sept.	Obs. 107 Exp. 109.1	8 5.8	0 0.1	3.5	115

tioned are: Saratov, 53.9 per cent; Ivanovo, 37.2 per cent; Erivan, 21.2 per cent; the towns of Noginsk and Michurinsk, 12.1 per cent and 8.7 per cent respectively; Voronezh, destroyed by war, 0.9 per cent as in rural areas; the gardenlike city of Alma-Ata in Turkestan, 1.48 per cent. The inversions themselves differ in their comparative frequency in city and country. In the Moscow region, the relative frequencies of the several inversions, are: II–1, urban = 55.4%, rural =

0.9%; II–2, urban = 19.2%, rural = 0.15%, II–3, both urban and rural = 0.45%; III–1, urban = 0.48%, rural = 0; IV–1, urban = 11.5%, rural = 0.05%. There is a north-south gradient for II–1 which is common in the north, where it is 80 per cent of all inversions present, while it falls off to 1.3 per cent of the inversions in Erivan. II–2 has the opposite distribution, for it is only 3 per cent of the inversions at Ivanovo in the north but 95 per cent of those found at Erivan in the south. These data and the other information presented clearly indicate that we have here the survival of different genotypes, marked by inversion, in different ecological niches.

GENIC BALANCE

Genic balance is directly involved in many of the phenomena of evolution. The theory that evolution occurs through the accumulation of gene mutations holds that an organism is an integrated and coordinated system and that, when enough mutant alleles have been substituted to form a new and different balanced system, a new species results. In this case we have a substitution of a number of genes to change one type of complex coordinated differentiation to another and different integrated system. The slow course of evolution is in part due to the fact that, not only must the initial and final gene systems work, but that the intermediate steps must also be functional. Goldschmidt's very different views on the mechanism of evolution will be discussed later. This concept implies that an allele beneficial in one system may be neutral or detrimental in another. The special adaptions of a species are ordinarily of this gene complex type, involving such differences as food requirement, sexual isolation, and hybrid sterility.

Bridges (1939) summarized his concept of genic balance as the result of the joint action of the entire complement of genes on the character and features of the adults. Some genes tend to drive development in a particular direction; others oppose this; still others act as plus or minus modifiers. Bridges did not believe that these genes were symmetrically distributed in the chromosomes, for aneuploids are abnormal in Drosophila and other forms. The relation between genic balance and sex determination has been studied extensively in *melanogaster*. Morgan and Bridges (1919) reviewed the information on sex determination in *melanogaster* obtained from gynandromorphs. Most

of these were mixtures of male and female tissues resulting from the loss of one of the X chromosomes from the cell(s) originating the male tissue. It was obvious from the work of these authors that sex differentiation of even individual cells was autonomous, determined by the male or female genotype of the chromosomes present. The Y chromosome does not influence sex determination in *melanogaster,* although it is necessary for male fertility.

Patterson (1931a, 1933) first demonstrated X-ray-induced modifications, and Patterson and Stone (1938) reviewed their work on the production of gynandromorphs, especially X-chromosome aneuploid types. Some gynandromorphs were reported in which the male tissue had one X plus a fragment of a second X. Certain cases included as the extra fragment either the left-hand third of the X (from *yellow* through *miniature* in one case) or the right-hand third (from *forked* through the centromere in several cases). When the middle section (the *garnet-pleated* region) was included in the fragment of the X, the aberrant tissue was female. In none of these cases was the hyperploid male tissue intersexual, so far as could be determined. Bonnier, Luning, and Perje (1949) reported their study of a number of gynanders and other aberrant mosaics. They did not report the presence of intersexual characters in these cases. Bridges (1922, 1925, 1939) reported triploid and tetraploid *melanogaster* females and the heteroploid offspring that survived. Those combinations with either two or three sets of both chromosomes 2 and 3 survived; those with two sets of one but three of the other were inviable. The dot chromosome 4 could be present two, three, or four times and the organism was viable, although some combinations were phenotypically aberrant. The progeny of triploids gave certain important information about sex determination. Bridges showed that an individual was not male if it had only one X or female if it had two X chromosomes, but rather that the sex of the individual was determined by a balanced gene system, so that 1X:2A (2A = two sets of autosomes) was male, but so was 2X:4A; while 2X:2A, 3X:3A, and 4X:4A had a different X:A balance and were females. Other combinations were more aberrant and 2X:3A were intersexes rather than females. Dobzhansky and Schultz (1934) and Pipkin (1940, 1947) extended the analysis of triploid combination to aneuploids, both for the X and the autosomes. Dobzhansky and Schultz showed that the addition of an X-chromosome fragment made a 2X + F:3A more nearly female; the

intersex type was shifted in the direction of femaleness, and fewer male characteristics were present. Pipkin (1940) proved that 2 1/2 X (either the left or right half of the X): 3A individuals were females rather than intersexes. On the other hand, all 1X + F:3A individuals were malelike, even though in two different tests the fragments added included about seven-eighths of the X. Pipkin (1947) tested the effect of small sections of the 2 chromosome on sex differentiation of both 2X:3A and 3X:3A individuals. No small section of the 2 chromosome, either hyperploid or hypoploid, shifted the sex of the aneuploid intersexes or aneuploid triploid females. Small sectors which together included the whole length of the 2 chromosome were tested in this fashion, showing that no region of this chromosome affects sex determination under these conditions. The *americana* triploids and intersexes (Stalker, 1942a) are discussed in Chapter 10.

In contrast to these triploid complexes, gynandromorphs showed no intersexual character. Furthermore, Patterson, Stone, and Bedichek (1935, 1937), Patterson (1938), and Crow (1946), using the series of X-chromosome translocations produced by Patterson, Stone, Bedichek, and Suche (1934), have shown that diploid aneuploid combinations which survive do not show intersexual characteristics. In diploids no hyperploid male or hypoploid female has been shown to have intersexual characteristics, although many types are phenotypically abnormal. All of the X chromosome has been tested in small sectors as duplications in hyperploid males, using a series of chromosomal rearrangements which allowed a test of each fragment of the X. Although some were sterile, none showed a shift toward femaleness. There is no single gene in the X or small cluster of genes as tested which will shift the developmental pattern so that females will develop when the remainder of the X is haploid and the autosomes diploid. Hypoploid females survived for only four of the sectors of the X as tested for regions between the breaks of the rearrangements. The genic unbalance seemed most serious in this type of aneuploid, although no surviving hypoploid females were intersexual or malelike.

Patterson, Brown, and Stone (1940), Burdette (1940), and Cumley (1940) have investigated the effect of aneuploidy of the autosomes both in hypoploids, hyperploids, and homozygous hyperploids (i.e., having the sector present four times when other regions were diploid). No autosomal aneuploid showed a change in any sexual character, although many were abnormal.

A very important development from Burdette's tests was the demonstration of the selection factor in genic balance. He showed that six out of seven tested regions, each roughly one-eighth of the X, survived in the homozygous condition (i.e., present four times) when the other genes in these hyperdiploids were present twice. Only one out of nine regions tested of comparable size in the autosomes was able to survive in this doubled duplicated condition. The greater flexibility of the X must have developed from the fact that the X normally alternates in frequency, whereas the autosomes are ordinarily present twice. The correlated activities of the genes are thus adjusted by the agency of selection in the establishment of the different balanced gene systems.

The balance controlling developmental correlation is so well coordinated that no intersexual aneuploids occur in diploids. The balance is more labile in triploid forms if a minimum of 2X chromosomes is present, for the extra fragments add to femaleness and reduce the intersexual character. This lability is further illustrated by the fact that those hypointersexes having 2X — F:3A which survive are strongly shifted so they are malelike instead of intersexual. In the more labile triploid derivatives, the shift in sex type caused by changes in the amount of X chromosome present, both 2X + F:3A and 2X — F:3A are in decided contrast to the lack of effect of such a shift in diploids. The flexibility of the genic balance involving X-chromosome balance is obvious from the fact that all the hyperintersexes and hypotriploids survive with either right-or left-hand fragment of any of a series of translocations tested. This flexibility is not shared by the autosomes, where only small sections of the 2 chromosome may be missing in hypoploids (Pipkin, 1947).

These triploids, and especially the aneuploid classes, illustrate another facet in the divergence between forms. Many of them are phenotypically different from normal. In some cases, this difference is extreme; in others, it is similar in effect to certain of the mutants that occur in the species. Thus the *normal* genes under unusual conditions of gene interaction can lead to changes in phenotype. The argument that mutations such as those commonly studied are only abnormalities is fallacious, for this would have to be extended to normal genes which can give similar effects in the different combinations. In fact, if the characteristics of two distinct species are compared individually, the normal character in one species would often be abnormal were it present by itself in the other.

There are several known cases where a single mutation disrupts to a serious degree the normal pattern of development. Those that interfere with normal sex determination illustrate how the substitution of one gene by a different allele can modify a process under the control of a large number of integrated gene controlled reactions. Lebedeff (1934, 1938, 1939) showed that *intersex, ix,* a recessive 3 chromosome (element D) gene in *virilis,* modified development. The XY:2A males are not affected by *ix* or its modifiers, but 2X:2A females may be intersexes, sterile males, or hermaphroditic forms when *ix* is homozygous, depending on the modifier genes present.

Newby (1942) studied another *virilis* mutant, the dominant 2 chromosome (E element) gene *Intersex*^{B}, *Ix*^{B}, found by Mr. Elwood Briles in a stock from Blanco, Texas. In this case also the XY:2A males are normal, but 2X:2A *Ix*^{B} individuals are intersexes rather than females. The effect of this gene homozygous is unknown, but Price (1949a) showed that it was close to *brick* (locus 248.0) by inducing crossing over in heterozygous males by X ray, using the method devised and first used by Patterson and Suche (1933, 1934).

Several genes have been detected in *melanogaster* which modify sex determination. Morgan, Redfield, and Morgan (1943) reported one in the 2 chromosome, Sturtevant (1945) reported another in 3 L (element D), and Gowen (1948) found several different genes which produce a modification of the usual sex system. For example, two of those reported by Gowen add male reproductive systems to the female, thus modifying the system toward a hermaphroditic condition. Another gene on the 3 chromosome reacts to cause death in X-bearing eggs. Here progenies of any female receiving this gene from their male parent are all male. There is another gene (or genes) on the 2 chromosome which is lethal to diploid females, with a greater effect in the homozygous than in the heterozygous condition. Gowen and Nelson (1942) have reported a case where females carrying a certain gene had only male progeny, no matter what kind of male they mated—a case somewhat similar to *grandchildless* (Spurway, 1948). Dobzhansky and Spassky (1941) found a dominant mutant in *pseudoobscura* which changed chromosomal (2X:2A) females into intersexes. Such mutations are known for a number of species, and in each case affects chromosomal females.

Goldschmidt (1948) found that *Beaded* on the 3 chromosome of *melanogaster* caused part of the chromosomal males (XY:2A) to be intersexual when present with several different *Minutes.* The *Minute*

classes of aberrations are lethal homozygous and are often, but not in all cases, demonstrable as deficiencies. Sturtevant (1949) has disagreed with this interpretation and suggested that these flies are abnormal but not intersexual. Goldschmidt (1949a,b,c) has vigorously upheld his interpretation. These cases represent mutations that are lethal or semilethal in one genetic system, or cause abnormal sex development in one gene balance without affecting the alternative system. We will not discuss further the mechanism and implications of sex determination. This has been adequately reviewed by Goldschmidt (1934, 1949), Patterson (1938), Stone (1942), and Seiler (1949). These cases illustrate how drastically a single mutant can alter the normal course of development, often shifting from one toward the other of the male or female developmental systems.

One case mentioned above allows some further analysis of genic balance. Sturtevant (1945) tested several mutants which show phenotypic differences in males and females, using the gene *transformer* (*tra*) which transforms homozygous XX:2A individuals (chromosomal females) into phenotypic males. The testis size is variable, but smaller than normal; and these transformed males are sterile, even with a Y chromosome present. One interesting point is that an XXX:2A *tra/tra* individual was slightly less male than the XX:2A *tra/tra* genotypes, indicating that the extra X did interfere very slightly with the action of *tra*. Triploids homozygous for *tra* are similar to diploids. Three genes showing sexual dimorphism were compared in females, males, and transformed males (XX:2A *tra/tra*). The mutant *scute*^{3} which is lethal in the female but viable in the male was also lethal in transformed males (2X:2A, *tra/tra*). Furthermore, transformed males resembled their sisters in phenotype for *eosin,* which is paler and more yellowish in the male, and for *facet,* which is more extreme in the male. These genes acted in relation to X-chromosome gene frequencies rather than sex.

Muller (1932) has studied the problem of gene frequency and gene action using the *white* alleles, *eosin* and *apricot*. Only *eosin* shows sexual dimorphism, for while the eyes of *eosin* females are darker than those of *eosin* males, *apricot* females and males have eyes more nearly the same color. Muller obtained a deleted fragment of the X chromosome carrying the gene *eosin* and added this fragment both to the male and female genotypes. In each case the color darkened. In similar experiments with *apricot* in another fragment, both hyper-

ploid males and females had darker eyes. The chromosomal normal *apricot* male and female eyes were alike due to dosage compensation balance in the X. Although the results found for *eosin* were alike in the two experiments, Sturtevant did not report tests of *apricot*. Muller (1950), in a long discussion of dosage compensation of genes in the X chromosome, reported that he and Lieb tested *apricot, scute, forked,* and *Bar* in 2X:2A *transformer* males and found gene compensation as in females; hence gene compensation is not related to the sex of the organism as such. This type of information, together with the marked difference in the several aneuploid X chromosome combinations, demonstrated by Patterson, Stone, and Bedichek (1937), show that there is a marked internal balance of X-chromsome genes. Another important example of gene balance combinations necessary for survival is the X-chromosome female viability gene demonstrated by Patterson (1932a).

POSITION EFFECT

Goldschmidt (1940) has ascribed much importance to position effect as a factor in evolution. Position effect was first worked out by Sturtevant (1925), who proved that *Bar* was due to a position effect or gene association effect. In this case *Bar* changed to + and *BB,* by unequal crossing over. Bridges (1936), Muller (1936), and Muller, Prokofyeva-Belgovskaya, and Kossikov (1936) showed the *B* was due to a duplication, a repeat in place, and that *BB* was due to a triplication, in agreement with Sturtevant's genetic evidence. Since then a number of other cases of *Bar* effect have been found, Bridges and Brehme (1944) list both mutations and reversions. Sutton (1943) analyzed and reviewed the information on this case and concluded that the *Bar* locus was included in bands 16A1.2 (Bridges' 1935 map of the chromosomes in salivary gland nuclei). All known *Bar* effects were due to changed associations of these genes. Several different loci from different chromosomes induce a *Bar* effect if brought into contact with this 16A1.2 region. *Bar* position effects may be either increased or modified partially or all the way to normal by further changing the association. Also, mutation in one or the other component of the gene association may decrease the effect, in some cases all the way to normal. Deficiencies of the *Bar* locus have no phenotypic effect in the heterozygous female, and no point mutation giving a *Bar* effect is known.

A large number of rearrangements associated with change in phenotype are known in *melanogaster,* as well as a few in *pseudoobscura* and *virilis.* Some of these are very small deletions associated with the rearrangements; others are true position effects. The only critical way to differentiate between the two is by further mutation or by changing anew the association and determining how the genes then function. Muller (1930) reported on several "eversporting" mutants in *melanogaster* and pointed out that these cases all involved rearrangements at or near the eversporting locus. The position effects of unstable mutations are connected with rearrangements.

Unstable genes with high germinal or somatic mutation rates are known in a number of forms. Demerec (1941) reviewed several cases he had studied in *virilis* where the unstable genes were not connected with rearrangements. He showed that both somatic and germinal stability or mutability of a gene could vary just as that of eversporting displacements. On the other hand, these cases all involve instability of a single locus. Indeed, several of Muller's (1930) cases, as well as those discussed by Schulz (1936) and by Demerec (1940), had shown that euchromatic segments inserted into heterochromatic regions may exhibit a marked instability of a number (but not necessarily all) of the euchromatic genes near the point of rearrangement. Position effect caused multiple instabilities in several cases that involve rearrangement of heterochromatic material.

Not all position effect is connected with the heterochromatin. Although not many cases can be tested by crossing over as was the *Bar* case, Griffen and Stone (1940) showed that, by irradiation of a *white* mottled, the *white* locus (3C2 on Bridges', 1935, map) could be shifted to numerous positions in the chromosome system. In some of these positions the gene gave a normal phenotype, but in others it gave mottling. Two seemingly contradictory facts are known about the relation between position effect and heterochromatin: genes moved near heterochromatic regions sometimes become eversporting, but an extra Y chromosome suppresses variegation of the *white* locus in *melanogaster* (Gowen and Gay, 1934). Schulz (1936) and others have shown that the suppression effect on variegation is the same for other such loci; that variegation is more drastic in XO males and suppressed to some degree by extra Y chromosome(s), as well as by heterochromatic regions of other chromosomes. However, Girvin

(1949) could not detect such a suppression by the Y chromosome of the *yellow* mottling associated with a translocation in *virilis*. Whatever hypothesis or hypotheses to explain variegation should prove correct, and none is now satisfactory, the concentration of some substance or substances controlled by the Y chromosome must be important in the cellular chemistry of the gene activity. Stern (1948) has reviewed briefly his and other work on the *cubitus interruptus* position effect. Lewis (1950) has reviewed the subject of position effect in Drosophila. The remarkable cases reported by McClintock (1950) in maize indicate that position effect may be of general importance in organisms.

Goldschmidt (1944, 1946) reviewed and extended his theories on the nature of the chromosome and of mutation. He prefers the term "rearrangement effect" to position effect, as he does not consider that the evidence for localized particular genes is satisfactory. He reviews the evidence which to him indicates that many mutations can be caused by different changes in a localized region, rather than at one place. Goldschmidt proposes that all point mutations are very local rearrangements, too small to be detectable even in the salivary gland chromosomes. In addition, there are the large visible rearrangements which may be of major importance. He believes there are different types of activity of the chromosome as a unitary system, both local, regional, and chromosome as a whole in ascending importance. Most changes so far studied by the geneticists he considers are the result of such structural shifts. Goldschmidt (1946) does mention the possibility of occasional chemical changes which would have more far reaching and decisive effect.

We are in decided disagreement with this hypothesis of Goldschmidt's, particularly with his opinion that the normal genes in ordinary genetic terms and genic balance, in the general sense as we have described it, are not real but part of the action of the whole chromosome. His hypotheses do point out the weakness of our analyses of position effect, but this does not seem the place to review the question extensively. Without regard to the merits of the several views of the mechanisms of evolution, it suffices here to point out that chromosomal variability is plentiful, as shown in Chapters 4 and 5, so that position effect may be involved in some species differences.

HYBRIDIZATION TESTS

The detailed information from crossing different forms will be presented in later chapters. Certain examples will be given here to illustrate general principles. A number of cases illustrate that species and subspecies differences involve genic balance. This is very well demonstrated in the *azteca-athabasca* crosses. Sturtevant and Dobzhansky (1936 a) showed that these two similar species cross to give nearly normal female and abnormal male hybrids. The cross *azteca* ♀ × *athabasca* ♂ gives F_1 males that are larger than their sisters or parents, with large wings. The reciprocal cross produces F_1 males that are dwarfs, with relatively small wings. All hybrids are sterile. This is a case of X-autosome unbalance. The F_1 females with an X and autosomal set from each species are nearly normal. The reciprocal types of abnormal male hybrids each show the unbalance of one X with a heterozygous set of foreign autosomes.

The obscura group has several other interesting examples of genic balance change. Judged by *subobscura,* the basic chromosome pattern is X, Y, four pairs of large rod-shaped autosomes (elements B, C, D, E), and a pair of dots (element F). Philip, Rendel, Spurway, and Haldane (1944) report a very interesting trisomic condition for one of the large autosomes. They found three such trisomic males, of which two were normal and one only slightly abnormal in phenotype. Such a trisomic condition for a large autosomal element is not known in other species, except in *americana* (Chapter 10). This lability may account for the fact that the American representatives of this species group have element D fused to element A. Furthermore, so far as is known, the D element of this V-shaped X chromosome is haploid in the male as is the primitive X, the A element. The Y may carry the normal alleles of several genes, but this has not been demonstrated. Thus Krivshenko's (1949, 1950, and earlier) evidence that the Y of *busckii* contains a section necessary to viability of the males, together with mutations in this section, is the only case where the Y is known to carry several genes other than the normal allele of *bobbed* and male fertility factors. The European *subobscura* is not known to produce hybrids with any of the obscura complex having this A-D fusion, so no further investigation of this genic balance system is possible.

The cross of the species *pseudoobscura* and *miranda* does give addi-

tional information. Dobzhansky (1935 b, 1937 a) showed that *miranda* has a complex X-Y chromosome system. In addition to the A-D fusion of the related forms, there is an X_2 which is equivalent to the third chromosome (element C).

Dobzhansky (1937 a) and Koller (1939) investigated the problem of segregation of these elements at meiosis and suggested some form of determinate disjunction causing the Y to separate regularly from X_1 and, despite lack of association, X_2 goes to the same pole with X_1, so that all sperm were X_1 plus X_2, or Y. They give no explanation for this segregation. Cooper (1946) showed that a trivalent was formed regularly at the first division of meiosis and that the Y segregates to one pole and the $X_1 + X_2$ to the other in from 97 to 99 per cent of the cases without chiasma formation. MacKnight (1939) had shown that the Y chromosome of *miranda* has segments of euchromatic material homologous to parts of X_2 (element C). There had occurred so many chromosomal rearrangements with this Y-C element during the evolution of *miranda* from the ancestral translocation form that only very limited synapsis could occur. Parts of the Y are homologous to both the X_1 and X_2, thus explaining the regular synapsis and segregation of these chromosomes.

The regular functioning of this multiple sex chromosome mechanism suggests plausible explanations for other unchecked cases of the regular disjunction of multiple sex chromosomes. The most remarkable feature of *Drosophila miranda* is the change in genic balance. If we begin with the *subobscura* chromosome type with an XY pair, four autosome pairs, and a pair of dots as our primitive type, roughly one-fifth of the genes in element A are sex-linked and haploid in the male. In the *pseudoobscura* type, roughly two-fifths of the chromosomes are male haploid, the fused A-D elements. Finally, in *miranda,* nearly three-fifths (elements A, D, and C for the most part) fall into this category. It cannot be determined whether the male haploid system in Hymenoptera originated in this way, but certainly *miranda* is nearly halfway between the primitive sex determining chromosome complex for Drosophila and a Hymenopteralike system.

The reciprocal hybrids between *pseudoobscura* and *miranda* show the extent of gene divergence. Hybrid females are produced in both reciprocal crosses. Hybrid males survive only if both a *pseudoobscura* 3 chromosome and its *miranda* homologue X_2 are present. Therefore, the cross *miranda* ♀ × *pseudoobscura* ♂ gives both sexes in equal

numbers, but the reciprocal cross produces a great excess of females, over 200 ♀ : 1 ♂ (Dobzhansky, 1935 b, 1937 a). Dobzhansky gave evidence that the X_2 chromosome of *miranda* was present in these exceptional males and assumed that the Y was also. MacKnight (1939) used genetically marked chromosomes and proved that the *miranda* Y was not present, so that the exceptional males resulted from nondisjunction of X_1 and the Y in the *miranda* male parent. They received the *pseudoobscura* X with a set of autosomes and the *miranda* X_2 with the *miranda* autosomes. The latter males, although small, were nearly normal in appearance, and the summed data gave a ratio of 268 ♀ : 1 ♂. Other nondisjunctional classes are not formed, or else die in the egg stage. In fact, MacKnight showed that the expected males in this cross, which would have the *miranda* Y and lack X_2, died either in the egg or very early larval stages.

The males from the cross *miranda* ♀ × *pseudoobscura* ♂ were decidedly abnormal in phenotype, as well as less viable than their sibs. They differ genetically from the few exceptional males that survive from the reciprocal cross in having a *miranda* X_1 and *pseudoobscura* Y. Obviously the *miranda* X-autosome balance has been shifted by numerous changes. If we assume that the *pseudoobscura* Y does not affect the phenotype appreciably, as seems likely, the *miranda* X_1 is genetically unlike its homologue, the *pseudoobscura* X. This implies that, as the original Y-3 translocation changed into the present *miranda* Y, the X_1 made some of the compensatory balance changes. This type of change by the autosomes does not lead to phenotypic abnormality in the exceptional *pseudoobscura/miranda* hybrid males. The hybrid males formed from the regular *miranda* Y gametes die in the egg or very shortly thereafter, showing the serious effect of the hypoploidy caused by loss or loss of function of the genes originally in element C of the Y-3 translocation as it "degenerated" to become the *miranda* Y. The exceptional males which had both *miranda* Y and X_2 did not occur, whether because of the very low rate of this type of nondisjunction or because this class, hyperploid for 3 chromosome genes still present in the *miranda* Y, is lethal. All hybrid males are sterile with rudimentary testes.

The hybrid females from both crosses are sterile or only slightly fertile, depending on the strains crossed. Kaufmann (1940a) studied some of the sterile hybrids from both reciprocal crosses and found a similar abnormal behavior of the fertilized eggs, although the matura-

tion divisions were completed and the two pronuclei had come together. The early cleavage divisions were abnormal, and there seemed to be excessive proliferation of the chromosomes derived from polar nuclei. This led to the scattering of the chromosomes and chromosome masses through the cytoplasm without normal cell formation. Kaufmann points out that the hybrid sterility is gene-controlled. The cytoplasm of the eggs of hybrid females does allow regular normal development of a few gene combinations, and these only from certain parent strains.

A very different illustration of the interaction between genes in different systems can be illustrated in the mulleri group. In the pertinent case, Crow (1942) demonstrated the existence of a sex-linked gene (or closely linked genes) in a strain of one of these species, *aldrichi 2,* which acted as a hybrid lethal in crosses to *mulleri* females. The general discussion of all these cases will be presented later, but the necessary information is obtained from comparison of the following crosses. The *mulleri* males do not cross with *aldrichi* females, but *mulleri* females cross to both types of *aldrichi* males, as follows: *mulleri* ♀ × *aldrichi* ♂ give 54% ♀ and 46% ♂; *mulleri* ♀ × *aldrichi 2* ♂ give 9% ♀ and 91% ♂. Tests of the F_1 males from the reciprocal crosses of (*aldrichi* × *aldrichi 2*) with *mulleri* females prove the gene is sex-linked. The F_2 males obtained by inbreeding the F_1 from crosses between these *aldrichi* strains suggest that one gene or perhaps several closely linked genes are responsible. The F_1 females from *mulleri* by *aldrichi* are more viable than the F_1 males, although both classes are sterile. There is no lethal or abnormal class in crosses between the different *aldrichi* strains. The interspecies lethal is sex-linked and has no detrimental effect within its own species, for it was found several times in *aldrichi* populations. It has not been possible to prove conclusively whether this gene has a similar effect in other species crosses involving *aldrichi 2.*

The mulleri group gives another example of the drastic effect of genic unbalance. Patterson (1942) and Crow (1942) discuss crosses between *buzzatii* and other members of this group. There exists a major spatial geographical isolation between populations of *buzzatii* and its relatives, and the degree of gene difference is great. The only crosses that gave offspring were: (1) *mulleri* ♀ × *buzzatii* ♂ gave 1 sterile abnormal ♀ + larvae; (2) *arizonensis* ♀ × *buzzatii* ♂ gave larvae only. Here the hybrid genotypes were unable to develop

properly, with the exception noted in (1), so that this is an even more drastic example of gene divergence than the *pseudoobscura-miranda* case.

Wharton (1942) described a different type of result from genic unbalance in the melanopalpa group of the repleta complex. If *melanopalpa* females are crossed to a certain strain of *repleta* males, the offspring include intersexes in addition to phenotypically normal males and females. The genetic factors responsible for this condition have not been demonstrated. In her tests, *neorepleta* × *repleta* gave a few abnormal offspring. She noted that Sturtevant had obtained some intersexual forms in crosses of *neorepleta* and *repleta.* Sturtevant (1946) gave an account of his tests. In the crosses *neorepleta* females were mated to *white* or *white-singed repleta* males (the *white* is faintly tinged with color). The F_1 offspring are all normal in phenotype, except that many of the females have three anal plates, suggesting intersexuality, and the males are sterile with narrow testes. Some of the F_1 females backcrossed to *white repleta* males gave offspring, but the number of *white* females was too small: 70 wild type ♀, 9 *white* ♀, 42 wild type ♂, 58 *white* ♂. Repeated backcrosses of the wild-type females to *white* or *white-singed repleta* males through many generations probably resulted in replacement of most of the *neorepleta* genes by those from *repleta,* except for the region with the normal alleles of *white* and *singed.* Crossovers between these genes indicated that the gene from *neorepleta* which is responsible for the small testes condition in these crosses was located between *white* and *singed* very near to the locus of *white.*

The wild-type females in these backcrosses fall into two classes: those that give normal frequencies of classes, and those which are most often nearly sterile and produce too few *white* females among their progeny. In the latter class one female produced a more fertile line, presumably having lost a gene for semisterility by recombination. This line always produced two classes of daughters in equal numbers, for in one case sixteen such females gave the normal ratio of 1 + ♀ : 1 *w* ♀ : 1 + ♂ : 1 *w* ♂, while the seventeen females of the complementary class gave a total of 472 + ♀, 5 *w* ♀, 63 *w* intersexes, 482 + ♂, 339 *w* ♂. Sturtevant points out that the five *white* females probably were crossovers with a critical X-chromosome gene from *neorepleta.* This proved not to be the gene for narrow testes. The intersexes were chromosomal females with two *repleta* X chromosomes,

and were the extreme type which possessed no gonads or rudimentary ovaries. The external genitalia were missing or of abnormal male type. These weak individuals were otherwise somewhat abnormal and had a high mortality as pupae. If the *white* male offspring from this type female are mated individually to *repleta* females, they all give normal ratios of offspring. Approximately half the daughters from half of these males carry the dominant *neorepleta* gene responsible for this intersexuality, as they produce only intersexes (chromosomal females) and male offspring. The gene is dominant and functions in the formation of the eggs by these heterozygous females, for it does not have to be present in the zygote to cause intersexuality; nor does it act when brought in through the sperm.

This is a very interesting case, for the dominant autosomal *neorepleta* gene seems to be even more effective than the dominant Ix^B gene of *virilis* in changing chromosomal females into intersexes, as all eggs from mothers of this hybrid origin which were heterozygous for the gene produce intersexes. In *neorepleta,* the dominant autosomal gene is in balance with the dominant X-chromosomal gene for femaleness, so that no intersexes result within the species. The dominance relationships are such that one of each of these genes produces a female, although some of these have abnormal anal plates. This illustrates a case in which major changes in genic balance controlling sex determination have been accomplished by a few major allele replacements. The *melanopalpa* crosses (Wharton, 1942), in which part of the F_1 hybrids are definitely intersexual if certain *repleta* strains are used, indicate more extensive genetic unbalance of the sex-determining mechanism in those hybrids. Wharton found no intersexes in the hybrids between *neorepleta* and *melanopalpa,* nor any in crosses between different strains of *repleta,* although genetic differences in sexual preference were present in both cases. The data suggest that genetic differences existed between the *repleta* strains in genes responsible for the sex balance system which had no phenotypic effect in *repleta* crosses but did show up in crosses to *melanopalpa.*

DISCUSSION

The genus Drosophila possesses many varied and dissimilar forms. The first question to answer in explaining its evolution is that of availability of genetic variability which would allow this divergence. The

evidence leaves no doubt of the answer. There are available in natural populations abundant mutations which modify the organisms in all possible ways and effect every conceivable degree of change represented by divergence of a particular character in the species. Their cumulative action may be extraordinary in terms of taxonomic resemblances and differences. Professor W. P. Spencer (unpublished) has accumulated several mutants in a *hydei* stock so that it now resembles much more closely *D. pachea,* except that it almost entirely lacks the spotted thorax pattern so characteristic of the repleta group. Even mutations that can accomplish such changes as these are not necessarily the mutants used in the differentiation of any particular species. Patterson, Brown, and Stone (1940) showed that aneuploid conditions in *melanogaster* often caused phenotypic changes similar to mutations. A change in genic balance that accompanied the evolution of the species may be responsible for the change in phenotype, rather than a major change by a single mutation. Genetic differences associated with sexual isolation, hybrid sterility, and death of hybrid genotypes have been studied, so that we consider all types of gene variability necessary for species differentiation and evolution to be present in abundance.

Wright (1949) stated that: "Taking into account the rates at which many mutations are known to occur, the estimates of the number of loci (thousands in higher organisms) and the possibility of multiple alleles at each locus, it would appear that there should be unlimited variability in any moderately numerous species." The situation presented in the Drosophila species studied shows the presence of genetic variability sufficient to justify a similar description. Cases of sibling species like *pseudoobscura-persimilis, azteca-athabasca,* and *mulleri-aldrichi* show that the divergence between species is a change in genic balance with many factors involved. In these cases, the drastic shift in balance was not accompanied by many changes in phenotype. In other cases, such as *mojavensis-arizonensis,* the reverse is true. The latter, and especially the differences in genic balance between *pseudoobscura* and *miranda,* show that, as one mutation is fixed in a population, it helps to determine and limit the other mutations that can be added as beneficial mutants. Such mutations accomplish a new balanced state, and in so doing many mutations are fixed as "good" which would be "bad" in another balanced condition. The normal alleles which are responsible for these differences are not

homologous. Abnormal hybrids, and especially F_2 segregation hybrids which are sterile or abnormal, illustrate the importance of balanced gene combinations and illustrate the differences between the genes normal to the several species.

Hereditary control has been demonstrated for the basic genetic systems, such as crossing over, with the C3G factor described by Gowen (1933), and chromosome reduplication, with the sex ratio factor reviewed above. Other mutations with extreme effects that cause changes equivalent to the aberrations found in some hybrids have been mentioned. Whatever selective agent may be responsible for the selection into an evolving species of those genes which cause hybrid abnormalities, the major modifications during the evolution of a species must be the accumulation of a different balanced constellation of genes which fit the species to survive. This means the modification of the direction and type of development, metabolism, and activity patterns of the organism. Many examples of all sorts of diverse changes through mutation have been recorded and a few listed in this chapter. The very marked mutational changes and in addition the phenocopies, which modify the organism drastically, are important in showing the direction in and degree to which the organism can be modified. Many slight changes in these same directions are also known. The importance of the effect of a mutation will depend, not only on the extent of the difference between the actions of the mutant and its normal allele, but also on the system or systems dependent directly or indirectly on that gene.

Reverse mutation from a mutant allele to the normal allele is difficult or impossible to demonstrate in wild populations but occurs in experimental material, as proved by Patterson and Muller (1930) and by other workers, for example, Timoféeff-Ressovsky (1930) and Johnson and Winchester (1934). It must occur in wild populations; but in such populations, with two alleles present, it is impractical to demonstrate a particular mutation from one to the other. The similarity of the pattern of mutations in experimental and wild populations makes the assumption that such reverse mutations occur in wild populations inherently probable. Similarly, Patterson (1928, 1929a, b) demonstrated the several effects of X rays, including the occurrence of somatic mutations. Although these do not influence the evolution of a population, unless the gonads are involved, several somatic mutations have been recovered from wild populations.

Goldschmidt (1940) holds that major mutations due to position effect are responsible for the formation of new species. Drastic point mutations are known, as well as drastic changes accompanying position effect. Position effect has caused simultaneous mutations or changes simulating mutations for a number of genes moved close to heterochromatic regions of chromosomes in *melanogaster*. There are hundreds of thousands of gene mutations for every demonstrable chromosomal rearrangement present in wild populations. Some related species differ by few or no major chromosomal rearrangements, while others differ by very numerous changes in gene sequences. There is no proof that any one of the rearrangements present in wild populations induce position effect. Such position effects would be subjected to selection, just as are mutations. Perhaps the best evidence that the chromosomal rearrangements which occur in natural populations cause position effects that are subject to selection is to be inferred from the fact that only a few inversions are known to involve an obviously heterochromatic region other than the relatively few pericentric inversions, whereas hundreds of paracentric inversion differences have been reported in the species and their hybrids. A mutation causing a reduction in selective value of a rearrangement would be much more probable in a position effect on a number of genes at once, as is sometimes caused by shifts of genes next to heterochromatin. It is impossible to prove that any particular gene rearrangement has been incorporated into a species because of its position effect, but similarly it is very difficult to prove that a particular allele was incorporated for its beneficial effect in the population.

In our opinion, major mutations, either gene mutations or position effects causing radical departures from normal, are seldom important in evolution. It seems more probable that major evolutionary changes are the sum of the effects of many mutations which, selected to fit together, produce a new balanced genotype and, by accumulating further mutations and new combinations of genes, form a new species. In comparison, Goldschmidt's macromutations are postulated to so radically change an individual or group of individuals as to make it a different species (or genus). The equivalent change, in our opinion, is most often accomplished by the replacement of a number of genes by alleles, selected to produce together a new balanced genotype differing enough from the old genotype sometimes to be a new subspecies, sometimes enough to be a new species.

One important fact in the evolution of living forms is the increase in gene number. As polyploidy is uncommon in animals and unknown as a difference between species in Drosophila, we need to find other mechanisms which allow such an increase. Sturtevant (1925) proved that *Bar* was due to a position effect, and later cytological analysis proved it to be a duplication. The importance of such a duplication became obvious, for it was later shown that the *Bar* eye position effect could be modified to normal without the loss of the repeat duplication. Furthermore, Bridges (1935) has shown cytologically that several small duplications existed in the chromosomes in the salivary gland nuclei of *melanogaster*. Similar small duplications have been observed in the salivary gland chromosomes of other species also. Warters (1944) found one present in some wild *melanogaster* populations.

Genetic data indicating that small duplications had increased the available genes forming two or more duplicate loci in place were developed by Lewis (1941, 1945), Oliver and Green (1944), and Green and Green (1949, 1950). Serebrowsky (1938) proposed the hypothesis that the adjacent genes, *achaete* and *scute,* arose as a duplication repeat in place and that the two loci diverged by mutation to have complementary functions. One would judge from Green's latest report that the *lozenge* locus in *melanogaster* is a similar triplication repeat.

The addition of such a small duplication should preferably be reversed in sequence or in a different place in the chromosome, such as those that can be produced by crossing over between two not quite identical inversions. If followed by divergent mutations of duplicate loci and the rebalancing of the system, they will increase the available genetic divergence and so can allow for the evolution of a more complex system. This proceeds more slowly and in a different way from polyploidy, which involves the rebalancing of a whole new complex system. In fact, the addition of duplication makes possible greater potential variability without the loss which must exist, at least temporarily, of mutational flexibility that occurs in polyploids.

Ecological evolution between related species was demonstrated on the basic genetic and at the physiological level as differences in food requirements in the mulleri group (Wagner, 1944, 1949). Such divergence in ecological characters is necessary before two species derived from a common ancestor can occupy the same region. This proof of

the several divergent requirements in these closely related species is important, especially in showing how related or sibling species can occupy the same geographical area.

Timoféeff-Ressovsky (1934) showed with *funebris* mutants that some combinations of genes were superior to others, and similar combination effects are known in *melanogaster*. Certain races of *funebris* from different regions of the Palaearctic were proved to have different viabilities at different temperatures, with a decided correlation between temperature factors in the region of origin and the tolerance of the *funebris* strains to different temperatures (Timoféeff-Ressovsky, 1935).

Stone (1942) proved that heterosis resulted from crosses between strains of Drosophila from different localities. There was a heterosis effect on both fertility (number of pairs tested fertile) and fecundity (number of offspring per pair per day). Heterosis occurred in both *hydei,* which has a world-wide distribution and builds up very large populations, and *virilis,* which has a more restricted distribution, with large populations in Asia but very small scattered populations elsewhere. This heterosis proves the existence of mutational differences between populations and, further, that different mutant combinations are beneficial. This is one type of evidence for beneficial mutations in wild populations and demonstrates slightly different adaptive peaks of the genotypes.

Dobzhansky (1950) has demonstrated an interesting type of heterosis in *pseudoobscura* which has been important in proving the existence of natural selection in wild populations. In most populations of *pseudoobscura,* there are present several different gene arrangements (paracentric inversions as compared to Standard). Dobzhansky and Epling (1948) have shown that there is very little gene exchange between the several gene sequences usually found together. Dobzhansky and Levine (1948) have reexamined the data on heterozygosity versus homozygosity for these 3 chromosome arrangements in *pseudoobscura* and have shown that there is a significant excess of heterozygotes and deficiency of homozygotes. Dobzhansky and coworkers proved that a large percentage of the 3 chromosomes carry lethals or mutations detrimental to viability or fertility. This excess of heterozygotes does not depend on these detrimental genes alone but there is an excess of heterozygotes in populations set up with strains lacking gross abnormalities, as shown by the population cage

tests. When the several gene orders from the same region are tested in pairs, most, but not all, combinations show heterosis for the heterozygote sufficient to retain both gene orders, even if one is very weak homozygous. In other cases, the retention of a particular order in the the population is due to the very marked heterosis of one of the possible combinations. Dobzhansky showed that, in these heterozygous populations, in some localities there was a seasonal cycle with changing frequency of the several arrangements with respect to each other. He and his coworkers demonstrated that this change was due to different adaptive values of the particular arrangements with respect to particular seasons, so that the frequency of an arrangement reflected its adaptive value at that time in the seasonal cycle. Heterosis of the heterozygote retained some of the alternate gene arrangements through their low phase until the season when they were more effective. This is a type of adaptive polymorphism depending on gene sequence differences which accumulate combinations of genes that give particular adaptive values when homozygous.

Marshall and Muller (1917), using *melanogaster,* performed the pioneer experiments on the effect of natural selection in laboratory populations. They established stocks, both heterozygous and homozygous, for several visible mutations. The homozygous stock acquired a number of modifiers which reduced the deleterious and visible effects of these mutations during the course of a number of generations. The heterozygous stocks did not accumulate modifiers that reduced their detrimental effects and, in fact, some showed more extreme effects. Here natural selection had been able to accumulate beneficial mutations where it was able to work in the homozygous stock, but none where heterozygosity prevented the development of the selection pressure necessary to modify the genotype.

Dobzhansky and Spassky (1947) performed somewhat similar experiments with *pseudoobscura.* They used strains collected in the wild which had been tested for effect of a single second (or fourth) chromosome. This chromosome homozygous had some detrimental effect, so that if tested with a balancer chromosome, which carried a dominant marker as well as an inversion to prevent crossing over, the ratio of normal to dominant was less than the 1:2 expected if viability was normal. Dobzhansky and Spassky (1944) published tests showing that a number of the second and fourth chromosomes, if isolated homozygous, possessed different viabilities, fertilities, and so forth,

and so showed the extensive genetic variability that existed in these *pseudoobscura* populations. Dobzhansky (1946a) had used some of these 2 and 4 chromosomes to show the effect of recombination from crosses between them. He demonstrated that different gene combinations formed in this manner varied greatly in genetic characteristics. This was an excellent example of the importance of recombination in the formation of gene complexes, both good and bad.

Dobzhansky and Spassky (1947) set up four lines of a number of selected strains of both the 4 and 2 chromosomes. These strains were not isogenic for the other chromosomes, so that considerable recombination was possible, which may have been important in the results. Two sets of twin strains were established, and one set had the males treated with one thousand *r* units of X rays each generation. One pair of strains was homozygous for the second or fourth chromosome under test, while the other strain was carried heterozygous by breeding only from heterozygous flies each generation. Both sets were carried fifty generations, except those balanced strains that acquired a lethal in the chromosome under test. The homozygous strains were carried with twenty to twenty-five pairs per bottle each generation, which insured overpopulation of the bottles and very vigorous selection for viability and other factors. The balanced strains had only five pairs of heterozygous parents each generation, so that conditions were different and selection was much less intense. Undoubtedly the irradiation increased the number of mutants of all types present in the strains subjected to X rays, although the results did not show any consistent effect.

It seems to us that the data demonstrate the following points: The homozygous lines show that intense selection for mutations and combinations in populations kept large enough to provide opportunity for their effect to be evident will insure an increase in fitness. This is an example of the effectiveness of natural selection in increasing fitness. The experiments indicate decided effects of sampling error, but the over-all results for the homozygous strains show the effectiveness of selection. This selection was effective even when irradiation increased the number of mutants and chromosomal rearrangements in the population. The intensity of selection with these populations seemed great enough to increase fitness even under these conditions.

The evidence from the balanced stocks bears on another problem. Wright (1931) has demonstrated that chance sampling of gametes

can fix even detrimental genes in very small populations. The procedure with the balanced flies, of using only five pairs, did not therefore make this a control for the other test. Rather, it demonstrated that in small populations, when selection was low in intensity, detrimental or lethal genes would be fixed by chance. Even the low spontaneous rate of mutation was sufficient to provide enough detrimental genes for this chance effect to cause reduction of viability in most strains. It is exceedingly difficult to set up experiments to test and prove the effect of single variables, much less to evaluate the relative effectiveness of several variables in tests to prove natural selection.

Goldschmidt (1945) and his associates have described an interesting mutation phenomenon, namely, multiple or mass mutations. In these cases there occurred several mutations at one time, rather than a single mutation, as would usually be expected. It is not known how general or how frequent this phenomenon occurs. Goldschmidt considers that it is related to position effect, and McClintock (1950) describes a somewhat similar phenomenon in corn. This is important in that it makes immediately available to the organism a considerable genetic variability in addition to that normally present, and so increases the types of recombinants possible. In view of the importance of recombination in evolution, this may be of considerable interest.

Mather (1941, 1942a, 1942b) and others of his group have published their findings on the effect of selection on multiple factors. For example, Mather and Harrison (1949) showed that the chaeta number on segments four and five of the abdomen in *melanogaster* could be increased or decreased by selection. There is no doubt that Mather was able to demonstrate the effect of selection, just as did Payne (1918, 1920), Sturtevant (1918), Zeleny (1922), and others. Mather makes much of the inhibition of recombination due to linkage as a factor in selection. Anderson (1939, 1949) developed the fact that linkage decidedly affects the recombination possible in species crosses and gave some interesting examples of this effect. Wright (1931) developed at length the importance of gene combinations in evolution and natural selection, and pointed out that linkage only temporarily affects the formation of recombinations. The great importance of gene combinations has already been developed to apply in a much more general way to the problem of the genotype (see also Patterson, Brown, and Stone, 1940).

There are some factors in this work of Mather's that need com-

ment. Mather has expressed the opinion that certain genes may be regarded as polygenes, those quantitative factors usually referred to as multiple factors, while others are oligogenes, the genes having the usual qualitatively different alleles. He presents this hypothesis despite the fact that there exists a great body of data to disprove it. The same idea is also presented by Darlington and Mather (1949) in their volume, *The Elements of Genetics.* Fabergé and Singleton (1950) have criticized this concept. We quote from Mather (1943):

> Intercrossing two strains results in bringing together unlike polygenic combinations in the hybrid. If the histories of the two strains are separate, the two combinations received by the hybrid will not previously have been together and hence will not have been selected for good relational balance. The phenotype of the hybrid will be likely to show a greater departure from the optimum than does either parental strain, because combinations within the same strain will have been selected for relational balance. The departure of the hybrids from the optimum, or optima, to which the parental strains are adapted, will, if in the direction of increased size, be hybrid vigor or heterosis. We can thus recognize that in nature heterosis is a sign of poor adaptation and must be selectively disadvantageous.

This shows a remarkable misconception of the implications of gene recombination with allele replacement. It in fact suggests that a species, or a part thereof, might have the best of all possible combinations of alleles, which is an obvious absurdity. A case in point is heterosis in *hydei* (Stone, 1942). Here crossing strains collected from wild populations produced heterosis. *Drosophila hydei* is a world-wide form which builds up tremendous populations. It is an exceedingly well adapted and vigorous species, so that one could hardly say strains collected from wild populations were poorly adapted. They are well adapted but not so well adapted as other gene combinations which can be produced.

Dobzhansky (1950) reviewed his contributions to the theory of heterosis, using the data from his work on *pseudoobscura.* In earlier papers, he and his coworkers had shown that heterozygotes for different gene sequences are at a considerable selective advantage. When gene sequences from different localities are crossed, the selective advantage of the heterozygote is not so pronounced. Dobzhansky believes that this is due to the fact that particular mutations in gene sequences from the same area have been selected to give the beneficial

effect in the heterozygotes in relation to each other. He attempted to prove this by the following experiment. Standard from Mather (ST^M) was crossed to Arrowhead from Piñon Flats (AR^P), and Standard from Piñon (ST^P) was crossed to Arrowhead from Mather (AR^M). The F_1 were then crossed $ST^M/AR^P \times ST^P/AR^M$ in such large numbers in population cages that there would be very extensive selection among the several combinations for viability. The survival results of males and females from two cages made from reciprocal crosses of F_1's were $ST^M/ST^P = 114$, ST^P/AR^P and $ST^M/AR^M = 246$, $AR^P/AR^M = 63$. The Arrowhead gene arrangement homozygous, but containing genes heterozygous from the two localities, is obviously less viable than the other combinations; whereas the Standard arrangement homozygous, with genes heterozygous from Mather and Piñon, is about in the expected 1:2 ratio to the Standard/Arrowhead heterozygotes (114:246). There is no doubt that heterozygosity produces heterosis and that this heterosis depends on genes which act in the heterozygote (i.e., dominant genes). There is also no doubt that these populations have had such dominant genes selected into them and benefit by heterozygosity, nor that different dominant genes are present in different arrangements at the same locality, as well as between the same arrangements from different localities. This lone experiment does not prove that there has been co-adaption of these dominant genes, even though this is likely, especially in view of the fact that ST^M/ST^P is as well adapted as $ST^P/ST^P + ST^M/ST^M$. In fact, the data might be used to argue that no coordinated adaption exists. We believe that this conclusion would not be justified, and that more extensive and critical data are needed.

It seems to us that the problem of variation in gene arrangement in *pseudoobscura* and in other species where several alternative gene arrangements are present in any particular chromosome allows us to demonstrate one of the peculiarities of the selective mechanisms working on polymorphic systems. If we examine the genus Drosophila, we find in general that the multiple-inversion system is found to build up to serious proportions in the known populations of *pseudoobscura* and some of the members of the affinis complex, and of *willistoni* (also studied by Dobzhansky). In both *pseudoobscura* and *willistoni,* the populations where inversion heterozygotes are the rule are those which build up tremendous populations; for example, *willistoni* is the most numerous species of Drosophila in Brazil. Here populations

are so large that heterozygosity of genes is the rule and the incidence of inbreeding is comparatively small. If, on the other hand, we examine records of gene arrangements in *pseudoobscura* from the barren plains of Utah, Arizona, and New Mexico, we find that Arrowhead is in such large majority that there is very little opportunity for inversion heterosis. In fact, we might argue that where conditions are such that selection is most stringent, enforced heterozygous heterosis dependency is not as effective as the free recombination with homozygosity of all combinations possible when all or nearly all gene orders are the same.

If we check members of the subgenus Drosophila, we find that the funebris group affords supporting evidence. Dubinin and Tiniakov showed that there was a difference in selective advantage of the normal and inversion types, with inversion types having advantage in the cities, but normal with the selective advantage in the country. In comparing the *macrospina* subspecies, we find that *macrospina* and *ohioensis,* which build up large populations east of the Rocky Mountains, have a series of different inversions present part of the time, but that *limpiensis,* which is found in limited numbers in western United States, has no known chromosomal variability (Warters, 1944). In smaller populations, the increased inbreeding necessitates that each gene combination be satisfactory and that detrimental results from homozygosity be kept at a minimum, presumably because it is necessary to make the most of all mutations and recombinations, both dominant and recessive. In populations that are so large that heterozygosity is the rule, selection will be for genes that make the heterozygous fit, and balanced inversion polymorphism may have selective advantage.

7 ISOLATING MECHANISMS

GENERAL PROBLEMS

There are numerous types of habitats available all over the world for a small form such as Drosophila. As these habitats are variable and noncontiguous, the resulting isolation between segments of populations has certain effects on evolution in the genus. Furthermore, discontinuity of gene exchange exists in a group of species that live together, and the reason for and mechanisms of maintenance of this isolation must be determined. The isolating mechanisms belong in two classes, geographical isolation and genetic isolation. As these are sometimes casually related, much confusion has developed.

Geographical isolation consists of isolation between populations in the form of some physical barriers, such as the sea for land forms. The degree of isolation imposed varies from complete, or nearly so, to slight barriers, such as a river which may restrict migration of a particular form. It does not depend on the presence of genetic differences between the strains so isolated, although these may also exist. In contrast to geographical isolation, all other types of isolation are of genetic origin and maintained by gene differences.

The genetic isolating mechanisms may be separated into ecological and reproductive. Any or all types of isolating mechanisms may be present to separate two forms, but any one may interpose a complete barrier to crossing. For example, ecological isolation may be a complete barrier to crossing; for if two forms have such different ecological needs that mixed ecological regions do not exist, they may never come together. Usually several types of genetic isolating mechanisms coexist, to insure isolation of any form.

GEOGRAPHICAL ISOLATION

Our information on geographical isolation may be inferred from the material on geographical distribution in Chapter 3. The information is pertinent in relation to the origin of genetic isolation mechanisms. Both ecological and reproductive isolation between forms depends on the fixation of gene differences which determine the type of barrier between the isolated strains. Fixation of gene differences necessitates geographical isolation or the prior presence of some of the other effective genetic isolating mechanisms. There is no difference in freely interbreeding populations. Mayr (1947), in discussing the geographical and ecological aspects of species formation, concluded that strict sympatric speciation was not necessary or proved from the data. We still know of no valid evidence in Drosophila for sympatric speciation. The argument that ecological difference would allow reproductive isolation to develop begs the question, for populations would have to differentiate effectively the ecological needs of the strains.

The Drosophila species in the Hawaiian Islands illustrate the effect of geographical isolation where other associated conditions allowed rapid genetic change. A Drosophila species, or several of them, reached the Hawaiian Islands and there gave rise to a number of distinct and often specialized species. Some other species, such as the regular wide-ranging forms, have probably been introduced by human transfer. The islands are sufficiently isolated geographically that migrants have been too few to prevent the evolution of the numerous and very distinct native Hawaiian species of Drosophila. The Hawaiian Islands are an example of extreme isolation.

We have many examples where species or even species groups are mostly restricted to one continent, except for the very few widely distributed forms. As has been pointed out, the very great number of endemic species, together with the well-developed but often localized species groups, indicate separation followed by diversification. The evidence does not suggest wide and frequent intercontinental migration, but rather that species developed in much more restricted environments than whole continents. We presume that the necessary spacial isolation was provided by the diverse geography. Occasional transfer across such barriers as the Rocky Mountains or a desert might be accomplished by high winds, for these flying forms, even in

immature stages, could be so transported. Glick (1938) has collected Drosophila at several thousand feet altitude in an airplane, showing that they possess this dispersal potential. Such differential barriers are not crossed so often that differentiation and evolution of new species were prevented.

ECOLOGICAL ISOLATION

There are several general ecological divisions in the genus Drosophila, as well as a number of more restricted specializations. The following represents the major specializations: (1) wide spread or cosmopolitan forms; (2) forest forms; (3) fungus-feeders; (4) desert forms, succulent feeders for the most part; (5) specialized forms, such as flower or pollen feeders. Many or perhaps most species have some differential ecological specializations, at least those that live together. This is a general classification. As we are concerned with the evolution of ecological isolation, we would like to know how two closely related species are differentiated.

There are two problems that concern us. One is the development of genetic differences which cause preference for different ecological habitats, so that the two forms do not occur together except in intervening mixed habitats, if at all. The other consists in the development of differences in ecological needs such that two forms can live in the same area which contains a mixed habitat, either at the same time or in different seasons. Two forms which share some food and differ slightly in other ecological needs may replace each other but be unable to survive in the same area. This situation is characteristic of subspecies; for if two subspecies in contact with each other were identical, they would mix and lose their separate identity. We do not have much exact information on the ecological similarities and differences between species. In the obscura group, *pseudoobscura* and *persimilis* and *miranda* overlap in their distribution. In this case, *pseudoobscura* has a much more extended range than the others and seems to be more tolerant of heat and low humidity. The species *pseudoobscura* is restricted to regions west of the 98th parallel, while *affinis* occurs east of this division. Both occur in the Austin, Texas, region, but the range of *pseudoobscura* is to the west and that of *affinis* to the east. Both occur in forest areas, although some strains of *pseudoobscura* are found out in the desert. Along the northern por-

tion of the United States, *athabasca* coexists with both of these species. We can infer that *athabasca* has sufficiently different ecological needs to occupy the same area with both *pseudoobscura* and *affinis,* but that these last two are too seriously in conflict to live together and, owing to other ecological differences, can replace each other to the east and west of the zone of contact. We have no critical information on the genetic mechanisms responsible for the ecological similarities and differences involved.

The repleta group shows the effect of a major ecological adaptation. Here a group ancestor made the necessary transition and adaptation from forest or generalized form to a desert form adapted to live in the succulents, such as *Opuntia lindheimeri.* There followed an explosive diversification of species, so that the repleta group is the largest known group in the genus. It is very interesting and suggestive that *carbonaria,* which is an offshoot from the repleta group, has become adapted to live in the mesquite. We believe that this was a subsequent specialization and separation to occupy another available niche in the desert vegetation.

Wagner (1944, 1949) has obtained the only critical evidence on differences in food requirements which exist between closely related species of this genus. He has shown that there existed differences in the ability of the developing forms of several members of the mulleri subgroup to utilize and survive on different yeasts collected with their larvae. These differences in food requirements explain at least in part how two species can survive together in the same habitat, e.g., *mulleri* and *aldrichi.* It further suggests reasons for the fact that *mulleri* is a more widely distributed species than its sibling *aldrichi.* Table 18 (Chapter 3) shows the growth of the larvae of five members of the mulleri group on eight different strains of yeast. The five species show differences in their ability to utilize one or another strain of yeast and fall into four different classes, for only *mulleri* and *arizonensis* are similar in the tests. Figure 60 shows that *mulleri* has a somewhat different growth rate, as measured in larval length on the several yeasts, and further indicates the differences in adaptability of the organism to several possible sources of food. Wagner (1944) had shown that bacteria would not support normal growth of Drosophila, but that some strains would support growth if from thirty to three hundred grams of cholesterol were added to the medium. Probably other similar sterols would also be satisfactory. The very marked

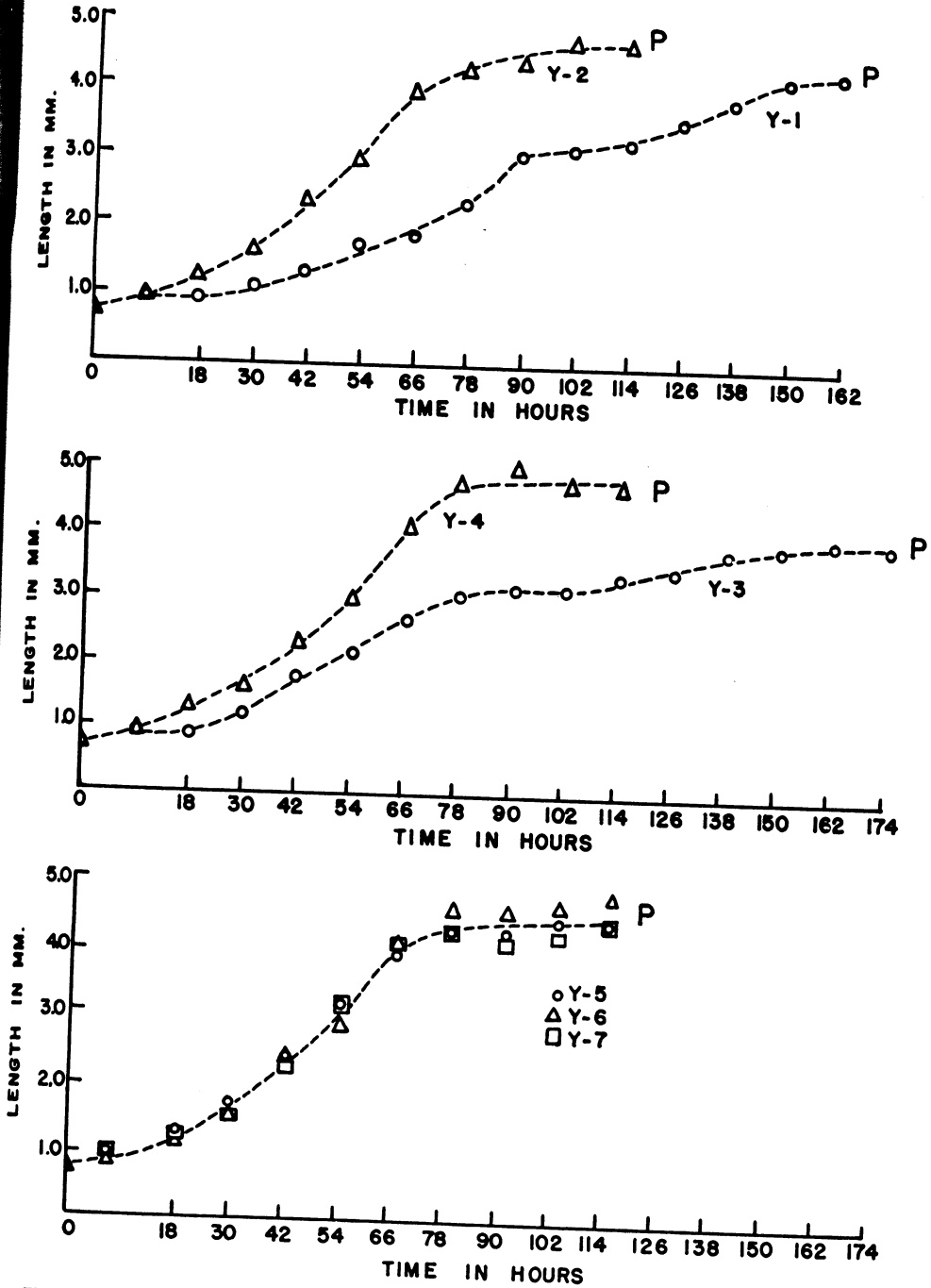

Fig. 60 Growth of *mulleri* larvae on different yeasts. (After Wagner.)

variation in food requirements indicated by this work presents an interesting problem for further exploration.

Another mechanism which reduces competition and allows the survival of two species in the same region is also illustrated in the repleta group. At the Aldrich Farm near Austin, Texas, both *longicornis* and *hamatofila* have their population peak in the spring, whereas those of *mulleri, aldrichi,* and *meridiana* occur in the fall (Patterson, 1943). These differences in population peak undoubtedly imply differences in ecological needs, and allow all these forms to survive in the same region. The existence of population peaks at different times of the year acts as an ecological barrier to crossing between species (Table 17).

Patterson and Wagner (1943) recorded the differences in species which came to trap cans placed only about one hundred feet apart, one set of traps along the desert stream and the other set of traps in the desert proper near Magdalena, Sonora, Mexico.

Desert stream	Desert proper
hydei	*hydei*
pseudoobscura	*pseudoobscura*
limpiensis	*arizonensis*
victoria	*hamatofila*
munda	*longicornis*
other Drosophilidae	*nigrospiracula*

The species *hydei* and *pseudoobscura* are more generally distributed, but other members of the repleta group are desert forms, whereas the fungus-feeders are restricted to the stream where the vegetation is suitable.

Dr. Herman Spieth has obtained additional evidence on this question. In a personal communication, he writes:

I have two interesting observations to report to you: I found an American elm that was bleeding at a point about six feet above the ground. Considerable slime flux had accumulated on the trunk of the elm, and I have been catching Drosophila adults in this vicinity. On the tree itself I have now taken *victoria, robusta, busckii,* and *paramelanica.* The latter two have been very rare, but *victoria* and *robusta* are consistently present. About ten feet away from the tree I placed a trap baited with banana. This trap has attracted the following species: *melanogaster, affinis, algonquin, paramelanica, funebris, hydei, transversa,* and one specimen of

victoria. Whether the trap was present or absent, the tree attracted only the four species first mentioned in this paragraph. In the slime flux itself I have recovered pupae of *robusta,* as yet none of *victoria,* and numerous larvae of Drosophila. Larvae of *Nosodendron unicolor* (a beetle) were present in great numbers. Near the point where the sap is flowing from the tree, the Drosophila adults collect in greatest numbers, but not the beetle larvae, wherever the beetle larvae are numerous, I find only a few very young Drosophila larvae. The larvae removed from the slime flux later produced *robusta.*

This shows that ecological differences of a fundamental nature are present and of the sort that determines food preference and food attraction. If the several species of flies seldom go to the same food source, there is a reduced opportunity for crossing.

Dobzhansky (1939) stated that samples of Drosophila species taken at certain localities, often no more than one or two hundred feet apart in Mexico or Guatemala, were different, indicating a differential distributional density. Timoféeff-Ressovsky (1940) collected systematically on an experimental plot in Berlin. The distributions of *melanogaster* and *funebris* were not uniform, indicating focal distribution points of micropopulations. Dobzhansky and Pavan (1950) have provided some interesting information on micropopulation distribution and on food attractiveness for a number of tropical species. Using one kilogram of fermenting banana, they placed forty-one baits in a cross-shaped distribution, with baits ten meters apart. Species from the same set of individual stations were collected on December 29, 1948, February 5, 1949, and June 11, 1949, every one or two hours. Some species were present on all three occasions; others were frequent on only one or two of the intervals. The distribution was not necessarily the same on the three different days of collection. We do not know if this was the result of migration to local preferred food sources or local areas of hatching from larval food source concentrations. Tables 48 and 49 show that the several species of Drosophila reported have different food preferences, sometimes very marked. These data represent Drosophila collected from the several sources indicated. Some species are rather general; others have definite preferences in their attraction to a particular form of bait. They demonstrated the difference in frequency of the several species or species groups in collections made at different seasons. Vila Atlantica has a superhumid tropical climate, with an excess of one hundred milli-

meters of rain in the dry season. Pirassununga has a dry season with less than thirty millimeters in the fairly warm (ca 19° C.) winter months—June, July, and August—but nearly two hundred millimeters of rain with mean temperature of 23.5° C. in January. There is a seasonal variation in species frequencies, similar to that demonstrated by Patterson (1943). We presume that both the seasonal fluctuations and the preference for foods, as shown by the discrimination in coming to the several baits used, are the result of the particular and individual ecological adaptation of the several species.

The virilis group illustrates ecological divergence and specialization. *Drosophila virilis* is found almost entirely in domestic habitats in

TABLE 48

Percentages of various species of Drosophila collected on different fruits at Iguassu National Park, Parana (from Dobzhansky and Pavan)

Species or Group	Jara-catia	Co-quinho	Canela amarela	Aguahi	Alecrim	Cauna	Pitanga	Goiaba
willistoni	68.0%	87.2%	..	48.5%	40.2%	31.9%	77.9%	62.7%
nebulosa	0.3	0.3	0.9%	14.9	17.1	28.1	3.9	0.6
mediogroup	20.3	1.6	16.4	11.1	4.9	5.0	3.9	1.8
guaramunú	3.0	5.0	81.9	17.2	23.5	32.6	14.3	6.4
polymorpha	8.3	6.0	0.9	8.4	14.3	2.1	..	28.3
Others	..	..	..	..	..	0.2	..	0.1
Individuals collected	300	367	116	586	286	427	154	3088

TABLE 49

Percentages of various species of Drosophila collected on different fruits at the Instituto Agronomico do Norte, Belem do Para (from Dobzhansky and Pavan)

Species or Group	Terra Banana bait	Firme Bread-fruit	Banana bait	Bacaba	Muru-muru	Igapo Clausia species	Hura	Matisia	Lu-cuma
willistoni group	79.2%	64.0%	58.1%	15.0%	40.9%	56.7%	12.6%	92.4%	75.9%
fumipennis	11.1	..	2.0	..	..	20.0	1.0	..	11.8
nebulosa	0.3	..	3.0	..	0.7	..	0.5	..	..
simulans	..	..	0.1	..	4.7	..	..	..	..
sturtevanti	1.1	..	15.2	..	0.3	..	..	..	..
mediogroup I	0.3	13.5	0.7	55.0	..	..	81.6	..	..
mediogroup II	0.3	9.6	0.8	..	..	..	0.5	..	5.6
camargoi	6.7	6.1	6.6	5.0	..	..	..	6.7	..
fumosa	..	3.8	0.6	..	..	..	..	..	..
peruviana	..	..	0.1	..	51.1	..	..	..	..
repleta group	..	0.4	6.0	..	1.8	..	..	0.4	0.3
canalinea	0.6	..	4.1	10.0	0.3	..	0.5	..	0.6
calloptera group	0.1	..	0.1	..	..	..	..	..	2.9
Others	1.7	2.6	2.7	15.0	..	23.4	3.2	0.7	2.3
Number of flies	360	261	712	40	276	30	191	284	341

North America. The other members of the group occurring on this continent are always found in wild habitats. We can presume that *americana* is better adapted to the cold and more northern environment, whereas its subspecies, *texana,* is better adapted to the warmer and different ecological region of the south. Although they overlap slightly and hybridize, it is along a boundary which delimits ecologically different regions. The other forms are also separate, for *lacicola* is restricted to the lake region of the northern United States, perhaps extending into Canada. The species *montana* occurs in the Rocky Mountains rather generally at altitudes above six thousand feet. The species *novamexicana* occurs along desert streams, and even though it extends into the same regions as *montana* in the southwest it is always found in the low river-bank areas and does not occur up in the mountains. These are all relatively small populations for Drosophila, but they show distinctive ecological specialization.

Spieth (in press) found very convincing evidence for ecological specialization and adaptation of the species *lacicola* and *borealis.* The material was collected in the summer of 1950 by a small pond two hundred feet long by thirty feet wide in Itasca Park, Minnesota, which had aspen, American elm, basswood, and ash growing around it. A number of large aspen had been felled, the wood cut in three-foot lengths, corded at the edge of the pond, and the brush piled in it. Spieth set traps at the edge of the pond and at localities thirty and seventy feet away from the pond. The traps at the pond usually attracted *lacicola,* but not those set away from it. He observed that most of the specimens collected were young flies. They were often carrying a bright orange-colored mite, similar to that found on mosquitoes and dragonflies captured in the same area, whereas other species of Drosophila were infested with brown mites. He concluded that the adults must remain very close to the same wet marshy places where the mosquitoes and dragonflies live. Spieth found that he could get *lacicola* to come to mashed banana-yeast mixtures, but that they did not oviposit in this food. He did not find *lacicola* larvae in the slime flux on American elms, nor in the flowers of bladderwort or in rotted wood. He writes:

> I next pulled a piece of aspen bark off a piece of cord wood. This bark was fairly tight, could be peeled away and the cambial layer was well rotted. The thick phloem tissue was a dark brown to black and very wet.

This was the jack pot. In the phloem tissue, but not in the cambial area, were numerous larvae of *lacicola.* In the frazzled ends of the bark where it had been sawed were the pupae. I found dozens of them. I then went from the lab back to the pond and pushed a piece of bark back from one of the stumps and left it hanging. That evening I took three sweeps of the net over this stump area and collected 87 flies, 85 of them were *lacicola,* one was a newly emerged *robusta* female, and one was a male of what I determined as *Scaptomyza* sp. The next day I removed the piece of loosely hanging bark and recovered about 20 *lacicola* eggs. Since then I have observed and collected *lacicola* adults from this exposed place on the stump at all hours of the day. Apparently the adults stay here all day. This bark, when peeled, has a very distinctive and penetrating odor. Men who have been about the pulp mills in this area tell me it is a typical pulp yard odor or aspen odor. Dr. Clyde Christenson, from the University of Minnesota Botany Department, teaches fungi here and he is now culturing this phloem tissue. He tells me that a small yeast is the dominant organism and at present he is attempting to isolate and culture this yeast. It has been known for some time that the phloem of aspen is extremely rich in sugars, so much so that the wood people have been trying to devise a method of collecting and utilizing commercially the sugars.

This establishes a definite ecological habitat for the species *lacicola.* We have since shown genetically that *lacicola* is mixed with a sibling species, *D. borealis.*

REPRODUCTIVE ISOLATION

The reproductive isolating mechanisms in animals such as Drosophila may be grouped as follows:

1. Mechanical isolation due to differences in genitalia.
2. Sexual isolation or failure to mate.
3. Seasonal isolation, in Drosophila probably due to ecological isolation.
4. Insemination reaction and perhaps failure to inseminate sufficiently normally (too few sperm).
5. Death or sterilization of cross-mated individuals.
6. Gamete mortality.
7. Zygote mortality = F_1, F_2, etc. recombinations.
8. Zygote sterility = F_1, F_2, etc. recombinations.

There are really two phases to the problem of reproductive isolation. The reduction of gene exchange must be considered in terms

of separate species and also in terms of subspecies and strains. Drosophilas do not form amphidiploid hybrids, and all hybrids between species so far tested have been at considerable disadvantage in viability or fertility. This is not true of crosses between strains which often produce heterosis. Subspecies are rare, or else are rarely recognized in Drosophila. Partial seasonal separation which allows strains to extend further into the available environment of the species might be useful between strains. In fact, partial sexual isolation which reduces but does not stop gene flow between populations might be of some advantage. These factors would be useful in case the genes concerned did not cause any other detrimental effects and if they were recessive or not detrimental in the heterozygotes, so that these also would be fertile.

When species descended from a common ancestor have separated sufficiently that their hybrids are at appreciable selective disadvantage, isolating mechanisms which reduce the frequency of hybridization to a sufficiently low level to be useful are of selective advantage to each species. The several different systems which act as reproductive isolating mechanisms have different value and efficiency. In Drosophila, we know of no cases of strict mechanical isolation due to major differences in genitalia among those forms which still show enough sexual response to attempt mating. This cannot be considered an important mechanism in the group. However, mechanical isolation, sexual isolation, and seasonal isolation are the most effective isolating mechanisms, as they prevent too much wastage of reproductive effort. The other mechanisms are useful and may be necessary in a particular case to insure enough isolation for the separate existence of a particular species. We will consider the useful effects of limited hybridization in another section.

ROLE OF SEXUAL ISOLATION IN SPECIATION

One of the most important factors in speciation is that of sexual isolation. Like other isolating mechanisms, it acts as a barrier to interbreeding between individuals belonging to different populations. It often prevents crossbreeding between many races and species, and may even reduce the frequency of mating between geographical strains of the same species. In preventing crosses between different strains, sexual isolation may preclude a wastage of reproductive effort. Were

it not present in a highly mixed population, reproductive effort might be so wasted as to cause the destruction of the species involved. While the phenomenon of sexual isolation is widespread throughout the genus Drosophila, yet it is not necessary to assume that it is the only isolating mechanism involved in the prevention of interbreeding between a given pair of species. It has been abundantly demonstrated that it is the total effect of several such mechanisms which finally brings about the more or less complete isolation of two forms, by reducing or preventing the exchange of genes between them.

According to the theory of sexual isolation, it is assumed that the failure of copulation to occur is due to a lack of mutual attraction between the sexes. The basis of this mechanism must relate to genetic factors rather than to extrinsic environmental influences. The exact nature of sexual isolation varies in different forms, and the lack of attraction between the sexes may be due to such causes as differences in courtship behavior, sexual recognition signs, and other forms of stimulation.

The modern experimental study of sexual isolation is a natural outcome of interest in Darwin's theory of sexual selection. Immediately following its publication in 1871, and for a long time thereafter, many studies were undertaken with a view either to support or disprove this theory of sexual selection. Most of these early studies were observational rather than experimental in character; and while they brought to light many remarkable cases of mating habits and of secondary sexual characters, especially among insects, yet they failed to elucidate the underlying basic cause of what is now called sexual isolation. With the development of modern genetics, particularly in Drosophila, interest has become focused on the question of the role of this mechanism in evolution. Moreover, modern genetic methods now make it possible to subject this question to a critical experimental analysis.

Different methods have been used to determine the possible occurrence of sexual isolation in Drosophila. The three most important of these are direct observation, pair-mating tests, and the multiple-choice technique. By watching cultures containing males and females of two different species, it is possible to determine whether crossbreeding is taking place. This method is rather tedious and time-consuming where it is desired to obtain sufficient data for statistical treatment, although it does have one advantage over the other two methods in

that a study of the courtship behavior will sometimes reveal which of the two sexes is exercising a choice of mate.

In using the pair-mating test, it is customary to make up one hundred or more cultures, each containing a male and a female belonging to different forms (species, subspecies, or strains). After a certain period of time, the cultures are checked and the number failing to produce offspring recorded. From the data thus obtained, the degree of isolation may be determined. However, for an accurate evaluation of the results in terms of sexual isolation, it is necessary to dissect the females from sterile cultures and examine their sperm receptacles for the presence or absence of sperm. Dissections have shown that not all females from sterile cultures, upon which the magnitude of sexual isolation must be based, have remained unimpregnated. This is especially true in interspecific crosses, for the sperm may have been inactivated, or even killed, in the reproductive tract of the alien female (Patterson, Stone, Griffen, 1942). Moreover, the sperm may penetrate the egg without development taking place, thus representing an initial form of zygotic mortality.

The method of multiple-choice technique is a test designed for the detection of sexual preference, rather than one intended primarily for use in the discovery of sexual isolation. Sexual isolation, if present, will usually be revealed in the results obtained in the experiment. Sexual preference may reduce the exchange of genes between two forms, but will not necessarily prevent such exchanges, whereas sexual isolation as strictly defined will prevent these exchanges. In tests involving two species with the use of the pair-mating technique, only heterogamic matings are possible; and if none of the females has been inseminated, it is evident that complete sexual isolation exists. On the other hand, in sexual preference tests both homogamic and heterogamic matings are possible.

METHOD OF DIRECT OBSERVATION

There are numerous incidental references in the literature on Drosophila reporting observations on sexual behavior. Most of these are concerned with courtship and mating habits, and only the more important ones will be considered in this treatise. One of the earliest articles dealing with sexual selection is by Lutz (1911), who carried out a series of experiments on *D. ampelophila* (synonym of *D. melano-*

gaster). Lutz studied the inheritance of abnormal wing venation and the effect of sexual selection and found that this character was strongly selected against. He reports that the fly, whether male or female, normal or extra-veined, chooses the normal mate much more frequently.

In a series of articles, Sturtevant (1915, 1920b, 1921b, 1942) has reported his observations on the courtship and sexual behavior of Drosophila, and has described the various elements of behavior which are exhibited by the flies. In the first paper (1915), he made extensive observations and tests on the courtship and mating habits of *D. ampelophila* and, from the results obtained, drew several definite conclusions. He concludes from his experiments that sight is not essential in sex recognition in Drosophila, but that olfactory and tactile senses are probably both concerned. The results from tests with males from which the wings had been removed indicated that the function of these organs in courtship is the production of sexual excitement in the female. The results from experiments carried out with four mutants (white eyes, vermilion eyes, yellow body color, and curved wings) made it seem probable that these characters have no selective value, "except in so far as results from the fact that at least two of these classes are less active than normals." Finally, he concludes that probably neither sex exercises any choice in the selection of a mate. The next paper by Sturtevant (1920b) is Part I of his two articles on the genetics of *Drosophila simulans* and includes an account of hybridization between *simulans* and *melanogaster*. In a short section on sexual selection, Sturtevant reports observations which strongly indicate that females of either species are much more likely to mate with males of their own species than with those of the other species. Sexual selection is therefore one means by which these two species are kept from crossing. In this connection, it should be mentioned that Lancefield (1929) found that the two races of *pseudoobscura* exhibited preference for mating with members of their own kind in mixed cultures.

Of the last two articles by Sturtevant (1921b, 1942), the first is his well-known monograph on *The North American Species of Drosophila*. In one section he makes a comparative study of the mating habits of some twenty-odd species and presents an abstract covering the experimental results. One of the most interesting experiments was

an attempt to obtain cross-copulation between different species. He found that males of *melanogaster* will mate with females of *affinis, pseudoobscura,* or *simulans,* but the only combination from which hybrids could be obtained was that of *melanogaster/simulans.* Sturtevant again returns to the subject of the mating habits of Drosophila in the 1942 article. He gives a brief account of observations on twenty additional species and cites several strikingly new types. He concludes that, in general, mating habits do not seem to furnish useful species group characters.

There are many other references to observations on sexual isolation in Drosophila, but most of these were made in combination with experiments designed to test some particular phase of the subject and will be referred to in subsequent sections of this chapter. There are two recently published articles by Spieth (1947, 1949) which belong under the heading of this section. In his first paper, Spieth reports extensive observations on the intraspecific mating habits of six members of the willistoni group (*fumipennis, nebulosa, capricorni, sucinea, willistoni, equinoxialis*). He found that, while all six species are similar with respect to the initiation of courtship, mounting, and termination of copulation, yet certain specific qualitative differences exist between each of them. These differences, which are restricted to the posturing behaviors of the male, to the female activity of signifying acceptance of the male, and to the actual insemination period, roughly parallel the morphological differences existing between the species, with the exception of *willistoni* and *equinoxialis,* between which the behavioral differences are greater. Hence the evolution of the sexual behavior approximates quantitatively the evolution of the morphological characters. One point of unusual interest is the fact that acceptance or nonacceptance in intraspecific courtships of this group is a function of the female. She can rebuff the male, especially by the extrusion of the genitalia; and unless she is receptive, the male is unable to copulate.

In the case of interspecific mating behavior, reports on direct observations are relatively few. Among these may be cited the observations of Stalker (1942) on the interspecific mating behavior of *virilis* and *americana,* the study of Wallace and Dobzhansky (1946) on *subobscura, persimilis* and *pseudoobscura,* and Mayr's (1946 a, b) observations on matings between *persimilis* and *pseudoobscura.* To

this method of study belong also the observations reported in the second paper by Spieth (1949). He employed the same series of six species of the willistoni group as used in his first paper.

In this study, males of each species were tested to the females of the other five species; and, conversely, females of each species were tested to the males of the other five species. In all, 399 males were employed for ninety-two observations in studying the possible thirty interspecific crosses, with only one copulation resulting (*sucinea* ♂ × *nebulosa* ♀). In all other cases, the courtship broke down at some point between the "tapping action" of the male and the acceptance response of the female. Spieth suggests that the single copulation observed indicates that "accidental" copulations between species may take place in nature. His final conclusion is as follows:

> The species differences in sexual biology of this group can best be accounted for by assuming that evolutionary divergence has occurred by accumulation of minor differences that have arisen independently throughout the group. In general the degree of differences of the sexual biology parallels the morphological differences, i.e., the evolution of the morphological differences and that of the sexual differences have proceeded at about the same rate.

Streisinger (1948), using a technique devised by Dr. Irwin Herskowitz, showed that males of *pseudoobscura* and *melanogaster* will mate both with their own and alien females when these females were etherized. If females of *pseudoobscura* or *persimilis* were etherized, their ovipositor plates squeezed open, and they were placed in vials with *pseudoobscura* males, 55 per cent and 45 per cent respectively were fertilized while anesthetized. In a comparable time 90 per cent and 10 per cent of normal females were fertilized. If *melanogaster* males were used with *melanogaster* and *persimilis* females, 45 per cent and 55 per cent respectively were fertilized, whereas 100 per cent and 0 per cent were fertilized in normal motile controls. Spieth (personal communication) has pointed out that the spreading of the genital plates is an acceptance reaction on the part of the female, so we know that these males will copulate if given this opportunity.

Miller (1950b) recounted his observations on the mating behavior of *affinis* and *algonquin,* both homogamic and heterogamic. From six to twelve individuals of each sex were placed together in an observa-

tion chamber, and their courtship activities determined. The species *affinis,* but not *algonquin,* undergoes a period of disorganized excited activity when moved about, much as Stalker and Spencer (1939) noted for *macrospina.* The courtships of both *affinis* and *algonquin* agree with the description given by Sturtevant (1921b). Courtship is usually brief, with the following steps: orientation (recognition) of the individual to be courted; vibration of the wings and circling; attachment of genitalia and mounting. Miller did not observe tapping, and the males could not discriminate between the sexes. The females avoid the males by decamping, or extruding the genitalia if recently mated. These species do not usually cross. The males will initiate courtship with the alien female, but break off without attempting to copulate. There is no physical contact, so Miller suggests that the stimulation from the female is chemical, as Spieth (1949) had suggested from similar observations of courtship discrimination between *willistoni* and *capricorni.*

Mayr (1950) presented very convincing evidence that the antennae of Drosophila females acted as a receptor in the chain of stimuli-response courtship reactions. He showed that removing the antennae of normal *melanogaster* females reduced their receptivity to courtship to a very marked degree, but that they could still discriminate against *yellow* males. Not only does the removal of the antennae reduce the effectiveness of courtship to stimulate the female, but it also removes sexual isolation between *pseudoobscura* males and *persimilis* females. Mayr (1946b) has shown that discrimination between *pseudoobscura* and *persimilis* is exercised by the females. He placed *pseudoobscura* males with *pseudoobscura* and *persimilis* females, allowing multiple choice. With normal flies 86 per cent of the *pseudoobscura* but only 2.5 per cent of the *persimilis* females were fertilized. When antennaeless females were used under optimum conditions, 33.0 per cent of the *pseudoobscura* and 26.8 per cent of the *persimilis* females were fertilized. As this difference is insignificant, the removal of the antennae eliminated the species discrimination of the *persimilis* females against *pseudoobscura* males.

Sears (1947) observed the mating behavior in the possible crosses between ten members of the quinaria group. He used one female and five males in a vial with food, observed their courtships for a period, and dissected at the end of three days to determine insemination. The number of each combination tested was small, but much of the

general pattern of sexual isolation could be inferred. As Spieth (unpublished) has studied the mating behavior in this group thoroughly, we will only briefly refer to some major points. Of the ninety possible cross-matings, in forty-five there was no interest shown by either sex; in thirty-three the male showed interest but the female was indifferent to the courting; in six cases the male showed great interest and often tried unsuccessfully to copulate, but the female was actively antagonistic to the courting male; while six crosses besides the controls resulted in insemination. There were some interesting patterns of sexual isolation shown, for four of the six successful cross-matings involved *occidentalis* males, which represented all combinations where the male courted. With this exception, indifference of both sexes, courtship by males of indifferent females, and active antagonism to the males on the part of the females was pretty well scattered at random throughout the combinations. Sexual isolation involves both sexes in this case.

Spieth (1951) published an extensive study of sexual isolation in the virilis group. He had made a thorough study of the sexual behavior of nearly one hundred species of this genus (unpublished), so that his wide knowledge of these mechanisms made the analysis much more complete. He proved conclusively that both males and females exercised choice and that a considerable range of sexual urge existed in these species.

The courtship mechanisms in the virilis group are simple. Courtship is initiated when two flies come in contact. If a male sees a walking fly, he immediately investigates and, on contact, taps with his foreleg. If the individual is a female, the male moves to her rear, licks her genitalia, and rubs the ventral surface of her abdomen with his forelegs. While doing this, the male intermittently extends one wing about 15° from the resting position and vibrates it rapidly. Unless disturbed, the courtship may last five to fifteen minutes with a nonreceptive female. A female, in accepting, raises and spreads her wings and genital plates. A nonreceptive female may decamp, lower her abdomen and wings, kick and rapidly flutter her wings if unfecundated; or, if fecundated, extrude her genitalia. Although none of these reactions will deter a courting male of the same species, some lack of proper stimulus sequence may be effective in crosses between species. The males of the virilis species seem to lack an effective countersignaling device, for males often will court each other in

preference to foreign females. In the presence of females, which always increases the sexual excitement of the males, *novamexicana* and some western *americana* males engage in fights, with the result that usually one male decamps. In this group, the vibrations of the male's wings seem to be one of the important stimuli for an acceptance reaction on the part of the female. The males always lick the inside of the genitalia of the female before mounting.

Spieth reviews the literature on sexual isolation and, in the light of his observations, points out that two factors enter into sexual isolation: sexual drive, and courtship discrimination. Differences in both factors are present in isolation between the virilis forms. Spieth tested the following strains of the virilis species group:

I. *D. virilis,* Sturtevant:
   1. Pasadena—mixed but inbred stock
   2. Hangchow, China
   3. Texmelucan, Puebla, Mexico (1801.1)

II. *D. americana americana,* Spencer—eastern:
   1. Independence 2, Defiance, Ohio
   2. Smithville, Ohio
   3. Millersburg, Pennsylvania (1882.6)

III. *D. americana americana,* Spencer—western:
   1. Chinook, Montana (1761.9s)
   2. Poplar, Montana (1760.8i)
   3. Chadron, Nebraska (1773.4i)

IV. *D. americana texana,* Patterson:
   1. New Orleans, Louisiana (1128.10)
   2. Lake McKethan, Florida (1148.9)

V. *D. novamexicana,* Patterson:
   1. San Antonio, New Mexico (1714.4)
   2. White Water, Colorado (1954.3a)

The several strains of each species and subspecies allowed a good check of the variability that exists in the species group, both within and between species. Spieth checked most of these as unknowns, including duplicate Chinook strains, to be sure the differences discovered would be objective. In all cases the presence of females in a tube stimulated the males to active courtship.

He used flies aged at least ten days from emerging, but both young and old males were tested. Four females and four males were placed without etherization in an observation chamber with a free cylinder,

about twenty by twenty millimeters, between the food and the glass cover and observed for thirty minutes, unless all females accepted males before the end of this time. This procedure reduced the number of courtships (and perhaps copulations) of the forms which crossed readily as the virilis species group males will continue to court fertilized females, even if they refuse to mate. Table 50 is a summary of the averages on courtships and copulations. Usually the intrastrain control gave maximum courtships and copulations. As this is higher than the crosses between different strains of the same species, it is apparent that selection has been very effective in establishing positive sexual selection within the several strains.

The only consistent sexual isolation is that involving males of *virilis* and females of any other species. The *virilis* males usually failed to continue courtship after initial investigation by tapping any foreign female. The only marked exception was that of Pasadena males, which courted the Millersburg *americana* females a number of times. There were a few courtships with other combinations except by Hangchow males, which did not court alien females. Sexual isolation is very high for these males, and the combinations where courtships may occur are unpredictable (Table 50).

Table 50 shows the variation in number of courtships in the several combinations, and the comparative persistence and effectiveness of these in terms of the ratio of courtships to copulations. Both sexual drive and courtship discrimination are factors in the isolation of these forms. The *virilis* males are isolated from other forms because of courtship discrimination; and even though they may refuse to court foreign females and may perhaps court each other (apparently they can discriminate between their own and different species much better than between sexes of their own species), they will always court their own species' females vigorously. Table 51 does not show consistent similarity between the strains of any species or subspecies in the number of courtships. The *virilis* males showed a high degree of isolation, but females accepted alien males readily, judged by courtship-copulation ratios. The females of the eastern strains of *americana* were least receptive of all females as judged by the same ratios, even with eastern *americana* males from other than their own strain.

Spieth summarized his observations and his opinions, based on the activities of the flies under observation, on the relative sexual drive of these several forms. In his opinion, *virilis* males and females have

TABLE 50

Courtship and copulation averages, and ratios of courtships to copulations arranged according to taxonomic groupings (after Spieth)

Crosses	Average Courtships	Average Copulations	Ratio of Courtships to Copulations
Novamexicana Males:			
intrastrain novamexicana	59.85	11.00	5.44
interstrain novamexicana	43.00	5.00	8.60
americana western	42.00	4.75	8.84
americana eastern	15.88	1.00	15.88
texana	7.48	0.75	10.02
virilis	46.84	6.20	7.55
Americana Western Males:			
novamexicana	29.12	4.50	6.47
intrastrain americana western	38.25	9.50	4.02
interstrain americana western	33.84	7.58	4.46
americana eastern	29.05	1.81	16.00
texana	27.50	5.33	5.16
virilis	24.70	7.40	3.34
Americana Eastern Males:			
novamexicana	34.80	4.25	8.18
americana western	44.98	8.25	5.45
intrastrain americana eastern	54.60	8.00	6.82
interstrain americana eastern	54.32	4.83	11.65
texana	33.84	9.00	3.76
virilis	11.00	3.80	3.80
Texana Males:			
novamexicana	45.70	9.25	4.94
americana western	39.73	10.17	3.90
americana eastern	62.06	5.80	10.70
intrastrain texana	45.50	13.00	3.50
interstrain texana including first series Lk ♂ No ♀	63.00	5.50	11.45
interstrain texana including second series Lk ♂ No ♀	42.35	10.00	4.24
virilis	42.60	8.60	4.95
Virilis Males:			
novamexicana	0.48	0.20	2.40
americana western	0.00	0.00	0.00
americana eastern	4.28	0.75	5.71
texana	1.96	0.60	3.26
intrastrain virilis	89.57	13.00	6.89
interstrain virilis	48.78	13.75	3.55

TABLE 51

Total number of courtships for each of the 150 crosses observed (after Spieth)

♀\♂	Sa	Wh	Ch 1	Ch 2	Po	Cd	Id	Sm	Mi	No	Lk	Tx	Pa	Hg	Totals
Sa	61.0	61.0			60.5	26.0		34.1	72.0	50.0	42.0	1.1	0	0	407.7
Wh	25.0	58.7			20.0	10.0		7.5	25.6	46.8	44.0	1.3	0		238.9
Ch 1			18.1	29.0	59.0	26.6	30.0	29.9	67.4	51.0					
Ch 2			29.6	21.3	60.8	21.8	29.0	32.0	52.3	42.0					
Po	61.0	64.0	22.4	39.4	77.6	22.0	43.7	53.0	96.0	45.0	57.2	0	0	0	475.8
Cd	37.0	6.0	5.3	19.2	71.0	26.0	18.0	45.7	42.7	19.2	24.0	0	0	0	271.6
Id			32.0	13.9	53.9	24.6	32.0	45.5	76.8	60.0					
Sm	23.0	7.5	18.0	5.3	18.0	65.0	38.0	49.0	53.0	55.0	52.0	1.1	3.2		327.0
Mi	17.0	16.0	8.5	20.4	40.0	49.0	62.0	50.1	82.8	74.0	69.3	1.1	16.0	0	415.3
No	8.5	2.0	2.1	3.0	17.0	16.0	32.0	7.5	43.7	48.0	41.3	3.7	4.0		171.1
Lk	13.7	5.7			83.0	44.0		19.0	67.0	64.0	43.0	1.0	1.0	0	341.5
Tx	81.2	8.5			40.0	8.5		7.5	17.0	47.0	30.0	49.0	38.9	30.8	327.6
Pa	61.9	46.0			37.0	4.0		11.7	7.5	43.0	36.0	67.4	86.4		400.9
Hg	36.6				34.0				11.4		57.0	58.0		133.3	
Totals	389.3	275.4			464.1	270.5		330.8	507.6	492.0	418.2	125.7	149.6		

the greatest sexual drive, followed by *novamexicana* and *texana*. In *americana,* the eastern strains have aggressive males and lethargic females, whereas the western strains, except Poplar, have lethargic males and very receptive females. The variation between strains can be inferred in part from the summed number of courtships (Table 51), but variation in courtship discrimination complicates the results.

Spieth points out that, in this group, both sexes exhibit courtship discrimination or true sexual isolation. The *virilis* males were normally not sexually excited by the presence of alien females, although they might court each other. If *virilis* females were introduced, they immediately became extremely active in courting them, but not the alien females. The few completed courtships of *virilis* males and alien females were those where the males did not stop after tapping.

The other species' females accepted the *virilis* males when they courted normally. The *virilis* females show little, if any, courtship discrimination. There is some discrimination exercised by the females (Table 51); for although the Millersburg *americana* males courted as frequently as the very active New Orleans strain of *texana,* Spieth's data show only 104 copulations for the former but 138 for the latter, which were accepted somewhat more readily.

In this group the courtship discrimination is mainly the function of the males, for in general the females that were courted more mated more frequently. The spotted and unpredictable nature of this discrimination may be determined. The high courtship-copulation ratio of eastern *americana* is in part due to the high threshold of stimulation necessary before these females will copulate. There is no simple correlation between species relation or strain distribution that will explain the variation in active attempts to mate other than the general discrimination of the *virilis* males. For example, Table 50 shows that the least effective courtships of the eastern *americana* were between interstrain crosses. Unexpected discrimination shows up in such cases as Hangchow *virilis* females, which refused to mate with San Antonio *novamexicana* males, although Texmelucan and Pasadena females each accepted them ten times. There is some indication that strains go least well with those of most distant origin, but this is not always true. This is further complicated by the intrastrain mating balance whereby if the males are very persistent and aggressive the females may be lethargic, and if the males court less vigorously the females accept readily. Spieth recorded a number of cases

of individual differences between males in a strain, as well as strain differences.

Spieth believes that the general high sexual activity of this group, as well as the unpredictable nature of the variation in sexual isolation, is the result of the needs of the species in breeding in very small populations (high sexual activity), together with the more random fixation of differences leading to discrimination.

THE PAIR-MATING METHOD

The pair-mating method of testing for the presence of sexual isolation has been used extensively in the Texas laboratory of genetics. The results obtained from the interspecific crosses between members of the mulleri subgroup may be used to illustrate this method (Patterson, 1947a). Eight different species of the subgroup were selected for the experiment, as follows: *aldrichi, arizonensis, buzzatii, hamatofila, mojavensis, mulleri, peninsularis,* and *ritae.* In carrying out the tests, the usual procedure was followed of making reciprocal matings and then, after a certain elapsed time, dissecting at least one hundred females from each combination and examining their reproductive tracts for the presence of sperm. The results obtained from the fifty-six possible reciprocal crosses are given in Table 52. The data from other tests for hybridization have been interpolated in the second column for the ten crosses which yielded hybrids.

The dissections revealed that, in thirty of the fifty-six crosses, none of the females had been inseminated. In these thirty combinations, sexual isolation is complete. Sixteen of the remaining twenty-six crosses failed to produce hybrids of any kind, although varying proportions of the females had been fertilized. In several of the crosses the percentage of inseminated females is relatively high, and in two of them the percentage runs as high as 74 and 88 (crosses 37 and 28, respectively). The failure to produce hybrid offspring in such crosses must be attributed to causes other than that of sexual isolation.

Of the ten crosses producing hybrids, one gave a few abnormal flies (number 5); one, a few larvae only (number 27); two, sterile males and females (numbers 1 and 7); two, sterile females only (numbers 15 and 21); one, sterile males only (number 3); two, fertile females and sterile males (numbers 9 and 31); and one, fertile females and

TABLE 52

Results obtained from 56 reciprocal crosses (from Patterson)

Crosses	Types of Hybrids	♀ ♀ Dissected	Inseminated
1. mulleri ♀ × aldrichi ♂	Sterile ♀ ♀, ♂ ♂	100	32
2. aldrichi ♀ × mulleri ♂	None	100	18
3. mulleri ♀ × arizonensis ♂	Sterile ♂ ♂	100	8
4. arizonensis ♀ × mulleri ♂	None	100	0
5. mulleri ♀ × buzzatii ♂	Abnormal flies	100	2
6. buzzatii ♀ × mulleri ♂	None	101	0
7. mulleri ♀ × hamatofila ♂	Sterile ♀ ♀, ♂ ♂	100	10
8. hamatofila ♀ × mulleri ♂	None	109	0
9. mulleri ♀ × mojavensis ♂	Fertile ♀ ♀, st. ♂ ♂	100	21
10. mojavensis ♀ × mulleri ♂	None	105	0
11. mulleri ♀ × peninsularis ♂	None	104	0
12. peninsularis ♀ × mulleri ♂	None	104	0
13. mulleri ♀ × ritae ♂	None	110	0
14. ritae ♀ × mulleri ♂	None	104	0
15. aldrichi ♀ × arizonensis ♂	Sterile ♀ ♀	100	26
16. arizonensis ♀ × aldrichi ♂	None	100	0
17. aldrichi ♀ × buzzatii ♂	None	100	0
18. buzzatii ♀ × aldrichi ♂	None	105	0
19. aldrichi ♀ × hamatofila ♂	None	100	11
20. hamatofila ♀ × aldrichi ♂	None	103	0
21. aldrichi ♀ × mojavensis ♂	Sterile ♀ ♀	100	46
22. mojavensis ♀ × aldrichi ♂	None	100	15
23. aldrichi ♀ × peninsularis ♂	None	102	0
24. peninsularis ♀ × aldrichi ♂	None	105	0
25. aldrichi ♀ × ritae ♂	None	102	0
26. ritae ♀ × aldrichi ♂	None	106	0
27. arizonensis ♀ × buzzatii ♂	Larvae only	106	1
28. buzzatii ♀ × arizonensis ♂	None	100	88
29. arizonensis ♀ × hamatofila ♂	None	100	21
30. hamatofila ♀ × arizonensis ♂	None	100	5
31. arizonensis ♀ × mojavensis ♂	Fertile ♀ ♀, st. ♂ ♂	100	29
32. mojavensis ♀ × arizonensis ♂	Fertile ♀ ♀, ♂ ♂	100	93
33. arizonensis ♀ × peninsularis ♂	None	102	0
34. peninsularis ♀ × arizonensis ♂	None	100	43
35. arizonensis ♀ × ritae ♂	None	101	0
36. ritae ♀ × arizonensis ♂	None	100	0
37. buzzatii ♀ × hamatofiia ♂	None	100	74
38. hamatofila ♀ × buzzatii ♂	None	100	1
39. buzzatii ♀ × mojavensis ♂	None	100	18
40. mojavensis ♀ × buzzatii ♂	None	104	0
41. buzzatii ♀ × peninsularis ♂	None	103	0
42. peninsularis ♀ × buzzatii ♂	None	103	1
43. buzzatii ♀ × ritae ♂	None	100	0
44. ritae ♀ × buzzatii ♂	None	100	0
45. hamatofila ♀ × mojavensis ♂	None	100	19
46. mojavensis ♀ × hamatofila ♂	None	100	33
47. hamatofila ♀ × peninsularis ♂	None	103	0
48. peninsularis ♀ × hamatofila ♂	None	100	10
49. hamatofila ♀ × ritae ♂	None	100	1
50. ritae ♀ × hamatofila ♂	None	100	10
51. mojavensis ♀ × peninsularis ♂	None	103	0
52. peninsularis ♀ × mojavensis ♂	None	100	0
53. mojavensis ♀ × ritae ♂	None	110	0
54. ritae ♀ × mojavensis ♂	None	102	0
55. peninsularis ♀ × ritae ♂	None	103	0
56. ritae ♀ × peninsularis ♂	None	100	0

males (number 32). On the basis of these experimental results, *aldrichi, buzzatii, hamatofila, peninsularis,* and *ritae* cannot exchange genes with any other member of the subgroup, but gene exchange could occur through the reciprocal crosses of *arizonensis* and *mojavensis,* and also through the female hybrids of the *mulleri-mojavensis* cross. The extent to which sexual isolation is responsible for preventing gene exchanges between the different members of this subgroup can be determined approximately from the data in the table. A total of 5,690 females from the fifty-six crosses was dissected, and 636 of them were found to have been inseminated. This gives a percentage of about 11, which indicates that approximately 89 per cent of the opportunities for gene exchange are prevented by sexual isolation.

In addition to the results reported above for the mulleri subgroup, both Crow (1942) and Baker (1947) have determined the extent of sexual isolation among certain members of this subgroup. The first author tested five different species, using the pair-mating test, and then dissected all females failing to produce offspring. The seminal receptacles of such females were found to contain no sperm, thus making it possible to determine the degree of true sexual isolation present. Nearly all of the combinations of crosses showed definite sexual isolation. In his experiments, Baker employed five strains of *arizonensis* and two of *mojavensis.* His results from the interspecific crosses between *arizonensis* and *mojavensis* do not differ essentially from those obtained by Crow.

Baker made use of the insemination reaction (see Chapter 8) to determine the extent of mating, in addition to the presence of sperm in the seminal receptacles of the female. This allowed him to detect mating even if all the sperm had been used or exhausted. Table 53 shows a very appreciable reduction in number of offspring, as well as considerable sexual isolation between strains. These factors are shown to be very pronounced, for crosses of $F_1 \times F_1$ were 78 to 100 per cent fertile (average 92 per cent), with 83 to 100 per cent inseminated and 58 to 131 offspring per vial (78 average). Table 54 shows crosses between *mojavensis* and different strains of *arizonensis.* Both sexual isolation and other isolating mechanisms are operating to prevent free gene exchange in these crosses. These two species are the most closely related of any known in the mulleri subgroup, and the tests of Patterson, Crow, and Baker demonstrate all grades of isolation.

A number of investigations have been made in which the pair-

TABLE 53

Fertility relations in P_1 crosses between strains of *arizonensis* (after Baker)

Nature of Cross	Number of Pairs Tested	% ♀♀ Producing Progeny	Estimated % ♀♀ Inseminated	Number of Progeny Per Vial
A ♀ × A ♂	152	78	84	42
As ♀ × As ♂	87	26	37	39
Am ♀ × Am ♂	175	75	88	23
Ag ♀ × Ag ♂	141	55	62	38
As ♀ × A ♂	152	68	74	39
A ♀ × As ♂	128	54	70	36
Am ♀ × A ♂	165	60	69	66
A ♀ × Am ♂	134	63	71	62
Ag ♀ × A ♂	72	32	43	—
A ♀ × Ag ♂	112	70	75	60
At ♀ × A ♂	56	70	82	30
A ♀ × At ♂	59	86	90	22
As ♀ × Am ♂	148	34	48	27
Am ♀ × As ♂	164	66	77	13
As ♀ × Ag ♂	124	33	35	31
Ag ♀ × As ♂	95	32	47	29
As ♀ × At ♂	50	56	74	12
At ♀ × As ♂	54	72	88	16
Am ♀ × Ag ♂	123	71	76	39
Ag ♀ × Am ♂	117	37	50	6
Am ♀ × At ♂	69	72	77	26
At ♀ × Am ♂	—	—	—	15
Ag ♀ × At ♂	59	86	90	32
At ♀ × Ag ♂	41	59	88	24

TABLE 54

Fertility relationships in *arizonensis-mojavensis* crosses (from Baker)

Nature of Cross	Number of Pairs Tested	% ♀♀ Producing Progeny	Estimated % ♀♀ Inseminated	Number of Progeny Per Vial
Md ♀ × Md ♂	123	72	98	11
A ♀ × Md ♂	139	20	44	—
As ♀ × Md ♂	139	20	36	12.5
Am ♀ × Md ♂	134	27	41	14
Ag ♀ × Md ♂	133	38	44	16
Md ♀ × A ♂	105	9	67	—
Md ♀ × As ♂	118	8.5	41	6
Md ♀ × Am ♂	90	10	52	5
Md ♀ × Ag ♂	83	12	53	4.5

mating test has been used to determine the possible presence of sexual isolation in interspecific crosses. These studies included members of several of the species groups belonging to the subgenus Drosophila and should be referred to in this connection.

quinaria group

Sears (1947) has made an extensive study of ten members of the quinaria group and found that, of eighty-eight interspecific crosses tested, only fifteen proved to be fertile. He showed that the most important cause of this lack of cross-fertility was sexual isolation. In two of the crosses, *munda* ♀ × *occidentalis* ♂, and perhaps *munda* ♀ × *suboccidentalis* ♂, the hybrid males were fertile with their hybrid sibs, but failed to fertilize the parent type females. It is suggested that these two cases represent the origin of cross-sterility as being due to a new combination of genes present in the hybrid males.

At the time Sears made his investigations, one species of the quinaria group was not available. Since then a stock of this species, *D. tenebrosa,* has been developed in the laboratory, and Blumel (1949) has been able to make several important additions to the results obtained by Sears. It was found that *tenebrosa* would cross to over half of the other members of the group to which it was tested, but went more readily when used as the female parent. Of interest in this connection was the demonstration that sexual isolation was operating in these crosses. In one cross, *palustris* ♀ × *tenebrosa* ♂, sexual isolation was found to be complete.

virilis group

Tests for sexual isolation among members of the virilis group were first made by Spencer (1938), who showed that the cross of *virilis* ♀ × *americana* ♂ is more successful than the reciprocal mating. His observations were confirmed and extended in a series of articles from this laboratory, which included tests with all six of the then known members of the group (Patterson, Stone, and Griffen, 1940a, b, 1942; Patterson and Griffen, 1944a; Patterson, McDanald, and Stone, 1947; Patterson and Stone, 1949). Some of these publications include reports on sexual preference tests which are considered in the next section. In general, the results obtained demonstrate that sexual isolation is a potent factor in keeping the different forms from crossbreed-

ing. Nevertheless, there are several other factors involved, such as geographical and ecological isolation, gamete and zygote mortality, hybrid sterility, and others. The total effect of these several factors tends to keep the different species from exchanging genes.

funebris group

Mainland (1942b) has made a study of sexual isolation between three species of this group; the cosmopolitan *funebris, subfunebris* from California and *macrospina,* which has three recognized subspecies. All matings between *funebris* and both *subfunebris* and the three subspecies of *macrospina* were found to be incompatible. Not much evidence was found for the existence of sexual isolation in crosses between the *macrospina* subspecies. The interspecific crosses between *subfunebris* and the subspecies *macrospina* are cross-sterile, with the exception of strains from the western limits of the distribution range of the latter form. The exceptions occur only when *macrospina* is used as the female parent. All crosses between *subfunebris* and the western distributed subspecies *limpiensis,* with the exception of one strain, are fertile. Mainland found that, in general, sexual isolation between *subfunebris* and both of these subspecies increases with the increasing geographical separation between the points of origin of the parental strains.

repleta group

Wharton has investigated the degree of cross-fertility between several members of the repleta group. In her first article (1942), Wharton reports that sexual isolation exists between the species *repleta, neorepleta,* and *melanopalpa.* Perhaps of more significance was the demonstration that sexual isolation functions between different geographical strains of *D. repleta,* indicating that it might result in establishing the separation necessary for further divergence. Wharton suggests that this type of incipient isolation may function to establish within a large population smaller and more effective units which are more flexible for rapid evolutionary changes. Table 55 shows the results of pair matings and small mass matings, respectively. Sexual isolation exists between these strains, which are nevertheless all fertile to some third strain. All progeny are fully fertile when obtained. The degree of isolation indicated was checked by dissecting sterile females. As these were never fertilized, we infer that the isolation was sexual;

whether it was due to mate discrimination in every case is unknown, but the reciprocal cross went poorly in a number of cases which showed isolation, so mate discrimination is probably often present.

TABLE 55

Crosses between different strains of *repleta* (from Wharton)

♀♀ \ ♂♂	Fredericksburg	Elgin	New Haven	Guatemala	Eagle Pass	Ankara
Fredericksburg, Tex.:						
Pairs	+	−	−	+	+	
Mass	+	−	−	+	+	±
Elgin, Tex.:						
Pairs	±	+	+	±	+	
Mass	±	+	+	±	+	+
New Haven, Conn.:						
Pairs	+	−	+	+	+	
Mass	+	−	+	±	+	+
Guatemala:						
Pairs	+	−	−	+	−	
Mass	+	−	−	+	−	+
Eagle Pass, Tex.:						
Pairs	+	−	+	+	+	
Mass	+	+	+	+	+	+
Ankara, Turkey:						
Mass	+	+	+	+	+	+

When a choice of mates from the other strain was offered in the small mass matings of twenty-five pairs, only one cross went which failed to go in pairs. Thus reluctance to mate seemed to be a strain and not an individual discrimination. These tests are further examples in support of Spieth's assertion that strains have been selected and adjusted to insure mating. This tendency to develop sexually isolated populations may be one of the factors responsible for the development of the large repleta group.

In the second article, Wharton (1943) summarized the results which have been obtained from fifty-eight combinations, involving interspecific crosses between twenty-two different members of the repleta group. Sixteen of these have been successfully hybridized, of which ten could accomplish gene exchanges with some other member of the group through the production of fertile hybrids. However, in practically every case where the production of fertile hybrids occurred in the laboratory, potent isolating mechanisms operate to prevent such gene exchanges in nature.

melanica group

Three members of this group have been tested by Griffen (1942) for the presence of sexual isolation. The three forms tested were *nigromelanica* and the two subspecies, *melanica* and *paramelanica.* Small mass matings of ten pairs each were first used, followed by large mass matings for those combinations which did not show fertility. All fertile combinations were then tested by pair matings. The results obtained indicate that a rather strong degree of isolation exists between these three members, since the average was only about 1 per cent of fertility for each interspecific cross. In view of the fact that hybrids, when produced, are viable and fertile, sexual isolation in the P_1 crosses must be the type of mechanism operating to prevent gene exchange.

THE MULTIPLE-CHOICE METHOD

In all of the experiments discussed above, the flies did not have a choice of mate. If a successful copulation is to occur, the flies are forced to undergo heterogamic matings. In contrast to this condition, the method of multiple choice that has been used allows the males to exercise a preference. Hence, both homogamic and heterogamic matings are possible. In employing the preference test, it has been customary to make up cultures containing two kinds of females, together with males belonging to one of the two types of females. After the flies have been exposed to each other for a certain length of time, the females are dissected to determine if insemination has occurred. If the time of exposure has been properly determined and if not all of the females have been fertilized, it is possible to determine whether fertilization of the two types of females has taken place selectively or at random. As Dobzhansky and Mayr (1944) have pointed out, if fertilization has occurred selectively and not at random, sexual isolation will be indicated.

In making up cultures for sexual preference tests, different combinations of the sexes have been used by different investigators. Dobzhansky and associates have usually employed ten females of each of two forms and ten males of one of the forms for a single culture. Stalker (1942a), in his experiments with *virilis* and *americana,* used two males and ten females each of the two species per

culture. In our own work on members of the virilis group (Patterson, McDanald, and Stone, 1947), and on members of the mulleri subgroup (Patterson, 1947a), we employed a single male and only five females each of the two species under test. After a given period of exposure to the males, the sexes are separated and the females dissected and their seminal receptacles examined for the presence of sperm. In recording the experimental results in the tables, the number of females dissected and the percentages found to be inseminated are given for both the homogamic and heterogamic matings. The χ^2 values for the observed difference between the frequencies of these two types of matings are also given, as well as Stalker's *isolation index*. This index is obtained by dividing the difference in percentages between inseminated conspecific and alien females by the sum of these two percentages.

A number of tests for sexual preference in Drosophila have been carried out in recent years. Five different species groups have been examined, of which three belong to the subgenus Sophophora (saltans, willistoni, obscura) and two to the subgenus Drosophila (virilis, repleta).

saltans group

Dobzhansky and Streisinger (1944) carried out a series of sexual preference tests on seven geographic strains of *D. prosaltans*. Three of these strains originated from points in Mexico (Chilpancingo, Zopilote, Huichihuayan), near the northern limits of the range of this species; one came from Guatemala City, in Central America; another was from Belem, in Equatorial Brazil; and two came from the state of São Paulo, in southern Brazil (Bertioga, Iporanga). The results from the forty-two combinations tested are listed in Table 56.

All seven strains doubtless belong to the same species, since they intercross readily and produce fertile hybrids. Of the forty-two combinations listed in the table, twenty-seven gave positive isolation indices and fifteen yielded negative indices. Inspection of the data clearly indicates that preferential mating is the rule when geographic strains of *prosaltans* are brought together, and that strong preferences for both homogamic and heterogamic matings are common. The χ^2 values show that, within the limits of significance, thirty-five or thirty-four of the forty-two crosses give results deviating from random mat-

ing and that significant positive indices occur in twenty-three or twenty-four crosses, and significant negative ones in eleven crosses.

Dobzhansky and Streisinger explain these relationships by stating that the geographic strains examined form a hierarchic series extend-

TABLE 56

Number of females dissected (*n*) and percentage carrying sperm (%) in various crosses of *Drosophila prosaltans* (from Dobzhansky and Streisinger)

Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation Index
Chilpancingo, Zopilote	Chilpancingo	60	88.3	65	60.0	12.8	0.19
Chilpancingo, Zopilote	Zopilote	91	57.1	90	56.7	0.0	0.00
Chilpancingo, Huichihuayan	Chilpancingo	72	95.8	70	32.9	61.7	0.49
Chilpancingo, Huichihuayan	Huichihuayan	48	31.2	47	87.2	30.8	—0.47
Chilpancingo, Guatemala	Chilpancingo	60	96.7	63	28.6	60.7	0.54
Chilpancingo, Guatemala	Guatemala	93	40.9	106	71.7	19.3	—0.27
Chilpancingo, Belem	Chilpancingo	91	89.0	86	26.7	70.5	0.54
Chilpancingo, Belem	Belem	111	27.0	102	74.5	47.9	—0.47
Chilpancingo, Bertioga	Chilpancingo	48	91.7	48	6.2	70.0	0.87
Chilpancingo, Bertioga	Bertioga	103	17.5	111	65.8	51.0	—0.58
Chilpancingo, Iporanga	Chilpancingo	57	100.0	57	14.0	85.9	0.75
Chilpancingo, Iporanga	Iporanga	85	35.3	89	67.4	18.0	—0.31
Zopilote, Huichihuayan	Zopilote	71	71.8	72	34.7	19.7	0.35
Zopilote, Huichihuayan	Huichihuayan	105	26.7	114	69.3	39.7	—0.44
Zopilote, Guatemala	Zopilote	65	75.4	59	18.6	40.1	0.60
Zopilote, Guatemala	Guatemala	67	28.4	75	58.7	13.0	—0.35
Zopilote, Belem	Zopilote	46	95.6	42	26.2	45.5	0.57
Zopilote, Belem	Belem	112	49.1	113	61.1	3.2	—0.11
Zopilote, Bertioga	Zopilote	77	72.7	76	19.7	43.3	0.57
Zopilote, Bertioga	Bertioga	105	32.4	108	67.6	26.4	—0.35
Zopilote, Iporanga	Zopilote	50	92.0	53	32.1	38.8	0.48
Zopilote, Iporanga	Iporanga	82	26.8	80	70.0	30.1	—0.45
Huichihuayan, Guatemala	Huichihuayan	92	71.7	92	38.0	21.1	0.31
Huichihuayan, Guatemala	Guatemala	79	36.7	80	48.7	2.6	—0.14
Huichihuayan, Belem	Huichihuayan	86	77.9	82	13.4	70.1	0.71
Huichihuayan, Belem	Belem	106	57.5	100	30.0	15.8	—0.31
Huichihuayan, Bertioga	Huichihuayan	74	58.1	74	17.6	25.8	0.54
Huichihuayan, Bertioga	Bertioga	100	32.0	96	51.0	7.1	—0.23
Huichihuayan, Iporanga	Huichihuayan	76	81.6	75	48.0	18.7	0.26
Huichihuayan, Iporanga	Iporanga	100	33.0	95	45.3	3.1	—0.16
Guatemala, Belem	Guatemala	103	55.3	102	10.8	46.5	0.67
Guatemala, Belem	Belem	99	55.6	106	16.0	35.0	0.55
Guatemala, Bertioga	Guatemala	51	64.7	51	11.8	30.2	0.69
Guatemala, Bertioga	Bertioga	78	66.7	80	41.3	10.2	0.24
Guatemala, Iporanga	Guatemala	50	60.0	52	11.5	26.2	0.68
Guatemala, Iporanga	Iporanga	164	60.4	155	38.7	14.9	0.22
Belem, Bertioga	Belem	122	53.3	116	36.2	7.0	0.19
Belem, Bertioga	Bertioga	91	41.8	94	21.3	9.1	0.32
Belem, Iporanga	Belem	94	45.7	94	50.0	0.3	—0.04
Belem, Iporanga	Iporanga	85	43.5	80	41.2	0.1	0.03
Bertioga, Iporanga	Bertioga	70	42.8	67	38.8	0.2	0.05
Bertioga, Iporanga	Iporanga	99	61.6	108	48.1	3.8	0.12

ing along the north-south distribution range of this species. Their explanation is as follows:

> If males of a strain higher up in this series are confined with females of the same strain and of another strain lower down in the series, the frequency of homogamic matings exceeds that of the heterogamic ones, making the isolation index positive. But if males of a "low" strain are kept with a mixture of females of their own and of a "higher" strain, the frequency of heterogamic matings exceeds that of the homogamic ones, and the isolation index is negative. This regularity was found in thirty-six out of forty-two crosses.

The occurrence of negative isolation indices signifies that mating between individuals of different strains is more frequent than between those of the same strain in certain of these crosses. In all probability, this indicates that one is dealing here with sexual selection rather than with sexual isolation in such intraspecific crosses, and that sexual vigor is important.

Sexual preference tests have been carried out by Dobzhansky (1944a) on geographical strains of another member of the saltans group (*D. sturtevanti*). For these tests five different geographical strains were used, as follows: one from Mexico (Tamazunchale), one from Guatemala (Quiriguá), one from northern Brazil (Belem), one from Rio de Janeiro (Rio), and one from São Paulo (Bertioga). The results from these tests are given in Table 57. The same technique was employed as before, and the full twenty crosses were made. In every case a positive isolation index was obtained, indicating that males of all strains inseminated more of their own kind of female than of alien females. Values of χ^2 from 6.4 to 32.6 were observed in eleven of the crosses. The highest isolation indices were found in crosses of strains from the two most geographically remote localities (Mexico and São Paulo), although two other strains from almost as far apart (Mexico and Rio) failed to show statistically significant isolation indices. Dobzhansky concludes that, on the basis of these results, strains of *sturtevanti* show incipient sexual isolation, but the extent of this isolation is not strictly correlated with the geographical origin of the strains.

TABLE 57

Number of females dissected (*n*) and percentage carrying sperm (%) in various crosses of *Drosophila sturtevanti* (from Dobzhansky)

Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation Index
Tamazunchale, Quiriguá	Tamazunchale	194	60.3	192	37.0	21.0	0.24
Tamazunchale, Quiriguá	Quiriguá	104	46.2	107	24.3	11.1	0.31
Tamazunchale, Belem	Tamazunchale	111	54.1	109	32.1	10.7	0.25
Tamazunchale, Belem	Belem	86	65.1	82	41.5	9.5	0.22
Tamazunchale, Rio	Tamazunchale	104	52.9	112	46.4	0.9	0.06
Tamazunchale, Rio	Rio	124	61.3	130	49.2	3.7	0.11
Tamazunchale, Bertioga	Tamazunchale	115	73.9	104	35.6	32.6	0.35
Tamazunchale, Bertioga	Bertioga	108	58.3	108	24.1	26.2	0.42
Quiriguá, Belem	Quiriguá	106	48.1	97	25.8	10.9	0.30
Quiriguá, Belem	Belem	94	54.3	99	24.2	18.3	0.38
Quiriguá, Rio	Quiriguá	99	56.6	96	43.8	3.2	0.13
Quiriguá, Rio	Rio	116	50.0	113	35.4	5.0	0.17
Quiriguá, Bertioga	Quiriguá	99	49.5	103	32.0	6.4	0.21
Quiriguá, Bertioga	Bertioga	106	59.4	109	26.6	23.7	0.38
Belem, Rio	Belem	160	55.6	157	49.0	1.4	0.06
Belem, Rio	Rio	98	65.3	106	39.6	14.5	0.24
Belem, Bertioga	Belem	101	50.5	100	39.0	2.7	0.13
Belem, Bertioga	Bertioga	91	41.8	88	31.8	2.0	0.14
Rio, Bertioga	Rio	106	51.9	106	50.0	0.1	0.02
Rio, Bertioga	Bertioga	101	50.5	106	48.1	0.1	0.02

willistoni group

Five geographical strains of *D. willistoni* have been examined by Dobzhansky and Mayr (1944) for the presence of incipient isolation in intraspecific crosses. They used one strain from Guatemala (Quiriguá), one from Equatorial Brazil (Belem), and three from southern Brazil (Bertioga, Praia, Rio). They also tested a strain from Teffé, state of Amazonas, Brazil, but the results obtained are not included because it did not cross with any of the other strains and was later described by Dobzhansky (1946b) as a distinct species under the name *D. equinoxialis*. They employed the multiple-choice technique and tabulated their results as in the previous article.

Eight different combinations of crosses between the four Brazilian strains gave 62 per cent of homogamic and nearly 60 per cent of heterogamic inseminations among 1,090 dissected females, the difference not being statistically significant. The inseminations therefore occurred at random, and no trace of sexual isolation was observed. In contrast to these results, when the Guatemalan strain was crossed with the Brazilian strains a different result was obtained, for the isolation index is positive whenever Brazilian males are used and nega-

tive when Guatemalan males are used. This means that when mixtures of Brazilian and Guatemalan females are confined with Brazilian or Guatemalan males, Brazilian females are inseminated in preference to Guatemalan females. The authors conclude that the mating of Brazilian and Guatemalan flies is selective rather than random, but that the peculiar type of selectivity observed does not constitute a barrier to gene exchange.

Two strains of *D. nebulosa* were tested by Dobzhansky (1944a), one from Mexico and the other from northern Brazil. The results show that when Brazilian males were exposed to a mixture of Brazilian and Mexican females, more Brazilian than Mexican females were inseminated ($\chi^2 = 37.1$); but when Mexican males were used, an excess of Brazilian females was inseminated, giving a negative isolation index of -0.14, which does not indicate a significant difference.

Burla *et al.* (1949) tested sexual isolation in the four sibling species, *willistoni, paulistorum, tropicalis,* and *equinoxialis.* Insemination occurred in cross-matings between all forms except *equinoxialis* and *tropicalis,* at least one way. In fact 51.3 per cent of *willistoni* females and 65.0 per cent of the *tropicalis* females were inseminated by *paulistorum* males, with no choice of mate. In multiple-choice experiments, homogamic matings were always much in excess of heterogamic; but *tropicalis* males inseminated half the *paulistorum* females, while fertilizing all of their own type of females. Despite the lack of complete sexual isolation, no hybrids survive.

obscura group

Several articles dealing with the question of sexual isolation among members of the obscura group have been published. One of the first of these was by Dobzhansky and Koller (1938), who made numerous tests between strains of *pseudoobscura* (races A and B) and *miranda* of the pseudoobscura subgroup. They also made a limited number of tests between *azteca* and *athabasca* of the affinis subgroup. It was found that, in cultures containing mixtures of *pseudoobscura* and *miranda* females and males of either of these species, a strong preference for homogamic matings was exhibited; but they were not able to determine whether the preference is exercised by the males or by the females, or by both sexes. A similar, although less pronounced, preference was observed in mixed cultures of different geographic

strains of *miranda.* In one series of interspecific, reciprocal crosses between *miranda* and *pseudoobscura,* where heterogamic matings alone can occur, it was found that only a part of the females had been inseminated after an exposure of nine days. It takes an exposure of but four or five days for almost all of the females to become inseminated in cultures containing males and females of the same species.

In mixed cultures of *azteca* and *athabasca,* a preference for homogamic matings was observed. However, the sexual isolation exhibited between these two species is much less pronounced than that between *pseudoobscura* and *miranda.*

Under the general title, *Experiments on Sexual Isolation in Drosophila,* Dobzhansky, Mayr, and coworkers have published a series of eight articles, of which three have already been referred to above and the remaining five deal primarily with *pseudoobscura* and *persimilis* (Mayr and Dobzhansky, 1945; Mayr, 1946a, 1946b; Levene and Dobzhansky, 1945; Wallace and Dobzhansky, 1946). One of these may be considered here; the other four will be discussed in later sections.

It has been shown that males of either *pseudoobscura* or *persimilis,* when exposed to females of both species, inseminate a higher percentage of their own kind of female, and that the degree of preference can be altered slightly through conditioning (Mayr and Dobzhansky, 1945); but it is clear that preference is mainly controlled by genetic factors. For this reason Mayr (1946a) considered it desirable to test the hybrids between *pseudoobscura* and *persimilis* in regard to their position in the mating preference scale. Since the hybrid males are sterile, only the F_1 females of the reciprocal crosses were tested with males and females of their parental species. The results obtained from these tests are given in Table 58.

In the first two crosses, *persimilis* males inseminated more hybrid than their own kind of females, each giving a negative index. In the next two crosses, *pseudoobscura* males showed a slight preference for their own females. In both of these cases a maternal effect is apparent. As Mayr points out, direct observations of mating behavior of Drosophila indicate that the "ratio of preference" is in the main controlled by three factors: (1) species recognition; (2) physical compatibility of genitalia; and (3) degree of activity of the fly.

Mayr believes that the factor of activity is especially important in the case of hybrid females, because hybrid vigor may compensate for

any adverse influence of the other two factors. It was found that males of *persimilis* inseminate about twice as many of their own as of alien females if equal numbers of the two species are present, while, under

TABLE 58

Number of females dissected (*n*) and percentage carrying sperm (%) if males had the choice between females of their own species and alien female (from Mayr)

Male and Homogamic Female	Alien Female (Heterogamic)	Homogamic		Heterogamic		χ^2	Isolation Index
		n	%	*n*	%		
persimilis	*persimilis* × *pseudoobscura* H *	68	38.2	68	73.5	17.2	−0.32
persimilis	*pseudoobscura* × *persimilis* H *	142	33.8	143	45.5	4.04	−0.15
pseudoobscura	*pseudoobscura* × *persimilis* H	142	69.0	145	54.5	5.76	+0.11
pseudoobscura	*persimilis* × *pseudoobscura* H *	80	67.5	84	27.4	29.7	+0.42
persimilis	*pseudoobscura* C †	107	72.0	97	41.3	19.5	+0.27
pseudoobscura	*persimilis* C	202	77.3	205	6.8	207.7	+0.84

* H Hybrid females.
† C Control females.

similar conditions, *pseudoobscura* males fertilize more than ten times as many of their own kind as of *persimilis*. This might indicate that factors one and two are less important for males of *persimilis* than for those of *pseudoobscura*. The results obtained in the experiments with hybrids are consistent with this hypothesis.

Tan (1946) tested sexual isolation in several mutant combinations in *pseudoobscura*, as well as hybrids and recombination F_2 hybrids between *pseudoobscura* and *persimilis*. In *pseudoobscura*, some mutant combinations showed preferential matings. Both normal and *yellow* males inseminated more *yellow* than non*yellow* females, but both *aristapedia* and *Bare Curly* males prefer the latter type females.

The *pseudoobscura-persimilis* F_1 and F_2 hybrid females were tested with both types of males. When *persimilis* and hybrid females were placed with *persimilis* males, and when *pseudoobscura* and hybrid females were placed with *pseudoobscura* males, the hybrid females were inseminated more often. The hybrid females were much preferred by *pseudoobscura* males when mixed with *persimilis* females. Tan decided from the recombination tests that the X and 2 chromosomes were the most important in the preference, for these increased the mate selection for a hybrid female. It is clear from his results that

several gene pairs are involved in the sexual isolation between these species.

virilis group

Three main articles covering sexual preference tests between members of the virilis group have been published. The first by Stalker (1942) included the results from interspecific crosses between *virilis* and strains of *americana,* and also intraspecific crosses between four geographic strains of *americana* from Ohio. In his main experiments, Stalker used the multiple-choice technique, employing ten females each of the two species (or strains) and two males belonging to one of them for each culture. In these experiments, matings between *virilis* and *americana* showed that the males exercise a choice, and consequently they inseminated more conspecific than alien females. Similar matings between strains of *americana* demonstrated the presence of sexual isolation, although this isolation is less complete than that which occurred between *virilis* and *americana.*

Stalker also tested *virilis* for sexual isolation with the four strains of *americana* in experiments in which the males from one of the species were placed with an equal number of females of the other species. Under such conditions, *virilis* showed sexual isolation with all four strains of *americana,* but the crosses in which *virilis* entered as the male parent gave significantly greater isolation than the reciprocal ones. Similar experiments in which the male had no choice of mate showed no sexual isolation among any of the four strains of *americana.*

The second and third articles report the results obtained in sexual preference tests from interspecific crosses of the six members of the virilis group (Patterson, McDanald, and Stone, 1947; Patterson and Stone, 1949). The tests were made by using a mixed culture of five females of each of two species, together with a single male belonging to one of the species. The results obtained in the thirty possible crosses are listed in Table 59. In ten of the combinations (1, 13, 15, 16, 21, 25, 27 to 30) the isolation index is 1.00, which indicates that sexual isolation is complete. In eight other combinations (3, 5, 7, 9, 14, 19, 20, 22) the isolation index is close to unity, showing that sexual isolation in each of these several crosses is nearly complete. Even in the *lacicola-virilis* cross (8), which gave the lowest χ^2 value, the isolation index of 0.29 shows that the difference between homo-

gamic and heterogamic matings is significant. In all but four combinations the isolation indices are positive. The four exceptions with negative indices include males of *novamexicana* (10), *americana* (17), and *texana* (12, 23), which in each instance preferred the alien females to their own kind when choice of mate was possible. The χ^2 values of 13.6, 13.9, 28.2, and 12.0, respectively, for the four cases indicate that the differences are statistically significant.

TABLE 59

Sexual preference tests between members of the *virilis* group (data from Patterson, McDanald, and Stone, 1947; and Patterson and Stone, 1949)

Females	Males	Homogamic		Heterogamic		χ^2	Isolation Index
		n	%	*n*	%		
1. *virilis, americana*	*virilis*	102	92.1	103	0.0	175.3	1.00
2. *americana, virilis*	*americana*	105	49.5	106	24.6	14.1	0.33
3. *virilis, texana*	*virilis*	101	74.2	103	2.9	109.9	0.92
4. *texana, virilis*	*texana*	101	58.4	102	24.6	21.2	0.37
5. *virilis, montana*	*virilis*	109	80.7	115	1.8	145.3	0.95
6. *montana, virilis*	*montana*	111	58.5	108	14.8	44.9	0.69
7. *virilis, lacicola*	*virilis*	102	71.5	102	0.9	109.9	0.97
8. *lacicola, virilis*	*lacicola*	102	39.2	108	21.3	8.0	0.29
9. *virilis, novamexicana*	*virilis*	95	73.6	90	1.1	102.9	0.97
10. *novamexicana, virilis*	*novamexicana*	100	34.0	100	60.0	13.6	—0.28
11. *americana, texana*	*americana*	115	60.8	115	31.3	20.2	0.32
12. *texana, americana*	*texana*	111	30.6	112	55.3	13.9	—0.28
13. *americana, montana*	*americana*	120	50.0	102	0.0	70.6	1.00
14. *montana, americana*	*montana*	109	67.8	115	0.9	112.9	0.97
15. *americana, lacicola*	*americana*	107	50.4	109	0.0	92.6	1.00
16. *lacicola, americana*	*lacicola*	100	75.0	100	0.0	120.0	1.00
17. *americana, novamexicana*	*americana*	100	23.0	100	60.0	28.2	—0.44
18. *novamexicana, americana*	*novamexicana*	100	75.0	100	5.0	102.1	0.87
19. *texana, montana*	*texana*	107	83.1	104	0.9	145.7	0.97
20. *montana, texana*	*montana*	104	69.0	102	0.9	104.8	0.97
21. *texana, lacicola*	*texana*	101	60.4	102	0.0	88.1	1.00
22. *lacicola, texana*	*lacicola*	100	50.0	100	2.0	61.6	0.92
23. *texana, novamexicana*	*texana*	100	61.0	100	83.0	12.0	—0.15
24. *novamexicana, texana*	*novamexicana*	100	72.0	100	8.0	85.3	0.80
25. *montana, lacicola*	*montana*	100	52.0	105	0.0	69.6	1.00
26. *lacicola, montana*	*lacicola*	111	73.8	105	6.6	100.6	0.83
27. *montana, novamexicana*	*montana*	100	73.0	100	0.0	115.0	1.00
28. *novamexicana, montana*	*novamexicana*	100	65.0	100	0.0	96.2	1.00
29. *lacicola, novamexicana*	*lacicola*	100	51.0	100	0.0	68.5	1.00
30. *novamexicana, lacicola*	*novamexicana*	100	94.0	100	0.0	177.4	1.00

We also carried out a series of preference tests on four geographical strains each of *virilis, texana,* and *montana.* The *virilis* strains employed were Pasadena, Florida, Mexico, and Shenking (China). The four *texana* strains came from New Orleans, Louisiana; George-

town and Newton, Texas; and Okefenokee, Georgia. The *montana* strains originated from Yellowstone National Park, Wyoming (1211.-58); Cottonwood Canyon, Utah (1218.8); Puffer Lake, Utah (1220.2); and Bonita Canyon, New Mexico (1324.8). One strain each of these species was selected and tested with the other three; Pasadena for *virilis,* New Orleans for *texana,* and Cottonwood for *montana.* The results obtained from this series of tests are listed in Table 60.

The χ^2 values and isolation indices for the *virilis* series show that the differences between homogamic and heterogamic matings are significant in five of the six crosses (1, 3 to 6). In three of the combinations the isolation index is positive, and in three it is negative, including the one that is not significant (cross 2). The χ^2 values and isolation indices in the *texana* series show a significant difference between homogamic and heterogamic matings in three of the six crosses, including two (10, 11) with positive indices and one (12) with a negative index. Finally, in the *montana* series, five of the six crosses show a significant difference, although in two crosses (14,

TABLE 60

Number of females dissected (*n*) and percentage inseminated (%) in intraspecific crosses of the virilis group (from Patterson, McDanald, and Stone)

Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation Index
1. Pasadena, Florida	Pasadena	105	80.9	106	53.6	17.2	0.202
2. Florida, Pasadena	Florida	104	53.8	100	54.0	0.001	—0.001
3. Pasadena, Mexico	Pasadena	100	30.0	100	81.0	52.7	—0.459
4. Mexico, Pasadena	Mexico	100	61.0	100	18.0	38.7	0.544
5. Pasadena, Shenking	Pasadena	116	90.5	116	43.9	57.1	0.339
6. Shenking, Pasadena	Shenking	102	33.3	103	65.0	20.6	—0.322
7. New Orleans, Georgetown	New Orleans	103	34.7	101	46.5	2.37	—0.145
8. Georgetown, New Orleans	Georgetown	130	40.0	149	32.1	1.52	0.108
9. New Orleans, Newton	New Orleans	104	38.4	104	41.3	0.18	—0.036
10. Newton, New Orleans	Newton	104	44.2	112	25.9	8.00	0.261
11. New Orleans, Okefenokee	New Orleans	128	56.2	127	30.7	16.9	0.292
12. Okefenokee, New Orleans	Okefenokee	101	28.7	107	48.6	8.64	—0.257
13. 1218.8, 1211.58	1218.8	111	66.7	101	37.6	17.9	0.279
14. 1211.58, 1218.8	1211.58	98	44.9	98	61.2	5.24	—0.153
15. 1218.8, 1220.2	1218.8	104	63.4	106	33.9	18.29	0.303
16. 1220.2, 1218.8	1220.2	114	43.8	114	60.5	6.35	—0.160
17. 1218.8, 1324.8	1218.8	107	51.4	104	56.3	0.40	—0.045
18. 1324.8, 1218.8	1324.8	101	69.3	101	43.5	13.61	0.207

16) the χ^2 values and the isolation indices are at or just above the border line of significance. Again, three of the indices are positive and three negative, with two of the latter (14, 16) low, and one not significant (17). The occurrence of negative indices in this series of tests is not unique, for Dobzhansky *et al.* have reported similar cases among geographical strains of both *prosaltans* and *willistoni.*

repleta group

Only two papers dealing with sexual preference tests among members of this group have been published, and one of these includes tests on eight species of the mulleri subgroup (Patterson, 1947a). In the section on pair-mating tests, it was pointed out that thirty of the fifty-six crosses listed in Table 52 failed to produce offspring and that none of the dissected females had been inseminated. The experimental results indicate that sexual isolation is complete for the thirty crosses; for if the males failed to fertilize the females in such heterogamic matings, in which the males did not have a choice of mate, it is practically certain that they would not do so where a choice is possible. Consequently the multiple-choice tests were restricted to twenty-four of the remaining twenty-six combinations (Table 61).

The χ^2 values and the isolation indices for most of these crosses clearly indicate that the difference between the homogamic and heterogamic percentages are highly significant. In four of the combinations (4, 11, 20, 24) no heterogamic matings were observed, although in the corresponding interspecific crosses, in which the male had no choice of mate, some inseminations occurred in each case. In one cross (18) *hamatofila* males exhibited no preference between their own and the *buzzatii* females. In two crosses the isolation indices are negative. In one of these crosses (16) the difference between homogamic and heterogamic matings is insignificant, but in the other cross (12) the χ^2 value of 36.9 and the isolation index of —0.56 show a definitely significant difference in favor of the heterogamic matings. The results obtained from this series of experiments demonstrate that sexual isolation is a real barrier to gene exchange between the different species and represents one of the most important factors in the evolutionary divergence of this group of species.

Dreyfus (1948) has demonstrated that considerable sexual isolation exists between *paranaensis* and *pararepleta.* In multiple-choice and single-alien-choice experiments, the amount of insemination,

using *pararepleta* and *paranaensis* females with *pararepleta* males, varied from 2 to 20 per cent. The frequency of insemination increased with the increase in relative frequency of the alien male.

TABLE 61

Number of females dissected (*n*) and percentage inseminated (%) in various interspecific crosses of the mulleri subgroup (from Patterson)

Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation Index
1. *mulleri, aldrichi*	*mulleri*	100	78	100	6	118.4	0.85
2. *aldrichi, mulleri*	*aldrichi*	100	63	100	4	78.1	0.88
3. *arizonensis, mulleri*	*arizonensis*	100	61	100	5	70.9	0.85
4. *buzzatii, mulleri*	*buzzatii*	100	92	100	0	170.4	1.00
5. *hamatofila, mulleri*	*hamatofila*	100	77	100	3	114.1	0.92
6. *mojavensis, mulleri*	*mojavensis*	100	75	100	11	83.6	0.74
7. *arizonensis, aldrichi*	*arizonensis*	100	51	100	10	39.6	0.67
8. *hamatofila, aldrichi*	*hamatofila*	100	75	100	3	108.9	0.92
9. *mojavensis, aldrichi*	*mojavensis*	100	83	100	20	88.6	0.61
10. *aldrichi, mojavensis*	*aldrichi*	100	73	100	9	84.7	0.78
11. *buzzatii, arizonensis*	*buzzatii*	100	90	100	0	163.6	1.00
12. *arizonensis, buzzatii*	*arizonensis*	100	16	100	58	36.9	−0.56
13. *hamatofila, arizonensis*	*hamatofila*	100	63	100	6	74.0	0.82
14. *arizonensis, hamatofila*	*arizonensis*	100	74	100	2	110.0	0.94
15. *mojavensis, arizonensis*	*mojavensis*	100	89	100	10	124.8	0.79
16. *arizonensis, mojavensis*	*arizonensis*	100	60	100	61	0.21	−0.01
17. *arizonensis, peninsularis*	*arizonensis*	100	60	100	9	57.5	0.74
18. *hamatofila, buzzatii*	*hamatofila*	100	55	100	55	0.0	0.00
19. *mojavensis, buzzatii*	*mojavensis*	100	92	100	7	144.5	0.85
20. *buzzatii, peninsularis*	*buzzatii*	100	86	100	0	150.9	1.00
21. *mojavensis, hamatofila*	*mojavensis*	100	80	100	5	115.1	0.88
22. *hamatofila, mojavensis*	*hamatofila*	100	64	100	13	54.9	0.66
23. *hamatofila, peninsularis*	*hamatofila*	100	72	100	9	82.3	0.77
24. *hamatofila, ritae*	*hamatofila*	100	78	100	0	127.8	1.00

Bateman (1949) and Levene (1949) have discussed the measurement of isolation and joint isolation in the multiple-choice tests. Bateman differentiated between sexual isolation or mating discrimination, which he assumed incorrectly from the data then available to be exercised only by the female, and nonspecific mating propensity of the female, which neglected the role of differences in aggressiveness of the males. By assigning all choice to the females, he could calculate the two separately. Spieth's work, particularly on members of the virilis group, has shown that the effective isolation is dependent on the activity of both sexes. Bateman introduced a joint isolation index which is the average of the isolation index of the two types of male multiple choice mating. He also proposed a measure of mating propensity of the females, but the value and interpretation of this measure with different species groups is not clear. Bateman points out that the

evidence favors the development of this isolation as incidental to changes in small populations, rather than as a result of selection pressure. Levene points out certain limitations of these indices, and proposes some means of getting around the limits imposed by differences between different tests and some methods of calculating significance. He developed a *coefficient of isolation* which corresponds to the isolation index but is more independent of the variations in the experiment, and so allows a comparison between different experiments and is much better in calculating joint isolation. He also gives a simple means of calculating a *coefficient of excess insemination* of strain 1 over strain 2, which have been tested together. This shows the difference in gene flow in the two directions between the species under the conditions of the experiment.

ENVIRONMENTAL FACTORS AND SEXUAL ISOLATION

It is known that environmental factors influence the mating habits of Drosophila, and thus play a role in sexual isolation. Light and temperature are two such agents. One of the clearest cases of the effect of light has been reported by Philip, Rendel, Spurway, and Haldane (1944; also Rendel, 1945) for the European species *Drosophila subobscura.* They showed that this species will not mate in the dark and pointed out that, since visual stimuli are essential for mating, certain mutant forms with abnormal eye colors do not respond to moving contours and are male-sterile. The mutant *yellow* is at a disadvantage in mating, since normal females usually rebuff *yellow* males which attempt to copulate with them. These investigators were able to sellect a stock in which normal females were comparatively tolerant of *yellow* males, although the normal body color was still preferred. They were thus able to demonstrate "not only sexual selection of a more or less Darwinian type, but also the inheritance of a degree of preference in the female, such as Darwin postulated."

In view of these experimental results on *subobscura,* Mayr and Dobzhansky (1945) carried out a series of sexual preference tests on the American species *pseudoobscura* and *persimilis,* two relatives of the European *subobscura.* They also tested four geographic strains of *prosaltans.* From the results obtained, they conclude that in all three species mate discrimination is not influenced greatly by the presence or absence of light. However, in the case of *prosaltans,* light does in-

fluence the total number of inseminations which take place within a given period of time, since a significantly higher number of inseminations takes place in cultures exposed to light than in those kept in the dark.

In attempting to obtain copulations for use in studying the insemination reaction, Wheeler (1947) found that placing members of the quinaria group in bright light often resulted in matings otherwise difficult to obtain.

A much more extended series of experiments on *pseudoobscura, persimilis,* and *subobscura* were later carried out by Wallace and Dobzhansky (1946). In the first series of tests, ten to fifteen females of one species were placed with a similar number of males of the same or of a different species, and then part of the cultures were exposed to light and part to dark for different periods of time. As shown in Table 62, the intraspecific crosses of *subobscura* gave 80 per cent of the females inseminated in the light series and none in the dark. When *subobscura* females were crossed with either *persimilis* or *pseudoobscura* males, some of the females were inseminated in both series, with higher percentages in the light series. In the reciprocal crosses, 25 per cent of the *pseudoobscura* females were inseminated in the light and none in the dark. In the other cross, 15 per cent were fertilized in the light and 1.3 per cent in the dark. The authors state that the single inseminated female found in this cross is probably due to an experimental error.

Wallace and Dobzhansky made direct observations on the sexual behavior of these three species and also studied the effect of the intensity of illumination and sexual activity of *subobscura.* The nature of their results can best be presented by quoting their summary, which reads as follows:

> Mating takes place with or without light in *Drosophila pseudoobscura* and *D. persimilis,* while *D. subobscura* mates only in the presence of light. Males of *D. persimilis* and *D. pseudoobscura* inseminate some *D. subobscura* females, the frequency of this cross-insemination being greater in the light than in the dark. *D. subobscura* males inseminate some females of the other two species in light but not in dark. Light intensity of the order of 30 foot-candles was found to be close to the optimum for mating in *D. subobscura,* but no mating and no courtship take place in this species in red light of an intensity which permits observation. Direct observations disclose that, in the light, males of any one of

the three species court about equally frequently females of their own and of the other two species. However, interspecific copulation occurs only seldom, and if it does the female dislodges the male in usually less than 30 seconds, ordinarily before sperm ejaculation takes place.

TABLE 62

Number of females dissected (*n*) and per cent fertilized (%) after several days' exposure in light and darkness (from Wallace and Dobzhansky)

Crosses		Light			Dark		
Female	Male	Days	*n*	%	Days	*n*	%
subobscura	*subobscura*	3– 4	60	80.0	5– 9	72	0.0
persimilis	*persimilis*	3– 5	41	75.6	9	22	100.0
subobscura	*persimilis*	7–14	118	8.5	7– 9	136	2.9
persimilis	*subobscura*	7–13	80	15.0	7–12	80	1.3
subobscura	*pseudoobscura*	9–11	64	21.9	8–15	152	7.2
pseudoobscura	*subobscura*	9–13	96	25.0	10–15	128	0.0

Reports have been made in two articles on experiments which were designed to determine what, if any, effect varying the temperature might have on sexual preference in Drosophila. In the first of these articles, Mayr and Dobzhansky (1945) carried out sexual preference tests between *pseudoobscura* and *persimilis,* and between four geographic strains of *prosaltans.* The cultures were kept at four different temperatures for as long as necessary to obtain insemination of about half of the females. Their results are summarized in Table 63. The sexual behavior of *prosaltans* and *pseudoobscura* flies was about the same at the different temperatures tried. But *persimilis* showed clear sexual isolation from *pseudoobscura* at the higher temperatures, while its males seem to prefer *pseudoobscura* females at the lower temperatures, as is indicated by the negative isolation indices. The conclusion is reached that, if *persimilis* males are used, sexual isolation between *pseudoobscura* and *persimilis* appears to be weaker at lower than at higher temperatures.

Experiments on sexual preference between *virilis* and *americana,* in which the cultures were kept at two different temperatures, have been reported by Patterson, McDanald, and Stone (1947). Each culture contained five females of each of the two species and a single male of one of the forms. The cultures were then divided into two equal lots, one of which was kept at 72° F. and the other at 62° F. Fifty females were dissected at twenty-four-hour intervals for each homogamic and

TABLE 63

Mate discrimination at different temperatures (from Mayr and Dobzhansky)

t°	Females	Males	Homogamic n	Homogamic %	Heterogamic n	Heterogamic %	χ^2	Isolation Index
24½°	*pseudoobscura*, *persimilis*	*pseudoobscura*	30	83.3	28	3.6	20.4	0.92
18°	*pseudoobscura*, *persimilis*	*pseudoobscura*	21	85.7	18	0.0	15.4	1.00
16½°	*pseudoobscura*, *persimilis*	*pseudoobscura*	42	92.9	40	12.5	24.2	0.76
24½°	*pseudoobscura*, *persimilis*	*persimilis*	65	93.8	64	39.1	14.6	0.41
21°	*pseudoobscura*, *persimilis*	*persimilis*	56	53.6	63	12.7	15.4	0.62
18°	*pseudoobscura*, *persimilis*	*persimilis*	21	4.8	20	55.0	8.7	—0.84
16½°	*pseudoobscura*, *persimilis*	*persimilis*	86	32.6	90	52.2	4.0	—0.23
24½°	*prosaltans*-A, *prosaltans*-C	*prosaltans*-A	59	86.4	58	8.6	37.8	0.82
24½°	*prosaltans*-B, *prosaltans*-D	*prosaltans*-B	58	74.1	58	8.6	30.1	0.79
16½°	*prosaltans*-A, *prosaltans*-C	*prosaltans*-A	77	90.9	75	2.3	62.3	0.94
16½°	*prosaltans*-B, *prosaltans*-C	*prosaltans*-B	84	44.0	85	4.7	14.6	0.81

heterogamic mating. The results of these tests are summarized in Table 64.

The results obtained do show that temperature influences the degree of sexual isolation in these crosses. The most obvious effect is the retardation of copulation. When *virilis* is used as the male parent, homogamic matings begin during the forty-eight-hour interval at 72° and not until the 144-hour interval at 62°, while the heterogamic matings begin during the ninety-six-hour interval at 72° and not at all at 62°. It is clear from these results that *virilis* males prefer their own kind of female to the exclusion of the alien females at the lower temperature. When *americana* is used as the male parent in the 72° series, homogamic matings begin during the seventy-two-hour interval and the heterogamic matings during the ninety-six-hour interval. No matings of either kind occurred in the 62° series. Since none of the *americana* females in either cross was inseminated at the lower temperature, it is possible that the absence of fertilization is due to a failure of these females to respond to the courtship of either type of male.

OTHER FACTORS AND CONDITIONS AFFECTING SEXUAL ISOLATION

There are scattered throughout the literature several attempts to demonstrate by laboratory experiments the possible effectiveness of other factors and conditions on the degree of sexual isolation among species of Drosophila. Some of these have already been reviewed; others will now be considered briefly.

TABLE 64

Effect of temperature and length of exposure on the per cent inseminated in the *virilis/americana* crosses (from Patterson, McDanald, and Stone)

Temperature	Hours Exposed	(5V ♀ ♀ + 5A ♀ ♀) × V ♂		(5A ♀ ♀ + 5V ♀ ♀) × A ♂	
		% Homogamic	% Heterogamic	% Homogamic	% Heterogamic
72° F.	24	0	0	0	0
	48	8	0	0	0
	72	10	0	6	0
	96	94	4	62	6
	120	86	12	88	16
	144	100	4	42	16
	168	90	32	42	16
62° F.	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	0
	120	0	0	0	0
	144	6	0	0	0
	168	52	0	0	0
	192	56	0	0	0

Rendel (1945) found that males and females of *subobscura* do not mate until the third day after emerging, provided both sexes are of the same age. However, males will copulate from the time they are one day old, so that it is the females which determine the time at which copulation begins. He found that there is a correlation between the state of development of the ovaries and willingness to mate.

Levene and Dobzhansky (1945) tested the effect of varying the proportions of *pseudoobscura* and *persimilis* (also two strains of *prosaltans*) on the frequency of insemination in mixed populations. After a rather long mathematical treatment of the experimental data, the authors reached the following conclusion:

Results obtained by placing *D. persimilis* males with varying proportions of *D. persimilis* and *D. pseudoobscura* females admit of the hypothesis that the ratio of the probability of heterogamic mating to the probability of homogamic mating is a fixed constant independent of these proportions. However, the possibility of some decrease in this ratio when many *D. persimilis* females are present cannot be rejected. Because of the small numbers of heterogamic matings, similar experiments with two strains of *D. prosaltans* furnish little evidence on this point.

Another point of interest is the possibility of "conditioning" the males before making the sexual preference tests. Mayr and Dobzhansky (1945) did this by keeping some males of *pseudoobscura* for several days in bottles with an excess of females of their own species (proconditioning) and other males for the same length of time with females of *persimilis* (counterconditioning). Males of *persimilis* were conditioned in the same manner. Cultures were then made up containing ten of these males and ten freshly emerged females of each of the two species. For control experiments, freshly emerged males were confined with freshly emerged females. The results obtained were inconclusive in so far as *pseudoobscura* males were concerned. In the case of *persimilis* males, the conditioning seemed to be effective, because males that had been conditioned with their own females showed a higher isolation index than the controls or males conditioned with *pseudoobscura* females. The effects of counterconditioning were found to be more significant than those of proconditioning.

Spieth and Hsu (1950) tested several species in the *melanogaster* group of the Sophophora to see if mating was affected by light, as *subobscura* had been shown not to mate in the dark. The species *melanogaster, montium,* and *ananassae* mated as well in the dark as in the light, while *rufa* and *takahashii* were perhaps slightly affected. On the other hand, *simulans* mated very much less frequently in the dark than in light, while *auraria,* like *subobscura,* failed to mate in the dark. These latter species must depend in part or altogether on sight as a necessary step in the courtship cycle.

Koopman (1950) tested in population cages the effectiveness of selection on the isolation between *pseudoobscura* and *persimilis.* The two species carried different mutant genes, so that the hybrids were phenotypically normal and each class was separable by phenotype. The cages were kept at 16° C., where differences in sexual

isolation could be readily detected. The mutant strains of the two species were outcrossed to strains recently brought into the laboratory, to establish heterozygosity except for the mutant markers. Several population cages were established, using equal numbers of virgin females and males of each species. The larvae were removed, and the proportion of hybrids and pure strain males and females counted. Some of these pure-strain progeny were used to establish the second cage, and so on. The hybrids were removed each generation, although they would not have contributed progeny that would have survived the vigorous selection in these overcrowded population cages.

In two of three cages, the percentage of hybrids dropped from 22.5 per cent and 35.5 per cent to less than 10 per cent, and usually less than 5 per cent by the end of six generations, and remained there. In the third cage, the percentages of hybrids were F_1, 49.5 per cent; F_2, 17.6 per cent; then below 5 per cent except for F_9, which was high with 14.5 per cent hybrids. In each case the proportion of hybrids dropped from the 25 to 50 per cent range to a 1 to 10 per cent range in a few generations.

Selection under these conditions increased the sexual isolation against the *persimilis* males, as shown in multiple-choice tests with several different test generations during the experiment. The original strains were heterozygous and the stringent selection in the cages soon established strains which produced few hybrids and so wasted less reproductive effort. It is not clear whether the strains selected for vigor under these conditions were also isolated from each other or whether those isolated were selected because they wasted fewer gametes, or if both selective forces were acting.

DISCUSSION

The existence of geographical isolation of a major nature is demonstrated best by the fact that 553 out of 613 species are endemic to one of the major zoogeographic regions of the world and that only twelve species occur in more than two regions. Large species groups occur wholly or for the most part in one region. The only two regions which share many species are Nearctic and Neotropical, with thirty-five. A much more important problem is that of more subtle but less complete geographical isolation, which is in fact often ecological in nature, found within a zoogeographic region.

We have no real evidence on initial habitat invasion and selection in Drosophila. We do know that forms exist like *carbonaria,* which breeds on the mesquite only so far as we can tell, and that a large number of the members of the repleta group live on cactus. In each case the microorganisms present in the sap are probably a very important food constituent, as shown by Wagner's work. The genetic differences between species with different food requirements can be inferred from these data also. Patterson and Wagner (1943) showed that the species living along desert streams were in part different from those living only a hundred feet away in the desert proper. The cactus plants in the desert support members of the repleta group and *pseudoobscura,* whereas fungus-feeders, such as *limpiensis* and members of the quinaria group, occur along the stream. Dobzhansky and Pavan (1950) published the data on differences in collections on different fruits, while Spieth, and especially Sturtevant, had discussed these specializations.

Patterson (1943) and Dobzhansky and Pavan (1950) have shown large seasonal differences in population density. We do not know the reason for these, but they act as isolating mechanisms. Wagner (unpublished) raised in the laboratory specimens of *Chymomyza amoena* which were found as larvae and pupae in windfall apples buried under the snow in New York. Buzzatti-Traverso (1943) described hibernation in *nitens* which took place during the winter months. If collected in the fall, the adults ceased to reproduce, even in the laboratory, until spring; then they began again. Presumably *nitens* must regularly winter over this way as adults. We do not know how many forms have developed such specializations for a cold climate.

We cannot prove how ecological isolation developed. If organisms are in contact to more than a minor degree, genetic mechanisms that insure reproductive isolation must be present or the populations will grade into a mixed population. Therefore the population must be geographically (spatially) or reproductively isolated in some manner that prevents free gene exchange, if the genetic changes necessary to enforce ecological isolation are to become fixed in a population. Mayr (1947) and Blair (1950) have reviewed the evidence on ecological divergence. Both oppose the theory of sympatric evolution in the strict sense. We find no evidence of sympatric speciation in Drosophila through ecological divergence or any other mechanism.

The origin of related species which are found together might have occurred by sympatric divergence, but in no case is the alternate possibility of divergent evolution during separation ruled out.

Reproductive isolation between forms is necessary if they are to occur together in large numbers during the mating season without loss of identity. Numerous species are sympatric, and only a few such as *mulleri* and *aldrichi* hybridize (Patterson, 1947). Even here the number of hybrids produced is small, and they are sterile. The most efficient type of reproductive isolation is sexual isolation. It is the most effective form of isolation, for the species spend very little time in courtships of another species, as recognition of the form as alien stops the courtship. When sexual isolation is effective, reproductive effort is not wasted. In this case it is of advantage to a species for the females to refuse alien males, except occasionally closely related males, which will give fertile hybrids. On the other hand, as long as the males keep their own type females fertilized, there is a certain advantage in cases of any competition for the males to fertilize alien females and so reduce the reproductive efficiency of the second species. Species with a very high male sex drive with less mating discrimination, but with females which have a high sex drive and especially efficient mating discrimination, will have one type of advantage. This is not always the case, for *mulleri* females will mate males of several other species, while the *mulleri* males do not cross. This is true also with *virilis* females, which are receptive to other species of the group, whereas *virilis* males have a very high mating discrimination (Spieth, 1951). In fact, cross-mating is nearly always limited to members of the same species group; the only exception known to us occurs in the Sophophora, where *melanogaster* males will mate *affinis* and *pseudoobscura* females.

Patterson, McDanald, and Stone (1947) brought in females collected in a wild habitat and isolated them to determine if they were inseminated. Table 65 shows the number inseminated. The species *repleta* is rarely found other than in domestic habitats and was collected in a produce house, all others being taken from a wild habitat. The females of most species were inseminated, and most of these were fertile. The repleta group is exceptional in both respects, for only slightly over two-thirds of the females of *mulleri* and *longicornis* were inseminated. In addition, these two species were the least fertile of those tested. It is probable that this is associated with food

requirements, for many members of the repleta group are cactus-feeders and in general do poorly in the laboratory. There is no correlation between density of population and number inseminated, so

TABLE 65

Percentage of inseminated females in wild strains of Drosophila (from Patterson, McDanald, and Stone)

Species	Females Isolated	Percentage Fertile	Females Dissected	Females with Sperm	Percentage Inseminated	Percentage in Population
D. busckii	51	96.0%	2	1	98.0%	1.8%
D. putrida	203	92.1	16	1	92.6	15.4
D. melanogaster	547	95.0	27	3	95.6	23.2
D. simulans	302	92.0	24	3	93.0	18.1
D. affinis	88	84.1	14	0	84.1	5.5
D. pseudoobscura	331	96.6	11	5	98.5	27.0
D. tripunctata	42	92.8	3	0	92.8	0.89
D. macrospina	105	83.8	19	3	86.6	4.8
D. repleta	152	73.0	41	32	94.0	18.7
D. hydei	552	80.8	106	55	90.7	23.4
D. mulleri	201	50.2	100	40	70.1	12.7
D. longicornis	130	30.8	88	48	67.7	11.6
D. meridiana	63	85.7	9	5	93.6	3.2

there are very effective mechanisms other than density of population which insure males and females being together at the proper time for mating. Outside the repleta group, there is a very close correlation between the number of inseminated and fertile females; therefore there is little evidence for loss of reproductive effort from cross-mating.

Although Wharton (1944) and Patterson (1947a) showed some cross-mating in the mulleri subgroup, it is not possible to determine if the reduced fertility of certain repleta group species is due to cross-inseminations. The difficulty encountered in obtaining these in the laboratory argues against this factor. Patterson has presented evidence on the effectiveness of isolation between *mulleri* females and *aldrichi* males. The reciprocal cross does not go. The F_1 hybrid males can be recognized by their characteristically abnormal testes. These hybrid males do not occur in natural populations unless the proportion of *aldrichi* males to *mulleri* males is greater than a third of the male populations of the two species. Sexual isolation between the species is sufficient to prevent enough cross-mating to allow hybrid males to be present, unless the proportion of *aldrichi* males is quite high.

The work of Spett (1931), Diederich (1941), and Mayr (1950)

with *melanogaster,* and of Rendel (1945) with *subobscura,* demonstrated that *yellow* mutant males are discriminated against by *yellow* females, and especially by normal females. Rendel (1945) found that some strains of normal females would accept the courtship of *yellow* males nearly as well as that of normal males. Merrell (1949) found that males marked by the mutants *yellow, raspberry,* or *cut* were all at a disadvantage in courtship effectiveness, but that *forked* mutants were not. Presumably this shows that there is some factor responsible for the reluctance of females to mate with these males, other than the fact that the males were mutants. Since Merrell used only *melanogaster,* he decided that all mating activity was performed by the male to excite the female and that there was no mutual stimulus-response pattern, but Spieth's results with the virilis group demonstrated that such a pattern does exist in that group.

Reed and Reed (1950) tested normal and white-eyed mutants in *melanogaster.* With stocks otherwise isogenic by inbreeding, they showed that the *white-* and the normal-eyed flies were about equally viable. The *white* gene disappeared from the population bottles used to test competition because both normal and *white* females discriminated against *white* males in mating. The authors calculate that *white* males are only 0.75 times as effective in mating. These data all indicate that different types of gene mutations may have such unpredictable advantages or disadvantages in sexual selection. In these cases mating discrimination on the part of the females determines the number of successful courtships of males marked by obvious gene mutations. The differences between species which determine sexual isolation do not necessarily depend on major phenotypic differences, as shown by the tests involving sibling species.

The numerous tests within species groups show that complete sexual isolation exists between some species and incomplete isolation as sexual preference between others. The males of some species may lack mating discrimination with many foreign species, but no case has been found where a species is not well isolated when both sexes are considered. There have been several types of tests which measure the effectiveness of sexual isolation, but only direct observation shows us how or where the courtship is terminated. Spieth's work has demonstrated that there are marked differences in sex drive and courtship discrimination, both between species and subspecies and strains, and also between males and females. Both drive and discrimination con-

tribute to sexual isolation, as would any factor that interferes with the necessary stimulus-response sequence and prevents copulation. The evidence of the efficient adjustment of these factors to insure mating was given by Spieth and others. In fact, it is probably a result of maladjustment of these two factors that leads to negative isolation indices in many crosses. This is seen in the virilis group, for example (Patterson, McDanald, and Stone, 1947; Patterson and Stone, 1949), in the *mulleri* subgroup (Patterson, 1947a), in *sturtevanti* (Dobzhansky, 1944a), in the saltans group (Dobzhansky and Streisinger, 1944), as well as in other cases. It may be that some strains possess an excess of those factors which act as sex stimulants, but this is very difficult to prove. One point should be made concerning the value of our tests of isolation. The species *pseudoobscura* and *persimilis* occupy a very large area together where they can be collected at the same time on natural food or on bait. Despite an extensive and careful search, Dobzhansky and his coworkers have not found hybrids of these species in nature, although they cross readily and produce many hybrids in the laboratory. Sexual isolation appears to be completely effective in the wild. This of course raises some question as to whether Koopman (1950) obtained new isolating factors or sorted out new combinations that were more efficient under the laboratory conditions of his experiment. In either case, his experiments are examples of the effectiveness of selection for factors increasing sexual isolation between these species. On the other hand, the presence of hybrids between the subspecies *texana* and *americana* in the overlap zone is in agreement with the lack of isolation between them (Stone and Patterson, 1947). Furthermore, Patterson's (1947a) data on the occurrence of male hybrids between *mulleri* and *aldrichi* in nature is in agreement qualitatively and roughly quantitatively with the demonstrated isolation between them.

Dobzhansky (1941) has suggested that factors favoring sexual isolation have been selected into populations of different species in contact so as to increase their isolation. Such selection could be of value only if the hybrids were at a disadvantage compared with the two species. Some of the tests of isolation between *pseudoobscura* and *persimilis* or *miranda* have been in agreement with this hypothesis; others have not, for distant strains have proved to be more isolated. The members of the virilis group have an unpredictable distribution of sexual isolation barriers. Some species or strains which occur near

one another are quite isolated, but other strains are much more isolated from strains of other species of distant origin. Spieth presumed that the factors favoring sexual isolation toward other species were fixed in populations incidentally, rather than as a result of selection pressure in a zone of contact. This is in agreement with our opinion of the origin and fixation of factors favoring sexual isolation. For this reason the funebris group in this country shows the same absence of fixation of isolating factors due to selection. In fact Mainland (1942) found that the isolation between the species *subfunebris* and *macrospina* increased with distance, with some exceptional highly isolated strains. The increase of sexual isolation with distance favors the opinion that increasing genetic difference, as shown by other tests, is accompanied by increasing isolation.

8 THE INSEMINATION REACTION AND OTHER ISOLATING MECHANISMS

INTRODUCTION

Sexual isolation is a complete barrier between most species. However, closely related forms in the same species group may not be so isolated; for we find that various other mechanisms act as barriers to free gene exchange, and thus prevent the loss of separate identity by the species. These mechanisms are so effective that we do not know of sympatric species that produce an appreciable number of fertile hybrids.

Mechanical isolation might, theoretically, prevent successful mating. Hsu (1949) has shown that there is considerable variation in the external genitalia of Drosophila males. It may be that, in some cases, there is enough difference in the genitalia to prevent successful mating if attempted, but we know of no evidence for this. The case that is most suggestive of such isolation is one reported by Sturtevant (1921b). He found that *pseudoobscura* or *affinis* females would sometimes copulate with *melanogaster* males, although no hybrids or hybrid larvae occurred. This might be due to mechanical difficulties or to those which arose as a result of an abnormal reaction to foreign sperm.

There are several instances of incomplete copulation and of lack or reduction in the amount of sperm transmitted in crosses between species. Several examples of this type have been observed by Wallace and Dobzhansky (1946). They state that if, in spite of the negative reactions of *subobscura* females, *pseudoobscura* or *persimilis* males persist in courting and mount, the pair usually separates immediately. In fact very few *subobscura* females have been proved to be insemi-

nated by these males, even after ample time together. The mating of *subobscura* males and *pseudoobscura* or *persimilis* females is an even more striking example of unsuccessful copulation. Within a few seconds of the time the male mounts and initiates copulation, the female of either of the other two species begins a violent attempt to free herself. The pair often falls to the bottom of the container as the female strives to free herself by kicking with her hind feet. In spite of his efforts to retain his position, the male is usually dislodged in less than thirty seconds. Although *persimilis* females readily allow the initiation of copulations by *subobscura* males, only six out of fifty females were found to be inseminated on dissection. However, up to 15 to 25 per cent, respectively, of *persimilis* and *pseudoobscura* females were fertilized in from one to two weeks. Mayr (1946b) has described incomplete copulations between *pseudoobscura* and *persimilis,* but these matings were terminated within a few seconds after the beginning of copulation. In fact this is so common that he observed many more such attempts at mating than complete ones with successful insemination. He points out that Farris was unable to find any physical differences between the genital systems of the two species.

In addition to these cases of termination of copulation prior to insemination, there are others of partial insemination. Mayr and Dobzhansky (1945) state that the amount of sperm present in inseminated females varies greatly in *pseudoobscura* and *persimilis,* particularly in heterogamic crosses. Patterson, McDanald, and Stone (1947) have compared the results of homogamic and heterogamic crosses in the virilis group. No attempt was made to count the number of sperm, but an estimate was made as to whether there were many, moderate, or few present. Table 66 summarizes the results of

TABLE 66

Crosses	Spermathecae			Ventral Receptacle		
	Many	Moderate	Few	Many	Moderate	Few
Homogamic	1,022	109	135	996	95	129
	80.7%	8.7%	10.6%	81.6%	7.8%	10.6%
Heterogamic	125	19	43	118	30	52
	66.8%	10.2%	23.0%	59.0%	15.0%	26.0%

a number of crosses. It is clear that homogamic matings have been somewhat more successful in insuring a plentiful supply of sperm. This may be due to a greater sperm delivery, more repeated insemi-

nations, interrupted delivery in matings between species, or a deleterious physiological effect on the foreign sperm as a result of the insemination reaction or some more indirect effect.

THE INSEMINATION REACTION

Among the several isolating mechanisms which prevent or reduce the exchange of genes between different species of Drosophila and which are effective in evolutionary divergence is one that has been termed the *insemination reaction* (Patterson 1946). This reaction usually occurs soon after coitus, but in some species it may start before copulation is completed. It occurs in both intraspecific (homogamic) and interspecific (heterogamic) matings and is revealed in the vagina through a rapid secretion of fluid into its cavity, which results in causing an increase in the size of the vagina to three or four times its normal size as well as an increase in the size of the epithelial cells.

Figure 61 illustrates the main macroscopic features of the reproductive tract of a Drosophila female. It shows the ovaries, oviducts, azygous oviduct, parovaria, spermathecae, long coiled ventral receptacle, and vagina. The part of the vagina which extends anteroventrally is called the *vaginal pouch.* It is in this portion of the vagina that the enlargement takes place during the course of the insemination reaction. Its wall is capable of much distention, while the wall of the vagina proper remains practically unchanged.

The microscopic structure of the wall of the vaginae of virgin females has recently been studied by Lee (1950), who describes three distinct layers: (1) an external thick muscular layer which is much thinner on the wall of the vaginal pouch; (2) a middle layer composed of cuboidal epithelial cells, with oval-shaped nuclei; (3) an innermost transparent, noncellular cuticular intima, which is more prominent in the vagina than in the vaginal pouch. The intima and epithelium are often thrown into deep folds within the lumen, especially on the wall of the vaginal pouch.

INSEMINATION REACTION IN HOMOGAMIC MATINGS

A large number of species of Drosophila have been examined for the occurrence of the insemination reaction in homogamic matings (Patterson 1947b, Wheeler 1947). In this type of mating, the con-

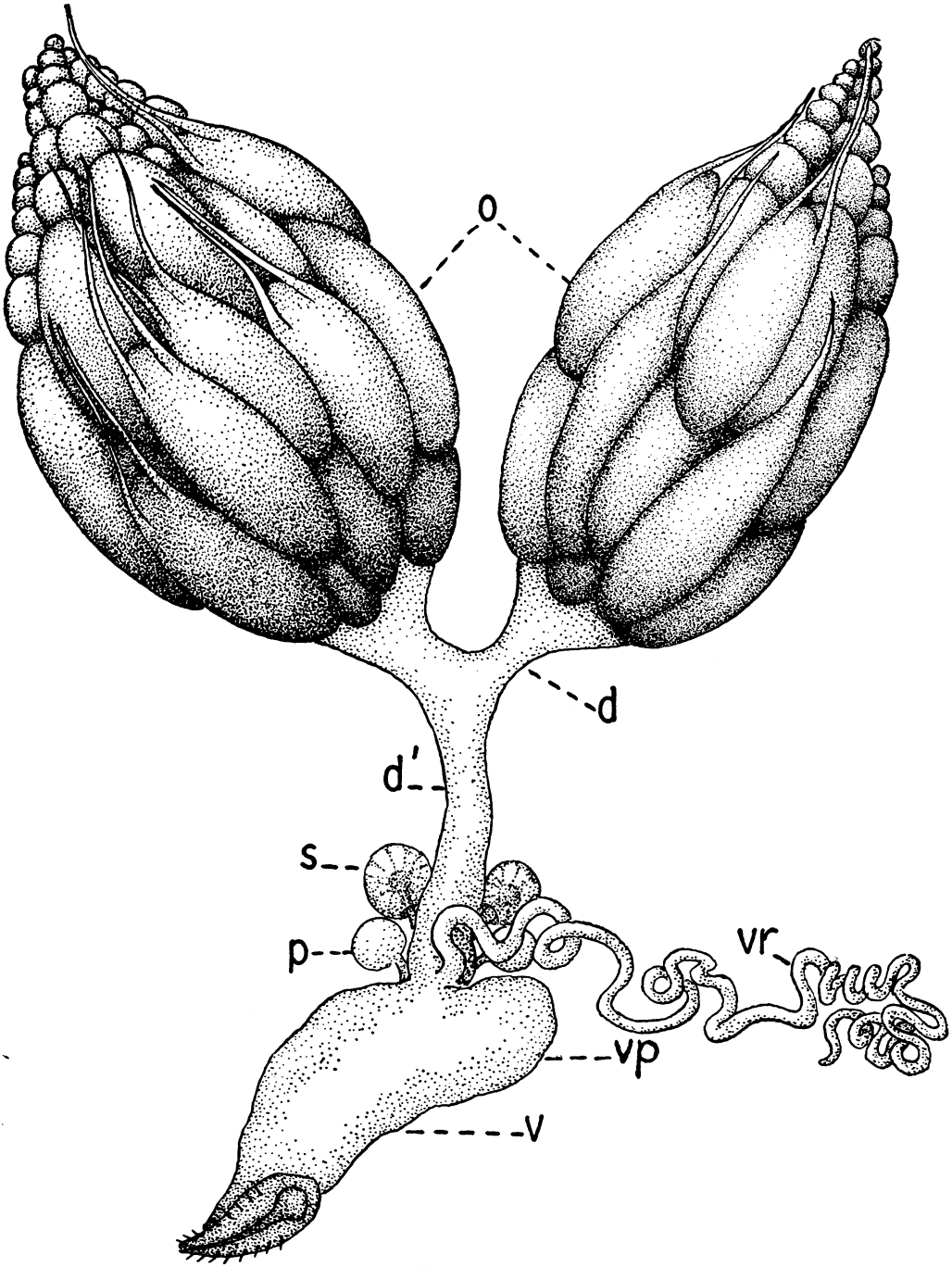

Fig. 61 Camera lucida drawing of female reproductive tract of *D. buzzatii:* d, oviduct; d′, median or azygous oviduct; o, ovaries; p, parovaria; s, spermathecae; vr, ventral receptacle; v, vagina; vp, vaginal pouch. (From Patterson.)

tents of the enlarged vagina remain soft and are soon expelled by the female, and this allows the vagina to return to normal size in the course of a few hours. Hence, in such matings, the insemination reaction does not prevent the production of offspring.

We shall use the intraspecific cross of *D. aldrichi* to illustrate the course of the reaction in this type of mating (Figure 62A to D). Figure A illustrates the condition of the reproductive tract of a virgin female. The vagina is nearly transparent, with the tracheal system appearing as fine dark tracings over its surface. Three and one-half hours after copulation, the vagina is enlarged and opaque, and photographs black by transmitted light (B). The enlargement is confined almost entirely to the vaginal pouch. This represents its maximum size, and soon thereafter it begins to clear (C). It gradually becomes reduced in size until about the eighth hour after copulation, when it resembles in size and transparency the virgin vagina (D).

The history of the insemination reaction in homogamic matings is essentially the same in all species examined and follows the same course as outlined for *aldrichi*. To be sure, there are certain variations in the process, such as the length of its duration, the time at which the reaction begins, and the character of the reaction mass, but these represent minor details. The reduction in size and clearing of the vagina is brought about gradually by elimination of the excess sperm and reaction material in the form of small droplets, which the inseminated female deposits on the surface of the food and the sides of the container.

The extent to which the insemination reaction occurs among Drosophila species has been investigated by Wheeler (1947). He studied homogamic matings and found that the reaction varied throughout the genus, from species in which it is apparently absent, through forms in which there is only a slight swelling of the vagina, to forms showing a strong reaction. He arranged seventy-five members of the genus Drosophila, two of the genus Chymomyza, and one of the genus Scaptomyza under three classes, with respect to the degree of expression of the insemination reaction (Table 67).

In class 1 are placed fifteen members of the genus Drosophila and one each of the genera Chymomyza and Scaptomyza. In none of these forms was there any apparent evidence of a reaction in the vagina. A very good example of this type is found in *D. polychaeta* (Figure 63A). Eighteen members of the genus Drosophila and one

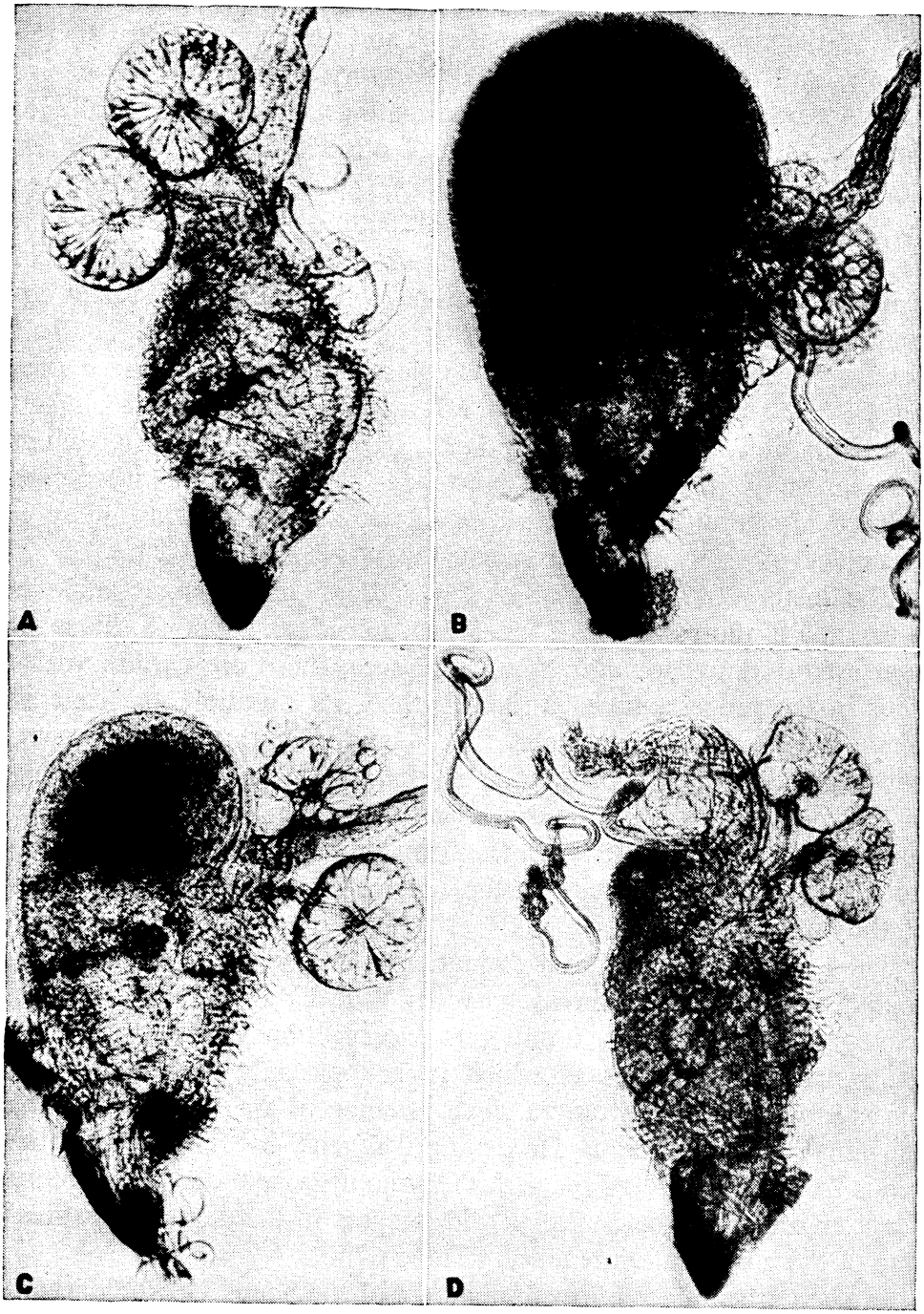

Fig. 62 Vaginae of virgin and three homogamic matings of *D. aldrichi:* A, virgin; B, at 3½ hours; C, at 5 hours; D, at 7 hours. (From Patterson.)

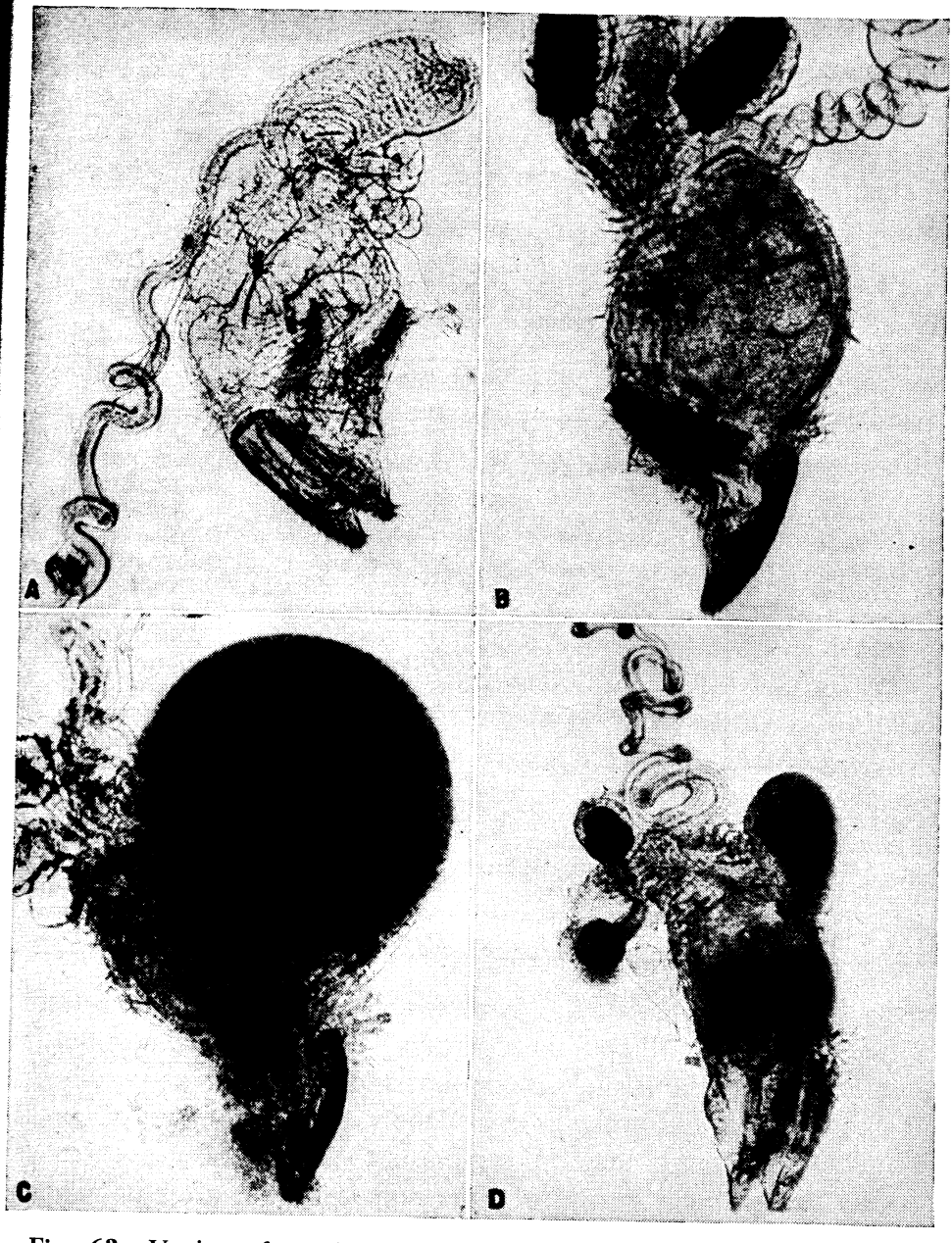

Fig. 63 Vaginae from homogamic matings of four different species of Drosophila: A, *D. polychaeta;* B, *D. neorepleta;* C, *D. mercatorum;* D, *D. carbonaria.* All from dissections made 30 minutes after copulation. (From Wheeler.)

of the genus Chymomyza are placed in class 2, which includes species showing a slight or moderate reaction. A typical example of this class is seen in *D. neorepleta* (Figure 63B). As Wheeler points out, the main distinction between members belonging to classes 1 and 2 is largely the speed with which the ejaculate disappears from the vaginal

TABLE 67

Classification of seventy-eight species with respect to the type of insemination reaction present (after Wheeler)

Class 1	Class 2	Class 3	
C. procnemis	*C. amoena*	*D. duncani*	*D. arizonensis*
S. graminum	*D. busckii*	*D. victoria*	*D. mojavensis*
D. sturtevanti	*D. prosaltans*	*D. transversa*	*D. buzzatii*
D. rectangularis	*D. cordata*	*D. munda*	*D. hamatofila*
D. nebulosa	*D. elliptica*	*D. quinaria*	*D. ritae*
D. melanogaster	*D. emarginata*	*D. subquinaria*	*D. peninsularis*
D. simulans	*D. willistoni*	*D. suboccidentalis*	*D. mercatorum*
D. tripunctata	*D. equinoxialis*	*D. innubila*	*D. m. pararepleta*
D. crocina	*D. sucinea*	*D. subpalustris*	*D. meridiana*
D. repleta	*D. fumipennis*	*D. guttifera*	*D. anceps*
D. melanopalpa	*D. ananassae*	*D. virilis*	*D. hexastigma*
D. hydei	*D. pseudoobscura*	*D. americana*	*D. gibberosa*
D. robusta	*D. persimilis*	*D. a. texana*	*D. melanica*
D. polychaeta	*D. affinis*	*D. montana*	*D. m. paramelanica*
D. cardini	*D. azteca*	*D. lacicola*	*D. nigromelanica*
D. guaraní	*D. tolteca*	*D. putrida*	*D. micromelanica*
D. subbadia	*D. neorepleta*	*D. funebris*	*D. carbonaria*
	D. limensis	*D. subfunebris*	*D. immigrans*
	D. canapalpa	*D. macrospina*	*D. parachrogaster*
		D. mulleri	*D. pallidipennis*
		D. aldrichi	*D. p. centralis*

cavity. If a female of class 1 is dissected at the proper time during coitus, the vagina will contain a certain amount of ejaculate; but frequently this material is slight in amount and disappears so rapidly that no sign of it is found in the cavity of the vagina by the end of copulation. A like amount of material present in the vagina of class 2 females persists for a longer period of time and is visible after copulation. Moreover, in none of the members of class 1 does the ejaculate appear to form a persisting reaction mass.

Class 3 contains forty-two forms, all but two of which belong to the subgenus Drosophila. The two exceptions are *D. duncani* (Hirtodrosophila) and *D. victoria* (Pholadoris). In this class the enlargement of the vagina is usually accompanied by the formation of a dense *reaction mass,* which largely involves the vaginal pouch, as in

D. mercatorum (Figure 63C), and *D. carbonaria* (Figure 63D).

The history of the insemination reaction is very similar to that described for *aldrichi* for species belonging to other species groups. A very good example of this is found in the quinaria group, in which Wheeler describes its manifestations for the following species: *innubila, munda, quinaria, suboccidentalis, subpalustris, subquinaria,* and *transversa.* Blumel (1949) has added *palustris* and *tenebrosa* to this list, and observed the results of the insemination reaction in three heterogamic crosses: *tenebrosa* ♀ × *palustris* ♂, *suboccidentalis* ♀ × *transversa* ♂, and *suboccidentalis* ♀ × *palustris* ♂. The results obtained in these three crosses were very similar to those reported for heterogamic crosses in the mulleri series, which are described in the next section.

The function of the insemination reaction in homogamic matings is not entirely clear. We have suggested (Patterson, 1946) that it might have the effect of preparing the reproductive tract for the fertilization mechanism which is to follow. It was pointed out that, even in forms which show no visible reaction, there still might occur a change in the wall of the vagina which could have the same effect. Wheeler has discussed this point further and suggests that this reaction may facilitate oviposition. His preliminary examinations indicate that females which lay large numbers of eggs as virgins usually do not show an obvious reaction, while those with a strong reaction lay a smaller number of eggs or none at all.

INSEMINATION REACTION IN HETEROGAMIC MATINGS

The first stages of the insemination reaction in heterogamic matings are very similar to those which occur in homogamic matings. About the same changes take place in the vagina in both types of crosses during the first few hours following insemination. The formation of the reaction mass is somewhat more rapid in the heterogamic type, and the vagina is frequently more densely opaque. Another difference is seen in the length of time it takes the sperm to reach the opening of the ventral receptacle after they have been deposited at the posterior end of the vaginal cavity. In homogamic matings, this takes from five to ten minutes, in contrast to forty-five minutes in heterogamic matings. Still another difference relates to the number of sperm which finally succeed in gaining entrance to the lumen of the ventral

receptacle. The number found in this receptacle after heterogamic matings is usually only a fraction of the number present following homogamic matings. A most important difference concerns the consistency of the reaction mass, which remains soft in homogamic matings and is soon expelled. In contrast to this, the reaction mass in heterogamic matings eventually becomes hard and may be retained for a long period of time. Its long retention in the vagina interferes with normal fertilization of the eggs in the reproductive tract and may prevent the production of hybrids altogether. It is this effect of the insemination reaction which makes it a very definite and potent mechanism in Drosophila speciation. It is especially effective in preventing hybridization in crosses in which sexual isolation is too weak to prevent insemination of the females.

The matings between *buzzatii* females and *arizonensis* males will illustrate the nature of the insemination reaction in interspecific crosses. Figure 64A to D shows different stages of this reaction in the *buzzatii-arizonensis* cross. The specimen illustrated in A was photographed twenty-seven hours after the female had been inseminated. The reaction material occupies the entire cavity of the vagina and is densely opaque. This condition prevails for the next twenty-five to thirty hours, after which the contents of the vagina become pear-shaped, with the broad end directed anteriorly. At the seventy-two-hour stage the reaction material begins to drain out through the vaginal orifice (B) and continues to do so up to the ninety-six-hour stage. By this time the reaction mass has become a very discrete object, sharply outlined and largely confined to the vaginal pouch, which occupies the anteroventral part of the vagina (C). It may remain in this condition for a long time (D) and has been observed as late as 192 hours after copulation. The reaction mass may eventually become smaller through further drainage or by disintegration, especially should the female attempt to lay eggs.

We have examined all successful heterogamic matings between eight members of the mulleri subgroup, including the series from the *buzzatii-arizonensis* cross. Certain differences were found in the history of the insemination reaction among the several combinations. In general, however, the results indicate that the course of the reaction is much the same in all nonfertile combinations like that of the *buzzatii-arizonensis* case. Such crosses never produce hybrids, no matter what percentage of the females has been inseminated. In

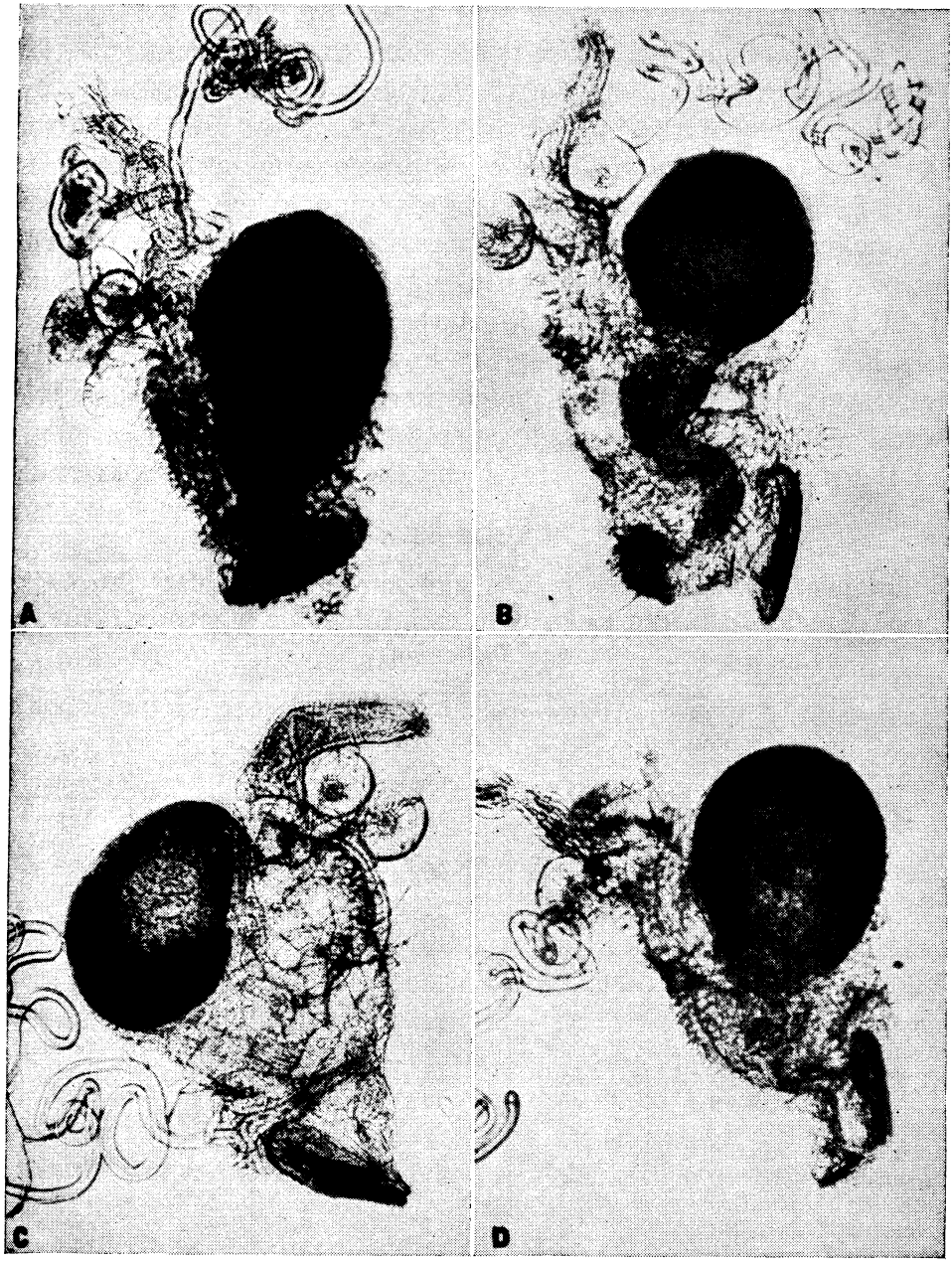

Fig. 64 Four stages of the reaction mass from heterogamic matings of *D. buzzatii* ♀ × *D. arizonensis* ♂: A, at 27 hours; B, at 72 hours; C, at 96 hours; D, at 120 hours. (From Patterson.)

fertile combinations, in which hybrids are produced, the reaction mass may be eliminated before it has done too much damage to the reproductive tract, thus allowing the female to lay fertile eggs.

In twenty-six of the fifty-six possible reciprocal crosses between the eight species of the mulleri subgroup used in the tests, dissections of the females after ninety-six hours exposure to the males showed that one or more females in each of the crosses had been inseminated. But only ten of the twenty-six crosses produced hybrids. These data (Table 68) reveal that, in each of these ten crosses, the reaction mass had disappeared by ninety-six hours, at least in some of the inseminated females, and that motile sperm were still present in their ventral receptacles. It is such females that produce hybrid offspring. But the number of progeny produced from these crosses is much

TABLE 68

Condition of reaction mass, vagina, and motile sperm after the males and females had been exposed to each other for ninety-six hours (from Patterson)

Crosses		In-seminated Females	Reaction Mass	Vagina Normal	Motile Sperm	Hybrids Produced
Females	Males					
aldrichi × *arizonensis*		36	28	8	24	Yes
aldrichi × *hamatofila*		14	14	0	0	None
aldrichi × *mojavensis*		66	46	20	40	Yes
aldrichi × *mulleri*		24	24	0	5	None
mulleri × *aldrichi*		36	20	16	18	Yes
mulleri × *arizonensis*		13	9	4	8	Yes
mulleri × *buzzatii*		2	1	1	1	Few
mulleri × *hamatofila*		13	12	1	2	Yes
mulleri × *mojavensis*		32	22	10	10	Yes
arizonensis × *buzzatii*		1	0	1	1	Larvae
arizonensis × *hamatofila*		27	27	0	3	None
arizonensis × *mojavensis*		39	16	23	19	Yes
buzzatii × *arizonensis*		156	156	0	97	None
buzzatii × *hamatofila*		129	129	0	2	None
buzzatii × *mojavensis*		25	25	0	14	None
hamatofila × *arizonensis*		7	7	0	2	None
hamatofila × *buzzatii*		1	1	0	1	None
hamatofila × *mojavensis*		24	11	13	16	None
hamatofila × *ritae*		1	1	0	0	None
mojavensis × *aldrichi*		24	24	0	10	None
mojavensis × *arizonensis*		154	97	57	80	Yes
mojavensis × *hamatofila*		46	46	0	0	None
peninsularis × *arizonensis*		52	52	0	24	None
peninsularis × *buzzatii*		1	1	0	0	None
peninsularis × *hamatofila*		19	19	0	0	None
ritae × *hamatofila*		10	10	0	2	None

below that derived from homogamic matings of the parent species. For example, in the *mojavensis-arizonensis* cross, which is the most fertile one of the entire series, only about 75 per cent of the females produced hybrids, while 93 per cent of the dissected females were found to have been inseminated. There is not only a reduction of 18 per cent in the number of females producing hybrids, but also the number of offspring per fertile culture is only about one-tenth of that derived from homogamic matings of the parent species.

Of the sixteen nonfertile crosses listed in the table, the vaginae of the females in fifteen at ninety-six hours still contained reaction masses, and motile sperm were absent from the ventral receptacles of five. In the *buzzatii-arizonensis* cross, 88 per cent of the dissected females showed reaction masses; and in 62 per cent of the inseminated females, a few motile sperm were present in the ventral receptacle at the ninety-six-hour stage. Later dissections demonstrated that, in this and similar crosses, such live sperm eventually become inactivated and may disappear altogether. The *hamatofila-mojavensis* cross was the only exception to the rule that the vagina of some females from nonfertile crosses may appear to be free from reaction material within ninety-six hours after copulation. In thirteen of the twenty-four inseminated females from this cross, the vagina appeared to be normal, and a few motile sperm were still present at the distal end of the ventral receptacle. This cross never produces hybrid offspring. There are two possible explanations for this case: either harmful aftereffects of the reaction prevent the fertilization of eggs, or the sperm have become inactivated in the reproductive tract of alien females—a phenomenon which has been observed in a number of other interspecific crosses in Drosophila.

The effect of the insemination reaction on the reproductive tract is not necessarily confined to the vagina. In some cases, the reaction material extends up into the median oviduct and may even enter the ventral receptacle, thus occluding the lumen at its proximal end. One of the most striking effects is seen in a change in consistency of the reaction mass and even in the wall of the vagina, which may become very hard and crystallinelike in character (Figure 65 A). This is especially true in matings in which *hamatofila* was used as the male parent. The males of this species inseminate the females of the other seven species of the subgroup used in the experiments. The results recorded in Table 68 show that in six of these crosses, all nonfertile,

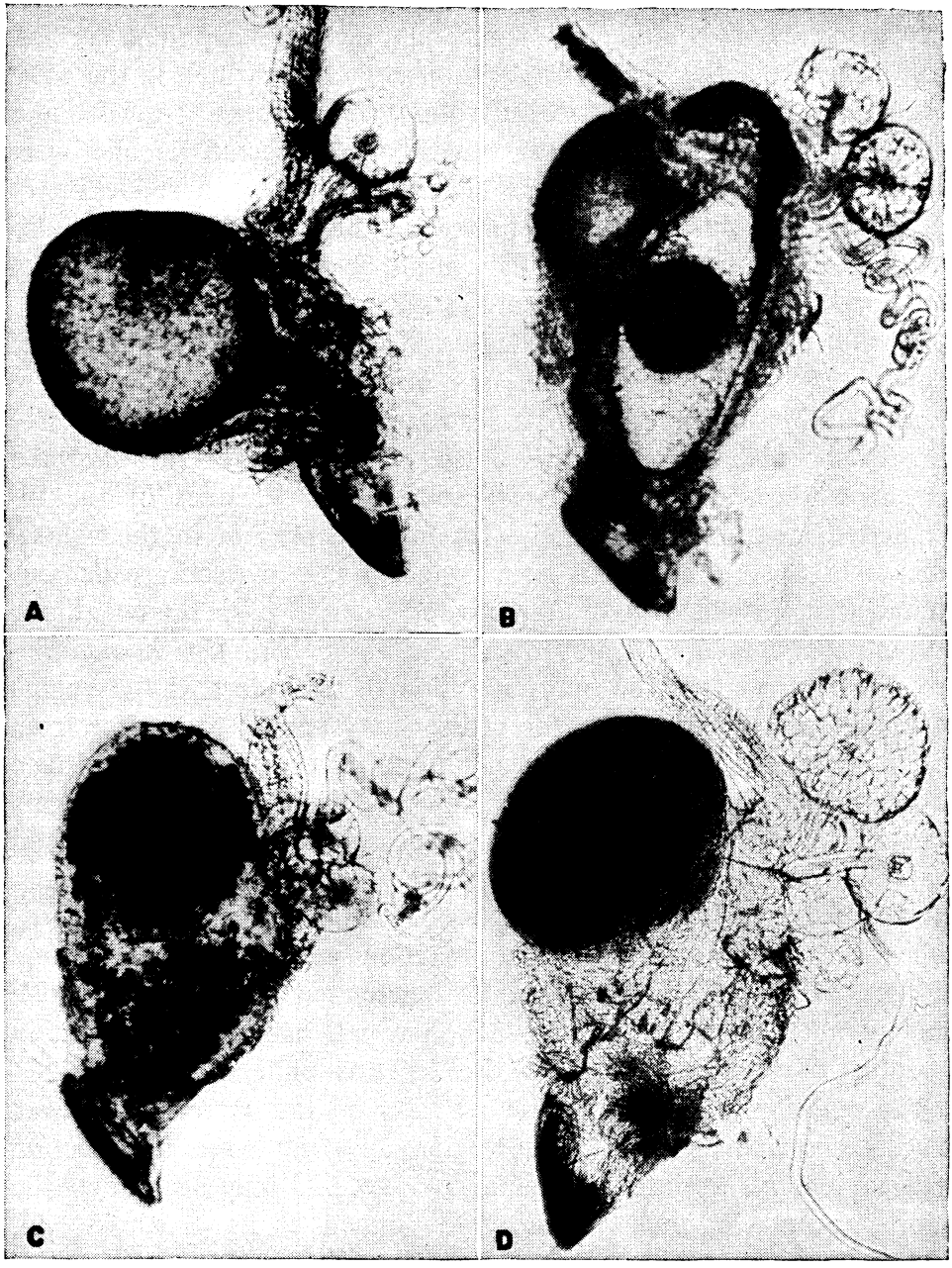

Fig. 65 A, a reaction mass in vagina of female from heterogamic mating of *D. mulleri* ♀ × *D. hamatofila* ♂ at 96 hours; B, degenerating egg imbedded in reaction mass of female from the cross of *D. mulleri* ♀ × *D. arizonensis* ♂ at 150 hours; C, vagina of *buzzatii* female from the *buzzatii-arizonensis* cross remated to a *buzzatii* male and dissected at 3¾ hours; D, *D. aldrichi* female mated to a sterile F_1 male from the *arizonensis/mojavensis* cross and dissected at 24 hours. Such males deliver sperm-free semen. (From Patterson.)

the reaction mass was present in all inseminated females, but that sperm were present in the ventral receptacle in only seven of the 245 dissected specimens. In the seventh cross *(mulleri-hamatofila),* one of the thirteen inseminated females showed a normal vagina with motile sperm in her ventral receptacle. It must be such females that are responsible for the few sterile hybrids produced in this cross.

As pointed out above, the long retention of the reaction mass in the vagina, together with its harmful effects on the reproductive tract, is chiefly responsible for the failure of hybrid production in these nonfertile matings. The damage to the female apparatus is such that normal ovulation, insemination, and oviposition of the eggs are practically impossible. It has been observed that a recently discharged egg in the upper part of the oviduct may appear normal, but by the time it has reached the vagina signs of abnormality, or even disintegration, may have set in. Many cases of disintegrating eggs have been recorded during our studies. If the female is unable to lay eggs, the dissolution process may have advanced to the point where only the hexagonally sculptured chorion and egg filaments are left. In one case fragments of five eggs were found in the vagina. If the female is able to lay eggs, they are usually abnormal, and a study of such eggs by the smear technique demonstrates that they have not been fertilized.

Figure 65 B illustrates an early stage in the deterioration of an egg in the vagina. It is imbedded in the reaction mass, which is seen to extend up into the median oviduct, at the upper right-hand corner of the figure. Cytolysis has already begun in this egg, and in later stages the "digestive process" will completely dissolve the contents of such eggs.

REMATING OF FEMALES WHICH HAVE ONCE BEEN INSEMINATED

These experiments raise the question as to whether a female which has been inseminated will mate a second time and, if so, whether the insemination reaction will occur again. Answers to these questions will depend on whether the matings have been homogamic or heterogamic in species which show the reaction. It is known that females belonging to class 1 (Table 67), in which the reaction does not occur, will remate two or more times, often at frequent intervals. In contrast, females of species showing a strong reaction once they have been

inseminated do not immediately respond to the courtship of the males and frequently do not mate again for a considerable period of time, especially if the first mating happened to be heterogamic.

Two series of tests were carried out in an effort to find answers to the questions raised above. In the first series, homogamic matings of *buzzatii* were studied. Females which had been observed mating were isolated and exposed to males of their own species at intervals extending from fourteen to 135 hours after the first mating. Not a single copulation took place, although the females were vigorously courted by the males. In the second series of tests, females in the *buzzatii-arizonensis* cross were employed. These inseminated females retain the reaction mass for at least 192 hours after coitus. They were exposed at intervals to males of both *arizonensis* and *buzzatii*. Remating did not take place with the *arizonensis* males within the time limits of the experiment, but a series of twenty-seven remated females was obtained with the *buzzatii* males. The first remating occurred 120 hours after the first copulation, and others were obtained up to the 168-hour period. These females were isolated and then dissected at intervals, beginning after fifteen minutes and extending through the eighth hour. It was found that the old reaction mass was present in all but one female. In this female only the fresh reaction material from the homogamic mating was present in the vagina, indicating that the copulation in the heterogamic mating had been unsuccessful (Table 69, No. 5).

Since the second mating was homogamic, we were interested to determine what effect, if any, the presence of the old reaction mass might have on the history of a second reaction. The dissections revealed that it had no apparent effect. Immediately following the second mating, the new reaction material soon surrounds and covers up the old mass; but by the end of about four hours the new material is in the process of being eliminated, and thus the old reaction mass in the vaginal pouch again becomes visible (Figure 65C). By the end of eight hours all of the new material has disappeared, leaving the old mass intact. The interesting point is that the new reaction exactly follows the same history of homogamic matings in *buzzatii,* both in the sequence of events and in the time it takes the female to expel the reaction material.

TABLE 69

D. buzzatii females from the *buzzatii-arizonensis* cross remated to *buzzatii* males (from Patterson)

Female	Time of Remating	Interval of Dissection	Reaction Mass Old	Reaction Mass New
1	144 hours	15 minutes	Present	Present
2	168 hours	15 minutes	Present	Present
3	120 hours	20 minutes	Present	Present
4	120 hours	20 minutes	Present	Present
5	144 hours	30 minutes	Absent	Present
6	168 hours	30 minutes	Present	Present
7	144 hours	30 minutes	Present	Present
8	144 hours	32 minutes	Present	Present
9	144 hours	35 minutes	Present	Present
10	124 hours	1 hour	Present	Present
11	124 hours	1 hour	Present	Present
12	125 hours	1 hour	Present	Present
13	144 hours	1 hour	Present	Present
14	144 hours	1 hour	Present	Present
15	144 hours	1 hour	Present	Present
16	144 hours	1 hour	Present	Present
17	160 hours	1 hour	Present	Present
18	120 hours	2¾ hours	Present	Present
19	120 hours	3 hours	Present	Present
20	144 hours	3¾ hours	Present	Present
21	144 hours	6 hours	Present	Present
22	168 hours	7 hours	Present	Present
23	168 hours	7⅓ hours	Present	Present
24	168 hours	7½ hours	Present	Present
25	168 hours	7¾ hours	Present	Present
26	168 hours	7⅚ hours	Present	Present
27	168 hours	8 hours	Present	Cleared

RELATION OF THE SEMEN TO THE INSEMINATION REACTION

From the beginning of our study of the insemination reaction in Drosophila, it was clear that the rapid secretion of fluid by the wall of the vagina must be due to its response to some component of the ejaculate. Whether the reaction was due to the motile sperm, the semen *per se,* or both was not known. The first evidence that live spermatozoa might not be involved was found in a few cases in which copulation had been incomplete. In these cases the otherwise fertile males had delivered sperm-free semen, and yet the typical reaction followed mating.

It has been shown that sterile hybrid males from certain crosses owe their sterility, at least in part, to a failure of spermatogenesis, in which the meiotic divisions are either abortive (Dobzhansky, 1934; Patterson and Dobzhansky, 1945), or else the maturation process stops just short of motile sperm formation (Patterson and Wheeler, 1947; Stone, 1947; Baker, 1947). Several such cases have been found in crosses between members of the mulleri subgroup (Patterson, 1946). The first indication that such males could be used to determine some of the functions of the ejaculate in the reaction came from observations that they sometimes would copulate with normal females.

Two crosses in particular (*arizonensis-mojavensis* and *mulleri-mojavensis*) yield a sufficient number of hybrid males for making the tests. The hybrid males from each of these crosses appear rather normal, except that those of the second cross have small abnormal testes. In neither type are spermatozoa produced. Both types of males will sometimes mate with the parent and other kinds of females. Males from the *arizonensis-mojavensis* cross were tested to seven different types of females, but failed to mate with those of *hamatofila, mulleri,* and *peninsularis.*

Twenty-three successful copulations were obtained in the backcross to *mojavensis* females by males derived from the *arizonensis-mojavensis* cross. Dissections of these females at regular intervals showed that, from the end of coitus to the twenty-sixth hour, the history of the insemination reaction exactly parallels that of homogamic matings, both in the sequence of events and in the behavior of the reaction mass. Sometime between the twenty-sixth and thirtieth hours after copulation the vagina becomes clear and normal in appearance. The chief difference in the reaction between pure homogamic matings and those in backcross with the hybrid males is a matter of time. It takes approximately three times longer for the vagina to return to normal in the latter type of mating (Table 70).

It was much more difficult to obtain backcross matings between the hybrid males and the females of *arizonensis.* In fact only six successful copulations were secured. The history of the reaction was the same as for the other backcross. In the outcross of the sterile males to *aldrichi* females, a single successful copulation was obtained. The female was dissected twenty-four hours after coitus, and the vagina was found to contain a typical heterogamiclike mass (Figure 65 D). In the outcross matings of the sterile males to *buzzatii* females,

only two successful copulations occurred. One female, dissected after fifteen minutes, had a typically enlarged vagina; and the other, dissected after twenty-four hours, contained a reaction mass in the va-

TABLE 70

Sterile F_1 males from the *arizonensis/mojavensis* cross mated to *mojavensis, aldrichi, arizonensis,* and *buzzatii* females (from Patterson)

Types of Females	Interval of Dissection	Character of Reaction Mass
mojavenesis	0 minutes	Beginning to enlarge
mojavenesis	12 minutes	Well-formed, oqaque
mojavenesis	1 hour	Maximum size
mojavenesis	2 hours	Densely opaque
mojavenesis	3 hours	No change
mojavenesis	5 hours	No change
mojavenesis	10 hours	No change
mojavenesis	16 hours	No change
mojavenesis	22 hours	Clearing
mojavenesis	24 hours	Mass reduced to ½
mojavenesis	26 hours	Mass reduced to ¼
mojavenesis	30 hours	Vagina normal
arizonensis	0 minutes	Beginning to form
arizonensis	15 minutes	Well-formed
arizonensis	30 minutes	Large, opaque
arizonensis	35 minutes	Maximum size
arizonensis	23 hours	Mass reduced to ½
arizonensis	24 hours	Mass reduced to ¼
aldrichi	24 hours	⅔ cleared
buzzatii	15 minutes	Well-formed
buzzatii	26 hours	¾ cleared

ginal pouch similar to the one illustrated in Figure 65 D. Efforts to backcross the sterile F_1 males from the *mulleri-mojavensis* cross proved to be especially difficult, but after many attempts two matings to *mulleri* females were secured. The vagina of each of these contained a typical reaction mass.

Baker (1947) reports a very interesting result obtained with sterile F_1 hybrid males from the cross of *mojavensis* ♀ × *arizonensis* ♂, which is the reciprocal of one of those cited above. These hybrid males are partially sterile, as only about 75 per cent of the females in pair matings produce offspring, and that with a very low fecundity. In one test in which the hybrid males were backcrossed to *mojavensis* females, dissections revealed that only about half of the exposed females

contained motile sperm in their reproductive tracts and that thirty-five others had a reaction mass in the vagina but no sperm. This means that some of the males were sterile, owing to a failure to produce motile sperm. This conclusion was proved by a study of smear preparations of the testes of fifteen males which had failed to deliver sperm at the time of copulation. Thirteen of them contained no motile sperm in their testes, one had a small group of sperm, and one showed an abundance of sperm in the gonads.

The conclusion to be drawn from the results obtained in crosses between the sterile hybrid males and various types of females is that live sperm are not necessary for the induction of the insemination reaction. As we have pointed out elsewhere (Patterson, 1947), this does not mean that the active agent inducing the reaction is absent from the semen in the sterile males. It was further pointed out that the absence of spermatozoa from the testes does not exclude the possibility that some degenerative product of the abnormal gonads might constitute the inducing agent. Unfortunately, the composition of the semen in Drosophila is not very well known. Nonidez (1920) states that secretions from the paragonial accessory glands are added to the testicular products to form the fluid part of the ejaculate. The inducing factor may therefore be derived from several different sources.

Lee (1950) undertook to determine what particular substance might be responsible for the insemination reaction. She employed *Drosophila gibberosa* and, by means of a fine syringe, injected into the vagina the following substances: brei of testes in saline; brei of male accessory glands plus testes in saline; brei of entire male fly; brei of male accessory glands in saline; Yeager's cockroach saline; 2 per cent gelatin in cockroach saline; 2 per cent NaCl solution.

The injection of testes brei is followed by macroscopic changes in the vaginae similar to those of normal mated females, and the same is true for injections of testes brei plus male accessory glands, except that the distention is of a lesser degree. Injection of saline also brings about the same amount of distention. In contrast to these results, injection of male accessory gland brei, entire male fly brei, 2 per cent gelatin, and 2 per cent NaCl may bring about an insignificant increase of vaginal size, as a result of mechanical distention by the fluid injected into the lumen, but the vaginal pouch does not thin out as in the other experiments. The vaginae injected with saline undergo a peculiar histological change, in which there is a moderate

thinning out of the vaginal pouch, but no reaction mass is formed in the lumen. Lee suggests that increase in cell volume here is the result of the uptake of water from the injected saline because of the lower osmotic pressure of the saline used as compared to the protoplasm of the epithelium. The conclusion is reached that the active component is probably from the testes and that it is perhaps protein in character.

RELATED REACTIONS

The insemination reaction is the most obvious and immediate reaction occurring after copulation. Certain other adverse reactions may occur in heterogamic matings, perhaps related to this one but which may be of independent origin, since they occur as well in forms that do not show a marked insemination reaction.

A reaction between sperm and female is common, namely, the death or incapacitation of sperm in the receptacles of alien females. An examination of Table 68 shows that there are many more combinations in which females are cross-inseminated than produce hybrids. Part of this failure is due to sperm mortality, part to failure of the hybrid zygote to develop properly (see Chapter 9).

This mechanism is very effective in the virilis group. Patterson, Stone, and Griffen (1942) reported it for crosses of *virilis* females and *americana* males (Table 71). In this case, the sperm are viable and effective in fertilization for at most two days; but Patterson and Stone (1949) showed that, in crosses of *virilis* females to *novamexicana* males, a few sperm were functional for as long as nine days, although with much reduced effectiveness after about four days.

TABLE 71

Egg hatch from females mated once (from Patterson, Stone, and Griffen)

Cross		First Day		Second Day		3 to 10 Day	Total
		Smear	Hatch	Smear	Hatch	Hatch	Hatch
V × V	Sperm in eggs	15/15	47/66	8/10	30/34	82/93	159/193
	%	100	71	80	88	88	82
A × A	Sperm in eggs	20/22	49/70		38/43	145/167	232/280
	%	91	70		88	87	83
V × A	Sperm in eggs	8/52	7/215	0/13	0/71	0/964	7/1,250
	%	15	3	0	0	0	0.6

There is little evidence for an insemination reaction in the obscura group. However, Miller (1950a) reports both fewer sperm and evidence of sperm inactivation in crosses of *affinis* females to *athabasca* males.

DISCUSSION

The purpose of this chapter was to present any evidence obtained from a study of the insemination reaction and related phenomena which might have some bearing on the question of speciation. In attempting to evaluate such evidence, one must keep in mind that evolutionary divergence is rarely ever brought about through the operation of a single isolating mechanism. It is usually the total effect of several such mechanisms that is responsible for the more or less complete separation of two diverging forms, by reducing or even preventing the exchange of genes between them.

Among such mechanisms may be mentioned the following: (1) sexual isolation, or the failure of the sexes to mate; (2) gametic mortality, or the death or inactivation of sperm in the reproductive tract of an alien female; (3) zygotic mortality, or the death of the zygote during developmental stages; (4) hybrid sterility. All four of these general isolating mechanisms operate among members of the mulleri subgroup, as well as among members of other species groups.

With this number of isolating mechanisms operating in the mulleri subgroup, it is somewhat difficult to make a precise determination of the proportion of the sum total of isolation that should be attributed to the effects of the insemination reaction. It is clear, however, from the experimental results that this reaction constitutes a potent isolating mechanism in the interspecific crosses of the mullerilike forms, as well as among members of other species groups. Of thirteen such crosses which did not yield hybrids, 557 inseminated females were found among 2,600 dissected females, or slightly more than 21 per cent (Table 68). In the *buzzatii-arizonensis* cross, a total of 156 out of 200 dissected females had been inseminated (78 per cent). All of these females had a reaction mass in the vagina, and motile sperm were present in the ventral receptacles of 62 per cent of them. It is possible that, in the absence of a reaction mass in the vagina, many of them would have produced offspring. Their failure to do so was in most cases due, not to the absence of live sperm, but rather to their inability to lay eggs in the presence of a reaction mass. Moreover, the

insemination reaction adversely affects reproduction in fertile interspecific matings by reducing the possible number of hybrids. For example, in the *mojavensis-arizonensis* cross, only 75 per cent of the females produced hybrids, although 93 per cent of them had been inseminated.

In all nonfertile crosses, the duration of the effects of the insemination reaction is longer than in fertile ones. This is due to the deleterious effects caused by the prolonged retention of the reaction material; or, to express it in another way, the quicker this material is eliminated from the vagina, the better are the chances that hybridization will follow. What value should be placed on these data in attempting to estimate the degree of relationship between members of a group is difficult to determine. There is very little reliable experimental evidence to show whether hybridization occurs between two species belonging to different species groups, and still less relating to the question of insemination. Nevertheless it is reasonable to assume that, in crosses between any two species in which a large number of females had been inseminated in nonfertile heterogamic matings, such species would be more closely related than two other species in which only a few females had been inseminated. The possible relationship between species based on the results from nonfertile crosses is considered elsewhere in this volume.

At least two possible functions of the insemination reaction may be suggested. The first of these refers to its occurrence in homogamic matings, where it may possibly have the effect of preparing the reproductive tract of the female, either for the fertilization mechanism (Patterson, 1946) or to facilitate egg-laying (Wheeler, 1947). A second function is revealed in the results obtained in interspecific crosses among forms which have the reaction, for here it has the effect of either reducing gene exchanges or preventing such exchanges altogether. An example of the complete elimination of gene transfer has already been referred to in connection with the *buzzatii-arizonensis* cross, in which sexual isolation is weak and mating occurs almost as frequently as in intraspecific crosses; and yet, as a result of the insemination reaction, the females are unable to produce offspring. The widespread occurrence of this reaction in the genus would suggest that it may occur in other insects.

A function of the insemination reaction that is of importance in matings both within and between species lies in the prevention of

remating. This would be of selective advantage in two ways. It would more nearly insure that all females are fertilized within a species. The prolonged reaction in heterogamic matings would prevent remating of the female to males of her own species, and so select for more complete sexual isolation.

Patterson (1946) pointed out that a mutation which would cause the semen of a male to induce this reaction would spread in the population, as a male with such a mutation could follow other males and remate with a female. The normal males could not remate with females after such a male for a considerable period; thus the sperm effective in fertilizing the eggs would come from the latter type male. This mutation would also be beneficial in conserving reproductive effort, yet keep the females fertilized. Its effect is in some respects similar to the vaginal plug formed in some rodents.

We pointed out in the preliminary paper that this reaction follows almost immediately after the introduction of the semen into the lumen of the vagina and is visibly expressed in the form of a rapid swelling of that organ. This increase in size is caused by a secretion of fluid by the epithelial cells of the vagina. The reaction is evidently brought about by a response of the epithelium to the presence of foreign material, perhaps protein. This would imply that the epithelium is hypersensitive to the foreign substance; and, if so, its hypersensitiveness must be inherited, since the reaction occurs after the first copulation and without any previous sensitization. In some respects the insemination reaction resembles certain features of an immunological reaction, except that it does not require any preconditioning. All of the evidence indicates that the active inducing agent is secreted by the testes, but does not come from live sperm.

Prevention of hybridization is found to be a complex mechanism. The phenomenon of sexual isolation affords the most effective isolating mechanism, that is, it involves the least wastage of reproductive effort. There are a few cases that suggest some mechanical isolation. The failure of *pseudoobscura-melanogaster* pairs to separate after copulation may be such a case. The interruption, violent with *subobscura* males, of crosses between *pseudoobscura, persimilis* and *subobscura* might be due to this cause. This type of incomplete copulation may result from mechanical difficulties with the genitalia or from an abnormal physiological stimulation from some component in the seminal or vaginal fluids.

The insemination reaction with its seriously adverse effect on the fertility of heterogamic matings prevents hybridization in many cases where cross-matings occur. This reaction or one allied to it causes the death of the female in some cases. Even in cases where no violent and obvious insemination reaction occurs, sperm may die or become nonmotile and nonfunctional in the seminal receptacles of alien females. We do not know if this is due to toxins or lack of the proper physiological habitat in these cases. The fact that repeated matings are successful each for a short time suggests that this is not a case of building up "immunity" against a foreign protein. The sum of these mechanisms reduces to a relatively small number the successful hybridizations.

9 HYBRIDS AND HYBRID STERILITY

INTRODUCTION

It has long been recognized that interspecific hybrids furnish indispensable material for the study of comparative genetics and phylogeny. The fact that certain crosses between different species sometimes result in the production of progeny has been known since ancient times, but only in comparatively recent years has the number of such cases been greatly increased, both among plant and animal forms. It is somewhat surprising to know that the discovery of hybrids in the genus Drosophila did not occur until 1920, and even as late as 1934 only two such cases were known. Since that time 101 cases of hybridization have been discovered among members of this genus.

This great increase in the number of cases is due chiefly to the recent interest in the problem of evolution which has resulted in the use of more intensive and better methods of collecting Drosophila, and in an improvement of technique for the detection of hybridization. Several investigators in this and other countries have been engaged in taking a census of Drosophila populations within their own particular regions. This has naturally resulted in the discovery and description of many new species, as well as subspecies and other intermediate forms.

Another factor that has been of great help in obtaining hybridization is the fact that many of the species of Drosophila can be arranged into species groups, each composed of one or more similar forms. It was soon discovered that it was practically useless to attempt crosses between species belonging to different species groups, which at best could only be regarded as remotely related. In our experience, such cross tests were found to be incompatible. We have therefore followed the plan of selecting members from the same

species group in making tests for the detection of hybridization. This of course implies that, as far as these tests go, they confirm the relevance of the morphological characters used to establish the species groups.

In carrying out the tests between two forms, as illustrated by our usual procedure, we first make reciprocal crosses in small mass cultures in vials (about ten pairs to the vial). If larvae do not appear in the culture within a few days, the flies are transferred to fresh food vials; and, if necessary, this procedure is repeated until it can be determined whether or not this method is adequate as a test of the cross-fertility of the two forms. If the results are negative, the experiment is repeated by using larger mass cultures in half-pint bottles (from fifty to a hundred pairs to the bottle); then, if the results are still negative, it is assumed that the cross is incompatible. Often a number of the females will be dissected to check for foreign sperm in their reproductive tracts. If the results are positive, the experiment is continued by using pair matings and carrying the test through the F_3 generation. This procedure and a study of the salivary gland chromosomes of the F_1 larvae will give a measure of the degree of genetic divergence between them.

In testing for the production of hybrids between related forms, a number of additional barriers or isolating mechanisms have been found to function after fertilization of the egg by a foreign sperm. The following may be mentioned: (1) zygotic mortality, which may act to insure that the eggs do not develop into larvae or occur during larval or pupal stages, and which may apply to F_1 or later generation recombinations; (2) hybrid inviability, which may so reduce the viability of the hybrids that they cannot mature or function; (3) hybrid sterility, which may insure that one or both sexes of the F_1 or later generation hybrids will be sterile or semisterile. Some species groups do not produce hybrids, while others are much less well isolated.

The list of known cases of Drosophila hybrids presented in the following pages includes several for which the parent forms have been ranked as subspecies. These cases have been included because they frequently show important steps in evolutionary divergence. We shall designate as a single case of hybridization the cases where hybrids are produced by either one or both reciprocal crosses.

HYBRIDS PRODUCED IN DIFFERENT SPECIES GROUPS

melanogaster-simulans cross

We shall first consider the *melanogaster-simulans* cross, since it represents the first known case of hybridization in the genus Drosophila. It was analyzed by Sturtevant (1920a, b). These two species, which belong to the melanogaster group, have the same metaphase chromosome pattern and are strikingly similar morphologically. They show a few minor differences, but the only satisfactory character by which they may be separated is the external male genitalia. The posterior process of the genital tergite appears like a clamshell in *simulans* and like a small hook in *melanogaster*. The genes analyzed have proved to be alleles in the two species (Sturtevant and Novitski, 1941b).

In the cross of *melanogaster* ♀ × *simulans* ♂, only female offspring are produced, unless the composition of the female is XXY, in which case nondisjunction may produce exceptional sons. In the reciprocal cross, *simulans* ♀ × *melanogaster* ♂, usually only males are produced, but sometimes a small number of female hybrids appears in the cultures. From the nature of the matings, the regular females have the same chromosome constitution; but in one case they live, while in the other they fail to emerge. Sturtevant (1920b) accounts for this relation by supposing that the egg cytoplasm influences the result and points out that hybrids survive only if they carry a *simulans* X chromosome. In the presence of the *simulans* egg cytoplasm and a *melanogaster* X, survival usually does not occur, even if a *simulans* X is also present. Sturtevant presented evidence indicating that, in the cross of *melanogaster* ♀ × *simulans* ♂, the regular males die in larval stages. It was found that all hybrids had small, undeveloped gonads and were completely sterile. Several studies have been made on the salivary gland chromosomes of these hybrid larvae (Pätau, 1935; Kerkis, 1936, 1937; Horton, 1939). Horton found ten chromosomal rearrangements, of which six were classed as inversions and four as changes of one or a few bands at the free ends of certain chromosomes. He also found fourteen short areas where the chromosomes do not ordinarily synapse.

The main difficulty which stands in the way of further genetic

analysis of species differences in such cases of hybridization is the sterility or inviability of the F_1 hybrid. However, Muller and Pontecorvo (1940a, 1940b, 1942) found a means of circumventing the sterility of these F_1 hybrids and were able to obtain certain "recombination" types of the kinds that would be produced in a backcross of the hybrid were it fertile to *melanogaster*. They used triploid *melanogaster* females carrying recessive mutant genes in all of their major chromosomes and crossed them to heavily irradiated *simulans* males bearing the dominant normal alleles of these genes. Many of the eggs produced by the triploid female will have one or more of their chromosomes in diploid number, while the X-ray treatment of *simulans* males will cause the loss of individual chromosomes, presumably by breakage. When an egg with an extra chromosome or chromosomes is fertilized by a sperm in which the homologous chromosome or chromosomes have been incapacitated, a viable diploid results. The chromosomes in question in such diploids are of homozygous *melanogaster* type; and the others are heterozygous, as in the species hybrid. The kind of recessive character revealed in the individual indicates which chromosomes are of the homozygous *melanogaster* type.

A study of the different types derived from this cross showed that those differences between the two species which cause their hybrids to be sterile, morphologically abnormal, or inviable are dependent upon properties of their chromosomes. Their demonstration that the effects of sterility and inviability are wholly chromosomal in origin was made possible by obtaining a single fertile "recombinant" female, which had received all of its major chromosomes from *melanogaster* and only minor chromosomes (Y and one 4, the dot chromosome) from *simulans*. By breeding this female, they were able to establish the minor chromosomes in stocks which otherwise were purely *melanogaster*. They found that the various genes of the minor chromosomes failed to act in precisely a normal manner in this *melanogaster* setting. This supported their inference "that the number of cryptic genetic differences between species far outrun those causing obvious differences between them or those working in a 'complementary' way to cause abnormalities of their first-generation hybrids" (1940b, page 418).

In their third article, Muller and Pontecorvo (1942) summarize the results from their studies on the effects of minor chromosomes

of *simulans* after they had been transferred into the *melanogaster* genotype. Males which carry the *simulans* Y were found to be sterile. This Y chromosome somewhat suppresses variegation and undergoes nondisjunction with attached *melanogaster* X's. The *simulans* homozygous 4 in *melanogaster* permits fair viability, but produces a complex of slight, variable phenotypic peculiarities. Males homozygous for the *simulans* 4 chromosome, unlike the females, are sterile. They found that this sterility depended upon a small localized chromatin region, which almost certainly represents a single gene. In their final conclusion, they state, "The fact that even these minor chromosomes exhibit so many gene differences indicates that the reaction system producing the similar phenotypes of apparently closely related species may be highly divergent. Hybrid sterility is but one expression of this cryptic divergence, which need not in itself have had a selective value" (1942, page 157).

obscura group hybrids

As pointed out in Chapter 2, this species group includes a series of forms which can be arranged under two subgroups. Two of the main diagnostic characters of the first subgroup are the presence of several teeth in the distal sex comb and eight acrostichal rows of hairs; those of the second subgroup have a single tooth in this sex comb and six acrostichal rows of hairs. Representatives of the first subgroup occur both in North America and Europe, while those of the second are confined to North America (Table 72).

The hybridization cases reported for the first subgroup of species occur between the three American forms, *pseudoobscura, persimilis,* and *miranda,* and one of them constitutes the second reported case of hybridization in the genus. This was discovered by Lancefield in 1929, in testing two stocks which had been developed from flies collected on the Pacific coast. The two stocks used by him were mistakenly identified as *Drosophila obscura* Fallén. At about the same time, Frolova and Astaurov (1929) showed that the American form was different from Fallén's European species and named it *D. pseudoobscura.* Lancefield, in his paper, refers to his two stocks as race A and race B, and he did so without implying that they were not entitled to specific rank. These designations for the so-called races remained in the literature for about fifteen years, when Dobzhansky and Epling (1944) brought the nomenclature into line with the bio-

logical species concept by retaining the term *pseudoobscura* for race A and describing race B as new under the name *persimilis*. Throughout our discussion we shall use these two specific names.

TABLE 72

Hybrids between members of the *obscura* group

Crosses Female × Male	Females F_1 Hybrids	Males F_1 Hybrids	Possible Gene Exchanges	Author Reporting
1. *pseudoobscura* × *persimilis*	Fertile	Sterile	Yes	Lancefield, 1929
2. *persimilis* × *pseudoobscura*	Fertile	Sterile	Yes	Lancefield, 1929
3. *pseudoobscura* × *miranda*	Fertile	Sterile	Yes	Dobzhansky, 1935, 1937
4. *miranda* × *pseudoobscura*	Fertile	Sterile	Yes	Dobzhansky, 1935, 1937
5. *persimilis* × *miranda*	Fertile	Sterile	Yes	Dobzhansky, 1935, 1937
6. *miranda* × *persimilis*	Fertile	Sterile	Yes	Dobzhansky, 1935, 1937
7. *athabasca* × *azteca*	Sterile	Sterile	None	Sturtevant, Dobzhansky, 1936
8. *azteca* × *athabasca*	Sterile	Sterile	None	Sturtevant, Dobzhansky, 1936
9. *affinis* × *athabasca*	Sterile	Sterile	None	Miller, 1941
10. *algonquin* × *athabasca*	Fertile	Sterile	Yes	Miller, 1941
11. *ambigua* × *pseudoobscura*	Pupae	Pupae	None	Buzzati, 1950
12. *ambigua* × *perisimilis*	Pupae	Pupae	None	Buzzati, 1950

Lancefield found that crosses between *pseudoobscura* and *persimilis* gave semisterile daughters and completely sterile sons in the F_1 generation (Table 72, 1, 2). In the cross *pseudoobscura* ♀ × *persimilis* ♂, the testes of the hybrid males were of normal size; but in the reciprocal cross, *persimilis* ♀ × *pseudoobscura* ♂, the hybrid males have small testes. He was also able to obtain offspring in backcrosses of the F_1 females to males of either species, but the backcross seems to go more readily to *persimilis* males than to *pseudoobscura* males. It was found that the offspring from backcrossing the F_1 hybrid females differed from those obtained in intraspecific crosses, as revealed by differences in sex ratios, in size of testes of hybrid males, and in the amount of crossing over between certain regions of

the unlike X chromosomes as compared to the value obtained in either species. With reference to differences in the size of the testes of sons of backcrossed hybrid females, about half of them had gonads of less than half normal size and were sterile. A correlation was found to exist between the nature of the backcross and the type of the sons with small testes. Thus a son who had received from his mother an X chromosome derived from the same species as his father usually has gonads of normal size, while a son who has received the other X chromosome from his mother usually has small gonads. The interesting fact is that the two reciprocal backcrosses give results which are the opposite of each other in this respect.

Dobzhansky and Beadle (1936) transplanted larval testes of male hybrids of *pseudoobscura* and *persimilis* into *pseudoobscura* male larvae, as well as the reciprocal transplant. The pure *pseudoobscura* testes were normal and functional, but the hybrid testes were not. The abnormal spermatogenesis of these hybrid males was not corrected by change of environment. Dobzhansky (1934) showed that the sterility of these male hybrids was not due to chromosomal differences. He (1936, 1941) proved that the sterility was due to gene differences and that there were at least two genes on each of the major chromosomes influencing this difference and sterility. He showed that F_2 or F_3 backcross individuals were more fertile as these chromosomes came increasingly from one of the species. The effects of these genes on testes size are cumulative; and furthermore the recombination males having both 3 and 4 chromosomes from the other species are sterile, although either chromosome by itself as a replacement allows the hybrid male to be fertile.

There are other barriers to the exchange of genes between *pseudoobscura* and *persimilis*. Despite careful and extensive search, no hybrid or cross-fertilized individual has been found in nature. Furthermore, although F_1 hybrid females are fertile back to either type male, Dobzhansky (1945) and Koopman (1950) point out that these hybrid larvae are comparatively inviable, and so no F_2 individual is produced in mixed population cages where larval selection is very intense. From this we can infer that the eggs of the F_1 hybrid females and/or the genetic recombinations are not as viable as those of either of the species.

The discovery of *D. miranda* by Dobzhansky (1935b, 1937a) made it possible to obtain two additional cases of hybridization among

members of the first subgroup. This new species crosses reciprocally without great difficulty to either *pseudoobscura* or *persimilis*. The cross *miranda* ♀ × *pseudoobscura* ♂ produces offspring of both sexes in about equal numbers. The reciprocal cross, *pseudoobscura* ♀ × *miranda* ♂, produces mainly females, and only a very few exceptional males (crosses 3 to 6). Apparently the *miranda-persimilis* crosses follow the same pattern. It was at first stated that all F_1 hybrids were sterile, but MacKnight (1939) later found that female hybrids from crosses with certain strains of *miranda* males were slightly fertile.

Drosophila miranda and its hybrids with *pseudoobscura* and *persimilis* allow a very interesting analysis of both gene and chromosome evolution. Dobzhansky showed that this species had a complex sex chromosome system of X_1 X_2 Y 2A males (nine chromosomes) and $2X_1$ $2X_2$ 2A females (ten chromosomes), so that the male had one chromosome less than the relatives with which they hybridize. Dobzhansky thought that the X_2 chromosome, which is equivalent to the 3 chromosome of *pseudoobscura,* lacked a homologue in the male. MacKnight (1939) showed that a large portion of this homologue was present interwoven in the Y. He presumed that a Y-3 fusion had, through subsequent rearrangement and mutation (as well as position effect) resulted in the present Y-3 complex. He demonstrated that two of three recessive mutations available for testing had no normal alleles in the Y-3 complex, while all had normal alleles in X_2. It is not possible to tell how many of the loci originally present at the time of origin are missing or inactive. In crosses to *pseudoobscura* females, no F_1 males having the Y of *miranda* survived. The exceptional males that survive have *miranda* X_2 and lack the Y. The genes in the Y of *miranda* are no longer able to replace those in X_2 or in the 3 of *pseudoobscura* in the heterozygote. The cytological mechanism for disjunction of both X_1 and X_2 from the Y worked out by Cooper (1946) has given a good basis for understanding the retention of sex chromosome complexes in populations. The male hybrids between *miranda* and *pseudoobscura* or *persimilis* are sterile, with rudimentary testes. A few females are fertile. Kaufmann (1940a) published an account of the aberrant egg development from these hybrid females responsible for this sterility. None of the hybrids between the strains used by Kaufmann was ever fertile. The eggs from the hybrid females occasionally, at least, had a full set of chromosomes. Presumably,

sometimes this might have included only *pseudoobscura* or *miranda* chromosomes, so that many of the gene combinations should have been like the original hybrid. Kaufmann points out that there were irregular and supernumerary divisions of the polar nuclei, and that aberrant chromosome masses and cell masses were formed. In many cases the eggs laid by these hybrid females do not allow normal development.

Three different cases of hybridization have been reported for the second subgroup. The first of these was found by Sturtevant and Dobzhansky (1936). They made various crosses between members of this subgroup, as follows: *affinis* with the other five members described as new at that time; *algonquin* with *azteca, athabasca,* and *narragansett; seminole* with the subspecies *mahican;* and *azteca* with *athabasca.* The only successful cross was between *azteca* and *athabasca,* which goes in both directions (crosses 7, 8). The F_1 hybrids are sterile and have rudimentary gonads. The testes of the hybrid males are variable in size, depending somewhat on which strains of the parent species are used, and the ovaries of the hybrid females have egg chambers only. The F_1 males from the reciprocal crosses were found to be different. Those from the *azteca* ♀ × *athabasca* ♂ cross are larger than their sisters or parents and have large wings; those from the reciprocal cross are dwarfs with small wings and low viability. In contrast to this condition in the males, the F_1 females are of about the same size and proportions as the parent species.

The other two cases of hybridization in this subgroup were found by Miller (1939, 1941, 1950a, b). He obtained hybrids in the cross between *algonquin* females and *athabasca* males. Although the frequency of insemination is low, yet a goodly number of viable offspring result whenever mating occurs (crosses 9, 10). The male offspring produce no sperm and are naturally sterile, but the F_1 females are fertile in backcross to *algonquin* males. The other cross-producing hybrids was between *affinis* females and *athabasca* males. The production of hybrid offspring is low, but the hybrids are quite viable, though sterile. A study of the spermatogenesis showed that the maturation process was highly abnormal, with the formation of large, multinuclear masses within the testes (1941). This series of crosses represents six different cases of interspecific hybridization.

We have mentioned the matings described by Wallace and Dobzhansky (1946) of *subobscura,* a member of the obscura group from

Europe, with *pseudoobscura* and *persimilis,* which are American species of the same subgroup. These matings do not produce hybrids.

Buzzati-Traverso (1950) has found a very interesting case of hybridization between *ambigua* females (European species) and males of either *pseudoobscura* or *persimilis.* Adult hybrids have not been reported, but the larvae mature and allow a check of the salivary gland chromosomes. The metaphase plate of *ambigua* has four pairs of V-shaped chromosomes, which is the result of one fusion and three pericentric inversions, for the salivary preparations show eight arms. The metaphase plate of either *pseudoobscura* or *persimilis* has one fusion V and three rods, which appear as five long arms in the salivary gland nuclei. These nuclei of the hybrid larvae have thirteen free arms, with no synapsis. This represents a case where chromosome reorganization has progressed to such a degree that no residual similarity shows as synapsis, despite the fact that the genetic systems are sufficiently compatible for these hybrid larvae to develop as far as they do.

quinaria group hybrids

The species belonging to this group are among the most difficult of the genus to breed on laboratory food. Spencer (1942) was the first to call attention to the fact that *palustris* could be successfully hybridized with *subpalustris,* and *occidentalis* with *suboccidentalis.* Sears (1947) was able to carry out a series of tests on the crossibility of a majority of the species of this group. He used ten different members of the group and made all ninety possible reciprocal matings, of which eighteen gave some type of hybrid offspring. Subsequently Blumel (1949) reported nine additional successful matings, mostly by the use of *tenebrosa,* which was not in stock at the time Sears carried out his investigations. The twenty-seven successful crosses are tabulated in Table 73.

A general summary of these results is as follows: (1) in eight cases, both male and female hybrids were fertile (crosses 1, 2, 7 to 11, 24); (2) in seven cases, only the female hybrids were fertile (crosses 5, 6, 16, 17, 20, 21, 23); (3) in seven cases, both male and female hybrids were sterile (crosses 3, 4, 12, 14, 18, 19, 26); (4) of the five remaining cases, two produced only larvae or pupae (13, 15) and the other three died soon after emerging from the pupae (22, 25, 27).

There is some question about the specific ranks of *occidentalis* and

TABLE 73

Hybrids between members of the *quinaria* group

Crosses Female × Male	F_1 Hybrids Females	F_1 Hybrids Males	Possible Gene Exchanges	Author Reporting
1. *munda* × *occidentalis*	Fertile	Fertile	Yes	Sears, 1947
2. *munda* × *suboccidentalis*	Fertile	Fertile	Yes	Sears, 1947
3. *munda* × *subquinaria*-A	Sterile	Sterile	None	Sears, 1947
4. *subquinaria*-A × *munda*	Sterile	Sterile	None	Sears, 1947
5. *occidentalis* × *palustris*	Fertile	Sterile	Yes	Sears, 1947
6. *occidentalis* × *subpalustris*	Fertile	Sterile	Yes	Sears, 1947
7. *occidentalis* × *subocciden-talis*	Fertile	Fertile	Yes	Spencer, 1942; Sears, 1947
8. *suboccidentalis* × *occiden-talis*	Fertile	Fertile	Yes	Spencer, 1942; Sears, 1947
9. *palustris* × *subpalustris*	Fertile	Fertile	Yes	Spencer, 1942; Sears, 1947
10. *subpalustris* × *palustris*	Fertile	Fertile	Yes	Spencer, 1942; Sears, 1947
11. *palustris* × *innubila*	Fertile	Fertile	Yes	Sears, 1947
12. *quinaria* × *subquinaria*-B	Sterile	Sterile	None	Sears, 1947
13. *subpalustris* × *munda*	Pupae	Sterile	None	Sears, 1947
14. *innubila* × *occidentalis*	Sterile	Sterile	None	Sears, 1947
15. *suboccidentalis* × *munda*	4 pupae, 2 larvae		None	Blumel, 1949
16. *suboccidentalis* × *palustris*	Fertile	Sterile	Yes	Sears, 1947
17. *subquinaria*-A × *sub-quinaria*-B	Fertile	Sterile	Yes	Sears, 1947
18. *subquinaria*-B × *sub-quinaria*-A	Sterile	Sterile	None	Sears, 1947
19. *transversa* × *innubila*	Sterile	Sterile	None	Sears, 1947
20. *tenebrosa* × *innubila*	Fertile	Sterile	Yes	Blumel, 1949
21. *tenebrosa* × *munda*	Fertile	Sterile	Yes	Blumel, 1949
22. *munda* × *tenebrosa*	4 flies	died	—	Blumel, 1949
23. *tenebrosa* × *palustris*	Fertile	Sterile	Yes	Blumel, 1949
24. *tenebrosa* × *quinaria*	Fertile	Fertile	Yes	Blumel, 1949
25. *tenebrosa* × *suboccidentalis*	12 flies	died	—	Blumel, 1949
26. *suboccidentalis* × *tenebrosa*	Sterile	Sterile	None	Blumel, 1949
27. *tenebrosa* × *transversa*	1 ♀	died	—	Blumel, 1949

suboccidentalis, and about those of *palustris* and *subpalustris.* The present status of the two forms recorded as *subquinaria* A and B also needs comment. The first pair of forms closely resemble each other morphologically, and the cross tests indicate their closeness of kinship. Thus, in the reciprocal crosses of *occidentalis* and *subocciden-talis,* the resulting hybrids were 100 per cent fertile (7, 8). Furthermore, the males of both forms produce fertile offspring when crossed to *munda* females (1, 2). These two forms show certain differences. In addition to the morphological variations noted by Spencer (1942), their metaphase chromosome patterns differ slightly in that the proxi-

mal end of the X chromosome of *suboccidentalis* bears a small knob, which is absent in *occidentalis* (Wharton, 1943). They behave somewhat differently in crosses to other members of the group. The crosses between the females of *occidentalis* and the males of either *palustris* or *subpalustris* produce fertile female and sterile male hybrids (5, 6), while the females of *suboccidentalis* go only to *palustris* males, also producing fertile females and sterile males (16). Again, *suboccidentalis* females crossed to *munda* males produce a few abnormal zygotes, while the cross of *occidentalis* females to *munda* males is incompatible (15). Finally, the reciprocal crosses of *suboccidentalis* and *tenebrosa* are slightly fertile, while the corresponding crosses of *occidentalis* with *tenebrosa* are incompatible (25, 26). On the basis of this evidence, it is clear that *occidentalis* and *suboccidentalis* have developed a considerable degree of evolutionary divergence; yet, in view of their high cross-fertility, we are inclined to agree with Sturtevant (1942) that this is a border-line case and that the two forms might be ranked as subspecies.

The ranking of *palustris* and *subpalustris* as full species would seem to be justified by the following facts. In the first place, the two forms exhibit greater morphological differences than is the case with the *occidentalis-suboccidentalis* pair, although in reciprocal crosses they likewise give fertile offspring (9, 10). In crosses with other members of the group, they show the following genetic differences: (1) the cross of *palustris* females to *innubila* males yields fertile male and female hybrids (11), while the corresponding cross of *subpalustris* is incompatible; (2) *subpalustris* females crossed to *munda* males give a few sterile flies and pupae (13), but the corresponding cross of *palustris* is incompatible; and (3), *palustris* males crossed to *tenebrosa* females give fertile female and sterile male hybrids (23), while a similar cross with *subpalustris* males is incompatible.

The status of the two forms listed in the table as *subquinaria* A and B (referred to by Sears by their stock numbers 1202.3 and 1210.5, respectively) needs clarification. These two stocks were originally identified as belonging to *D. subquinaria* Spencer, but cross tests reveal that they are genetically quite different. It will be sufficient to point out that stock A crosses reciprocally with *munda* and yields completely sterile hybrids (3, 4), while stock B does not cross with *munda*. Again, B males produce sterile hybrids with *quinaria* females (12), while A does not. Finally, the cross between A females and B

males gives fertile female and sterile male hybrids, while the reciprocal cross produces sterile offspring only (17, 18). It is clear that these forms represent two distinct species.

An analysis of the data may now be made with reference to the possibility of gene exchanges among the eleven tested forms. First of all, any possible gene exchange in crosses in which the F_1 males were found to be sterile would have to occur through the hybrid female line. The F_1 flies were first tested by inbred crosses. These crosses are listed in Table 74, where twenty-three such matings are shown. In one of these (13) a single hybrid male was produced, and in another (23) the F_1 inbred test was not made. Of the remaining twenty-one, eight were fertile (crosses 1, 2, 7 to 11, 24) and thirteen sterile (crosses 3 to 6, 12, 14 to 21, 26).

The inbred matings were then followed by backcrossing the F_1 flies to the parent species. These tests have brought out some very interesting facts. There are nine crosses indicated in column 3 of Table

TABLE 74

Number of hybrids obtained and their fertility in inbred tests

Crosses Female Male	Number of Hybrids ♀♀	♂♂	Total	$F_1 \times F_1$
1. *munda* × *occidentalis*	88	110	198	Fertile
2. *munda* × *suboccidentalis*	8	6	14	Fertile
3. *munda* × *subquinaria*-A	172	169	341	Sterile
4. *subquinaria*-A × *munda*	29	13	42	Sterile
5. *occidentalis* × *palustris*	24	23	47	Sterile
6. *occidentalis* × *subpalustris*	45	39	84	Sterile
7. *occidentalis* × *suboccidentalis*	256	178	434	Fertile
8. *suboccidentalis* × *occidentalis*	635	540	1,175	Fertile
9. *palustris* × *subpalustris*	325	342	667	Fertile
10. *subpalustris* × *palustris*	55	40	95	Fertile
11. *palustris* × *innubila*	24	25	49	Fertile
12. *quinaria* × *subquinaria*-B	25	25	50	Sterile
13. *subpalustris* × *munda*	0	1	1	———
14. *innubila* × *occidentalis*	1	2	3	Sterile
16. *suboccidentalis* × *palustris*	18	20	38	Sterile
17. *subquinaria*-A × *subquinaria*-B	27	32	59	Sterile
18. *subquinaria*-B × *subquinaria*-A	13	12	25	Sterile
19. *transversa* × *innubila*	272	256	528	Sterile
20. *tenebrosa* × *innubila*	27	19	46	Sterile
21. *tenebrosa* × *munda*	19	14	33	Sterile
23. *tenebrosa* × *palustris*	24	7	31	Not tested
24. *tenebrosa* × *quinaria*	26	30	56	Fertile
26. *suboccidentalis* × *tenebrosa*	14	34	48	Sterile

73 in which gene exchanges are not possible because of the sterility of the hybrid offspring, and in addition there are three other crosses in which the hybrid flies died before further tests could be made. This leaves fifteen combinations in which gene exchange is possible. In the cross between *munda* females and *occidentalis* males (1), the hybrid males did not fertilize the parent type females, but were fertile to their hybrid sibs. It is suggested that this case represents the origin of a cross-sterility due to a new combination of genes present in the hybrid males. The cross of *munda* females to *suboccidentalis* males (2) is somewhat similar to the preceding one, although in this case the hybrid males were fertile to *munda* females but not to those of *suboccidentalis*.

In the crosses of *occidentalis* females to the males of either *palustris* or *subpalustris* (5, 6), the F_1 females from both crosses were fertile in backcross to the parent species, but the corresponding matings of the F_1 males were incompatible. In the reciprocal crosses between *occidentalis* and *suboccidentalis* (7, 8) and those between *palustris* and *subpalustris* (9, 10), the F_1 hybrids of both sexes were fertile in both inbred and backcross tests.

In the cross of *palustris* females to *innubila* males (11), the inbred test was fertile, but the backcross tests showed that the hybrids of both sexes were fertile to *innubila* flies, while the matings to *palustris* flies proved to be incompatible. Consequently any transfer of genes would have to occur into the *innubila* line.

In the cross of *suboccidentalis* females to *palustris* males (16), the inbred matings were incompatible; but in backcross the F_1 females were fertile to *palustris* males only, while the other three backcross matings were incompatible. Hence gene exchange here would have to transfer genes from *suboccidentalis* into *palustris*. In the cross between *subquinaria*-A females to *subquinaria*-B males (17), the inbred matings were incompatible; but in backcrosses only the F_1 females were fertile, and to both parent species.

There are four remaining crosses in which the possibility of gene exchange is indicated. In the first three of these (20, 21, 23), with *tenebrosa* females crossed to either *innubila, munda,* or *palustris* males, the F_1 females were fertile in one direction only, back to *tenebrosa* males; all other crosses were incompatible. In the fourth cross, *tenebrosa* females to *quinaria* males (24), both male and female hybrids were fertile in backcross, but to *tenebrosa* only.

The cytology of the members of this group has been worked out by Wharton (1943), and Sears confirmed her observations. There is considerable variation in the metaphase chromosome pattern, as a result of fusions that form V-shaped chromosomes in four species and constrictions at the proximal ends of the X chromosomes, also found in four species. The salivary gland chromosomes are not especially favorable for study, but they show many inversions in hybrid larvae. Sears has shown that both the chromosome patterns and the constrictions are properties of the chromosomes themselves, since the two haploid patterns of chromosomes persist in the female hybrid (Figure 66).

Sears found that numerous phenotypic differences exist among the different species and that some hybrids were intermediate in appearance between the parent forms, while others showed mixtures. The hybrids reveal that both blending and dominant relations exist between the several gene systems which determine these phenotypes.

There must be several different isolating mechanisms operative in this group of species, for less than 25 per cent of the 110 possible reciprocal crosses exhibited cross-fertility. One must look for evidence which would account for the failure of so large a proportion of the matings to produce offspring. In his studies in ten of the species, Sears examined all of the crosses for possible causes and, using direct observation and dissections, found that sexual isolation was the principal cause. About half of the crosses that go are cross-fertile in one direction only, with sexual isolation preventing the occurrence of the reciprocal mating. This failure is often due to the behavior of the female, which sometimes shows a decided antagonism to the advances of the male. He cites several cases in which sexual isolation prevented mating, but the cross went when this mechanism was broken down by giving the partners no choice of mate. For example, without choice of mate *palustris* will cross with *subpalustris* reciprocally and the F_1 and F_2 hybrids are fertile, but with choice of mate only homogamic matings occur.

The investigations of Sears were carried out prior to the discovery of the insemination reaction, and we now know that the deleterious effects of that reaction were in part responsible for some of the failure of hybrid production in this group. This suggestion is supported by the results obtained in heterogamic matings between members of the mulleri subgroup, where it was found that the insemination reaction

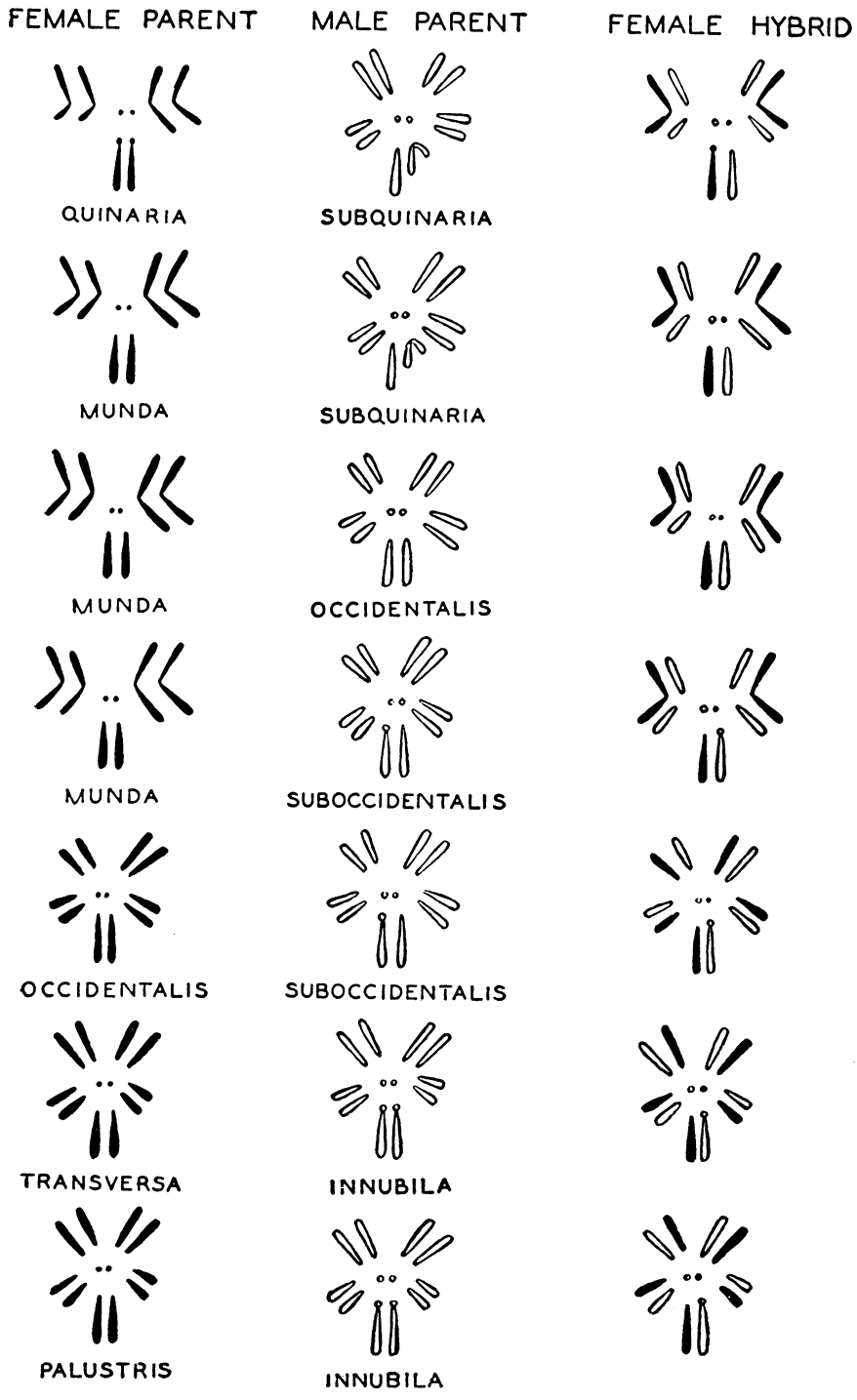

Fig. 66 Diagrams showing that the haploid patterns of chromosomes persist in the female hybrid. (After Sears.)

often prevented altogether the production of offspring even though the females had been fertilized (Patterson, 1946, 1947 b.). It is further supported by the work of Wheeler (1947), who found that this reaction was very pronounced in the quinaria group of species, and by Blumel (1949) in her work on the same group.

What has been termed "gametic mortality," or the inactivation or death of the sperm in the reproductive tract of an alien female, is a form of isolating mechanism (Patterson, Stone, Griffen, 1940 b.). Sears made a similar study in the quinaria group and found, for example, that in crosses between either *palustris* or *subpalustris* females and *occidentalis* males, copulation took place but hybrids were never produced. Dissections of the females showed that in each case the sperm had become inactivated, and consequently they were incapable of inseminating the eggs.

Hybrid sterility is another isolating mechanism extensively represented in the quinaria group, as we have already shown. There are eight inbred crosses that proved to be fertile (Table 74), and this means that the hybrids of both sexes were fertile, at least on inbreeding. Of the thirteen inbred crosses that were sterile, backcross tests showed that the sterility was confined to the hybrid male in seven cases (Table 74, 5, 6, 16, 17, 20, 21, 26) and involved both sexes in the other six (3, 4, 12, 14, 18, 19).

Other possible factors operative in the evolutionary divergence of the quinaria group of species are geographic and ecological isolation. The North American members of this group can be divided roughly into eastern and western divisions, as regards their geographic distribution. The eastern division includes *deflecta, palustris, subpalustris, quinaria,* and *transversa.* The first four species occur in the northeastern quarter of the United States, and the fifth is widely distributed over that portion of the country lying east of the ninety-eighth meridian. The western division includes *innubila, munda, occidentalis, suboccidentalis, tenebrosa, subquinaria* A and B and *suffusca.* The distribution ranges of these eight forms fall within the western part of the United States, with those of the first five extending down into Mexico. There is some evidence that the distribution areas of some of the forms belonging to the two divisions are in contact in the region of the Great Plains. The habitats of the western division species are located within the Rocky Mountain system, while those of the

eastern division species are found mainly within the forest areas of eastern United States.

If we consider the eleven forms listed in the table (*deflecta* and *suffusca* were not available for testing), there were only two successful crosses which represent one hybridization of the six possible combinations between the four available species of the eastern division. These were the reciprocal crosses of *palustris* and *subpalustris,* both of which produced fertile male and female hybrids (Table 74, 9 + 10). In the western division there were fifteen successful crosses, which represent ten cases of hybridization out of a possible twenty-one between the seven species, and of this number six produced fertile hybrids (1, 2, 7 + 8, 17 + 18, 20, 21). Ten out of a possible twenty-eight hybrid combinations were produced between the four eastern and seven western members of the group, and of these ten combinations six were fertile (5, 6, 11, 16, 23, 24). If the eastern and western groups are considered as separate units, there is no obvious positive or negative correlation between crosses within a group versus crosses between the two groups. In so far as the evidence may be used, it shows no correlation between geographic isolation and other mechanisms which prevent gene exchange.

If we consider the fact that only twenty-seven out of 110 possible matings yield hybrids and that only fifteen of these produce fertile hybrids, the amount of gene exchange which could take place in nature would be limited between members of the quinaria group, even though some of them may have overlapping ranges. The following facts support this suggestion: (1) in some crosses, hybridization does not occur except in the absence of choice of mate; (2) in several crosses, the number of hybrids produced is small and some of them are abnormal; (3) in others, the males are completely sterile, so that the only channel for gene exchange would be through the female hybrid; (4) some hybrids are fertile only on inbreeding, others only in backcrosses; (5) some hybrids are fertile in backcrosses to only one of the parent forms.

The greatest opportunity for gene exchange to occur would be with *occidentalis* and *suboccidentalis,* which not only produce completely fertile hybrids in their reciprocal crosses but between them are also cross-fertile to five other species, producing fertile hybrids in three cases. These two forms are the most closely related ones of

the group and have the least divergence. Perhaps next in order in the matter of isolation would be *palustris* and *subpalustris*. The hybrids from their reciprocal crosses are fertile; and together the two species are cross-fertile with five other forms, in three of which the hybrids are also fertile. It is possible that these two species have a certain amount of ecological isolation from the other members of the group.

Of the remaining species, *munda* is cross-fertile with six other forms, but the hybrids from three of the crosses are sterile. The species *innubila* is cross-fertile with four other species, with the hybrids from two of the crosses fertile. Of the two species listed under the name *subquinaria,* A and B are cross-fertile in reciprocal matings, but all of the hybrids were sterile except the females from the cross A♀ × B♂. Stock A also crosses reciprocally with *munda,* but all of the hybrids were sterile; and B males cross with *quinaria* females, producing sterile hybrids. The only other cross including *quinaria* is *tenebrosa* ♀ × *quinaria* ♂, which gives fertile male and female hybrids. In addition to crossing with *quinaria, tenebrosa* is cross-fertile with five other species, and the hybrids from three of these five crosses are fertile. The only one of the eleven tested forms which has developed complete reproductive isolation with the other ten is *transversa,* and while it crossed with *tenebrosa* and *innubila,* the resulting hybrids either died soon after emerging or else were completely sterile.

An inspection of the data given in Table 73 shows that, of the 110 possible crosses, twenty-seven produced some kind of hybrid. However, if we consider the fifty-five possible cases of hybridization without regard to the direction of the cross, twenty were obtained. In this species group at least, reciprocal crosses are not usual, for they are present in only seven of the twenty cases of hybridization.

virilis group hybrids

It will be recalled that the virilis group includes eight known Nearctic forms and that *D. virilis* lives in domestic habitats, at least in America, but in Asia is said to live in wild habitats. So far as known, seven other forms are endemic to the United States and have never been found in other than wild habitats. Five of these forms, *lacicola, borealis, flavomontana, novamexicana,* and *montana,* are ranked as full species, while the remaining two, *americana* and *texana,* are now

regarded as constituting a pair of subspecies (Stone and Patterson, 1947). The Palaearctic forms will be discussed separately.

All of the successful crosses between these eight forms are listed in Table 75. We shall first consider the cross-fertility of *virilis* with five other members. Spencer (1938) was the first to report that the reciprocal matings of *virilis* and *americana* are cross-fertile and that the cross goes better when *virilis* is used as the female parent. However, the degree of cross-fertility varies with different strains of the two forms. The F_1 hybrids are partially fertile, both in inbred and backcross tests.

The cross of *virilis* ♀ × *lacicola* ♂ produces hybrids, but the reciprocal cross is incompatible. The F_1 hybrids failed to produce offspring in inbred tests, but one hybrid female gave a single sterile female in the backcross to *lacicola* males. Hybrid females backcrossed to *virilis* males, and the hybrid males backcrossed to both parental types of females; all failed to give offspring. It is evident from the results of these tests that *lacicola* is completely isolated from *virilis* and that gene exchange between them is not possible (cross 3).

In the reciprocal matings between *virilis* and *montana,* the cross went better when *virilis* was used as the female parent and produced 542 fertile cultures in 2,700 tested pairs. In the reciprocal cross, with *montana* used as the female parent, only five cultures among 2,969 tested pairs were fertile, and these gave three sterile males and five larvae which died before undergoing pupation (Patterson and Griffen, 1944a). The F_1 hybrids from the first cross failed to produce offspring when inbred, but five hundred hybrid females mated in pairs to *virilis* males gave forty-three fertile cultures, and an equal number of these hybrid females backcrossed in pairs to *montana* males gave only ten fertile cultures. Of the 1,062 hybrid males backcrossed to the two types of parental females, only five fertile cultures were obtained, all from *virilis* females, and these yielded six females and four males. Any possible gene exchange between these two species would have to occur through the *virilis* female line (crosses 4, 5).

The cross of *virilis* ♀ × *novamexicana* ♂ gave 20 per cent fertile, but the reciprocal cross yielded only 2 per cent fertile. In the inbred tests, the F_1 flies gave 2 per cent fertile for each cross. However, in backcrosses, the hybrid VN females were 35 and 43 per cent fertile to the parent stocks, but the hybrid males were only slightly fertile.

TABLE 75

Hybrids between members of the virilis group

Crosses Female × Male	F_1 Hybrids ♀♀	♂♂	Possible Gene Exchanges	Author Reporting
1. *virilis* × *americana*	Fertile	Fertile	Yes	Spencer, 1938 P.S.G., 1940
2. *americana* × *virilis*	Fertile	Fertile	Yes	Spencer, 1938 P.S.G., 1940
3. *virilis* × *lacicola*	Fertile	Sterile	Slight	P. & G., 1944a
4. *virilis* × *montana*	Fertile	Fertile	Yes	P. & G., 1944a
5. *montana* × *virilis*	Larvae	Sterile	None	P. & G., 1944a
6. *virilis* × *novamexicana*	Fertile	Fertile	Yes	P. & S., 1949
7. *novamexicana* × *virilis*	Fertile	Fertile	Yes	P. & S., 1949
8. *virilis* × *texana*	Fertile	Fertile	Yes	P.S.G., 1940
9. *texana* × *virilis*	Fertile	Fertile	Yes	P.S.G., 1940
10. *americana* × *montana*	Sterile	Sterile	None	P. & G., 1944a
11. *montana* × *americana*	Sterile	Sterile	None	P. & G., 1944a
12. *americana* × *novamexicana*	Fertile	Fertile	Yes	P. & S., 1949
13. *novamexicana* × *americana*	Fertile	Fertile	Yes	P. & S., 1949
14. *americana* × *texana*	Fertile	Fertile	Yes	P.S.G., 1940
15. *texana* × *americana*	Fertile	Fertile	Yes	P.S.G., 1940
16. *texana* × *lacicola*	Sterile	Fertile	Slight	P. & G., 1944a
17. *texana* × *montana*	Fertile	Fertile	Slight	Patterson, 1942
18. *montana* × *texana*	None	Sterile	None	P. & G., 1944a
19. *texana* × *novamexicana*	Fertile	Fertile	Yes	P. & S., 1949
20. *novamexicana* × *texana*	Fertile	Fertile	Yes	P. & S., 1949
21. *montana* × *lacicola*	Fertile	Fertile	Yes	P. & G., 1944a
22. *lacicola* × *montana*	Fertile	Fertile	Yes	P. & G., 1944a
23. *montana* × *novamexicana*	Sterile	Sterile	None	P. & S., 1949
24. *novamexicana* × *montana*	Fertile	Sterile	Slight	P. & S., 1949
25. *lacicola* × *novamexicana*	Fertile	Sterile	Slight	P. & S., 1949
26. *imeretensis* × *virilis*	6 sterile flies		None	Sokolov, 1948
27. *littoralis* × *virilis*	Fertile	Sterile	Yes	Patterson, 1952a
28. *virilis* × *littoralis*	Fertile	Sterile	Yes	Patterson, 1952a
29. *littoralis* × *montana*	Fertile	Sterile	Yes	Patterson, 1952a
30. *montana* × *littoralis*	None	Sterile	None	Patterson, 1952a
31. *littoralis* × *lacicola*	Fertile	Sterile	Yes	Patterson, 1952a
32. *lacicola* × *littoralis*	Sterile	Sterile	None	Patterson, 1952a
33. *littoralis* × *americana*	Sterile	Sterile	None	Patterson, 1952a
34. *flavomontana* × *virilis*	Sterile	Sterile	None	Patterson, 1952b
35. *virilis* × *flavomontana*	Fertile	Sterile	Yes	Patterson, 1952b
36. *flavomontana* × *montana*	Fertile	Sterile	Yes	Patterson, 1952b
37. *montana* × *flavomontana*	Sterile	Sterile	None	Patterson, 1952b
38. *flavomontana* × *lacicola*	Fertile	Sterile	Yes	Patterson, 1952b

TABLE 75 (*Cont.*)

Crosses Female × Male	F_1 Hybrids ♀♀	F_1 Hybrids ♂♂	Possible Gene Exchanges	Author Reporting
39. *lacicola* × *flavomontana*	Fertile	Sterile	Yes	Patterson, 1952b
40. *flavomontana* × *littoralis*	Fertile	Sterile	Yes	Patterson, 1952b
41. *borealis* × *virilis*	Sterile	Sterile	None	Patterson, 1952b
42. *virilis* × *borealis*	Sterile	Sterile	None	Patterson, 1952b
43. *borealis* × *montana*	Fertile	Sterile	Yes	Patterson, 1952b
44. *montana* × *borealis*	Larvae only		None	Patterson, 1952b
45. *borealis* × *littoralis*	Abnormal flies		None	Patterson, 1952b

The NV hybrid females were 71 and 18 per cent fertile in backcross, whereas the hybrid males were 8 per cent or less fertile (Patterson and Stone, 1949). It is possible, but very improbable, that gene exchange could occur between these two species because of ecological isolation (crosses 6, 7).

The cross-fertility between *virilis* and *texana* follows very much the same pattern as in *virilis-americana* crosses, and the results are very much the same (crosses 8, 9). All F_1 hybrids are partially fertile, both in inbred and backcross tests. There would be considerable opportunity for gene exchange to take place between *virilis* and either of the other two forms were it not for the fact that it is rather effectively isolated from them by ecological factors.

The crosses between *americana* and *lacicola* were all nearly incompatible, but large mass matings yielded a few sterile offspring. Hence these two forms are completely isolated reproductively from each other.

The crosses between *americana* and *montana* produce a few hybrid offspring. In one test in which two geographical strains of *americana* females (Smithville, Georgetown) were crossed to *montana* males, not a single fertile culture developed in 1,954 tested pairs. However, when the Anderson strain of *americana* females was used, four fertile cultures were found among 982 tested pairs. These cultures yielded seventeen sterile F_1 hybrids. In the reciprocal cross, 2,880 tested pairs gave twenty-six fertile cultures, which yielded sixty-

six hybrids. These were all found to be sterile in inbred and backcross tests (crosses 10, 11). These two forms are isolated by the incompatibility of the initial cross and by the sterility of their hybrids.

In the reciprocal matings between *americana* and *novamexicana* (crosses 12, 13), the cross went much better when *americana* was used as the female parent, giving 15 per cent fertile. In the reciprocal cross, 116 tested pairs failed to produce offspring, but a few hybrids were obtained in mass matings. In the backcrosses, AN hybrid females gave 4 and 17 per cent fertile respectively to *americana* and *novamexicana* males, while the AN males gave 19 and 1 per cent fertile respectively for the corresponding crosses. The NA hybrid females in backcrosses failed to produce offspring when mated to *americana* males, but gave 25 per cent fertile to *novamexicana* males. The NA hybrid males in backcross gave 28 and 10 per cent fertile respectively for the corresponding crosses.

The reciprocal crosses between *americana* and its subspecies *texana* are fertile and produce fertile offspring (crosses 14, 15). The percentage of fertile cultures when *americana* was used as the female parent was higher than in the reciprocal cross (44 vs. 28). Another point of interest is that these two forms hybridize in nature within the zone of overlap of their distribution areas (Stone and Patterson, 1947).

The crosses between *lacicola* and *texana* go only when *texana* is used as the female parent (cross 16). With one strain of *texana* females (Georgetown) the cross was incompatible, but with two other strains (Lake McKethan, New Orleans) the percentages fertile were 1 and 12, respectively. In inbred crosses the F_1 hybrids failed to produce offspring.

The cross of *montana* ♀ × *texana* ♂ usually produces only male hybrids, which are completely sterile (crosses 17, 18). This situation is brought about through a system of complementary factors which are sex-linked in *texana* (Patterson and Griffen, 1944b). This case is described elsewhere in the volume. The reciprocal cross is fertile and produces male and female hybrids in equal numbers.

The P_1 crosses between *texana* and *novamexicana* behave very much like those between *americana* and *novamexicana* (crosses 19, 20). In the cross of *texana* ♀ × *novamexicana* ♂, 16 per cent of the cultures were fertile, but in the reciprocal cross only 1 per cent was fertile. In the backcrosses, the TN hybrid females gave 29 per

cent fertile to the males of both *texana* and *novamexicana;* and the TN hybrid males also gave 29 per cent fertile to the *texana* females, but only 1 per cent to the *novamexicana* females. The NT hybrid flies gave percentages varying from 8 to 67 in the backcrosses. Gene exchange would therefore be possible between these two species if their distribution ranges should overlap.

The crosses between *lacicola* and nine geographical strains of *montana,* when the former was used as the female parent, gave percentages varying from 0 in one strain to as high as 26 in another strain, with an average of about 6 per cent fertile. In the reciprocal crosses the per cent fertile was slightly over eighteen (crosses 21, 22). These two species are fairly closely related, and would exchange genes in case they had overlapping distribution ranges.

The reciprocal crosses between *novamexicana* and *montana* went very poorly, yielding only a few offspring (crosses 23, 24). In the cross of *novamexicana* ♀ × *montana* ♂, mass matings plus a single fertile pair mating gave sixteen females and seven males. The F_1 flies were inbred and also backcrossed to parental types. All cultures were sterile except one (F_1 ♀ × N ♂), which produced a single pupa. In the reciprocal cross, two cultures out of 118 tested pairs each produced a single female, both of which soon died. Four mass matings produced a few larvae which died without undergoing pupation. Gene exchange between these two species is not possible.

In the cross of *lacicola* ♀ × *novamexicana* ♂ 121 tested pairs failed to produce offspring, but four mass matings yielded six females and three males. Two of the females and all three males died soon after emerging. The four remaining females were backcrossed to both parental types of males, and one culture gave a single sterile female (cross 25). The reciprocal matings were incompatible. Gene exchange between these two species is not possible.

Brief accounts of the two Palaearctic members of the virilis group recorded from Europe will be found in Chapters 2 and 3. One of these was recently described by Sokolov (1948) under the name *Drosophila imeretensis.* He made many attempts to hybridize this species with *virilis* and finally succeeded in obtaining six hybrid individuals which proved to be completely sterile. Sokolov does not state whether the cross went one or both ways; nor does he indicate the sex of the hybrids (cross 26).

The second species reported from Europe is *littoralis,* which was

described by Meigen in 1830. The results from the preliminary tests of this species with other members of the group are recorded at the end of Table 75. It crosses both ways with *virilis,* producing fertile females and sterile males (crosses 27, 28). As in many other reciprocal matings of *virilis* with other species of the group, the cross goes better when *virilis* is used as the female parent. The cross *virilis* ♀ × *littoralis* ♂ produced 1,962 hybrids from a total of 920 tested pairs, while the reciprocal cross gave but 40 hybrids from 700 tested pairs. In the matings with *montana* (crosses 29, 30), the cross *littoralis* ♀ × *montana* ♂ yielded 180 hybrids from 410 tested pairs, while the reciprocal cross was practically incompatible, producing but a single sterile male from 450 tested pairs. In the matings with *lacicola* (crosses 31, 32), the cross *littoralis* ♀ × *lacicola* ♂ yielded 26 hybrids from 220 tested pairs, and with the same number of tested pairs the reciprocal cross gave one female and one male, both sterile. Finally, *littoralis* was tested to all three members of the americana complex (*americana, texana, novamexicana*). Five out of six of the possible reciprocal matings proved to be incompatible. In the fertile cross, *americana* ♀ × *littoralis* ♂, 220 tested pairs yielded one sterile male and one sterile female (cross 33).

In the seven fertile crosses between *littoralis* and the other members of the group, there are represented three cases of hybridization in which exchange of genes would be possible through the hybrid females. These are the reciprocal matings with *virilis,* and the crosses of *littoralis* females with *montana* and *lacicola* males.

Patterson (1952b) has shown the relationship between the two newly described members of the montana complex and the other members of the group. The pertinent crosses are added (34 to 45) to Table 75 to show the seven new hybrids.

funebris group hybrids

It was stated in Chapter 2 that this group included four species, namely, *funebris, subfunebris, macrospina,* and *trispina.* The first of these is widely distributed and is regarded as a cosmopolitan species. The second, or *subfunebris,* has thus far been reported only from California. Three different subspecies have been recognized within the species *D. macrospina,* as follows: (1) *limpiensis,* which has been recorded from Utah and Arizona to as far east as west Texas, and in the northern half of Mexico; (2) *macrospina,* which ranges from cen-

tral Texas east to Florida and north as far as Missouri; (3) *ohioensis,* which replaces the subspecies *macrospina* in Ohio, and perhaps also in Michigan. The fourth species, *D. trispina* has been collected at a single locality near Earp in southeastern California. The results obtained from tests for hybridization among these different forms are listed in Table 76.

In a series of articles, Mainland (1941, 1942a, 1942b) has reported the results obtained in his studies on the cross-fertility of all the species and subspecies with the exception of *trispina.* He found that all crosses between *funebris* and other members of the group were incompatible. Crosses between the different strains of the same subspecies of *macrospina* showed variations in the degree of cross-fertility, but the offspring are fertile. Crosses between the three subspecies go somewhat less readily than crosses between strains of the same subspecies. However, when *limpiensis* is used as the female parent, the F_1 males are sterile or semisterile, depending on the geographical strains employed in the test (cross 2). He found that, in general, there was a decrease in the amount of male sterility as the distance between

TABLE 76

Hybrids between members of the funebris group

Crosses		F_1 Hybrids		Possible Gene Exchanges	Author Reporting
Female	Male	Females	Males		
1. *macrospina* × *limpiensis*		Fertile	Fertile	Yes	Mainland
2. *limpiensis* × *macrospina*		Fertile	Semi-sterile	Yes	Mainland
3. *macrospina* × *ohioensis*		Fertile	Fertile	Yes	Mainland
4. *ohioensis* × *macrospina*		Fertile	Fertile	Yes	Mainland
5. *limpiensis* × *ohioensis*		Fertile	Semi-sterile	Yes	Mainland
6. *ohioensis* × *limpiensis*		Fertile	Fertile	Yes	Mainland
7. *macrospina* × *subfunebris*		Fertile	Sterile	Yes	Mainland
8. *subfunebris* × *limpiensis*		Fertile	Sterile	Yes	Mainland
9. *limpiensis* × *subfunebris*		Fertile	Sterile	Yes	Mainland
10. *limpiensis* × *trispina*		Fertile	Sterile	Yes	Wheeler
11. *trispina* × *limpiensis*		Fertile	Sterile	Yes	Wheeler
12. *subfunebris* × *trispina*		Fertile	Sterile	Yes	Alexander

the points of origin of the parental strains decreases. At least part of the sterility of these hybrid males is due to a *limpiensis* X-autosome complementary factor (or factors) and to a *limpiensis* Y-autosome complementary factor (or factors).

The interspecific crosses between *subfunebris* and the three sub-

species of *macrospina* give an interesting series of cross-fertility relationships. With two exceptions, crosses between either *macrospina* or *ohioensis* and *subfunebris* were found to be incompatible. The exceptions were two strains of *macrospina* from near the western limits of its distribution range. Females from these strains crossed to *subfunebris* males produce fertile female and sterile male hybrids (cross 7). These results are in sharp contrast to those obtained from crosses between *subfunebris* females and *limpiensis* males, which, with the exception of one strain, produce progeny in at least one direction. The female hybrids are fertile, but the male hybrids are sterile (cross 8).

In the reciprocal cross of *limpiensis* ♀ × *subfunebris* ♂, all except three of the tested strains proved to be incompatible. The three exceptions were strains of *limpiensis* from Magdalena, Mexico; Zion National Park, Utah; and Silver City, New Mexico. These crosses gave fertile female and sterile male hybrids (cross 9). The points of origin of the first two strains are near the western limits of the range of *limpiensis,* and therefore nearest to the distribution area of *subfunebris* in California.

The *subfunebris-macrospina* series of species, subspecies, and geographical strains forms a distribution pattern which extends across the United States from California to central Texas, and then east and north to Florida and Ohio. At the western end, *subfunebris* is connected with *macrospina* of central Texas by an intergraded series of geographical strains of *limpiensis,* and *macrospina* continues east and north by geographical strains to connect up with *ohioensis.* The fertility tests show that, by starting at either end of the series, cross-fertility between strains decreases as their distance apart increases. Mainland noted this trend both in the case of semisterility of males from the *limpiensis-macrospina* cross and in the degree of sexual isolation which various strains of the species *macrospina* have in respect to *subfunebris.*

Preliminary cross tests were made by Wheeler (1949b) between *trispina* and both *subfunebris* and *limpiensis.* In the cross with *subfunebris* no offspring was obtained, but with *limpiensis* fertile female and sterile male hybrids were produced (crosses 10, 11). He found that when *limpiensis* was used as the female parent the cross went very well, but the reciprocal cross went poorly or sometimes not at all.

Alexander (unpublished) was able to obtain hybrids between *sub-*

funebris females and *trispina* males (cross 12) in mass cultures, although the reciprocal cross proved to be incompatible, as did the pair matings. In the small sample tested, the hybrid females were fertile in backcrosses to *subfunebris* males. Ward (unpublished) found one inversion present between *trispina* and *limpiensis,* but several rearrangements when compared with *subfunebris.*

repleta group hybrids

This very large group of species has been extensively tested for possible cases of hybridization. Almost every possible combination between species in stock has been made, but a large majority of the matings proved to be incompatible. Thus far thirty-five combinations have been found to be cross-fertile, although the degree of fertility between some of the forms is very small (Table 77). Hybridization was found to occur among at least some members of each of four established subgroups. The number of forms involved in each of these is three for the hydei subgroup, seven for the melanopalpa subgroup, nine for the mulleri subgroup, and three for the mercatorum subgroup. The hydei subgroup has been investigated by Wharton (1944), who found that, although in reciprocal matings *nigrohydei* exhibited considerable sexual isolation with *hydeoides,* yet both crosses go in mass matings and produce fertile F_1 and F_2 hybrids (crosses 1, 2). Hybrids between the females of *nigrohydei* and the males of *hydei* were obtained only after many unsuccessful attempts. In one test with a strain of *hydei* males from Mexico, each of two vials out of forty mass matings showed one larva (3). Both larvae developed to the pupal stage, when one disintegrated and the other yielded a small, abnormal female which died shortly after emerging. As Wharton points out, such residual fertility is significant only in demonstrating that the recent ancestors of the two species were more closely related and have now become separated through sexual isolation and hybrid inviability.

Members of the melanopalpa subgroup have been tested mainly by Wharton (1942, 1944). Of the thirteen species assigned to this subgroup, only six have been successfully hybridized in thirteen different combinations (4 to 16). These are *melanopalpa, canapalpa, neorepleta, limensis, repleta,* and *fulvimacula.* The first six combinations (4 to 9) represent the reciprocal crosses between the first three species. All produced fertile hybrids of both sexes. However, Wharton (1944) found a high degree of sexual isolation to exist in certain matings.

TABLE 77

Hybrids between members of the repleta group

Crosses Female	Crosses Male	F_1 Hybrids Females	F_1 Hybrids Males	Possible Gene Exchanges	Authors Reporting
1. *nigrohydei*	× *hydeoides*	Fertile	Fertile	Yes	Wharton, 1944
2. *hydeoides*	× *nigrohydei*	Fertile	Fertile	Yes	Wharton, 1944
3. *nigrohydei*	× *hydei*	Sterile	+ pupae	None	Wharton, 1944
4. *melanopalpa*	× *canapalpa*	Fertile	Fertile	Yes	Wharton, 1944
5. *canapalpa*	× *melanopalpa*	Fertile	Fertile	Yes	Wharton, 1944
6. *melanopalpa*	× *neorepleta*	Fertile	Fertile	Yes	Wharton, 1944
7. *neorepleta*	× *melanopalpa*	Fertile	Fertile	Yes	Wharton, 1944
8. *canapalpa*	× *neorepleta*	Fertile	Fertile	Yes	Wharton, 1944
9. *neorepleta*	× *canapalpa*	Fertile	Fertile	Yes	Wharton, 1944
10. *canapalpa*	× *repleta*	Sterile	Sterile	None	Wharton, 1944
11. *melanopalpa*	× *repleta*	Sterile	Sterile	None	Wharton, 1944
12. *neorepleta*	× *repleta*	Fertile	Sterile	Yes	Wharton, 1944 & Sturtevant, 1946
13. *limensis*	× *repleta*	Fertile	Sterile	Yes	Wharton, 1944, Ward & Stone, 1952
14. *limensis*	× *canapalpa*	Sterile	Larvae	None	Ward & Stone, 1952
15. *fulvimacula*	× *flavorepleta*	Fertile	Fertile	Yes	Patterson, 1952c
16. *flavorepleta*	× *fulvimacula*	Fertile	Fertile	Yes	Patterson, 1952c
17. *mulleri*	× *aldrichi*	Sterile	Sterile	None	Patterson & Crow, 1940
18. *mulleri*	× *arizonensis*	None	Sterile	None	Crow, 1942
19. *mulleri*	× *buzzatii*	Abnormal Flies	Abnormal Flies	None	Patterson, 1942 & Crow, 1942
20. *mulleri*	× *hamatofila*	Sterile	Sterile	None	Patterson, 1947a
21. *mulleri*	× *longicornis*	Sterile	Pupae	None	Patterson & Wharton, 1944
22. *mulleri*	× *mojavensis*	Fertile	Sterile	Yes	Patterson & Crow, 1940
23. *aldrichi*	× *arizonensis*	Sterile	None	None	Crow, 1942
24. *aldrichi*	× *mojavensis*	Sterile	None	None	Patterson & Crow, 1940
25. *arizonensis*	× *buzzatii*	Larvae	Larvae	None	Crow, 1942
26. *arizonensis*	× *mojavensis*	Fertile	Sterile	Yes	Crow, 1942
27. *mojavensis*	× *arizonensis*	Fertile	Fertile	Yes	Crow, 1942
28. *meridiana*	× *rioensis*	Fertile	Fertile	Yes	Patterson, unpublished
29. *rioensis*	× *meridiana*	Fertile	Fertile	Yes	Patterson, unpublished
30. *mercatorum*	× *pararepleta*	Fertile	Fertile	Yes	Wharton, 1944
31. *pararepleta*	× *mercatorum*	Fertile	Fertile	Yes	Wharton, 1944
32. *mercatorum*	× *paranaensis*	Sterile	Sterile	None	de Barros, personal communication
33. *paranaensis*	× *mercatorum*	Sterile	None	None	de Barros, personal communication
34. *pararepleta*	× *paranaensis*	Fertile	Sterile	Yes	Dreyfus & de Barros, 1949
35. *paranaensis*	× *pararepleta*	Fertile	None	Yes	Dreyfus & de Barros, 1949
36. *wheeleri*	× *mulleri*	Sterile	None	None	Patterson & Alexander, 1952
37. *mulleri*	× *wheeleri*	Fertile	Sterile	Slightly	Patterson & Alexander, 1952

TABLE 77 (*Cont.*)

Crosses Female	Crosses Male	F_1 Hybrids Females	F_1 Hybrids Males	Possible Gene Exchanges	Authors Reporting
38. *wheeleri* × *mojavensis*		Sterile	None	None	Patterson & Alexander, 1952
39. *mojavensis* × *wheeleri*		None	Sterile	None	Patterson & Alexander, 1952
40. *wheeleri* × *arizonensis*		Sterile	None	None	Patterson & Alexander, 1952
41. *aldrichi* × *wheeleri*		Fertile	Sterile	Yes	Patterson & Alexander, 1952
42. *wheeleri* × *aldrichi*		Fertile	Sterile	Yes	Patterson & Alexander, 1952
43. *buzzatii* × *wheeleri*		Larvae	Larvae	None	Patterson & Alexander, 1952
44. *wheeleri* × *buzzatii*		Sterile	None	None	Patterson & Alexander, 1952

Thus, out of forty mass matings of *canapalpa* females to *melanopalpa* males, only two cultures showed fertility, and in the reciprocal cross under the same conditions only one culture was fertile.

In the next two combinations (10, 11), *canapalpa* or *melanopalpa* females, when crossed to *repleta* males, produce sterile hybrids, but the reciprocal crosses are incompatible. The females of *canapalpa* cross only with great difficulty to *repleta* males, and only one out of many mass matings produces a few weak offspring. Hence the isolation between these two forms is almost complete. The females of *melanopalpa,* were hybridized with males of five different strains of *repleta,* and in one cross (Connecticut strain) some intersexes were produced. In her first paper, Wharton (1942) reported that females of this same species in a cross with *repleta* males from Guatemala produced, in addition to phenotypically normal males and females, malelike, femalelike, and mixed types of intersexes. Wharton states that the malelike intersex had very small, rudimentary claspers, and that the vaginal plates of the femalelike intersex were greatly reduced and crossed. The anal valves of the extremely mixed type intersex were poorly formed, with only one vaginal plate and a large "genital knob."

In the next combination (12), females of *neorepleta* crossed to males of a Guatemala strain of *repleta* produced a few abnormal offspring which within the limits of the tests appeared to be sterile (Wharton, 1942). In a private communication, Sturtevant reported at this time that, in a cross of *neorepleta* females to a *repleta* strain,

hybrid offspring were produced: "sterile males, and females slightly fertile but with anal plates suggesting intersexuality." Later, Sturtevant (1946a, b) reported that some of the F_1 females from this cross were fertile in backcross to the *repleta* males of the stock he used, but never fertile to *neorepleta* males. In one series of tests, 500 mass cultures yielded 532 female and 635 male hybrids from 74 of the cultures. Many of these females had three anal plates instead of the usual two, a character which suggests intersexuality. The F_1 males were found to be completely sterile, but about 400 of the hybrid females tested to *white repleta* males gave 179 offspring, thus demonstrating that some of the F_1 females are fertile.

In the next cross (13) *limensis* females were mated to *repleta* males. This cross does not go well, and usually produces only a few hybrids. Tests show that the F_1 females are fertile and the F_1 males sterile. The *limensis* females will also cross with *canapalpa* males (14), but the few female hybrids have been sterile. Most hybrids including all males have died as larvae or pupae. It is interesting to note that *limensis* does not cross with a new stock of *melanopalpa* from Michoacán, Mexico. Wharton (1942, 1944) used a stock of *melanopalpa* from Cave Creek, Arizona, which has been lost. The Michoacán strain of *melanopalpa* is not genetically equivalent to the Cave Creek strain and may represent a different subspecies or species, for its males do not cross with *canapalpa* females, and only one hybrid male was obtained in the reciprocal cross, most hybrids dying in the larval or pupal stages.

The Mexican subspecies *Drosophila fulvimacula fulvimacula* crosses readily with the subspecies *Drosophila fulvimacula flavorepleta* from Brazil. The two subspecies differ somewhat in phenotype but produce fertile F_1 hybrids. An interesting type of isolation develops here, for the $F_1 \times F_1$ crosses were fertile, giving a normal sex ratio in F_2; but backcrosses of the F_1 females or males to *fulvimacula* produce few offspring, although they crossed readily to *flavorepleta.* This seems to be another good example of isolation due to newly formed gene combinations.

The remaining combinations listed in the table include only the successful crosses between members of the mulleri and mercatorum subgroups. Nine of the seventeen forms assigned to the mulleri subgroup are represented. These data have been compiled from the following publications: Patterson and Crow, 1940; Patterson, 1942c, 1947a;

Crow, 1941, 1942; and Wharton, 1944. The thirteen combinations listed (17 to 29) represent only 20 per cent of those tested between the various members of this subgroup. This means that 80 per cent of the matings were incompatible. As an example of the failure of many matings to produce hybrids, we may cite the tests of the fifty-six possible crosses between eight species of this subgroup (see Table 53, Chapter 7). There were but ten successful matings, which are listed in crosses 17 to 27 (exclusive of cross 21). In this series of tests, there were sixteen additional crosses in which copulations were successful, but the inseminated females were unable to produce hybrids because of the harmful effects of the insemination reaction. In only three crosses are gene exchanges possible between the species. These are the cross of *mulleri* ♀ × *mojavensis* ♂ and the reciprocal crosses between *arizonensis* and *mojavensis*. Such exchanges in the other seven combinations, as well as in the one with *longicornis,* are not possible because of the sterility or the abnormality of the hybrid offspring.

At the bottom of Table 77 are recorded eight crosses, in six of which the hybrids were fertile. The first two (28, 29) are the reciprocal crosses between the subspecies *meridiana* and *rioensis*. The F_1 hybrids were inbred in mass cultures and produced offspring, thus indicating that both sexes are fertile. The last six crosses are between members of the mercatorum subgroup. The first two (30, 31) are the reciprocal crosses between the subspecies *mercatorum* and *pararepleta*. These are cross-fertile and produce F_1 hybrids which exhibit hybrid vigor, but Wharton noted a great reduction in fertility, fecundity, and viability among the F_2 flies.

The species *Drosophila paranaensis* de Barros found in Brazil hybridizes with the two subspecies of *mercatorum,* although the hybrids differ in fertility. The hybrids show that there is a very considerable degree of genetic difference between *paranaensis* and either *pararepleta* or *mercatorum*. The results of the crosses are similar, although de Barros (personal communication) did not report any fertile hybrids with *mercatorum*. If *paranaensis* is used as the female parent, only female hybrids are produced, while the reciprocal cross gives both males and females. The former are all sterile and are often abnormal in pigmentation and weak, so that they die within a few days. The female hybrids are somewhat fertile in backcrosses to both parent type males. The backcrosses of the hybrid females to *paranaensis* males gave a three female to two male ratio. Dreyfus and de

Barros (1949) have published studies which showed that there were several cytological differences involved in the autosomes. The species *paranaensis* has two simple independent inversions in one chromosome (II), (Dreyfus and de Barros, 1948, 1949), which are present in some strains but not in others. It differs from *pararepleta* by two other simple independent inversions in the same chromosome. If the strain of *paranaensis* with the two inversions is used, there are formed four overlapping (two and two) inversions. The two related forms from South America may differ by four inversions. The repleta group in general does not have many rearrangements between related species which cross.

Several important points are revealed in the tabulated data in the mulleri series. One of the most interesting is the fact that *mulleri* females are cross-fertile with the males of six other species of the subgroup, while the reciprocal matings are, without exception, incompatible. In three crosses, all or part of the resulting zygotes are either abnormal or else die in larval and pupal stages. In the *mulleri-buzzatii* cross, most of the zygotes die in larval stages, and the few that reach maturity are abnormal and sterile. The *mulleri-longicornis* cross produced one sterile female and three pupae. Finally, in the *arizonensis-buzzatii* cross, all zygotes die in midlarval stages. Still another point of interest is the failure of *mulleri-arizonensis* cross to produce female hybrids and the failure of *aldrichi* females to produce male hybrids in crosses to either *arizonensis* or *mojavensis* males.

The *mulleri-aldrichi* cross, which produces sterile hybrids, represents the first instance in which anyone was able to detect natural hybrids in field collections. This is made possible by the aberrant character of the F_1 male, which has rudimentary testes with but little coiling that are visible through the abdominal wall. Such males are identical with hybrid males produced in the laboratory from this cross and are likewise completely sterile. During July and August of 1940 and 1941, collections containing these two species taken from their overlap zone in Texas were examined for the presence of these hybrid males. Hybrid females must also be present in such collections, but they cannot be detected with certainty because of their close resemblance to the parent female. In all, twenty-two collections were made, and the results from the examination of these are listed in Table 78. All of the collections were made in the country, frequently at roadside parks (R.P.) where cacti were present.

The first thirteen collections contained a total of 2,317 normal and 30 hybrid males. The last nine collections contained a total of 927 normal males and no hybrids. If the two groups were alike, one

TABLE 78

Percentage of hybrid males found in nature from the cross *mulleri* ♀ × *aldrichi* ♂ (from Patterson)

Date	Place, County	*mulleri* Males	*aldrichi* Males	Percentage of *aldrichi* ♂♂	Hybrid Males
6/13/40	Selma, Bexar	34	188	84.6%	1
7/16/40	R.P., Fayette	27	26	49.0	1
7/19/40	R.P., Guadalupe	115	147	56.1	4
8/2/40	Selma, Bexar	100	99	49.7	1
8/2/40	R.P.(A), Comal	279	340	54.8	2
8/2/40	Cactus, LaSalle	5	11	68.7	1
8/2/40	Devine, Medina	24	16	40.0	1
8/9/40	R.P., Williamson	56	212	79.1	9
8/9/40	Troy, Bell	92	50	35.2	2
8/9/40	R.P.(A), Bell	153	148	49.1	3
8/9/40	R.P.(B), Bell	73	41	35.1	1
7/27/41	R.P., Travis	14	36	72.0	3
7/27/41	R.P.(A), Williamson	16	15	48.4	1
6/7/40	San Antonio, Bexar	19	6	24.0	0
8/2/40	R.P., Hays	38	15	28.3	0
8/2/40	R.P.(B), Comal	80	32	28.5	0
8/2/40	R.P., Guadalupe	177	64	26.5	0
8/2/40	Laredo, Webb	19	1	5.0	0
8/9/40	R.P., McLennan	52	3	5.4	0
8/12/40	Franklin, Robertson	190	72	27.4	0
8/12/40	Palestine, Anderson	60	15	20.0	0
7/28/41	R.P.(B), Williamson	68	16	19.0	0

would expect about 12 such hybrids among the total in the second group. The size of the individual collection evidently does not determine the presence or absence of hybrids. For example, the collection from Williamson County (8/9/40) contained 268 normal and 9 hybrid males, while the one from Guadalupe County (8/2/40) contained 262 normal and no hybrid males. From the sample presented in the table, the presence or absence of hybrids would seem to depend on the proportion of *aldrichi* males in the male population of the two species. It will be observed that, in every collection in which the percentage of *aldrichi* is above one-third of the male population, hybrids are present (first thirteen), but that hybrids are absent where the percentage is less than a third (last nine).

There should be mentioned in this connection the case of two

geographical strains of *D. peninsularis,* which is a member of the repleta group. The original flies of the two strains in question were collected some thirty-odd miles apart in Florida, at Lake McKethan and Tarpon Springs. The two strains showed certain morphological differences, and in addition various cross tests revealed that they had developed unilateral sexual isolation, a certain amount of hybrid sterility as a result of failure of spermatogenesis to produce functional gametes, and inviability of the sperm in the reproductive tract of the alien female (Patterson and Wheeler, 1947). This can hardly be regarded as a case of hybridization, but nevertheless it is of interest because the two strains show the beginning of evolutionary divergence.

Patterson and Alexander (1952) have described a new species of the mulleri subgroup, under the name *Drosophila wheeleri.* The successful tests of hybridization have been added to Table 77 (36 to 44). This species is especially interesting as an intermediate form between *aldrichi* and *mulleri.* The various crosses between members of the repleta group listed in Table 77 give a total of thirty cases of hybridization, of which twenty-six represent the interspecific type and four the intraspecific type.

melanica group hybrids

Hybridization between members of the melanica group has been reported by Griffen (1942), Miller (1944), and Sturtevant and Novitski (1941a). Griffen obtained hybrids from the reciprocal crosses of *melanica* and *paramelanica,* and from those of *paramelanica* and *nigromelanica.* In his investigations he used two geographical strains of *melanica,* five of *paramelanica,* and three of *nigromelanica,* and made all possible reciprocal matings. Of the twenty possible crosses between the several strains of *melanica* and *paramelanica,* seven were incompatible, twelve showed cross-fertility percentages of less than 1 per cent each, and one gave 3.2 per cent. In the crosses between *paramelanica* and *nigromelanica* strains, twenty-seven proved to be incompatible and only three were cross-fertile, each with a percentage of less than 1. All matings between *melanica* and *nigromelanica* were found to be incompatible (Table 79).

These results indicate that the main cause underlying the incompatibility of so many of these crosses, together with the low cross-fertility percentages, is sexual isolation. This conclusion is supported by the fact that all hybrids, although limited in number, are viable

and fully fertile, and that the sexes appear in equal numbers. Griffen (1942) found that the geographical strains of *melanica, paramelanica,* and *nigromelanica* form three distinct breeding groups, in which there is considerable fertility within the groups but very low fertility between them.

Geographical isolation must also have played some role in the divergence of *melanica* and *paramelanica,* since the main distribution

TABLE 79

Hybrids between members of the melanica group

Crosses Female	Male	F_1 Hybrids Females	Males	Possible Gene Exchanges	Author Reporting
1. *melanica* × *paramelanica*		Fertile	Fertile	Yes	Griffen, 1942
2. *paramelanica* × *melanica*		Fertile	Fertile	Yes	Griffen, 1942
3. *paramelanica* × *nigro-melanica*		Fertile	Fertile	Yes	Griffen, 1942
4. *nigromelanica* × *para-melanica*		Fertile	Sterile	Yes	Griffen, 1942
5. *melanica* × *melanura*		Fertile	Sterile	Yes	Miller, 1944
6. *paramelanica* × *melanura*		Sterile	Sterile	None	Miller, 1944
7. *micromelanica*-A × *micro-melanica*-T		Fertile	Fertile	Yes	Sturtevant & Novitski, 1941a
8. *micromelanica*-T × *micro-melanica*-A		Fertile	Sterile	Yes	Sturtevant & Novitski, 1941a
9. *euronotus* × *paramelanica*		Fertile	Sterile	Yes	Patterson & Ward, 1952

area of the former extends across the southern half of the United States, while that of the latter occurs in the northern half of the country. In contrast to this, the distribution area of *nigromelanica* overlaps those of both the other forms. There is some evidence to indicate that *nigromelanica* may be separated from the other forms by a certain amount of ecological isolation. Throughout the southern states, and perhaps elsewhere, this species is a forest-dweller and has been observed feeding on fungi, especially those growing in cavities of trees, logs, and stumps.

Miller (1944) tested his new species *D. melanura* to the other three forms and obtained hybrids between the females of *paramelanica* and *melanica* and the *melanura* males (crosses 5, 6), but none with *nigromelanica.* In the *melanica-melanura* cross only the female hybrids were fertile, but in the *paramelanica-melanura* cross both the female

and male hybrids were sterile. The cross-fertility tests among all of these forms represent four cases of hybridization.

There is one other member of the melanica group which has been tested to the first three forms listed in the table, but all matings proved to be incompatible (Griffen, 1942). This is *D. micromelanica,* which has, however, produced a very interesting case of intraspecific hybridization. Sturtevant and Novitski (1941a) tested strains of this species from Arizona and Texas, and found that Arizona ♀ × Texas ♂ yielded fertile hybrids of both sexes but that, in the reciprocal cross, only the F_1 females were fertile. Further tests indicated that the sterility of the male hybrids is dependent on their having a Texas X and an Arizona Y. By the use of mutant gene markers (*white* and *echinus*) in linkage tests, they were able to show that the sterility of these hybrid males was due to the presence of a particular portion of the Texas X chromosome and (probably) an Arizona Y. The case is especially interesting since it represents a possible beginning of divergence.

A new species, *Drosophila euronotus,* belonging to the melanica group has been described by Patterson and Ward (1952). This species is well isolated from the other members of the group. Only one successful cross (Table 79, cross 9) between it and the other members was obtained.

guaraní group hybrids

King (1947b) tested the various crosses between the six members of this group for possible cases of interspecific hybridization. He reports that hybrids between members of the guaramunú subgroup are never produced, and he was not able to obtain hybrid offspring between members of this subgroup and those of the guaraní subgroup. In fact, sexual isolation tests revealed that interspecific inseminations did not occur in any of these several crosses. He therefore concludes that the three species of the guaramunú subgroup are effectively isolated from one another and from the flies of the guaraní subgroup.

Within the guaraní subgroup only one combination failed to produce inseminations, and this was the cross between *guarú* females and *guaraní* males. In the other five combinations within this subgroup interspecific inseminations occur, but only the reciprocal crosses between *guarú* and *subbadia* result in the production of hybrid offspring. The cross between *guarú* females and *subbadia* males gives

large numbers of hybrid offspring, but the F_1 males are completely sterile. The F_1 females are fertile in backcrosses to males of either parent species. Microscopic examination of the testes of the hybrid males demonstrated that the spermatozoa develop to an advanced state and then completely degenerate.

In the reciprocal cross, *subbadia* ♀ × *guarú* ♂, about 90 per cent of the eggs fail to hatch. The larvae which do hatch fall into two classes. In one class they develop normally and are morphologically indistinguishable from those of the first cross, and their fertility is exactly the same. The other class of larvae dies before pupation. These larvae have no imaginal discs, and their nervous systems are highly abnormal. Apparently, the abnormal larvae which die before pupation include individuals of both sexes, since the adult hybrids arising from the normal larvae of this cross show a normal sex ratio. As the female hybrids of both crosses have identical chromosome sets, King concludes that the production of abnormal larvae in a certain percentage of cases in one of the crosses must be due to the interaction of the *subbadia-guarú* chromosomes and the cytoplasm of the *subbadia* egg.

It was found that the hybrid females from the *guarú-subbadia* cross, or its reciprocal, are to a limited extent fertile when mated to *guaraní* males. Most of the eggs in such cultures do not hatch, but those that do give viable larvae which develop into adult flies. The most striking feature of these offspring is their sex ratio. Only about 4 per cent (fourteen in 327 flies) were females. Both sexes proved to be sterile. The testes of the males of these triple hybrids fail to produce sperm, but the females, although sterile, have normal appearing ovaries and lay apparently normal eggs which never hatch. The near absence of females may be due to an incompatibility between the *guaraní* X and the X of either *subbadia* or *guarú,* and King suggests that such females may be the result of unusual gene combinations, or they may represent XXY individuals which have been produced by a nondisjunctional egg fertilized by a Y-bearing *guaraní* sperm.

These results demonstrate that *guaraní* is reproductively isolated from the other two members of the subgroup. They indicate, however, that some gene exchanges could take place between *subbadia* and *guarú,* but only through the hybrid female line. The reciprocal crosses between *subbadia* and *guarú* represent a single case of hybridization

in the guaraní group, and the *guarú-subbadia-guaraní* probably should be regarded as a second case.

pallidipennis-centralis cross

These two forms have been ranked as subspecies and originated from Brazil in South America and Veracruz in Mexico, respectively. The two forms are very similar morphologically, but measurements show that *pallidipennis* has a larger body size and a more rounded wing shape than *centralis*.

Patterson and Dobzhansky (1945) have carried out a series of tests on these subspecies and found that they cross and produce viable hybrids in both directions, with little evidence of sexual isolation. The F_1 males are completely sterile, but the females are fertile in backcrosses to males of either subspecies. A few of the males obtained in the offspring of the first backcross are fertile, and the proportion of fertile males increases in the progeny of succeeding backcrosses. The authors found that, in the progeny of the several backcrosses, the males derived from two backcrosses to the same subspecies were more likely to be fertile than those derived from backcrosses to different subspecies. This means that males carrying half of the chromosomes of one subspecies and half of the chromosomes of the other are sterile. Hence the greater the preponderance of the chromosomes of one of the parental subspecies, the greater the chance that a male will be fertile. These facts are compatible with the assumption that the sterility of the hybrids is due to the interaction of several genetic factors furnished by the two subspecies.

A cytological study of the F_1 males showed that spermatogenesis failed to produce functional gametes. The first and second meiotic divisions are abortive, resulting in the formation of groups of abnormal spermatids, which finally degenerate to form granular masses.

The reciprocal crosses between *pallidipennis* and *centralis* constitute a single case of hybridization.

DISCUSSION

Only a little over a decade ago, Dobzhansky (1937a) reviewed the known cases of hybridization in the genus Drosophila. These included *melanogaster* × *simulans, pseudoobscura* × *persimilis, pseudoobscura* × *miranda, persimilis* × *miranda,* and *azteca* × *athabasca.*

Dobzhansky went on to say, "Although further cases of interspecific hybridization in the genus Drosophila will, no doubt, be discovered by future studies, the conclusion is justified that in this genus the isolation of species from each other is more thorough than in many other animal and particularly plant genera." We are fortunate that this prophecy was wrong, as Dobzhansky would be the first to agree. At that time the tests were for the most part between members of the subgenus Sophophora, and the large virilis, quinaria, and repleta groups in the subgenus Drosophila had not been investigated. Table 80 summarizes the crosses which produced viable hybrids in the

TABLE 80

Summary of hybridization cases in the genus Drosophila

Species Groups	Number of Crosses	Reciprocal Crosses	One-way Crosses	Interspecific Crosses	Possible Gene Exchange	Cases of Hybridization
melanogaster	2	1	0	1	0	1
obscura	12	4	4	8	7	8
quinaria	27	7	13	20	15	20
virilis	45	19	7	22	30	25
funebris	12	5	2	3	12	7
repleta	44	14	16	26	23	30
melanica	9	3	3	3	8	6
guaraní	3	1	1	3	1	3
pallidipennis	2	1	0	0	1	1
Totals	156	55	46	86	97	101

species groups that have been investigated. This shows that only four more cases of hybridization have been added to the Sophophora, seventy-seven cases of interspecific hybridization have been added in the Drosophila. We have divided the total number of crosses into reciprocal crosses and one-way crosses where the reciprocal mating does not produce offspring, although sometimes the females are fertilized and a few produce larvae only. In fact, only about half the crosses go both ways (55:46). We have designated the number of interspecific crosses in each species group, but have also included the intraspecific crosses between some of the subspecies. This is necessary, since these cases contribute to our knowledge of genetic and chromosomal evolution. We have included all cases which show any residual fertility of the hybrids in the column "possible gene exchange," even though the probability of such exchange may be slight.

Hybrids illustrate certain further isolating mechanisms, for example, sterility and inviability. The more important role of hybrids is that of demonstrating the degree and type of genetic differences which have accumulated during the evolution of these forms. If evolution has occurred and still is occurring, there should be forms with all degrees of relationships, with both slight and great genetic differences, and with all kinds of combinations of isolating mechanisms.

The genus Drosophila exhibits a series of forms ranging from strains, races, and subspecies to species, species groups, and subgenera that illustrate all types and degrees of difference. We shall discuss the virilis group in detail in the next chapter, but can illustrate certain factors from the subspecies *texana* and *americana*. In a series of publications, we have shown that *texana* and *americana* differ in their chromosome arrangements and, to some degree, in their genotype, as shown by differences in crosses to other forms. Nevertheless, these two subspecies hybridize readily at their zone of contact (Stone and Patterson, 1947). The several inversion differences and the X-4 fusion do not have an appreciably detrimental effect in the hybrids. Patterson, Stone, and Griffen (1942) showed the F_1 and F_{10} inbred hybrids were both quite fertile. There are some genetic differences present between the western strains of *americana* from Montana and *texana* (Stone, 1947), but there is perhaps as much genetic difference between eastern and western strains of *americana* (Spieth, 1951). In fact, *texana* and the eastern strains of *americana* are phenotypically similar, although the western strains of the latter may be light and may somewhat resemble *novamexicana*. We presume that *texana* and both eastern and western strains of *americana* are adapted to the southern, northern, and northwestern temperature ranges and ecological conditions. Were it not for the chromosome differences, it would be difficult to separate these subspecies by crosses to each other. There is no indication of serious genetic isolation or genic balance differences between them.

There are three subspecies of *macrospina* in the funebris group (Mainland, 1942b). There are several inversions in the subspecies *macrospina* and *ohioensis,* but none in *limpiensis* (Warters, 1944). As Mainland points out, there is a gradation of subspecific character differences between Ohio and Texas, making it difficult to define the range of *macrospina* and *ohioensis*. The range of *limpiensis* lies west of the Pecos River in Texas, and the other subspecies are not found

west of Del Rio, Texas. There are some genetic differences involving the degree of isolation between strains of *macrospina* and *ohioensis,* in addition to the phenotypic differences. For example, a stock from Florida showed considerable sexual isolation to other *macrospina* strains, as well as to *limpiensis.* Also, males from a Mississippi stock of *macrospina* are partially isolated from females of *ohioensis.* The western subspecies *limpiensis* shows a much greater genetic difference from the other two subspecies, for hybrid males from a cross of *limpiensis* females to *macrospina* (or *ohioensis*) males are sterile or semisterile. The dissection of females inseminated by these males showed that the sperm were only infrequently motile. This is an example of one of the least drastic reductions from fully functional gamete formation. None of the hybrid females in these subspecies crosses shows any resultant sterility. There seem to be several factors contributing to the sterility of the hybrid males between *limpiensis* females and *macrospina, ohioensis,* or hybrid males. It may be that *limpiensis/macrospina* hybrid males are sterile because of a Y-autosome dependency with the genes in the *macrospina* autosome(s) recessive. Only about 50 per cent of the hybrid males carrying both a *limpiensis* X and Y and heterozygous for some or all the *macrospina* autosomes were fertile. Some factors influencing genic balance were not properly combined in some of these hybrids. Mainland suggests that an X-autosome (recessive) factor relation, in addition to a Y-autosome (a dominant factor in the same autosome as the recessive X-autosome complementary factor) relation determines this sterility. Other types of unbalance might be involved.

Several interesting differences have been demonstrated between strains and subspecies of the repleta group. The subspecies *mercatorum* and *pararepleta* cross readily, and the F_1 hybrids are quite vigorous and fertile, but there is a marked reduction in viability and fertility of the F_2 (Wharton, 1944). This cross is complicated by the fact that the *mercatorum* strain used by Wharton was XO, or perhaps better X(YY), as the Y element is fused with the very small rod chromosome to form a small V. Some other strains of *mercatorum* have in addition a small rod-shaped Y, similar to the Y of *pararepleta.* With the complex duplicated Y present, males that are XO would be rare; hence the greatly reduced fertility of the F_2 hybrids cannot be due in any large measure to this cause. The complexity that may result from abnormalities in the Y and autosome system has been dem-

onstrated by de Barros (1949a), who used a strain of *pararepleta* from Jacarépaguá, Federal District, Brazil. This strain had a small rod Y with a satellite and a pair of small rods for the dot chromosomes. In this strain de Barros found both primary and secondary nondisjunction of both the Y and 5 chromosome (the dots). She recovered XO, XYY, XXY, and XXYY forms, as well as haplo-5, triplo-5, and tetra-5 individuals and their combinations—for example, an XYY tetra-5 male. She concluded that these aberrations were due to a mutant, having obtained nondisjunction at least once for either the Y or 5 chromosomes in the F_1 and F_2 from each of forty pairs of this strain.

Another strain of *pararepleta* from Caraguatatuba, São Paulo, Brazil, gave a very abnormal sex ratio. Tests of females from the sex ratio strain to normal males showed the presence of three types of females: (1) those that produce females but very few males; (2) those that produce a ratio of three females to one male; (3) those that gave the normal 1:1 ratio. The sex ratio factor may be carried through the females in this case. This genetic abnormality in *pararepleta* is very interesting in view of the fact that the cross between the different species, *pararepleta* males $\times$ *paranaensis* females, gives only female offspring (Dreyfus and de Barros, 1949). The reciprocal cross gives both sexes, but with a reduced number of males. The cross-lethality seems to be a case of X-autosome unbalance of a serious nature, so that only female hybrids, which have both an X and autosome from each species, are viable. It is not possible to tell whether the sex ratio stock of *pararepleta* represents a mutation related to this cross-lethality or whether it is an unrelated mutational difference.

This set of closely related species illustrates several types and degrees of divergence. Wharton (1944) showed that some difference in gene balance existed between the subspecies *pararepleta* and *mercatorum,* which became apparent as heterosis in F_1 and poor viability and fertility among the F_2 recombinations. She also demonstrated the effect of the Y-autosome translocation or fusion which allows some strains of *mercatorum* to have an XO, since the necessary Y gene system is on chromosome 5. The further tests of de Barros demonstrated how mutational differences between strains could make possible nondisjunction of the Y and small 5 chromosome. It is not clear whether nondisjunction of the other chromosomes occurs. Furthermore, there exist gene differences (no chromosomal abnormality was

demonstrable) which lead to an aberrant sex ratio that could be effective through the female. Finally, Dreyfus and de Barros demonstrated a serious genic balance difference between the related species *pararepleta* and *paranaensis.*

Some of the other subspecies in the repleta group show still other shades and variations in gene differences. Patterson (1952c) has cross tested *fulvimacula* and *flavorepleta.* In this case both reciprocal P_1 crosses are fertile, as are the F_1 inbred. However, the F_1 would seldom cross back to *fulvimacula,* although all mated to *flavorepleta.* This seems to be an example of the origin of isolation (sexual) due to the formation of a particular gene combination. It would have the effect of transferring genes between these forms for the most part in one way only. Patterson (unpublished) has shown that *meridiana* and *rioensis* will cross both ways to give fertile hybrids, despite the fact that these forms differ phenotypically and by an autosomal fusion. Thus this case in the repleta group resembles the *texana-americana* cross in the virilis group. A different type of isolation mechanism was demonstrated between two strains of *peninsularis* by Patterson and Wheeler (1947). Some phenotypic differences exist, including a difference affecting the shape of the spermathecal apparatus, a character much used in species diagnosis in Drosophila. Some of the hybrid males between these strains produced only immature nonmotile sperm bundles, while others produced only a few functional sperm. This represents a very low level of genetic divergence and partial isolation.

Patterson and Dobzhansky (1945) have reported a case of partial isolation between *pallidipennis* and *centralis.* These subspecies are only slightly different phenotypically, and the chromosomes differ only by a single major inversion. The F_1 hybrid males are sterile, as are the majority of males in F_2 and subsequent backcrosses. The testes of hybrid males show abortive meiotic divisions, which give rise to very abnormal spermatids that degenerate. Since there seems to be little if any sexual isolation between these forms, and since they occupy different geographical regions so far as is known, they are ranked as subspecies, despite the reduced fertility of the female hybrids and the sterility of the males. This is a border-line case in which the forms might be classed as separate species on the basis of reproductive and phenotypic differences.

The melanica species group contains several subspecies. Griffen crossed the subspecies *melanica* and *paramelanica* to each other and

to the separate species *nigromelanica.* The two subspecies of *melanica* resemble each other fairly closely, although there is a light southwestern form of *melanica.* The species *nigromelanica* differs phenotypically from the melanicas and is partly ecologically isolated from the others in food habit, it being primarily a fungus-feeder. Its distribution range overlaps those of the other two subspecies. All three forms are very strongly sexually isolated from one another, although some fertile hybrids have been obtained between certain strains of *paramelanica* and *nigromelanica,* as well as between *melanica* and *paramelanica.* If these forms bred better in captivity, we might class them as three separate species. Miller (1944) has crossed males of the separate species *melanura* to both *melanica* and *paramelanica* females. The female hybrids with the former are fertile, but the others are sterile; hybrid males do not form actively motile sperm.

Sturtevant and Novitski (1941a) found an interesting difference between two phenotypically similar strains of *micromelanica,* one from Texas and the other from Arizona. Crosses of Arizona females to Texas males produce fertile hybrids of both sexes, whereas the males in the reciprocal cross are sterile. They showed by the use of the sex-linked genes, *white* and *echinus,* that the sterility was due to a factor (or factors) close to *white* in the Texas X which interacted adversely with the Arizona Y. Males with a Texas X and Y could have most or all the Arizona autosomes. This illustrates the fact that a single mutational difference between the two X chromosomes can cause cross-sterility with one of the two different type Y chromosomes.

In addition to the measure of differences that can be made by testing subspecies, the hybrids between separate species, especially the fertile hybrids, allow us to study the type and degree of genetic differences. Here again much more of the pertinent information comes from the subgenus Drosophila.

The hybrids between *melanogaster* and *simulans* illustrate several types of interaction effects including sterility, lethality, and cytoplasmic effects. The comparatively low viability of female hybrids with *melanogaster* cytoplasm illustrates a gene cytoplasm reaction as well as interaction between genes. However, it is not a case of abnormal interaction between cytoplasms, for Muller and Pontecorvo showed that a sperm carrying the Y and 4 chromosomes and the cytoplasm of *simulans* produced a fertile hybrid. Although Sturtevant

pointed out the inviability of the hybrids lacking a *simulans* X, Muller and Pontecorvo showed that this was an X-2-3 interaction, for recombination hybrids could be obtained lacking the X but carrying either 2 or 3, but not both, *simulans* chromosomes. They showed that the best differential diagnostic character, the male genitalia, was determined in part by genes in the 4 chromosome. The genetic divergence exhibited by the Y and 4 of these species indicates that very extensive allelic differences exist, despite the phenotypic similarity of these sibling species.

The obscura group has provided some detailed studies on relations and divergence between species. The cross between the most distantly related species, the European *ambigua* and the North American *pseudoobscura* and *persimilis,* give no adult offspring. Buzzati-Traverso found that larvae develop sufficiently to give good preparations of the salivary gland chromosomes of the hybrids. There was no appreciable synapsis between the different chromosome sets in the hybrids. The chromosomes have diverged so thoroughly in structural sequence, perhaps also chemical composition, that they do not pair. The lethality of the hybrids suggests a correspondingly great divergence in gene systems.

Sturtevant and Dobzhansky (1936a) found that *azteca* and *athabasca,* members of the affinis subgroup, would cross to produce sterile hybrids of both sexes with rudimentary gonads. The males from the two reciprocal crosses are strikingly different and abnormal, although the females resemble those of the parent species. The X-autosome unbalance which gives this phenotypic manifestation is an example of a considerable gene diversification, intermediate between the forms which produce no male hybrids and those which produce both normal sexes. Bauer and Dobzhansky (1937) found that the chromosome rearrangements between these North American forms were so extensive that homologous chromosome parts seldom paired.

Miller's tests with *affinis, athabasca,* and *algonquin* in the same subgroup of species show that these forms have diverged much less genetically than *azteca* and *athabasca.* In fact, there is a residual fertility of the *algonquin/athabasca* hybrid females that might allow some exchange of genes. The analysis of the genetic differences is as yet incomplete.

The most extensive studies have been made on the North American pseudoobscura subgroup of the obscura complex. The genetic basis

for the sterility of the hybrid males between *pseudoobscura* and *persimilis* has been investigated extensively. Although the female hybrids are fertile, the incompatibility of the two gene systems is indicated by the fact that F_2 larvae are at a serious survival disadvantage in competition in population cages with pure strain larvae. The F_2 and F_3 backcross male hybrids are more fertile as they become more nearly homozygous for genes from one species. Sturtevant (1937) showed that there was a marked reduction in the frequency of F_2 males as compared with females in crosses of F_1 hybrid females to the parent type males from different localities. He proved that this effect depends on the particular Y chromosome and, in some cases, to autosomal factors. Certain Y chromosomes lead to a decided mortality of these recombination hybrid males. Dobzhansky demonstrated that the sterility of the F_1 and backcross hybrid males was determined by multiple factors, with several genes present in each major chromosome. Thus all tests agree that these two sibling species, almost indistinguishable by ordinary morphological criteria, actually possess very different balanced gene systems.

Both *pseudoobscura* and *persimilis* cross to *miranda* and give comparable results. The change in genic balance that occurred with the formation of the X_1X_2Y sex chromosome system of *miranda* was accomplished by combining the Y and 3 chromosome of the ancestral form into the present Y system and adjusting the gene balance between this new Y, the new X_2 (3 chromosome of the ancestral form), and the X_1 and autosomes. This rebalanced system results in the death of regular hybrid males from crosses between *pseudoobscura* females and *miranda* males. The Y-3 element has a different genotype, so that many of the genes originally present in the 3 chromosome are missing or without normal function. The regular hybrid males in the reciprocal cross are viable, so that the genic rebalance has not lead to a readjustment of activity on their part that is lethal in these hybrids. The semisterility of the females is too drastic to allow many offspring to be produced by the few hybrid females that are fertile. The hybrids between most strains are completely sterile. The change of balance in *miranda* is in part the result of the complex sex chromosome mechanism of this species. It illustrates how such a necessarily different selective system will lead to greater and greater divergence.

The quinaria group includes several border-line cases where two very closely related forms cannot be easily pigeon-holed, either as

subspecies or separate species. The recognition of the existence of more and more distantly related subspecies grading into these incompletely separable species is major proof that evolution is a continuing process. The forms *occidentalis* and *suboccidentalis* cannot be separated on the sterility of their hybrids, but they show genetic differences in crosses to other species. The forms *palustris* and *subpalustris* also give fertile hybrids, but their phenotypic difference is more obvious, and they show different patterns of fertility with other species. There is sufficient residual cross-fertility between the species of this large group to demonstrate all gradations of genetic divergence, as well as extensive chromosomal revision. The eleven species tested are so thoroughly isolated by sexual isolation, by death of the male gamete in the seminal receptacles of the alien female, or by failure of the hybrid zygote to develop properly that only twenty-six crosses yielded any F_1 adults to test. The different mechanisms which isolate these forms are not necessarily correlated, for *munda* females crossed to *suboccidentalis* males gave only fourteen offspring which were fertile on inbreeding, while *transversa* females crossed readily to *innubila* males to produce 528 F_1 hybrids which were sterile. Of the twenty-six hybrid combinations tested, there were fifteen cases of fertile hybrids which would permit gene exchange. In eight cases both sexes were fertile, while in seven the females were fertile and the males sterile. The other eleven hybrid types were sterile. The fertile types allowed Sears to show that some of the phenotypic differences between species were controlled by simple gene systems, while others seemed to be controlled by multiple factors.

The gradations of genetic difference found in this group may be summarized briefly. There are various degrees of sexual isolation controlled by different gene systems and reacting differently to the same sexual stimuli. This is evident from the unpredictable direction and extent of sexual isolation that exists; in other words, we could not predict from the fact that a form crossed with a particular species that it would cross with another species. The genetic similarities and differences that allow or prevent the set of genes from one species to coordinate with the set from a second species show their ranges of effect. Replacement and cooperation seems relatively complete with forms that give fertile F_1 and F_2 hybrids, such as crosses of *palustris* $\times$ *subpalustris* and *occidentalis* $\times$ *suboccidentalis*. Next, there are forms which differ but whose gene systems will work fairly satisfac-

torily together, such as *munda* to *occidentalis*. Here the F_1 inbred are fertile, but the F_1 males fail to cross back to either parent. There are forms, such as crosses between *subquinaria* A and B, that give fertile female but sterile male hybrids. Here genic balance systems are markedly incompatible. Other crosses show even less compatible combinations as both sexes are sterile. Then there are several crosses in which the genic disharmony leads to the death of the developing zygote, as in *suboccidentalis* females by *munda* males. It is not clear whether the crosses in which the sperm are destroyed in the reproductive tract of the alien female are actually more serious disharmonies or, as seems more probable, simply a specialized type of reproductive isolation dependent perhaps on relatively few gene differences.

There are two species of the funebris group, *subfunebris* and *trispina,* that are closely related to *macrospina*. Both occur in California in small populations. These three species give fertile female and sterile male hybrids. Cursory cytological examinations of the salivary gland chromosomes show that *trispina* differs from *limpiensis* by only one major inversion. We do not know if the small extra chromosome seen in somatic metaphase, which makes *trispina* a unique species with seven pairs of chromosomes, is connected with this hybrid sterility. The species *subfunebris* has several inversions in gene order when compared with *limpiensis;* hence the chromosomes have developed more differences. In both cases, the genotypes fail to coordinate properly. The genic balance systems differ to some degree with respect to functional and developmental characters, as well as sexual isolation factors and those responsible for the physical differences such as that of the genitalia.

The hybrids between members of the guaraní group show only that all members tested have diverged genetically and cytologically to a marked degree; they possess different balanced gene systems. This group gives us an unusual type of breakdown of isolation between separate species in that *guarú/subbadia* hybrid females will cross to *guaraní* males. We had demonstrated the recessive nature of the genes controlling the sexual isolation factors in crosses between strains of species of the virilis group. The guaraní group is too difficult to analyze, so we can infer that it is due to a genetic mechanism similar to that found in the virilis group. King described a mechanism which illustrated very well the ineffectual coordination of different geno-

types. Most of the hybrid larvae, both male and female, from the cross of *subbadia* females by *guarú* males die before pupation because they lack imaginal discs and their nervous systems are abnormal. Thus the necessary apparatus for adult differentiation is missing, except for a few hybrid larvae which may be special genotypes.

The repleta group is large and diversified, and can be broken into several subgroups which are more closely related. The hydei subgroup yields some information, but the crosses between most members are incompatible. For example, *nigrohydei* females by *hydei* males yielded only a few sterile female and larval-pupal forms. Here only a few of the sperm effect fertilization, and the zygotes formed are seldom viable. Thus, *nigrohydei* represents a different genotype from *hydei,* but is very close to *hydeoides.* These forms are sexually isolated to a large degree and possess some differences in phenotypic characters, but the F_1 and F_2 hybrids are quite fertile. They show a different ecological adaptation, for *nigrohydei* extends into the United States, while *hydeoides* is restricted to Mexico. Their gene systems are compatible, but there is no way to determine whether their hybrids would have a normal survival value in nature.

The melanopalpa subgroup has representatives from both North and South America. Figure 67 is a reproduction of Wharton's (1942) map of the *repleta* chromosomes, with the several inversions that have been found in this group indicated. These rearrangements are from determinations by Wharton (1942) and by Ward and Stone (1952). Wharton used a strain of *melanopalpa* (W) from Cave Creek, Arizona; and Ward used a different strain (or species?) from Michoacán, Mexico. As the W strain was lost, they could not be cross-tested, but crosses to other forms demonstrate their genetic and cytological differences. The *repleta* chromosomes can be used as the basic design in this group, for the other forms differ from each other through the repleta sequence. The species *melanopalpa* W and *neorepleta* differ from *repleta* by an included inversion pair in 2 chromosome. The strain *melanopalpa* M has an additional small inversion in 5, and *canapalpa* has this latter gene arrangement but lacks these rearrangements in 2. However, there is a long inversion in 2 heterozygous in the stock. The South American species *limensis* differs from *repleta* by a different small inversion in 2 and a very small inversion in 3. There is no correlation between chromosome differences and distribution except for those which must be of common origin. *Dro-*

sophila repleta seems more closely related to *limensis,* for they produce fertile hybrids. Although *repleta* females give sterile offspring with *melanopalpa* W and M, *canapalpa,* and *neorepleta, limensis* is almost completely isolated from *melanopalpa* M and *canapalpa,* for even the few zygotes formed despite the strong sexual isolation usually die as larvae or pupae. These combined lines of evidence strongly suggest that *repleta* is closer to the common stem of this subgroup.

To demonstrate that very extensive divergence can exist between forms that seem close together, Wharton showed that *melanopalpa* W gives fertile hybrids with *canapalpa* both ways, whereas *melanopalpa* M only crosses to *canapalpa* males and most zygotes die. Sturtevant's analysis of the relationships between *neorepleta* and *repleta* is important, for it demonstrates the fact that a particular gene difference, in balance with its own genetic system, can have drastic and detrimental effects in a different genotype, e.g., that of the hybrid. Other cases where single factors are responsible for serious effects are few: Sturtevant and Novitski showed such a factor to exist between *micromelanica* A and B; Crow demonstrated one in *aldrichi* 2, in crosses to *mulleri;* Patterson and Griffen showed one to be present in the X of *texana,* in crosses to *montana.* For the most part, the genetic differences are unknown or seem to be due to multiple factors.

The mulleri subgroup is perhaps the best for laboratory tests in the repleta complex. Patterson showed that *mulleri* crosses to *aldrichi* in nature if the males of *aldrichi* are present in sufficiently high relative frequency. Although these species are isolated by the complete sterility of their hybrids, there is present this unusual factor in *aldrichi* 2. This is a single sex-linked factor, judging by the ratios in which it is recovered from the heterozygotes between it and *aldrichi* 1. It has no detectable effect in the *aldrichi* population. Females of *mulleri* are not sexually isolated from closely related members of the group. Crosses to males of most strains of *aldrichi* produce male and female hybrids in equal numbers. Crosses to another strain, designated as *aldrichi* 2, produce male hybrids, but the female (XX2A) hybrids die as a result of a dominant factor in the X of these strains. Crow crossed representatives of the two types of *aldrichi* and showed that there was no detectable effect of the factor in *aldrichi.* The segregation ratios from heterozygous females indicate that it is a single factor, or several closely linked factors, on the X chromosome. This represents a case of a gene which is adjusted with the other genes in the one species,

but which produces some fatal interaction in crosses to a different species with another balanced genotype.

The species *mulleri, aldrichi, mojavensis, arizonensis, buzzatii, hamatofila,* and *longicornis* illustrate increasing degrees of divergence. Drosophila *hamatofila* and *longicornis* are so genetically distinct that they give only a few offspring and lethal zyzotes with *mulleri* females. The species *buzzatii* is found in South America and around the Mediterranean, yet crosses both to *mulleri* and *arizonensis* to give zygotes which die or develop into abnormal F_1 hybrids. Other inviable hybrids are formed in crosses of *aldrichi* to *arizonensis* and to *mojavensis,* which produce sterile females but no F_1 male hybrids. Another departure from normal sex ratios is found in the cross of *mulleri* to *arizonensis,* which gives only sterile male offspring. The difference between *arizonensis* and *mojavensis* is illustrated here, for *mulleri* females crossed to *mojavensis* males produce sterile male but fertile female hybrids. The western forms, *mojavensis* and *arizonensis,* differ markedly in phenotype but give fertile hybrids, except for the male hybrids from *arizonensis* females by *mojavensis* males. The various forms that give cross-insemination without offspring have already been illustrated. The various crosses within the repleta group illustrate all gradations of genetic isolation, from a few factors determining sexual isolation between strains to lethality of zygotes and to complete inability to effect fertilization of an alien species.

We do not know if all the differences between species illustrated here are due to chromosomal (rather than cytoplasmic) genes. The cross-sterility and lethality that have been tested seem to be due to chromosomal genes, but in some cases involve interactions with the cytoplasm. For example, there are interactions between the genes and the cytoplasm in *melanogaster* to *simulans* crosses and in *pseudoobscura* to *persimilis* crosses, but no hint of the involvement of cytoplasmic genes. The case in the virilis series is given in the next chapter. Thus our information indicates that the cytoplasmic effects are conditioned by the gene systems present, but in these many cases of hybridization, there is too seldom adequate evidence to rule out strict cytoplasmic inheritance, although no case requires it. The data from hybrids is in general agreement with Haldane's (1922) rule that the heterozygous sex tends to be more often inviable or sterile. In our opinion, this is due to the genic unbalance between X and autosome; for the genes in the autosome set from one parent do not have

their normal set of X-chromosomal genes to balance them, and is illustrated by crosses of *athabasca* by *azteca*. The exceptions that we can analyze often illustrate the X-autosome balance system just as well; for example, the cross-incompatibility factor in *aldrichi* 2 must be balanced in its own system. Here it may be both X and autosome genes acting in the egg cytoplasm that interact with the *aldrichi* 2 factor to cause lethality. In either case we are faced with genic unbalance effects, whether directly or through egg cytoplasm. The general analyses of hybrid fertility and sterility are incomplete, but the addition of ninety-six cases since 1937 provides much material for analysis and the promise of yet more material with further tests.

10 EVOLUTION IN THE VIRILIS SPECIES GROUP

The virilis species group illustrates the various intergradations of relationships that exist in closely related forms evolving from a common stem. Although *virilis* is the most widely distributed member and occurs in four different zoogeographic realms, Nearctic, Neotropical, Palaearctic, and Oriental, yet the group is primarily Holarctic. The species *virilis* is close to *Drosophila pinicola,* which Sturtevant considers to be the most nearly phenotypically representative of the ancestral form of the genus. We find it satisfactory to regard *virilis* as the modern form most like the ancestor of this group. On the basis of genetic tests, the ten known forms of the group may be classified under the following divisions or subgroups: (1) the most widely distributed member is *virilis,* which probably is native in the eastern Palaearctic and Oriental regions; (2) the closely related forms of *americana, texana,* and *novamexicana* occur in North America; (3) the more distantly related North American forms are *montana, flavomontana, borealis,* and *lacicola;* and (4) *littoralis* and *imeretensis* are both European forms.

Only a few genetic tests have been made with the western Palaearctic forms, but several strains of each of the other species have been investigated. Little is known about the ecology of these forms, but we do know that each occupies a different ecological environment, as indicated in Figure 23 for the North American species. As previously mentioned, *virilis* has been found only in domestic habitats in America, but in Asia it occurs both in urban and rural areas. All other North American forms have been found in rural localities and, in the main, occupy different regions of the country. The distribution areas of *montana* and *novamexicana* in part overlap, but the former species is found high up in the mountains, while the latter occurs in the lower

river valleys. The distribution area of *texana* and *americana* also overlap, forming a narrow zone of contact in which they hybridize. We have not been able to find even one dense population of any of these species in North America such, for example, as exists for *pseudoobscura, melanogaster,* and *hydei.*

Patterson and Wheeler prepared a complete modern description of *littoralis* from living specimens of a stock from Switzerland. A comparison of this with Dobzhansky's translation of Sokolov's description of *imeretensis* indicates that the two forms are very similar in phenotype and have metaphase chromosome patterns that are alike. Sokolov obtained only a few sterile hybrids from crosses between *virilis* and *imeretensis,* and we find that *littoralis* is also strongly isolated from *virilis.* In the absence of material from the U.S.S.R. with which to make tests, we do not know whether *littoralis* and *imeretensis* are only geographic strains or sibling species.

CYTOLOGICAL VARIATION

Chino and Kikkawa (1933) reported a spontaneous fusion of chromosomes 3 and 5 in *virilis,* but no spontaneous inversions have been found in that species. Fujii (1936, 1942), Hughes (1936, 1939), Griffen (Patterson, Stone, and Griffen, 1940), and Hsu (Figure 68) have made maps of the salivary gland chromosomes of *virilis.* Fujii (1942) gave the approximate cytological location of some genes and induced chromosomal rearrangements. Hughes (1939) compared the gene order in *americana* with that of *virilis.* Griffen (Patterson, Stone, and Griffen, 1940, 1942) analyzed several strains of *virilis, americana,* and *texana,* as well as the progeny of the one *novamexicana* male available at that time. He detected the pericentric inversion in *montana* in his preliminary test of this species. Warters (1944) studied the gene order for a number of strains in all of these forms. Hsu (Figure 68) has analyzed the gene arrangement in a number of strains of all known forms except *imeretensis,* and our designation of the inversions and the major analysis of the chromosome evolution in this group is based on his studies.

Table 81 lists the rearrangements thus far known in this species group as worked out by Hsu (1952). Table 82 lists strains of the species used in the genetic studies reported in this chapter and others necessary to indicate the types of combinations of gene arrangements.

TABLE 81

List of inversions in the virilis group (after Hsu)

X CHROMOSOME

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
a	T A N	Submedian	Qb-c	Ye-f
b	T A N	Overlaps a	Nf-g	Vc-b
c	N(T)(A)	Overlaps a, b	Lg-h	Te-f
d	(A)	Included in c	Td-c	Se-d
e	F	Median on XM	Unknown	Unknown
f	F	Overlaps e	Unknown	Unknown
g	F	Subterminal on XM	Unknown	Unknown
h	B	Median on XM	Unknown	Unknown
i	B	Basal on XM	Unknown	Unknown
"Li"	Li	X of *littoralis*	Many inversions	
	M	X of *montana*	Many inversions	
M, "Lc"	Lc	X of *lacicola*	Many inversions on XM	

CHROMOSOME 2

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
a	All except V	Median	Ki-j	Tl-Ua
b	N(A)	Subterminal	Ac-d	Dh-i
c	N(A)	Includes a	Jf-g	Ug-h
d	M, F, B, Lc, Li	Basal, overlaps a	Pn-o	Z
e	M, F, B, Lc, Li	Pericentric, overlaps ad	Near Po	Other side of centromere
f	M, F, B, Lc	Superimposed on ade	Terminal heterozygous 2R	Ub-c
g	M, F, B, Lc	Superimposed on adef	Le-d	?
h	M, F, B, Lc	Superimposed on adefg	Wa-Vj	Near Ub-c
i	Li	Distal 2L	Fj-k	Ig-h
j	M, F, B, Lc	Distal 2L	He-f	Near I-J
k	M, Lc	Distal 2L	Near I-J	Kg-h
l	(M)	Distal 2L	Unknown	Unknown
m	(B)	Base to subterminal 2R	Uh-i	Z
n	(F)	Subterminal 2L	Ai-j	Dl-m
o	(Lc)	Subterminal 2L	Ac-d	Cj-k
p	(Lc)	Included in o	Ci-h	Be-d
q	(Lc)	Subterminal 2R	Terminal heterozygous	V
r	(Lc)	Basal 2R	?	Z

CHROMOSOME 3

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
a	N(A)	Basal	Ng-h	Z
b	M, F, B, Lc	Median	Nb-c	Vd-e

TABLE 81 (*Cont.*)

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
c	M, F, B, Lc	Overlaps b	Hc-d	Tc-b
d	(M)	Overlaps c	Gi-j	Ub-c
e	F	Included in c	Mh-g	Kj-i
f	(B)	Basal, overlaps bc	Jd-e	Z
g	(B)	Subterminal	Ba-b	Ej-k
h	Li	Subterminal	Bc-d	Fe-f
i	(Li)	Includes h	Ak-Ba	Pf-g
j	Lc	Basal, overlaps bc	Oe-d	Z
k	Lc	Overlaps j	Kc-b	Ya-b

CHROMOSOME 4

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
a	N(A)(T)	Median	Fg-h	Wh-i
b	(A)(T)	Included in a	Pc-b	Nf-e
c	(A)	Distal	Db-c	Fe-f
d	M, F, B, Lc, Li	Subterminal	Cf-g	Fh-i
e	M, F, B, Lc, Li	Median	Ib-c	Qf-g
f	M, F, B, Lc, Li	Submedian	Near Qf-g	Yd-e
g	(M)	Subbasal, overlaps f	Xc-b	Yl-m
h	FB(M)(Lc)	Terminal, overlaps d	Ab-c	Dh-g
i	(M)	Included in e	Pb-a	Ma-Lm
j	F	Included in e	Oe-d	Ml-k
k	(F)	Basal, overlaps efj	Na-b	Z
l	(M)	Distal	Unknown	Unknown
m	Li	Subbasal, overlaps f	Xh-g	Yn-Za
n	(Li)	Included in e	?	?
o	(M)	Overlaps e, f	Mm-l	T
p	Lc	Overlaps e, f	Km-l	Id-c
q	Lc	Overlaps p	Ks-t	Pa-b

CHROMOSOME 5

Symbol	Found in	Description	Approximate Breakages	
			Distal	Proximal
a	(T)(A)	Median	Kb-c	Vf-g
b	N(T)(A)	Subterminal	Af-g	Dk-l
c	M, F, B, Lc	Submedian	Ik-Ja	Rb-c
d	M, F, B, Lc	Subterminal	Ac-d	Dj-k
e	F	Overlaps c	Pf-e	Tf-g
f	B	Included in c	Kn-l	Jj-i
g	(B)	Distal	Dl-Ea	Ff-g
h	Li	Large basal	Lk-l	Z
i	Li	Subbasal, included in h	Yd-c	Pl-k
j	Li	Subterminal	Ac-d	Fg-h
k	(Lc)	Basal	Wa-b	Z
l	(Lc)	Included in c	Qc-b	Og-f
m	(Lc)	Distal, independent	Eg-i	Hl-m
n	(Lc)	Subbasal, overlaps c	?	?

TABLE 82

The virilis group species

virilis strains

Number	Origin	Designation	X	2	3	4	5	Authority
		Basic Sequence	+	+	+	+	+	
	N.Y., probably mixed stock	*virilis*, V						Griffen, 1942; Warters, 1944
		Pasadena						Griffen, 1942; Warters, 1944
86.4	Henly, Texas	Henly, H						Griffen, 1942; Warters, 1944
472.4	Galveston, Texas	Galveston						Griffen, 1942; Warters, 1944
863e	Santa Barbara, California	California						Griffen, 1942; Warters, 1944
718.7a	Victoria, Texas	Victoria						Griffen, 1942; Warters, 1944
290.3	Blanco, Texas	Blanco Ix^{B}						Hsu, 1952
1801.1	Texmelucan, Mexico	Texmelucan						Griffen, 1942; Warters, 1944
	Otaru, Japan	Otaru						Griffen, 1942; Warters, 1944
	Mukden, Manchuria	Mukden						Griffen, 1942; Warters, 1944
	Hanchow, China	Hanchow						

TABLE 82 (*Cont.*)

texana strains								
		Basic Sequence	ab	a	+	+	a	Hsu
84.7	Georgetown, Texas	*texana,* T					*a/b*	Griffen, 1942; Warters, 1944
821.12b	Georgetown, Texas							Griffen, 1942; Warters, 1944
821.12c	Georgetown, Texas		*c*			*ab*	b	Griffen, 1942; Warters, 1944
825.13c	Palestine, Texas					*ab*		Griffen, 1942; Warters, 1944
841.10	Newton, Texas	Newton, T_N						Griffen, 1942; Warters, 1944
849.11	Belton, Texas		*c*			*ab*	*a/b*	Griffen, 1942; Warters, 1944
1128.10	New Orleans, Louisiana	New Orleans, T_o						Warters, 1944
1148.9	Lake McKethan, Florida	Lake McKethan, T_L						Griffen, 1942; Warters, 1944

TABLE 82 (*Cont.*)

americana strains								
		Basic Sequence	abc	+	+	+	a or b	Hsu, 1952
	Smithville, Ohio	*americana*, A or A_s				*AB*	a	Hughes, 1939; Griffen, 1942
	Anderson, Indiana	Anderson, A_A					a	Griffen, 1942; Warters, 1944
821.12a	Georgetown, Texas	Georgetown				*aB*	*a/b*	Griffen, 1942
1880.6c	Richmond, Virginia		*c*				a	Hsu, 1952
1761.9s	Chinook, Montana	Chinook, A_c		*b*		a	b	Hsu, 1952
1773.4e	Chadron, Nebraska	Chadron	D	*b*		aB	b	Hsu, 1952
1773.4c	Chadron, Nebraska		*D*			aB*C*	b	Hsu, 1952
1760.8f	Poplar, Montana			*b/c*	*a*	*aB*	b	Hsu, 1952
novamexicana strains								
		Basic Sequence	ab C	a BC	A	A	B	Hsu, 1952
1714.4	San Antonio, New Mexico	*novamexicana*, N						Hsu, 1952
1954.3a	Whitewater, Colorado	Whitewater						Hsu, 1952

TABLE 82 (*Cont.*)

			littoralis strains					
2000.1	Merligen, Switzerland	*littoralis*, Li	X"Li"	ade I	H*I*	def M*N*	HIJ	Hsu, 1952
			montana strains					
		Basic Sequence	XM	ade FGHJK	BC	def	CD	Hsu, 1952
1210.83	Grand Teton Park, Wyoming	*montana*, M			e	GH		Warters, 1944
1210.98	Grand Teton Park, Wyoming		efg		e	*H*	e	Warters, 1944
1211.51	Iron Creek, Yellowstone, Wyoming			L	D	HL		Warters, 1944
1218.8d	Cottonwood Canyon, Utah					GH		Warters, 1944
1862.2a	Lake Tahoe, California					I		Hsu, 1952
1942.6h	Reno, Nevada							Hsu, 1952
1942.6o	Reno, Nevada					*H*		Hsu, 1952
1767.5a	Little Salmon River, Idaho					*GH*I		Hsu, 1952
1769.1a	Grand Teton, Wyoming				*D*	HI		Hsu, 1952
2064.2d	Lander, Wyoming				*D*	HI*O*		Hsu, 1952

TABLE 82 (*Cont.*)

borealis strains

		Basic Sequence	XM HI	adefg hjk	bc	defh	cd F	Hsu, 1952
1950.1h	Chester, Idaho	*borealis,* B					G	Hsu, 1952
1755.4e	Superior, Wisconsin			M	F		G	Hsu, 1952
2077.5b	Itaska Park, Minnesota				*FG*		G	Hsu, 1952
1951.1y	Hamilton, Colorado				G			Hsu, 1952

flavomontana strains

		Basic Sequence	XM EFG	adefg hjk	bc E	defh J	cd E	Hsu, 1952
1950.1c	Chester, Idaho	*flavomontana,* F		*N*		K		Hsu, 1952
1951.1h	Hamilton, Colorado					*K*		Hsu, 1952

lacicola strains

		Basic Sequence	XM "Lc" (varies)	adefg hjk	bc JK	def PQ	cd	Hsu, 1952
1360.1	Fairbanks, Minnesota	*lacicola,* Lc		*OPQ*			K*LM*	Hsu, 1952
1756.2b	Fenske Lake, Minnesota			OP*Q*			N	Hsu, 1952
2077.4g	Itaska Park, Minnesota			*OPQR*			K	Hsu, 1952

The fusions present in the several forms are not listed in this table, but are indicated on Figure 74. No variation in gene sequence was discovered in the dot 6 chromosome (element F), although Fujii (1940) described an interesting abnormal staining reaction of that chromosome in a New Orleans strain. Figure 68 is Hsu's map of the chromosomes of *virilis*. Figures 69 through 73 are diagrams showing the approximate limits of the rearrangements that have been analyzed. These are diagrammed to indicate the points of breakage on this virilis map. The gene arrangements in *virilis, texana, americana,* and *novamexicana* are well analyzed; these in the montana complex and in *littoralis* have not been studied so thoroughly, owing to lack of material and to the complexity of the changes.

Figure 74 is a series of diagrams of the chromosome evolution in this group, indicating both fusions and inversions. Where the inversion first occurred, a capital letter is used. If a sequence is known to be present in only part of the population or species, it is given in italics. This allows one to determine the origin of a particular gene sequence, present known distribution, particular inversions characteristic of a form, and inversions scattered in it by hybridization or simply as possible types. The primitive gene orders are all present in modern forms. *Drosophila virilis* and primitive I differ by the 2a inversion and in the basic pupa color, which is gray or black for *virilis* and red for all others. The primitive II arose from I by two overlapping inversions in the sex chromosome, Xab, and this line produced *texana, americana,* and *novamexicana*. Primitive III also arose from I by two inversions in the 2 chromosome, 2de. Either d or e, which is the pericentric in 2, could have occurred first. This new sequence, 2ade, evolved into *littoralis* in Europe and separately into the montana complex in North America.

The sequence of chromosome changes based on primitive II is quite clear. This configuration evolved into *texana* by adding 5a and the 2-3 fusion. These are the chromosomes characteristic of *texana* in the gulf south from Newton, Texas; New Orleans, Louisiana; and Lake McKethan, Florida. A separate line of evolution from primitive II produced *novamexicana*. The inversions added in this species are Xc, 2bc, 3a, 4a, and 5b. These have been present in all representatives of the species examined. An examination of the chromosome sequences and their distribution in *americana* shows that this form possesses a mixture of *novamexicana* and *texana* chromosomes. The

eastern strains are much more like *texana,* and in fact these two subspecies are in contact and hybridize in an overlap zone, so that gene orders more characteristic of the one subspecies occur also in the other in this zone. This shows in both species, as can be seen in *texana* strains 84.7, 821.12c, 825.13c and 849.11. These strains have the

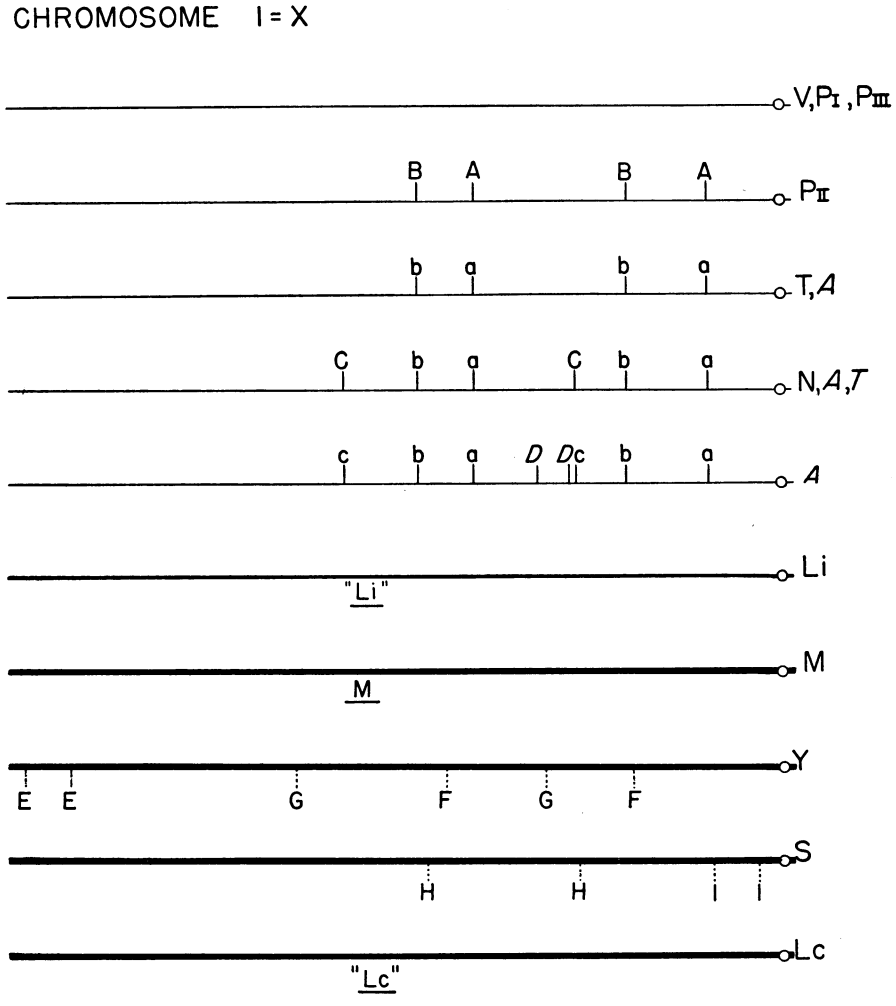

Fig. 69 X chromosome. Diagrams of inversions in proportion to Hsu's map (Fig. 68). Capitals indicate origin, italics, presence in some strains only.

Xabc, 4ab, or 5b inversions present heterozygous, although they are characteristic of *americana* or *novamexicana.* They occur in an area with *americana* which has been collected at Georgetown and Austin, Texas. The *americana* from Georgetown, 821.12a, is heterozygous for

5a/5b; and the stock from Richmond, Virginia, 1880.6c, had the Xab inversion characteristic of *texana* heterozygous in the strain when it was captured. In these cases the strains called *texana* had no X-4 fusion which those called *americana* possessed. The Texas strains of

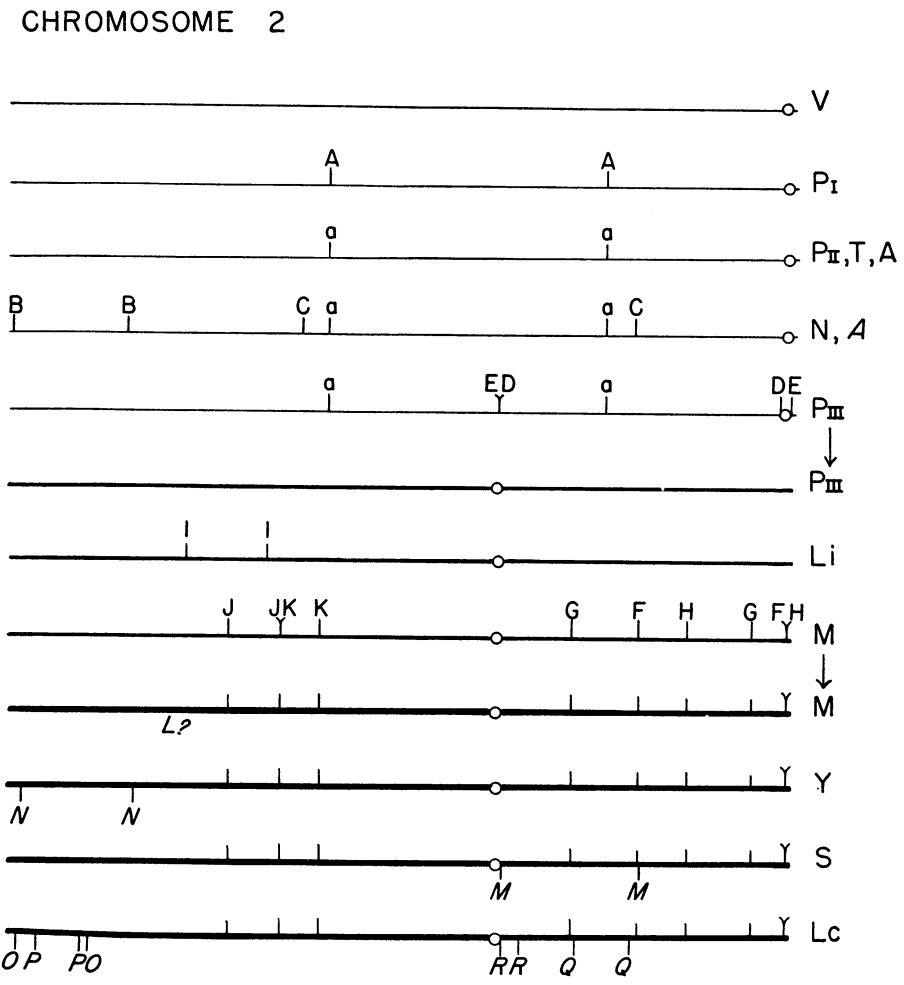

Fig. 70 Chromosome 2.

texana and *americana* often carried the 5b inversion characteristic of *novamexicana,* and this gene arrangement is characteristic of the typical western *americana* (Table 82).

All *americana* strains are characterized by the 2-3 fusion (from *texana*), but the X-4 fusion is specific for this subspecies. The inversions present in *americana* show that this form is a mixture of gene sequences from *texana* and *novamexicana.* It is in this sense a hybrid

form, whether or not *texana* and *novamexicana* were species or subspecies at the time their heterozygotes produced the basic configurations of *americana*. The chromosomes may be described thus: The Xabc (N), both in the east and west, while Xd (A) is present in only

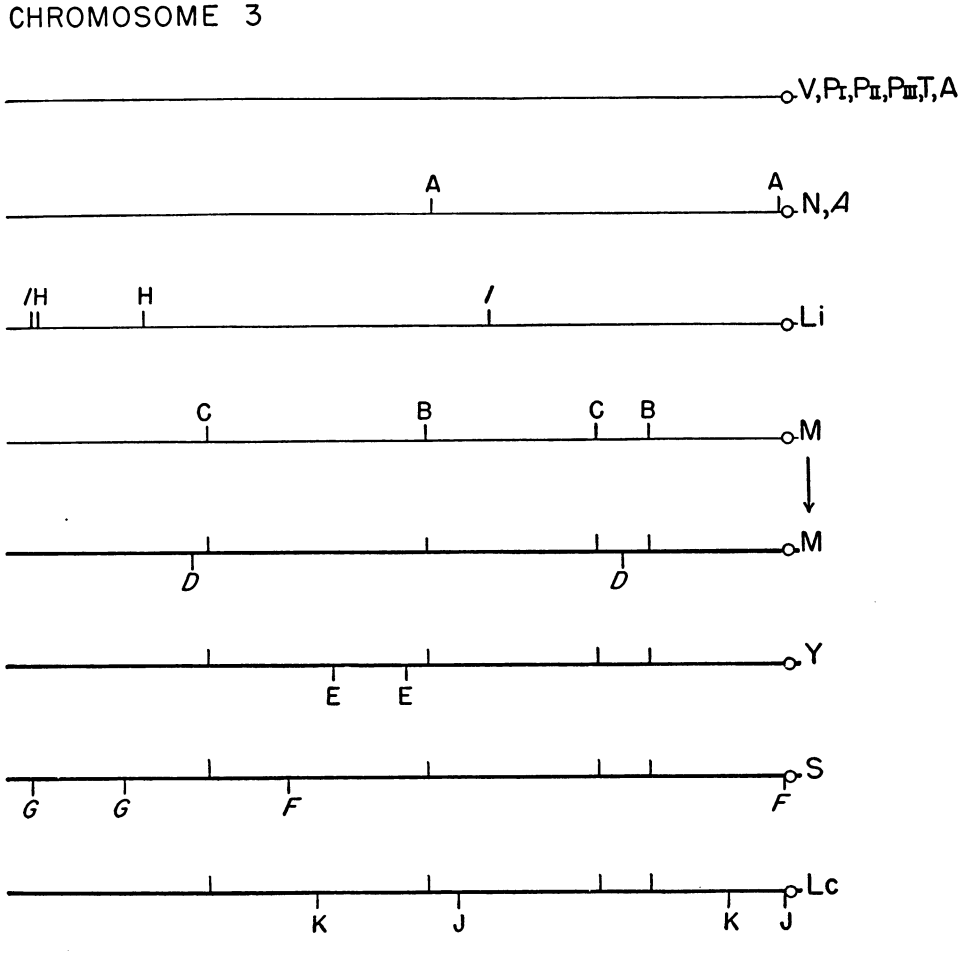

Fig. 71 Chromosome 3.

a few western strains; 2a (T) in the east, but 2b and c (N) also occur in the west; 3 (T) in the east, but 3a (N) in a few western strains; 4 + (T), 4a (N), and 4b and c (A) which have characteristic distributions. The 4ab, which is one of the usual gene sequences of *americana,* was formed by adding the included inversion b to 4a (N). This 4a is present only in western strains close to *novamexicana* and, together with 3a, suggests more recent or repeated

hybridization. The 5 chromosome is characterized by 5a (T) or 5b (N) in the east, but only by 5b (N) in the west.

The simplest explanation of the similarities and differences in gene arrangements in these forms demands the separate evolution

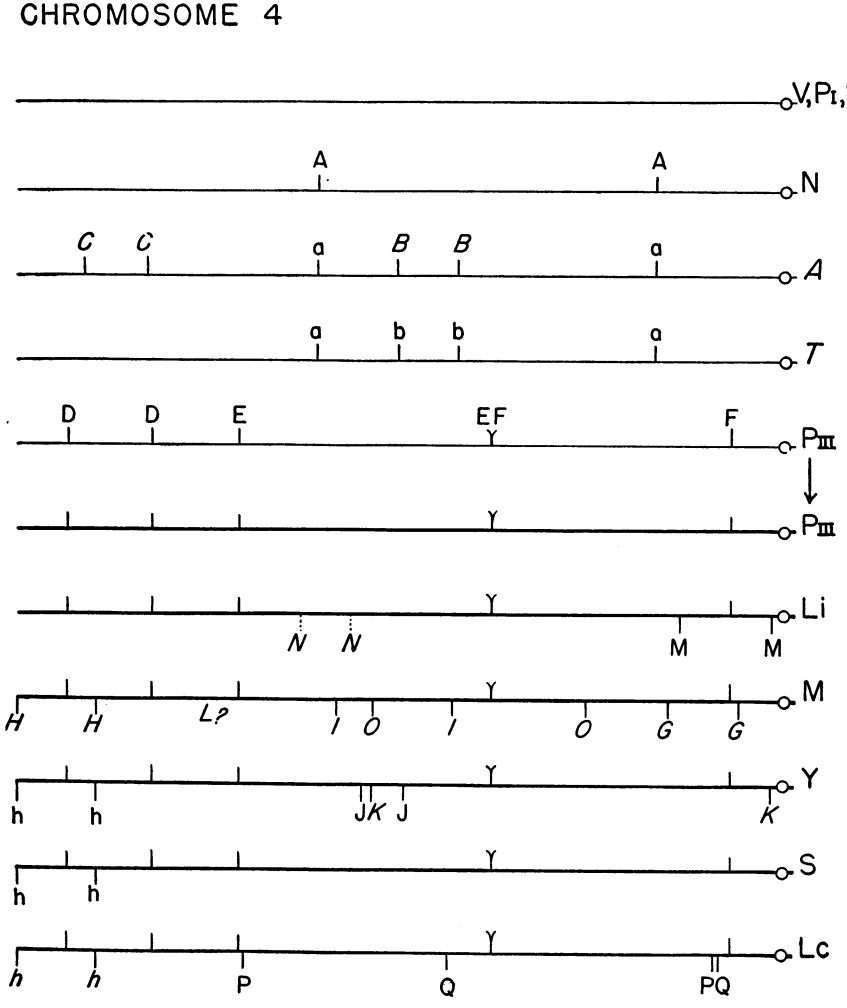

Fig. 72 Chromosome 4.

of the *texana* and the *novamexicana* inversions and the *texana* 2-3 fusion from primitive II, the combination of the sequences by hybridization (the forms may have been subspecies or species at the time of the initial hybridization), the separate population evolution of *americana* to fix certain gene orders, and the X-4 fusion. Even though *texana* and *americana* are now in contact and form hybrid combina-

tions in this zone, the bulk of their populations are separate and show certain distinctive differences. We have found no populations of *novamexicana* mixed with either *texana* or *americana*. Western *americana* is much more closely akin to *novamexicana*, both cytologically and genetically.

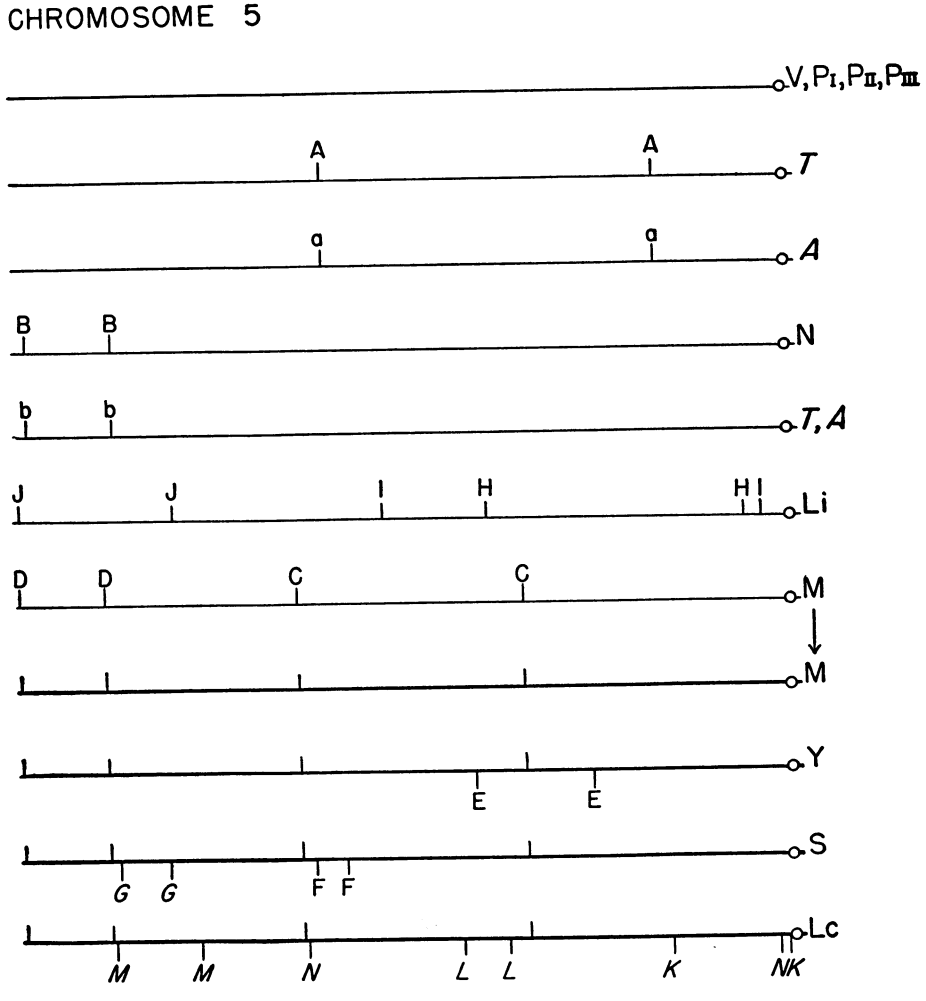

Fig. 73 Chromosome 5.

The descendants of primitive III have been much more difficult to analyze, especially since there are many more rearrangements when these strains are compared with *virilis*, the standard. The gene order in *littoralis* is closer to primitive II in the 2 chromosome and since it has both 2d and 2e, the pericentric inversion, it differs from primitive

II only in a small inversion (i). The analysis of the *littoralis* 2 chromosome gave the basic sequence in this chromosome. The only thing in doubt is the order of occurrence of 2d and 2e, for either could have been first. All the members of the montana complex had additional inversions, so that they could not be analyzed in hybrids to *virilis* or *texana*. However, Hsu had determined that the 2a inversion was present in *montana* by comparing the configurations in VM and TM hybrids. *Drosophila montana* is the basic species of that complex. The X chromosome, XM, has undergone a series of changes too extensive to allow analysis thus far. The inversions F, G, and H are added to 2ade in the short arm. The other inversions are indicated. Here, as in all the diagrams and tables, the large letter indicates the strain where the rearrangement originated. The species *lacicola* is descended from a gene sequence type like *montana* and still retains the same configuration in the 2 chromosome. The inversions in the other chromosomes of this species have been indicated except for the X.

The montana complex has not proved easy to classify taxonomically. There are three forms that occur in the Rocky Mountain area, but our samples of the populations are so small that we cannot plot their distribution with respect to each other satisfactorily. We know that *montana* and the *flavomontana* occur together at Grand Teton Park, Wyoming. Several of each species and hybrids were recorded by Warters (1944) and are illustrated in Table 82. The *flavomontana* collected in Colorado on the Yampa River are yellow, as was 1210.98, but some darker forms from Grand Teton Park, Wyoming, showed the *flavomontana* chromosome complex. Some strains tested by Warters were hybrids with both types of chromosomes; but all these strains were established from pairs, so that we cannot be sure how much these forms hybridize in nature. They cross to give fertile hybrids in the laboratory, but show differences in isolation to other forms. There are certain consistent differences in the chromosomes of *flavomontana* and *montana* which have not come from areas where both occur. The sex chromosome has three inversions, XMEFG; one inversion is present in the 2 chromosome in some strains, 2adefghjkN, and a new inversion in 3, 3bcE; the 4 chromosome has 4defhKJ, although J is present only in some strains, while all 5 chromosomes have one added inversion, 5cdE. The chromosomes

show the evolution from the basic *montana* stem, but so far have shown these consistent individualities.

The distribution of *borealis* is not well known. We have recovered it with *flavomontana,* to which it does not cross, but we have too few records to be sure that it occurs with *montana,* with which it does cross. These forms have local populations scattered through the Rocky Mountain chain, and the amount of geographical isolation between them is not clear. In addition, *borealis* is found in Wisconsin and Minnesota, where it is associated with *lacicola.*

The chromosomes of *borealis* have the following gene sequences: XMHI; 2adefghjk or 2adefghjkM, 3bc or 3bcF or 3bcG, or both; 4defh; and 5cdF and sometimes 5cdFG. Here again the basic pattern is standard *montana* with specific inversions added to the several chromosomes. There is no doubt that the stem of the montana complex had a basic pattern of gene arrangements which has evolved the several gene orders.

GENETIC RELATIONS

Metz, Moses, and Mason (1923) published an extensive study of the mutants of *virilis,* and Chino (1936, 1937, 1941) listed a great many mutants that occurred or were induced by X rays in Japanese strains of *virilis* and developed very extensive linkage maps. Thus this species had become a very useful genetic form as a representative of the subgenus Drosophila that bred well on laboratory food.

Spencer (1938, 1940a, b) reported the occurrence of *Drosophila virilis americana.* This was the second member of this species group to be recognized as a close relative of *virilis.* Spencer originally described this form as a subspecies of *virilis,* but it is now generally regarded as a separate species. Spencer described a number of phenotypic and physiological differences between *americana* and *virilis,* and showed that the former differs from the latter in having a more fusiform body, darker body color, larger eyes, finer eye pile, broader carina, and more clouded posterior cross veins. The number of branches of the arista is higher in *americana* than in *virilis,* and in addition the pupae of *americana* are red rather than gray or black. Moreover, they show different physiological adaptations, for the pupae of *americana* are formed on the surface or at the edge of the food,

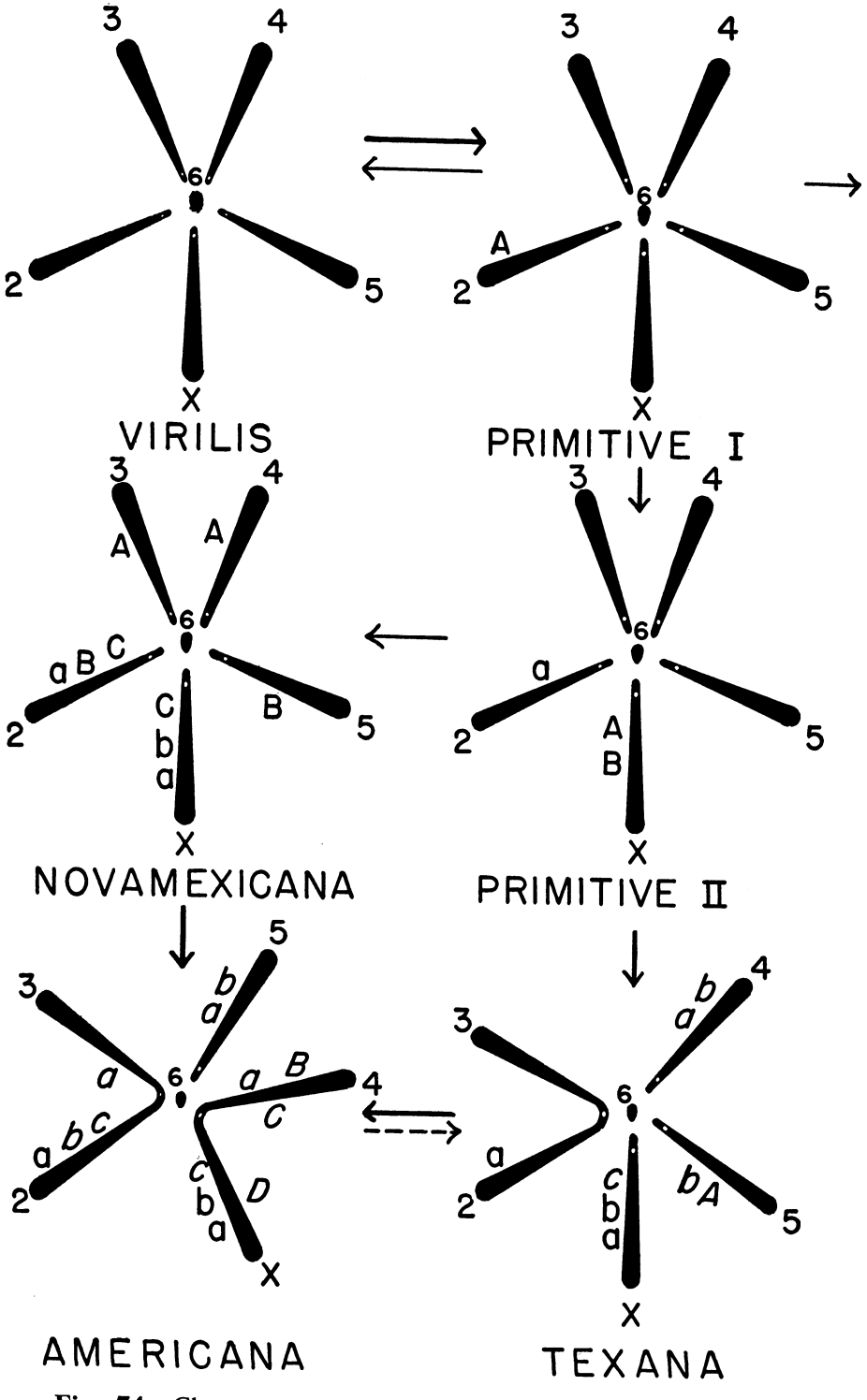

Fig. 74 Chromosome phylogeny of the virilis species group.

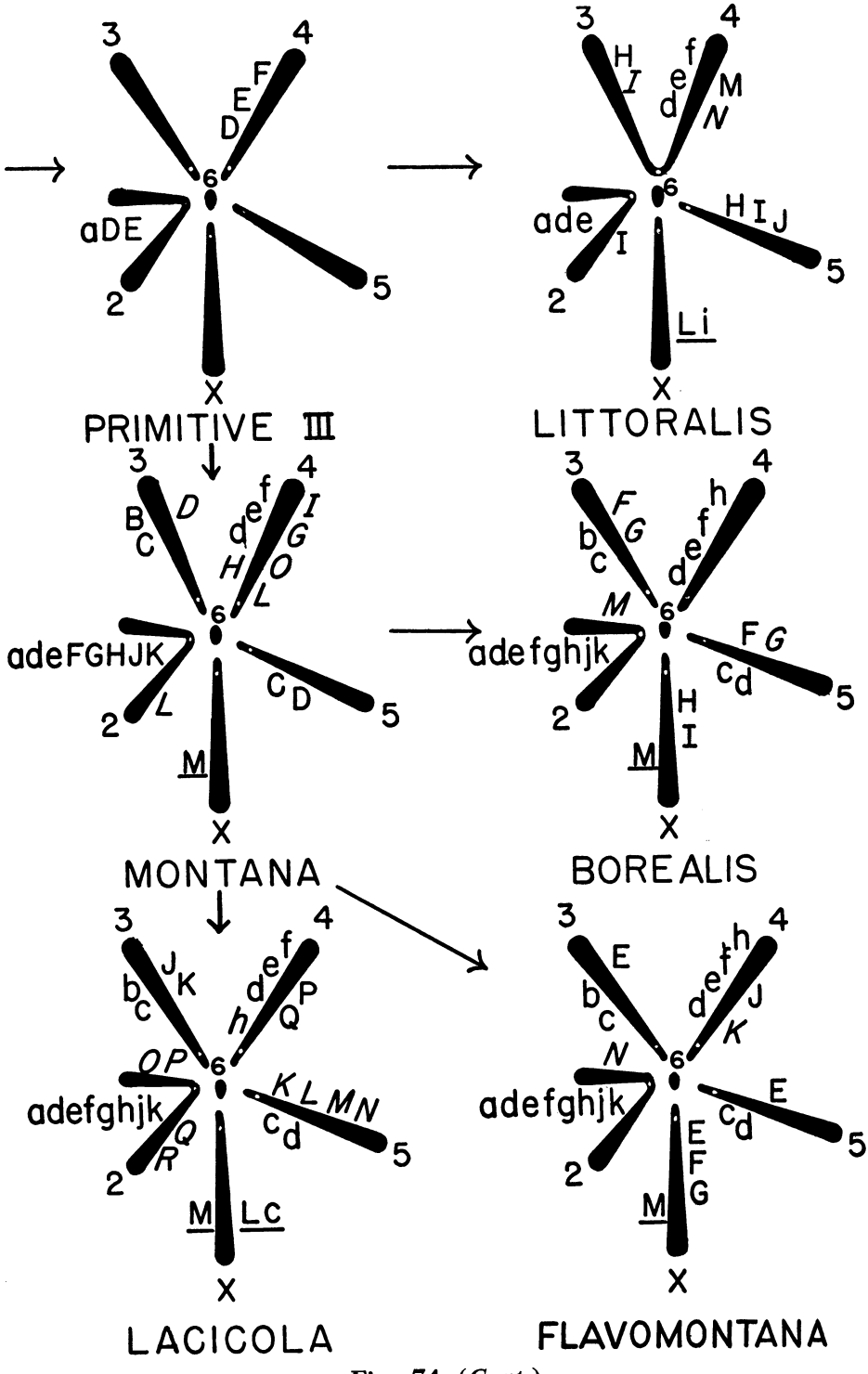
PRIMITIVE III
LITTORALIS
MONTANA
BOREALIS
LACICOLA
FLAVOMONTANA

Fig. 74 (*Cont.*)

while the larvae of *virilis* move out of the food and pupate on the side of the container or on the paper placed there for that purpose.

There is another consistent difference between these two species and the other tested species of the complex. *Drosophila virilis* is difficult to anesthetize with ether, whereas the others are easy, even requiring that care be taken to prevent death by overetherization. All other forms tested are somewhat less vigorous and productive on laboratory foods. Spencer points out that *virilis* adults tend to remain apart on the walls of the container, while those of *americana* are closely grouped aggregates. He made an extensive check of the number of branches on the arista of both males and females of these stocks and their F_1 and F_2 hybrids, and found that this number was significantly higher in *americana* than in *virilis,* while the hybrids were intermediate. The hybrids showed that the pattern of pupation was due to a dominant factor (or factors) in *americana.* An egg hatch count (Spencer, 1940a) from a cross of *virilis* females to *americana* males gave but sixteen offspring from 1,222 eggs. Part of the hybrid offspring tested were sterile. There was more sexual isolation between *americana* females and *virilis* males than in the reciprocal cross. Spieth's work (Chapter 7) has shown the reasons for the difference in sexual isolation in the courtship reactions in this group.

Stalker (1942b) found that the red pupa color depended on a main factor in the 5 chromosome and modifiers in the 2-3 fusion. Spencer had pointed out that there were degrees of crossability between *americana* and different strains of *virilis,* and Stalker extended this to show differences between *americana* strains. The data listed in Table 83 show that, in certain crosses, there was an appreciable reluctance to mate, even between members of this species. He also tested the Pasadena strain of *virilis* against four strains of *americana* and found that sexual isolation was marked in each cross. The isolation index, suggested by Dr. Donald Charles, varied from 1.00 for a mixture of *virilis* and *americana* females with *virilis* males to as low as 0.24 for a mixture of *virilis* and *americana* females with *americana* males.

Stalker (1942c) found triploids in *americana* and made some interesting observations on the aneuploid classes. The 2XY3A intersexes could be grouped into six classes, corresponding to the classes present in *melanogaster* (Dobzhansky and Bridges, 1928; Dobzhansky and Schultz, 1934; Pipkin, 1940). There was a somewhat higher proportion of malelike intersexes among the *americana* specimens,

but this may be irrelevant, since the proportion of different types varies with the *melanogaster* stock tested. One unusual characteristic of the malelike intersexes in *americana* is that they produce motile

TABLE 83

Sexual isolation between strains of *americana*, using multiple-choice method (from Stalker)

♂♂ ♀♀	Percentage of Own ♀♀ Inseminated	Percentage of Alien ♀♀ Inseminated	χ^2 of the Difference	P	Isolation Index	Number Examined
As × As & Ao	70.2%	67.5%	.44	.51	.02	507
As × As & Ad	68.5	51.0	16.36	<.01	.15	509
As × As & Al	60.7	57.1	.71	.44	.03	551
Ao × Ao & As	64.7	49.8	11.63	<.01	.13	514
Ao × Ao & Ad	67.9	46.3	24.03	<.01	.19	547
Ao × Ao & Al	65.2	49.1	5.32	.02	.14	534
Ad × Ad & As	60.4	47.5	16.91	<.01	.17	477
Ad × Ad & Ao	55.3	51.0	.88	.36	.04	489
Ad × Ad & Al	62.6	57.3	1.58	.21	.04	538
Al × Al & As	72.0	59.6	9.27	<.01	.09	548
Al × Al & Ao	66.1	63.8	.29	.61	.02	522
Al × Al & Ad	60.3	52.4	4.10	.06	.07	552

sperm. The intersex classes resembled the triploid intersexes of *melanogaster* more than the diploid *ix* intersexes found in *virilis* (Lebedeff, 1939). In Table 84 are listed the classes produced by crossing triploid *americana* females by diploid males. In these tests the small 6 chromosome might be either diploid or triploid, but the classes were judged on the basis of their major chromosomes. Stalker checked a large number of individuals in each of these classes, which were separated on the basis of differences in phenotype, including five of the nine hyperdiploid females.

TABLE 84

Types of offspring from triploid *americana* females (from Stalker)

Type	Number Observed	Percentage
Diploid ♀♀	243	25.63%
Diploid ♂♂	295	31.12
Triploid ♀♀	213	22.47
Intersexes	188	19.83
Diploid ♀♀ + Y and free 4	9	.95
Total	948	

Drosophila americana americana is one of the forms which has a complex Y chromosome. The X-4 fusion makes a large V-shaped chromosome, while the Y and 4 (male limited) are long rods. The 5 chromosome was a shorter rod in Stalker's cytological preparations. The following are some peculiarities due, presumably, both to regular disjunction of the sex chromosome complex and to genic balance relations in this species: No intersexes or diploids were found with an extra Y chromosome or an extra 4 chromosome, but nine hyperdiploid females with both the Y and the free 4, in addition to the normal chromosome complement, survived. It may be that the X-4 fusion disjoins so regularly from the Y and free 4 that few nondisjunction combinations are formed. All of the intersexes that survived had two X-4 fusions, the free Y and 4, three 2-3 fusions, and three 5 chromosomes. Obviously the unbalance created by the absence of the free 4 (and Y ?) resulted in a lethal condition. The only individuals that showed a 4 chromosome unbalance were the diploid females, which had an extra Y and free 4. These were few and abnormal, indicating that this hyperploidy caused developmental error. This is a case, somewhat like that in *subobscura,* of an extra whole chromosome surviving.

The tests with members of this group, other than *virilis* and *americana,* have been carried out by Patterson and his coworkers. Their studies have indicated that several types of isolating mechanisms exist between members of this group and that a considerable degree of genetic heterogeneity related to these factors is present between the different strains of any species. The main factors involved in sexual isolation have been presented in Chapter 7. Most of the tests given in these tables were obtained from pair matings where both male and female survived for all or much of the test period. This type of test measures the summed effect of the several isolating factors. It can be used to measure the amount of sexual isolation if the seminal receptacles of the females which produce no offspring are examined for the presence or absence of sperm. These tables are often given with two values. The one above is the percentage of pairs tested that were fertile. Usually this is based on one hundred or more pairs tested, although Table 93 was based on fifty pairs. The lower figure is the average number of flies to hatch per vial. Both figures are given as the nearest whole number.

The abbreviations, *st.* for sterile, *fert.* for fertile, and *lf.* for a lethal free stock are used in some tables. The designation *sterile* is used if no

offspring were obtained from the pairs tested, but in some cases large repeated mass matings may have produced hybrids which were further tested. Replicate experiments usually gave values in the same general range, so that the data represent comparable tests. Table 82 gives the pertinent data on the stocks employed including their origin and the symbols used for them in the tables. In these tests we have designated certain combinations by letters usually indicating certain strains. Also hybrids may be written thus: TV, for hybrid between *texana* female and *virilis* male, and so on. While the P_1 crosses measure the sum of all the isolating factors, the F_1 crosses test the effectiveness of the heterozygous alien genotypes and the F_2 crosses test the effectiveness of the recombinations among surviving offspring from the heterozygotes. Patterson, Stone, and Griffen (1940, 1942) tested strains of *virilis, americana,* and *texana,* and gave preliminary statements about *montana* and *novamexicana* (Tables 85, 88 to 94, 97, 99). The extensive tests of *montana* and *lacicola* were made by Patterson and Griffen (1944a, b; Tables 86, 95, 104 to 108). After pure strains of *novamexicana* had been isolated, Patterson and Stone (1949) published the results of the basic tests with this species (Tables 87, 96, 98, 100). Stone (1947, 1949) studied the fecundity of some recombination types (Tables 101, 102, 103).

Tables 85, 86, and 87 give the P_1 crosses between six members of this group. The new controls for *americana* and *texana* in Table 86 show that the improved food helped the fertility and viability of these stocks. There is no doubt of the difference between the separate species in their ability to cross and produce viable offspring. The subspecies *americana* and *texana* show genetic differences in addition to the fusion present only in the former, but they cross as well as different strains do within the subspecies. There is considerable variation in the amount of crossing between species, which demonstrates the genetic differences between the factors that determine this cross-sterility. The species show their several subgroups fairly well even in these tables. The subgroups contain: (1) *virilis;* (2) *americana, texana,* and *novamexicana;* (3) *montana* and *lacicola;* and (4) *littoralis.* Sokolov (1948) crossed *imeretensis* to *virilis,* but obtained only a half dozen sterile hybrids. No other tests with this form have been carried out. Patterson's (1952a) tests showed that *littoralis* is very strongly isolated from other forms in the group, but does give hybrids with some strains of *virilis, montana, lacicola,* and *americana*

(Table 75). The *virilis* strains cross more readily with strains of the second subgroup than with those of the third, whereas the second subgroup is very effectively isolated from the third.

In addition to sexual isolation, these forms are isolated by gametic and zygotic mortality. Tables 88 and 103 indicate the gametic mor-

TABLE 85

P_1 crosses between *virilis*, *americana*, and *texana* (from Patterson, Stone, and Griffen)

Stock	Control	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
virilis	100%			98%	96%	st.	79%	11%	44%
Pasadena (V)	67			80	90		2	7	5
Henly (H)	98%	96%	98%			3%	1%	1%	8%
86.4	83	90	80			2	1	6	1
Galveston	100%	95%	95%	85%	78%	47%	42%	35%	50%
472.4	94	122	111	88	82	5	4	24	9
Victoria	100%	98%	94%	99%	97%	4%	9%	2%	1%
718.7a	108	127	107	126	149	13	1	12	3
California	93%	79%	93%	96%	65%	10%	1%	53%	67%
863e	71	85	72	80	84	6	1	27	6
Hanchow	92%	89%	80%	92%	93%	27%	54%	44%	22%
	71	94	95	122	108	14	5	11	7
Mukden	85%	90%	88%	94%	92%	3%	17%	23%	22%
	98	80	58	81	112	21	6	6	4
Otaru	75%	85%	92%	80%	97%	9%	19%	41%	20%
	89	98	94	67	80	7	3	13	2
americana	74%	79%	st.	1%	3%			28%	44%
Smithville (A)	19	2		1	2			19	22
821.12a	47%	32%	3%	4%	st.	34%	59%	67%	34%
	51	5	9	1		78	60	79	70
texana	54%	44%	11%	8%	1%	44%	28%		
84.7 (T)	26	5	7	1	6	22	19		
821.12b	44%	33%	9%	6%	2%	81%	66%	71%	86%
	48	17	28	1	10	46	69	61	53
821.12c	55%	30%	3%	12%	st.	35%	89%	55%	23%
	35	7	13	1		67	51	48	35
825.13c	67%	51%	8%	2%	3%	22%	45%	45%	62%
	25	7	17	3	9	50	42	46	35
841.10	72%	56%	st.	2%	1%	66%	25%	57%	54%
	26	8		3	2	52	55	22	28
849.11	44%	57%	8%	2%	5%	68%	66%	78%	90%
	47	16	18	2	11	54	63	54	65

TABLE 86

Crosses between members of the virilis group (from Patterson and Griffen)

Species	Control	*montana*										*lacicola*	
		1210.83 87%, 58		1210.98 53%, 27		1211.51 73%, 61		1218.8d 87%, 36		1318.8a 40%, 50		1360.2	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
virilis Pasadena	100% 67	Sterile	36% 3	Sterile	29% 6	Sterile	48% 10	Sterile	54% 9	1% larvae	34% 22	Sterile	26% 16
Henly	98% 83	Sterile	2% 3	Sterile	4% 1	Sterile	1% 1	1% larvae	18% 4	Sterile	21% 4	Sterile	5% 6
Mukden	85% 98	Sterile	6% 2	Sterile	3% 1	Sterile	2% 6	Sterile	24% 6	1% 1	28% 3	Sterile	18% 6
texana Lake McK. 1148.9	81% 63	Sterile	1% 12	Sterile	Sterile	Sterile	Sterile	1% 3	12% 5	4% 2	4% 3	Sterile	1% 18
Georgetown 84.7	85% 76	Sterile	5% 10	Sterile	Sterile	Sterile	Sterile	1% 2	1% 14	1% 4	2% 8	Sterile	Sterile
New Orleans 1128.10	87% 76	Sterile	4% 6	Sterile	Sterile	Sterile	4% 4	Sterile	2% 8	1% 5	1% 9	Sterile	12% 21
americana Smithville	74% 30	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	4% 3	Sterile	Sterile	Sterile
Anderson	70% 55	Sterile	1% larvae	Sterile	Sterile	Sterile	Sterile	2% 1	Sterile	6% 2	3% 6	Sterile	Sterile
Georgetown 821.12a	76% 76	Sterile	Sterile	Sterile	Sterile	1% 2	Sterile	3% 2	Sterile	4% 4	Sterile	Sterile	Sterile
lacicola 1360.2 P_1	76% 65	5% 23	2% 16	2% 1	1% larvae	8% 18	3% 15	12% 15	14% 13	24% 3	15% 12		
1360.2 $F_1 \times F_1$		18% 11	24% 6	Sterile		8% 4	26% 12	14% 7	40% 11	18% 8	22% 6		

tality. In these tests, pairs of flies were allowed to mate; then the male was removed to prevent remating, and a daily egg hatch count was made. In the species *virilis, americana, texana,* and *novamexicana,* if

TABLE 87

Tests of *novamexicana* with other members of the group (from Patterson and Stone)

Crosses ♀ × ♂	Percentage Fertile	Average Per Vial	Sex Ratio ♀♀	♂♂	Percentage Fertile $F_1 \times F_1$	Average Per Vial
V × N	20% (60)	17	225	229	2% (7)	12
N × V	2 (2)	5	171	179	2 (94)	48
A × N	15	28	301	285	2	52
N × A	< 1	..	78	81	4	39
T × N	16	11	121	111	32	26
N × T	1	3	311	292	10	46
M × N	2	1	2	0	..	..
N × M	1	3	16	7	0	..
L × N	< 1	..	6	3	0	..
N × L	Sterile	..	...	...	..	..
N × N	80	52	571	518	..	..

a female is fertilized by her own type male, she will remain fecund for more than ten days with a normal egg hatch, since most or all eggs will be fertilized by sperm stored in the seminal receptacles. Practically no eggs hatch after the end of the first twenty-four-hour period following mating in the cross of *virilis* females by *americana* males. This was checked both by an egg hatch count (eggs laid versus offspring) and by examining freshly laid eggs for sperm. If the eggs are collected within a half-hour to an hour after they are laid, the sperm are easily detected in a smear preparation. Table 88 shows that a slightly higher percentage of the eggs are inseminated than hatch. In this test only seven flies emerged as adults from 1,250 eggs, and these came from the 215 eggs laid the first day. Table 103 shows that *virilis* females are much more compatible with *novamexicana,* for the sperm of the latter give about 15 per cent hatch for the first two days, then drop off toward zero hatch by the tenth day. Only the tests in which the females still had immobile sperm in their receptacles were included.

Tables 89 and 97 give some further tests that illustrate the effect of isolation; but here remating could occur, since the counts were made from pairs that remained together. In these tests mature males and

females, age six days, were mated and transferred daily thereafter. The egg hatch counts show that, even with remating, only 3 or 4 per cent of the eggs laid hatched in crosses of *virilis* females with *americana,*

TABLE 88

Egg hatch from females mated but once (from Patterson, Stone, and Griffen)

Cross		First Day Smear	First Day Hatch	Second Day Smear	Second Day Hatch	3- to 10-Day Hatch	Total Hatch
V × V	Sperm in eggs	15/15	47/66	8/10	30/34	82/93	159/193
	%	100	71	80	88	88	82
A × A	Sperm in eggs	20/22	49/70		38/43	145/167	232/280
	%	91	70		88	87	83
V × A	Sperm in eggs	8/52	7/215	0/13	0/71	0/964	7/1,250
	%	15	3	0	0	0	0.6

texana, or the heterozygotes from a cross of *texana* females by *americana* males. The genetic factors in *texana* and *americana* which cause their sperm to become immobilized in the reproductive system of *virilis* females must be alleles or present in most chromosomes. Table 97, which gives the fecundity of both the high and low productivity encountered in the cross, proves that remating occurs in the reciprocal crosses of *virilis* and *americana.* Table 89 indicates that *virilis* sperm are much more viable in the reproductive tracts of *texana* females, but the progeny counts in Table 97 show that this difference does not exist in the reciprocal types of *americana-virilis* crosses.

In considering the general problem of cross-fertility, we find that species cross with other species which are members of the same subgroup better than with members of other subgroups, but that many or most of the tested strains show generic variation in cross-fertility. This is shown by the differences between strains in the numerous tests mentioned as well as more direct tests. Tables 91 and 92 give tests of allelism of isolating factors and of their dominance, as judged by testing heterozygotes between strains. The crosses also test for maternal inheritance. Two strains of one species were crossed, and the heterozygote tested directly to another species (Table 91); or the heterozygote was inbred ten or fifteen generations, then tested to the other species (Table 92).

TABLE 89

Egg hatch tests from species and their hybrids (from Patterson, Stone, and Griffen)

♀ Cross ♂	Number of Pairs Tested	Number of Eggs	Number of Adults	Percentage Hatch
V × V	13	870	798	92.0%
T × T	13	1,137	644	56.6
T × A	5	618	320	51.8
A × T	5	864	410	47.4
T × V	8	690	153	22.2
V × T	13	1,361	51	3.7
V × A	8	1,448	45	3.1
V × TA	7	617	16	2.6
V × VT	11	1,834	714	39.0
V × V(V(VT))	14	2,366	0	0.0
	1	224	122	
	1	300	117	
	1	166	36	
	1	345	235	
	1	65	46	
	5	1,100	556	50.5
V(AV) × A	4	285	9	3.1
V(AV) × V	1	62	19	
	1	105	70	
	1	118	17	
	1	148	30	
	1	124	87	
	1	47	11	
	1	110	42	
	7	714	276	38.7
V × V(AV)	1	59	30	
	1	137	79	
	1	42	12	
	1	130	76	
	1	156	85	
	1	168	69	
	1	127	77	
	7	819	428	52.3

TABLE 90

Egg hatch with male replacement (from Patterson, Stone, and Griffen)

Cross	Day	Eggs	Pupa Color Red	Black	Eggs	Pupa Color Red	Black	Eggs	Pupa Color Red	Black	Eggs	Pupa Color Red	Black
V × V	2	25	0	24									
	3	31	0	28							44	0	39
	4				32	0	29	37	0	35	50	0	44
V male replaced by T male	5	31	0	31	21	0	21	26	0	25	39	0	39
	8							37	0	33			
	9	48	1	19	45	0	0	32	1	1	36	0	16
	10				28	1	1				42	3	0
	11	61	2	3				51	1	1	51	2	0
	12	45	1	0							38	0	0
	13	24	1	0							41	1	0
V × T	1				25	2	0	25	0	0	21	2	0
	2				26	0	0	31	0	0			
	3				27	0	0	48	1	0	23	1	0
	4	32	1	0	27	1	0						
	6	33	2	0	48	3	0	40	4	0			
T male replaced by V male	10	41	0	37	32	0	17	50	0	27	38	0	32
	11	34	0	32									
V × A	1				53	0	0						
	2							28	1	0	32	1	0
	4	37	0	0	26	1	0	32	2	0	46	1	0
	7										39	1	0
	8	38	0	0				39	0	0			
	9				25	3	0	50	0	0	23	0	0
	10	30	0	0	30	0	0	53	0	0			
	11	41	3	0				47	0	0			
A male replaced by V male	13	23	0	16				20	0	9	22	0	0
	14	47	0	43	58	0	19	59	0	48	36	0	0
	15	45	0	40	54	0	45	42	0	42	60	0	0
	16	31	0	31	42	0	39				42	0	0

TABLE 91

Tests for heterogeneity of isolating factors (from Patterson, Stone, and Griffen)

F_1 Heterozygotes Crossed to ♀ ♂	*americana* (A)						*texana* (T)					
	Female			Male			Female			Male		
	Pairs Tested	% Fertile	Average Per Vial	Pairs Tested	% Fertile	Average Per Vial	Pairs Tested	% Fertile	Average Per Vial	Pairs Tested	% Fertile	Average Per Vial
V × H	44	0		100	94	4	40	0		59	92	5
H × V	81	1	5	136	86	6	83	4	17	96	91	5
Hanchow × V	42	33	14	55	78	7	49	25	15	51	92	15
V × Hanchow	41	42	5	43	37	5						
Hanchow × H	33	49	25	50	32	3	50	42	18	48	35	4
H × Hanchow	27	41	14									
Mukden × V	39	Sterile		58	66	6	53	45	7	41	61	8
V × Mukden	40	10	11	52	64	4	54	48	18	47	45	6
Mukden × H	39	3	1	60	12	2	61	49	19	57	32	3
H × Mukden	51	31	9	58	12	2	50	48	13	50	32	3
Otaru × V	47	49	10	53	43	7	52	94	15	54	63	5
V × Otaru	55	62	11	53	70	7	56	91	8	56	64	9
Otaru × H	46	50	29	53	23	3	52	54	17	53	34	3
H × Otaru	46	33	4	59	56	6	50	36	11	54	48	4
863e × V	49	57	37	50	66	8	50	66	28	50	26	10
V × 863e	50	44	12	50	54	5	50	56	22	50	58	10
472.4 × V	54	24	15	50	64	4	50	38	22	52	89	11
V × 472.4	54	17	16	54	79	12	52	19	5	55	87	13
863e × H	55	35	17	50	8	2	50	76	28	38	13	3
H × 863e	47	9	4	51	10	5	45	58	15	38	16	4
472.4 × H	53	15	15	49	49	2	55	33	5	32	81	5
H × 472.4	27	41	7	51	46	7	51	18	7	52	52	7

TABLE 91 (*Cont.*)

	virilis, Pasadena (V)						*virilis*, Henly (H)					
T × A	78	91	10	46	9	18	77	13	2	49	4	10
A × T	42	76	10				41	17	3	45	2	2
A × 821.12b	100	86	6	100	10	21						
A × 821.12c	100	91	6	100	28	10						
A × 825.13c	100	75	8	100	27	11						
A × 841.10	100	82	8	100	24	9						
A × 849.11	100	62	5	100	12	12						
	A × T						T × A					
H × V	40	3	4	42	71	7	41	2	3	30	67	4

The Pasadena and Henly strains of *virilis* differ markedly in their ability to cross with *texana* (84.7 = T) and *americana* (Smithville = A). Henly is the most thoroughly isolated strain of *Drosophila virilis* that we have found. If we compare crosses of the F_1 from V ♀ × H ♂ and H ♀ × V ♂ to *americana* or *texana,* we find that they are alike. The reciprocal heterozygotes of A ♀ × T ♂ and T ♀ × A ♂ are alike in their crosses to these two *virilis* strains and to their heterozygote (H × V). These tests do not show maternal effects. There are some heterozygotes from reciprocal crosses in Table 91 which show consid-

TABLE 92

Tests for allelism of isolating factors (from Patterson, Stone, and Griffen)

Cross ♀ ♂	Cross Test of F_1 in Per Cent	Cross Test of F_{10} or F_{15} Inbred in Per Cent
A × (V × H)	Sterile	53%
(V × H) × A	94%	82
T × (V × H)	Sterile	70
(V × H) × T	92	79
V × (T × A)	91	73
(T × A) × V	9	21
H × (T × A)	13	4
(T × A) × H	4	13
T × (Hanchow × H)	42	4
(Hanchow × H) × T	35	40
T × (Otaru × H)	54	16
(Otaru × H) × T	34	0

erable differences in the amount of crossing. If these are compared to the original crosses (Table 95), they indicate that the discrepancies are probably due to sampling error of the small sample. For example, the (Hanchow × V) gave 78 per cent fertile to *americana* males, while the (V × Hanchow) gave only 37 per cent. This is a wide discrepancy, but it does not support the idea of maternal inheritance, for (V × Hanchow) with Pasadena (V) cytoplasm should be more like Pasadena (Table 85). There is evidence that some of the isolating factors are dominant, others recessive; some allelic, and others independent multiple factors which are capable of recombining with each other. For example, Tables 85 and 92 indicate that both the Pasadena and Henly strains of *virilis* are almost completely isolated from *americana* and *texana* females. Their F_1 (V × H) heterozygote is likewise

isolated, but the F_{10} inbred (V × H) heterozygote gave 53 and 70 per cent fertile with the females of these two species. The isolating factors here were dominant and probably nonallelic. On the other hand, the factors in Henly which isolate this strain from *americana* and *texana* males are recessive, for the (V × H) females cross readily to both types of males. In the reciprocal crosses, the factors isolating *texana* and *americana* from Pasadena and Henly seemed to be allelic, for F_1 and F_{10} were alike and quite similar to P_1 crosses (see also Table 85 for other *texana* strains). The factors in the Henly strain which isolate it from *texana* males and females are recessive but nonallelic to those in Hanchow and Otaru, for the F_1's (Hanchow × H) and (Otaru × H) tested to *texana* were as fertile or more so than Hanchow or Otaru, whereas the F_{15}'s of three tests of the four showed a greater isolation than the F_1's much like Henly. Table 92 demonstrates that, on inbreeding, the cross-sterility factors may be retained and fixed for little cross-fertility (the last examples) or for free crossing (the V × H). Even with the heterosis that may increase the amount of crossing which is characteristic of the F_1 heterozygotes in these *virilis* species as in *hydei* (Stone, 1942), the variation in genotypes is obvious.

A very effective isolating mechanism well illustrated in crosses between certain members of this group is zygotic mortality. Patterson and Griffen (1944b) made a series of tests (Tables 104 to 108) which showed that the significant crosses gave abnormal sex ratios. The several types of crosses that indicated the presence of abnormal ratios are listed in Table 104. These were made with *montana* females (strains 1318.82 and 1218.8d were used) and *texana* males (nine strains tested). In these tests, from 80 to 100 per cent of the progeny were males, depending on the strain employed (Table 105). As only one of the reciprocal crosses gave a sex ratio, it was obvious that the lethal effect occurred only in the *montana* eggs, a maternal effect in that sense, when it was fertilized by a *texana* sperm with the *texana* X. In the reciprocal cross, the same genetic female combination with an X and autosome set from both *montana* and *texana* was as viable as its male sibs. Tables 106 and 107 indicate that the effect is independent of the *texana* autosomes. The other subspecies *americana* does not carry this factor, for both *americana* and hybrid *americana-texana* males, with the X of the former, give good sex ratios or even an excess of females. The hybrid *texana-americana* males, or *texana-*

virilis or *texana-lacicola* males which have the *texana* X, all produce lethal female hybrid offspring with *montana* females. Table 108 shows that this is not true of females hybrid for *montana* and *virilis, lacicola,* or *texana.* Many of the double hybrids using heterozygous females and *texana* males must have had mainly *montana* and *texana* chromosomes, but were viable; therefore *texana* egg cytoplasm can be replaced by the egg cytoplasm from these hybrids. Thus the factors that produce the distinctive *montana* egg cytoplasm are recessive and the factor in the *texana* X is dominant, for it kills the heterozygous females. The main cross-lethal factor in the *texana* X was located near the locus of *echinus* (see Table 42) by testing crossover recombinants between a *texana* and marked *virilis* X to *montana* females.

Table 107 shows that there are few modifying factors in *virilis,* although the variation in percentage of females to survive in crosses to different strains of *texana* indicates that modifying factors can improve the viability of the aberrant females, which usually die during larval or pupal stages. Table 105 gives some evidence for genetic introgression between *americana* and *texana.* Eva, Tennessee, Morrilton, Arkansas, Hiwassee River, Tennessee, and Georgetown, Texas, are localities where *texana* and *americana* both occur and hybridize (Stone and Patterson, 1947). The Eva, Tennessee, stock shows a cross-sterility more characteristic of *americana,* while the other three stocks all give a relatively high percentage of females, and the presence of *americana* chromosomes increases markedly the viability of the female hybrids as compared with their male sibs (Tables 104 and 106).

An egg fertilization test was used as an additional check for the cause of the failure to produce viable hybrids. Males from a cross of *texana* females to *virilis* Henly males were crossed to *montana* females. A total of 680 eggs checked by the smear method for the presence of sperm showed that 67 were fertilized, but only a few larvae that soon died developed in 833 eggs kept to test development. In a similar test, except that the *virilis* Mukden strain was used, 14 eggs were inseminated of 198 smeared, or 7 per cent; while 4 males developed from 344 eggs kept to test development (1 per cent). Even the few fertilized eggs do not all develop in this type of isolating mechanism. The genotype with the dominant *texana* cross-lethal factor is very much more apt to die, and in fact dies 100 per cent of the time unless modifying factors are present. Tests with *montana* hybrid females

show that the maternal effect is not inherited cytoplasmically, but rather the *montana* egg cytoplasm does not establish the proper environment for this hybrid genotype to develop.

The F_1 tests are shown in Tables 93 to 96, and also 86 and 87. It is obvious from these tables that not all, and perhaps few if any, of the factors determining the initial isolation between the different strains of the different species are also responsible for hybrid sterility. The $F_1 \times F_1$ tests demonstrated that either or both male and female hybrids between the *montana* strains and the several *virilis* strains (884 pairs tested), the *texana* strains (150 pairs tested), and the *americana* strains (20 pairs tested) were sterile. Hybrids with *lacicola* substituted for *montana* were also sterile, with the numbers of pairs tested 127, 108, and 0, respectively. This hybrid sterility is not good evidence for the existence of different factors for the initial and the hybrid cross-sterility, as most of this latter sterility was due to failure to form functional gametes. The great fertility of a number of the F_1 hybrids when tested, even when their parent species are isolated by strong sexual isolation and gametic or zygotic mortality, is good positive evidence that the initial isolating factors do not function as sterility factors in these hybrids. The F_1 of the Henly strain with *texana* strains, other than those given in Table 93, included only two out of eighteen pairs fertile. This strain possesses very effective isolating factors, which act both in the P_1 crosses and in the hybrids. The *montana-lacicola* hybrids were not as fertile as the parent strains, although they were usually slightly more fertile than the P_1 crosses (Table 86). The *virilis-montana* and *virilis-lacicola* hybrids were tested in backcrosses (Table 95). Only one strain of *montana* (1211.51) gave fertile F_1 males. Here 5 per cent of the *montana* 1211.51-*virilis* Pasadena males were fertile to *virilis* females. All five strains gave fertile *montana-virilis* female hybrids when backcrossed to *montana,* but only in two cases, with 1211.51 and 1218.8d, were these hybrids fertile back to Pasadena males. The only hybrid fertility found in *virilis-lacicola* hybrids were a few females which crossed back to *lacicola* males. The number of fertile hybrids in each test is so small that we do not consider these tests to be convincing evidence of serious preferential isolation of these hybrids.

At least some of all types of hybrids between *virilis, texana, americana,* and *novamexicana* were fertile (Tables 87, 93, 94 and 96. The degree of hybrid fertility varied both between species hybrid types and

TABLE 93

F_1 inbred tests of hybrids (from Patterson, Stone, and Griffen)

Stock	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
virilis			98%	100%	72%	64%	66%	82%
Pasadena (V)			81	91	35	20	32	41
Henly (H)	100%	98%			34%	34%	34%	20%
84.6	91	81			27	16	29	20
Galveston	94%	96%	94%	98%	100%	90%	100%	100%
472.4	65	44	47	71	29	35	97	44
Victoria	94%	100%	96%	98%	88%		80%	92%
718.7a	54	143	58	75	45		53	43
California	100%	100%	98%	100%	88%	71%	88%	100%
863e	121	121	144	117	43	43	25	31
Hanchow	92%	100%	100%	98%	24%	58%	78%	86%
	59	134	82	80	37	52	35	49
Mukden	98%	100%	88%	100%	68%	40%	68%	60%
	88	78	87	104	27	11	54	68
Otaru	100%	100%	98%	90%	42%	43%	80%	74%
	90	104	71	73	30	14	89	43
americana	64%	72%	34%	34%			58%	60%
Smithville (A)	20	35	16	27			22	14
821.12a	60%	56%			92%	100%	96%	58%
	64	41			70	68	52	54
texana	82%	66%	20%	34%	60%	58%		
84.7 (T)	41	32	20	29	14	22		
821.12b	70%	30%			100%	94%	92%	90%
	61	35			48	76	75	59
812.12c	74%	80%			94%	90%	86%	96%
	82	46			78	63	74	65
825.13c	66%	58%			86%	78%	54%	64%
	56	75			72	57	50	38
841.10	64%				96%	88%	74%	64%
	48				60	66	35	42
849.11	96%	34%		14%	96%	90%	90%	98%
	87	67		11	95	77	90	86

even between the same type species hybrids from different strains. The Henly strain which goes well with other strains of *virilis* crosses very slightly to *americana* and *texana*. The Victoria strain showed an almost equally low initial cross-fertility (Table 85). The F_1 hybrids with Henly were also reduced in fertility, but those with Victoria gave a very high F_1 fertility (Table 93). The California strain, which

TABLE 94

F_1 backcross tests of species hybrids (from Patterson, Stone, and Griffen)

F_1 Backcross ♀ ♂	Number Tested	Percentage Fertile	Average Per Vial
H × AH	12	67%	35
TH × H	74	87	37
H × TH	36	33	42
TH × T	17	24	10
VT × V	121	95	42
V × VT	192	75	44
TV × V	43	91	48
V × TV	40	70	60
VT × T	65	65	29
T × VT	71	54	36
TV × T	18	89	29
T × TV	24	42	32
VA × V	83	90	44
V × VA	53	55	25
VA × A	64	88	35
A × VA	74	26	14
(T × Hanchow) × Hanchow	25	84	48
Mukden × (A × Mukden)	49	53	24
(A × Mukden) × Mukden	44	82	27
A × (A × Mukden)	35	63	24
(A × Mukden) × A	27	59	25
Mukden × (T × Mukden)	27	41	
(T × Mukden) × Mukden	27	85	
T × (T × Mukden)	15	67	
A × (Otaru × A)	9	56	15
Otaru × (T × Otaru)	56	82	—
(T × Otaru) × Otaru	60	20	—
T × (T × Otaru)	55	78	33
(T × Otaru) × T	50	90	37
(472.4 × A) × 472.4	8	100	49
T × (472.4 × T)	19	95	59
T × (T × 472.4)	24	79	81
863e × (T × 863e)	53	85	33
(T × 863e) × 863e	57	95	29
(T × 863e) × T	46	54	13
(V × 821.12b) × V	50	90	73
V × (V × 821.12b)	50	76	49
V × (821.12b × V)	50	34	21
(821.12b × V) × V	50	10	15
(V × 821.12c) × V	50	78	83
V × (V × 821.12c)	50	79	86
(V × 825.13c) × V	50	100	84
V × (V × 825.13c)	50	98	61
(V × 841.10) × V	50	88	84
V × (V × 841.10)	50	56	40
(V × 849.11) × V	50	94	118
V × (V × 849.11)	50	92	100

crossed poorly with *americana* but went well with *texana,* gave a very high F_1 fertility with both, but not quite as good as the F_1 fertility of their hybrids with the Galveston strain, which had high initial cross-fertility (Tables 85 and 93). The Asiatic strains included in Tables 85 and 93 went better with *texana,* both in P_1 and F_1 crosses. We have pointed out that the *montana* and *lacicola* hybrids were more often fertile in backcrosses. A comparison of the data in Tables 87, 93, 94, and 96 indicates that this is generally true, as would be expected if there is reduced fertility in both sexes of hybrids.

Since hybrids are often less fecund than the parent stocks, tests were carried out to measure this effect. Table 97 gives some tests of *virilis* Pasadena and *americana* Smithville. In these tests, the pairs

TABLE 95

F_1 backcross tests of *montana* and *lacicola* hybrids (Patterson and Griffen)

Crosses	Pairs Tested	Pairs Fertile	Average Per Vial
VM ♀ × V ♂ (Pasadena)	120	0	—
VM ♀ × M ♂ (1210.83)	100	7	3
V ♀ × VM ♂	109	0	—
M ♀ × VM ♂	108	0	—
VM ♀ + V ♂ (Pasadena)	100	6	1
VM ♀ × M ♂ (1211.51)	100	4	4
V ♀ × VM ♂	100	5	2
M ♀ × VM ♂	101	0	—
VM ♀ × V ♂ (Pasadena)	100	4	1
VM ♀ × M ♂ (1218.8d)	100	20	6
V ♀ × VM ♂	109	0	—
M ♀ × VM ♂	106	0	—
VM ♀ × V ♂ (Pasadena)	113	0	—
VM ♀ × M ♂ (1318.8a)	100	4	1
V ♀ × VM ♂	104	0	—
M ♀ × VM ♂	100	0	—
VM ♀ × V ♂ (Pasadena)	114	0	—
VM ♀ × M ♂ (1324.8m)	100	8	5
V ♀ × VM ♂	126	0	—
M ♀ × VM ♂	100	0	—
VL ♀ × V ♂ (Pasadena)	111	0	—
VL ♀ × L ♂ (1360.2)	100	1	1
V ♀ × VL ♂	108	0	—
L ♀ × VL ♂	107	0	—
VL ♀ × V ♂ (Mukden)	75	0	—
VL ♀ × L ♂ (1360.2)	76	7	11
V ♀ × VL ♂	72	0	—
L ♀ × VL ♂	65	0	—

TABLE 96

F_1 backcross tests of *novamexicana* hybrids (from Patterson and Stone)

Crosses ♀ ♂	Percentage Fertile	Average Per Vial	Sex Ratio ♀♀	Sex Ratio ♂♂
V × VN	1% (33)	2	37	41
N × VN	sterile (7)	—	—	—
VN × V	35 (98)	12	137	109
VN × N	43 (62)	22	257	191
V × NV	8	58	115	117
N × NV	<1	—	149	128
NV × V	71	30	366	235
NV × N	18	24	151	117
A × AN	19	27	265	284
N × AN	<1	—	194	185
AN × A	4	23	347	338
AN × N	17	36	381	333
A × NA	28	31	116	99
N × NA	10	17	31	19
NA × A	sterile	—	—	—
NA × N	25	19	43	51
T × TN	29	24	242	240
N × TN	1	6	243	185
TN × T	29	23	251	204
TN × N	29	15	291	282
T × NT	8	23	55	62
N × NT	67	17	180	169
NT × T	27	21	212	208
NT × N	50	20	202	196

which showed the high and low fecundity of the four tested are given. This represents a full-generation cycle, for the parents were mated when six days old, few of these species being fertile before this age, and by the twenty-third day progeny were emerging from the most vigorous pairs. Crosses involving *americana* are at some disadvantage as compared with *virilis* or their hybrids because they mature more slowly. As no egg count was made, this was a measure of fecundity in terms of viable offspring. *Drosophila virilis* was the most productive, while the A ♀ × V ♂ and V ♀ × A ♂ gave only a few progeny because of sperm mortality. There was considerable more variation in the tests with the hybrids than with *virilis* or even *americana*. This could mean that the factors responsible for partial

sterility of the hybrids were heterozygous in the parent species, or more probably that the hybrids were more susceptible to the detrimental effects of extrinsic factors in the environment. One important point is that the hybrids are as fecund on inbreeding as the *americana* parent, even if they are less fertile on backcrossing than the recurrent parent. Hence they could easily provide for exchange of genes between the species if the initial isolation failed to prevent their formation.

TABLE 97

Fecundity of *virilis, americana,* and their hybrids

Crosses	Days 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total
V ♀ × V ♂	48	59	49	31	41	61	37	61	57	59	40	38	64	83	44	69	43	884
	1	51	28	70	52	55	48	31	51	48	41	37	49	51	32	59	36	740
A♀ × A ♂	0	0	0	0	0	11	37	36	32	47	16	24	39	43	13	3	0	301
	0	0	0	0	0	20	26	11	11	24	12	0	0	8	28	7	0	147
V ♀ × A ♂	0	0	0	0	0	4	1	2	4	4	0	3	0	1	0	1	0	20
	0	0	0	0	0	0	0	3	1	4	0	0	1	1	1	0	0	11
A ♀ × V ♂	0	0	0	0	0	0	0	0	0	2	7	1	4	2	1	6	6	29
	0	0	0	0	0	3	0	0	1	1	0	0	0	1	0	1	0	7
V ♀ × AV ♂	0	17	0	3	15	36	1	24	15	20	14	28	19	25	48	43	41	349
	8	0	0	0	0	0	11	9	0	10	0	0	0	13	19	0	1	71
AV ♀ × V ♂	2	0	13	10	14	23	20	24	30	28	14	21	28	37	39	34	17	354
	3	7	4	14	10	9	13	9	7	29	2	12	21	25	10	19	11	205
A ♀ × AV ♂	0	2	15	17	11	20	3	27	4	13	15	4	28	30	29	35	31	284
	0	1	1	0	0	0	0	4	5	5	2	0	1	2	1	0	0	22
AV ♀ × A ♂	0	0	0	0	6	17	28	21	16	17	3	11	18	14	6	17	10	184
	0	0	0	0	0	0	1	1	6	12	2	3	7	18	3	11	7	71
AV ♀ × AV ♂	9	15	20	27	31	34	45	32	30	39	25	21	25	23	10	23	24	423
	5	4	0	14	5	4	11	12	9	8	6	9	11	14	10	15	14	152

Stone (1949) measured the detrimental effect of a heterozygous fusion on *texana-americana* heterozygotes. These subspecies differ by the X-4 fusion, as well as by several inversions. In order to minimize other sources of zygotic mortality, the crosses were made in such a way as to give the maximum heterozygosity by suitable crosses of unrelated stocks. A preliminary test to find the maximum possible egg hatch, i.e., the minimum negative selective effect, showed that the fusion had little detrimental effect under optimum conditions. The test results were as follows:

(1) T_oT_L ♀ × A_AT_N ♂ = 838 hatch/887 eggs or 94.6%
(2) A_AT_N ♀ × T_oT_L ♂ = 1877 hatch/1919 eggs or 97.7%

The females heterozygous for the fusion gave an egg hatch (as adults counted) much higher than that of an ordinary stock. These results were from a count of all the eggs laid over a three-day laying period from the six best pairs of twelve pairs tested, and thus showed the least effect that might be due to the fusion. Table 102 includes the data for all the eggs laid over a three-day period by twelve pairs of each cross, divided into the hatch from the six better pairs and the six poorer pairs. There is no doubt that the heterozygous fusion has little detrimental effect on disjunction and forms few, if any, aneuploid gametes. This table also indicates that the subspecies *texana* and the eastern strain of *americana* are so closely akin genetically that the majority of the gametes formed in an F_1 heterozygote produce viable offspring. The western Chinook strain of *americana* differs from *texana* more widely and produces a certain number of inviable zygotes among the recombinants.

Table 98 demonstrates that a very much more serious genetic difference exists when *americana* or *texana* is tested with *novamexicana*. A stock of *novamexicana* was established lethal-free, in the sense that 95 per cent egg hatch could be obtained from the pairs of F_1's from any pair in the stock. Certainly there was no balanced lethal condition, and the frequency of spontaneous lethal mutations present in the stock was very low. The same precautions were taken to insure maximum heterozygosity by testing with unrelated heterozygous strains. The *novamexicana* differs from the *americana* by several inversions, as well as the X-4 and 2-3 fusions, and from *texana* by several additional inversions, although the X-4 fusion is present in neither species. The segregation from F_1 male hybrids produced a reduction in the number of surviving zygotes. The F_1 female hybrids, which produce recombinations both by segregation and crossing over, gave a very much more serious reduction in number of viable offspring. There is no doubt that part of the gene combinations having some of the *novamexicana* genes heterozygous may act as a lethal. The gene systems are balanced both within and between chromosomes, so that increasing recombinations through crossing over increases the number of inviable gene combinations which act as dominant lethals. This is an excellent illustration of the extensive

reorganization of gene systems (change in balanced gene system) that has occurred during the separate evolution of these species. Other tests of the western *americana* strains indicate that they are more closely related to *novamexicana* than the eastern strains. This suggests the effect, expected from the spacial distribution, of gene exchange through introgression.

TABLE 98

Egg hatch tests of *novamexicana* hybrids (from Patterson and Stone)

	Egg Hatch	Percentage
P_1 1714.4 (lf) ♀ × T_N ♂;		
F_1 ♀ × T_oT_L ♂	140/428	32.7%
F_1 ♂ × T_oT_L ♀	411/558	73.7
P_1 T_N ♀ × 1714.4 (lf) ♂;		
F_1 ♀ × T_oT_L ♂	242/769	31.5
F_1 ♂ × T_oT_L ♀	452/523	86.4
P_1 1714.4 ♀ (lf) × A_A ♂;		
F_1 ♀ × T_oT_L ♂	218/824	26.5
F_1 ♂ × T_oT_L ♀	275/444	61.9
P_1 A_A ♀ × 1714.4 ♂ (lf)		
F_1 ♀ × T_oT_L ♂	152/452	33.6
F_1 ♂ × T_oT_L ♀	473/637	74.3

The data in Table 103 may be used to measure the production of inviable gene combinations in the offspring from both male and female *texana-virilis* or *americana-virilis* hybrids. Only the egg count during the time it is stable (first three days) may be used, because of the immobilization of sperm in the reproductive tract of an alien female. All the counts given in Table 103 are from females that still had (immobile) sperm in their receptacles at the end of the test. Both subspecies show greater difference from *virilis* than from *novamexicana*. A marked stock of *virilis* was used, and the viability of some classes may have been somewhat reduced by genes in the marker stock. The results are as follows:

P_1 *virilis* × *americana* (Chinook)
(1) F_1 ♂ × *virilis* ♀ = 14% hatch
(2) F_1 ♂ × *americana* ♀ = 21% hatch
(3) F_1 ♀ × *virilis* ♂ = 50% hatch
(4) F_1 ♀ × *americana* ♂ = 43% hatch

P_1 *virilis* × *texana* (New Orleans)
(5) F_1 ♂ × *virilis* ♀ = 17% hatch
(6) F_1 ♂ × *texana* ♀ = 17% hatch
(7) F_1 ♀ × *virilis* ♂ = 52% hatch
(8) F_1 ♀ × *texana* ♂ = 13% hatch

The reproductive efficiency of all these hybrids is considerably reduced.

Tables 87, 96, and 100 include results from crosses involving *novamexicana,* strain 1714.4. The data on percentage of fertility in the regular column for crosses were obtained with the Pasadena *virilis* strain. The figures in parentheses to the right under this heading are for crosses with *virilis* from Texmelucan. This strain gave hybrid progeny counts very similar to those for Pasadena and were omitted. There is no doubt that the initial isolation between Texmelucan *virilis* females and San Antonio *novamexicana* (1714.4) males is less effective, for 60 per cent of the pairs were fertile. As an additional test, we crossed Texmelucan *virilis* females to Whitewater *novamexicana* (1954.3a) males. There was about the same average number of offspring per vial, but 94 per cent of the pairs were fertile. Thus genetic differences between strains of *virilis* and strains of *novamexicana* appreciably affect their crossing as well as their mating, as is indicated by Spieth's tests of sexual isolation (Chapter 7).

These tests demonstrate that the genetic difference between strains determines the fertility or sterility of their hybrids in crosses. We have the comparative fertility of *novamexicana* hybrids with Pasadena or Texmelucan, and the most remarkable change is the fertility of the $F_1 \times F_1$ hybrids of *novamexicana* × Texmelucan *virilis.* These pairs are 94 per cent fertile, a value which was checked in several experiments, as compared with the 2 per cent fertility using the Pasadena strain of *virilis.* Furthermore, all classes of F_1 hybrids tested, (Table 96) and most classes of F_2 hybrids (Table 100) gave a significantly higher fertility if the Texmelucan rather than the Pasadena strain was used. It is clear that a genetic difference involving several genes exists between these *virilis* strains, as both males and females were affected. Thus possession of the Pasadena genes causes the *novamexicana-virilis* hybrids to be much more sterile, whereas the Texmelucan genes allow them to be much more fertile. This is a genetic difference between *virilis* strains that has no appreciable effect in crosses within this species, but the difference in genotype deter-

mines whether their hybrid offspring with *novamexicana* will be semi-sterile or fertile. Two egg counts were run with F_1 from a cross of Texmelucan *virilis* to San Antonio *novamexicana*. In these tests 30 per cent of 2,054 eggs laid by nine test pairs of F_1 hybrid females from a P_1 cross of *virilis* females to *novamexicana* males, backcrossed to *virilis* males, produced viable offspring. From a P_1 cross of *novamexicana* females to *virilis* males, 39 per cent of 1,738 eggs produced viable offspring when nine test pairs of the F_1 were inbred. This makes it very probable that some combinations of *novamexicana* genes survive much better if homozygous than heterozygous. It is not possible to tell whether the genes are sex-linked or autosomal or balanced groups. The original F_1 types have a set of genes from each species, but the survival of recombination sets depends on the gene combination.

Some preliminary tests with a strain of western *americana* (1773.4e) are presented in Table 109 because they show differences in cross-fertility with *novamexicana* (1714.4) and also illustrate the peculiar types of ratios that may occur in wide crosses. The *americana* stock came from a single fertilized female captured at Chadron, Nebraska. She produced a heterozygous stock showing several dark and light color phases. In these tests flies were classed as dark or light, and dark is dominant; for pairs of dark flies can produce light offspring, but the reverse does not happen. Table 109 lists the result of crosses to *novamexicana* (N). The symbol A_L indicates light-colored individuals, A_D dark individuals, and $(A_DN)_L$ light individuals from a cross involving a dark parent. Several facts are immediately evident. Both P_1 and F_1 crosses involving pairs of light flies are not fertile quite as often as corresponding classes involving dark flies. The average number of offspring per vial is not correlated with this color difference. The color phases are separated in the sex ratio column; and although males and females are fairly equal in frequency, invariably there are more light offspring than dark offspring, even when two dark hybrids are crossed together. Light flies bred together give only light offspring, whatever their origin, and hence the dark color depends on a factor from *americana* which suffers a decided survival disadvantage in the species hybrids. This western *americana* is much more cross-fertile with *novamexicana* than the eastern strain, both in P_1 and F_1 crosses. The higher the cross-fertility, the greater is the similarity of the chromosomes; and perhaps these

factors, together with the light color phase, in the Chadron stock all combine to indicate some introgressive hybridization, at least in the occurrence of more recent or more frequent gene exchange.

Patterson, Stone, and Griffen (1940, 1942) demonstrated the presence of a complementary Y-autosome fertility system in *texana* and *americana*. If males of these two species are crossed to *virilis* females carrying suitable genetic markers, the F_1 males backcrossed, and the F_2 males with the several chromosome combinations are tested for fertility, only those with both chromosome 5 and the 2-3 fusion are normally fertile. The Y of *texana* or *americana* must have with it these autosomes at least heterozygous for fertility. It is not a question of the heterozygous combination as such, for if the initial cross is made by using *texana* or *americana* females with *virilis* males, all these F_2 chromosome combinations are fertile (some combinations are not produced in both sexes with *americana* because of the X-4 fusion).

Tables 89, 99, and 100 list some F_2 tests of fertility and fecundity in which the different chromosome combinations are not known individually. Table 101 gives the percentage of eggs to develop into F_3 individuals (represented by the upper figure) from the eggs laid (represented by the lower figure), and thus measures the viability of these known gene combinations. The fertility of all these F_2 combinations varied from 70 to 95 per cent, except those combinations with a *texana* Y, and is illustrated by combination five in Table 101. In this case the two fertile male combinations, those having the *texana* chromosomes 5 and the 2-3 fusion, were 95 and 85 per cent fertile, respectively. The other male combinations were sterile or semisterile, for although from 1 to 8 per cent of the males produced a very few motile sperm, none produced more than two or three progeny (Stone, 1947).

Dissection of the sterile male combinations with a *texana* or *americana* Y revealed that those lacking chromosome 5 have small testes possessing many abnormal cells and much debris, and a few undeveloped sperm bundles. Those lacking the 2-3 fusion have testes nearly normal in size but usually filled with undeveloped sperm bundles, even though about 8 per cent produced a few progeny. Certain of the crosses in Table 99 have a fertility around 25 per cent as a result of this Y-autosome-conditioned fertility. The F_1 backcross to *texana* or *americana* females, which insures the presence of

the necessary autosomes in F_2, usually increased markedly the fertility of these males.

In every cross some of the F_2 hybrids between *virilis, americana, texana,* and *novamexicana* are fertile, although the amount of fertility varies markedly (Tables 89, 99, 100, and 101). Tables 89 and 101 show the variation in fecundity measured as percentage of offspring obtained from eggs laid. The data in Table 101 indicate why the different F_2 and F_3 pairs give such variable egg hatch counts in back-

TABLE 99

Tests of F_2 hybrids of *virilis, americana,* and *texana* (Patterson, Stone, and Griffen)

F_2 Crosses ♀ × ♂	Number Tested	Percentage Fertile	Average Per Tube
(VA)V × (VA)V	62	69%	40
V × (VA)V	83	63	62
(VA)V × V	48	94	45
V(VA) × V	51	98	54
V × V(VA)	110	10	41
(VA)A × (VA)A	21	67	30
A × A(VA)	7	29	25
V × A(VA)	25	28	25
(AV)V × V	35	97	46
V × (AV)V	37	57	61
(VT)V × V	47	100	73
V × (VT)V	80	88	57
V(VT) × V(VT)	123	25	30
V(VT) × V	50	96	63
V × V(VT)	336	25	54
T × V(VT)	15	20	20
T(VT) × T(VT)	18	75	30
V × T(VT)	40	88	30
T × T (VT)	70	57	29
(TV)V × V	49	98	42
V × (TV)V	82	78	40
V(TV) × V(TV)	102	67	30
V × V(TV)	153	84	40
T(TV) × T(TV)	22	86	30
(HT)H × (HT)H	35	83	30
H × (HT)H	15	0	
H(HT) × H	19	90	67
H × H(HT)	33	27	30
(TH)H × H	47	96	65
H × (TH)H	227	76	55
H × H(TH)	199	70	57
T × H(TH)	79	24	31

TABLE 100

F_2 tests of *novamexicana* hybrids (from Patterson and Stone)

Crosses ♂ ♀	Percentage Fertile	Average Per Vial	Sex Ratio ♀♀	Sex Ratio ♂♂
V × (VN)V	68% (90)	92	506	416
N × (VN)V	1 (26)	21	13	8
(VN)V × V	87 (86)	67	376	293
(VN)V × N	53 (46)	27	275	264
V × (VN)N	43 (56)	38	190	193
N × (VN)N	23 (45)	18	181	185
(VN)N × V	7 (59)	10	45	42
(VN)N × N	48 (37)	27	314	225
A × (AN)A	5	28	84	58
N × (AN)A	66	8	79	84
(AN)A × A	5	17	53	50
(AN)A × N	3	7	11	10
A × (AN)N	1	12	5	7
N × (AN)N	10	19	126	101
(AN)N × A	36	17	182	157
(AN)N × N	19	17	178	147
T × (TN)T	18	26	264	231
N × (TN)T	37	6	57	51
(TN)T × T	49	26	123	110
(TN)T × N	20	21	200	165
T × (TN)N	3	6	15	7
N × (TN)N	50	17	163	169
(TN)N × T	76	16	173	155
(TN)N × N	18	27	286	245

crosses. The V(AV·) females yield very different results in backcrosses to the two parent types. Even the recurrent cross to *virilis* showed the detrimental effect of abnormal gene combinations, for the females with crossing over to increase the number of recombinations, and so to increase the probability of detrimental types, produced fewer viable offspring. The four females of this combination tested back to *americana* must have been homozygous for the *virilis* factors which provide an internal environment in the seminal receptacles incompatible with *americana* sperm.

The male F_1 from crosses between *novamexicana* females (1714.4) and *virilis* males with each autosome, except for the dot, marked [*b*(2), *tb gp* (3), *cd*(4), *pe*(5)], were backcrossed to the marked

TABLE 101

Fecundity of F_2 recombinations of *virilis*, *americana*, and *texana* (from Stone)

A or T autosomes; see cross for X =	2–3,4,5	2–3,4	4,5	4	2–3,5	2–3	5	0
Phenotype of F_2 =	+	pe	R	R pe	cd	cd pe	R cd	R cd pe
R cd pe ♀ × A_A ♂	57%	39%	81%	79%	30%	28%	80%	65%
F_1 ♂ × cd pe ♀;	641	348	554	793	777	413	576	730
F_2 ♀ × cd pe ♂								
A_A ♀ × R cd pe ♂	50%	65%	35%	78%				
F_1 ♂ × cd pe ♀;	1,188	611	243	228				
F_2 ♀ × cd pe ♂			(1)					
A_A ♀ × R cd pe ♂					65%	65%	92%	96%
F_1 ♂ × cd pe ♀;					832	875	473	639
F_2 ♂ × Mexico ♀								
R cd pe ♀ × T_N ♂	52%	53%	87%	87%	54%	42%	86%	86%
F_1♂ × cd pe ♀;	484	106	722	607	353	295	615	449
F_2 ♀ × cd pe ♂								
R cd pe ♀ × T_N ♂	58%	0	2%	0	65%	0	0	0
F_1 ♂ × cd pe ♀;	412	806	437	610	1,301	1,152	984	1,278
F_2 ♂ × Mexico ♀							(2)	(3)
T_0 ♀ × R cd pe ♂	50%	49%	62%	79%	37%	46%	66%	78%
F_1 ♂ × cd pe ♀;	466	1,097	577	594	643	564	667	317
F_2 ♀ × cd pe ♂								
T_0 ♀ × R cd pe ♂	34%	36%	76%	78%	45%	56%	42%	78%
F_1 ♂ × cd pe ♀;	178	225	234	84	268	474	33	162
F_2 ♂ × cd pe ♀	(4)							

(1) 60% / 963 from cross to Mexico ♂
(2) 0.7% / 648 using New Orleans P_1
(3) 0.6% / 165 using New Orleans P_1
(4) 58% / 802 from cross to Mexico ♀

virilis stock. Each of the sixteen classes of F_2 males was fertile to some degree, slight in several cases, but many gave a poor egg hatch count when crossed to an unrelated *virilis* stock, a lethal free stock of Pasadena. The sixteen classes of F_2 female hybrids, all of which possessed the *novamexicana* X heterozygous, were fertile to a varying degree. The percentage of eggs to produce viable offspring was checked in a small test with counts of from 150 to 400 eggs for the several classes. If more than one *novamexicana* autosome was present, the hatch ranged from 25 to 40 per cent, while those classes with one or no *novamexicana* autosome ranged from 60 to 70 per cent hatch.

TABLE 102

Egg hatch from *texana-americana* hybrids (from Stone)

Cross	High Eggs	High Hatch	High %	Low Eggs	Low Hatch	Low %	Average Total	Average %
							971	
A_sT_N ♀	548	543	99.1	518	428	82.6	1,066	91.1
							1,141	
A_sT_N ♂	578	570	98.6	639	571	89.4	1,217	93.8
							964	
T_NA_s ♀	501	496	99.0	569	468	82.2	1,070	90.1
							1,058	
T_NA_s ♂	579	531	91.7	650	527	81.1	1,229	86.1
							807	
A_cT_N ♀	527	504	95.6	404	303	75.0	931	86.7
							844	
A_cT_N ♂	445	442	99.3	455	402	88.4	900	93.8
							825	
T_NA_c ♀	518	467	90.2	575	358	62.3	1,093	75.5
							1,024	
T_NA_c ♂	611	564	92.3	627	460	73.4	1,238	82.7
							763	
A_cA_A ♀ (Control)	346	336	97.1	489	427	87.3	835	91.4
							838	
A_cA_A ♂ (Control)	441	432	98.0	474	406	85.7	915	91.6

A more adequate test is needed to provide accurate measures of the effect of any single chromosome, but the effects of the several chromosomes seem cumulatively detrimental in terms of viable gene conbinations produced from the heterozygotes.

Another factor that must always be considered in describing differences between species is the effect of strain differences within the species. This difference is illustrated by the differences in fertility, but not viability, of the F_2 recombinants of *novamexicana* with the two strains of *virilis,* Pasadena and Texmelucan (Table 100). Despite the

fact that these classes come from F_1 hybrid females, which allowed both chromosome segregation and crossingover, several F_2 classes are decidedly more fertile when the Texmelucan strain is used.

DISCUSSION

The best approach to an understanding of the origin and relationships in the virilis group is through a combination of the cytological phylogeny and genetic tests. An examination of Figures 69 to 73 places primitive I at the head of all cytological evolution in the group. The strain differs from *Drosophila virilis* cytologically by the 2A inversion, and genetically by having a basic red color of the pupae and factors which allow its descendants to be anesthetized rapidly. The pupa color is a bright red in the descendants of primitive II, cytologically characterized by XAB and 2a, *texana, americana,* and *novamexicana,* but the pupae tend to darken in the descendants of primitive III, cytologically characterized by 2aDE and 4 DEF. It is not possible to prove whether primitive I or *Drosophila virilis* is the original form. As *virilis* retains its genetically central position in the group, we shall regard it as the primitive representative among modern forms. In discussing the cytological phylogeny, we shall assume that all changes were through two-break rearrangements, that is simple inversions or fusions. There is no evidence in the three fusions and 56 inversions so far analyzed in this group by Hsu that any of the changes took place through the occurrence of three or more breaks at one time leading to a complex rearrangement. This is in agreement with the evidence from analyses of the rearrangements found in the other species of Drosophila (Chapter 5).

If we look at the chromosome phylogeny, Figure 74, we find that *virilis* and primitive III are Holarctic. Strains of *virilis* have been found in both North and South America and this species has an extensive population in Japan and China. Descendants of primitive III are present in Europe (*littoralis*) and in the Nearctic (montana complex). Primitive I is ancestral to II and III, and in that sense is now Holarctic. Primitive II is represented by the americana complex in North America and can be considered as Nearctic. It may have underscribed descendants in the Palaearctic, or it may have originated there and migrated to North America, where it evolved further.

We do not know how often any one or all of these forms crossed the Bering Straits. We can only say that certain different gene sequences or their descendants are now on one or both parts of the Holarctic. It is very instructive to know certain particular differences in cytological behavior. Obviously *virilis* and primitive I are the oldest gene orders, and they differ only in the 2A inversion, while both have the primitive configuration consisting of six separate chromosome elements. At most, *virilis* has changed by one inversion during its separate evolution, and this one only if we assume primitive I to be the original form. In the *texana, americana,* and *novamexicana* complex, this same 2 chromosome with the 2A inversion is present as such in the first two forms. This complex has been studied extensively, and only twelve other paracentric inversions and two fusions have been found. The descendants of primitive I through primitive III have not been studied as extensively. In addition to this 2a inversion, there have been more than forty-five other known and analyzed paracentric and one pericentric inversions, as well as one fusion, Table 81. Furthermore, the X of *littoralis* has perhaps six inversions, which are indicated as XLi in the table, and that of *montana* (XM) even more, so that they pair so poorly with *virilis* that no analysis has been possible (also *lacicola* 2 and 3). The descendants of primitive III, the montana-littoralis complex, have perhaps sixty inversion differences from *virilis,* which has no inversions, while the americana complex differs from *virilis* by thirteen inversions. In view of the primary nature of the primitive I sequence in this species group, we can conclude that these three subdivisions changed cytologically at quite different rates, despite their necessarily very ancient origins and separation. The genetic variation also shows the same pattern.

Drosophila virilis has evolved slowly and has developed no separate species or subspecies. The second subdivision, consisting of *texana, americana,* and *novamexicana,* has an intermediate rate of genetic change; while the third cytological subdivision, consisting of the European littoralis complex, *littoralis* and *imeretensis,* and the American montana complex, *montana, lacicola, borealis,* and *flavomontana,* show the greatest rate of genetic change. Patterson (1952b) has described and analyzed the last two species. Their hybrids with the other members of the virilis group which survived to be tested are added to Table 75.

Many of the genetic mechanisms involved in and leading to evolutionary divergence are illustrated in this species group. The role of the genetic mechanisms in evolution is to provide the inherited basis for the necessary biological mechanisms involved in living and reproduction—or, to use one word, in survival—and to provide genetic variability necessary to allow modifications of the biological systems to better adapt the organism for survival in a constant or in changing environments. In sexual forms such as Drosophila, the genetic systems should provide for adequate crossing, to insure maximum utilization of both mutation and gene recombination. They also must provide mechanisms to insure sufficient isolation to retain their genetic identity.

We have shown that there are genes present which affect the survival of the organism by demonstrating that many mutations are lethal, and that therefore their normal alleles are necessary in that genic system. We have also shown that there are different mutations present in different populations that can effect basic survival. Combinations of these genes obtained by crossing different strains give heterosis, as expressed by better viability and fecundity in *virilis, americana,* and *texana* (Stone 1942a).

Alexander (1949, 1952a) has checked the incidence of mutation in a number of stocks of *texana,* western *americana,* and the seven *novamexicana* strains available. In comparing these species with *hydei* and *macrospina limpiensis,* she finds that the frequency of visible mutations carried in populations of *texana* and *americana* falls between the high number in *hydei* and much smaller number in *limpiensis.* The inadequate sample of *novamexicana* has yielded so few mutants that it may have the smallest incidence of mutations of any species studied. This is in general agreement with the size of the populations, for the smaller populations which are localized along streams would have the most inbreeding, and so most rapidly eliminate mutants if detrimental. The studies of Metz and his associates and of Patterson, Stone, and Griffen (1942) with American strains of *virilis,* and of Chino and his associates with Asiatic strains, indicate that this species has a large number of all types of mutations present in natural populations. This species group can be said to have all types of mutations, and therefore to possess the general genetic variability necessary for evolution.

The virilis group is an excellent example of a series of forms

showing varying degrees and kinds of differences from one another. This is true of phenotype, where, for example, body color varies from the black of *lacicola* and *borealis* to a rusty yellow in *novamexicana* and *flavomontana*. The ecology varies from the northern forest forms, *lacicola* and *borealis*, which Spieth found associated with aspen around lakes, to the more temperate forest forms, *americana* and *texana*, which occur along streams, the mountain forms, *montana* and *flavomontana*, the desert stream vegetation association of *novamericana*, and the domestic form, *virilis*.

We have mentioned the difference in chromosomal plasticity as measured by effected change. The most interesting and extensive variability is that of the genetic systems, and especially the genetic background for isolating mechanisms. This was obvious from the first, for Spencer pointed out that differences existed between strains which determined how much *americana* and *virilis* crossed, and Stalker demonstrated that sexual preference existed between strains of *americana*. Spieth's work demonstrated the variation in sexual isolation particularly well (Chapter 7). Some of the differences in mating are due to differences in sex drive, others to differences in sexual isolation, and the barrier to mating is the sum of these two.

Gamete mortality is the second in the sequence of types of isolating mechanisms. Here again there are marked differences in degree of effect. Crosses of *virilis* females to *texana, americana,* or TA hybrid males show that sperm live only a short period (less than twenty-four hours) in the reproductive tract of the alien female (Tables 88 and 89). Crosses of *texana* females to *virilis* males do not show such sensitivity; nor do *virilis* females to *novamexicana* males (Table 103). This mechanism is present in numerous tests and shows as few offspring per fertile pair in a number of P_1 crosses.

The next mechanism in order of effect is zygotic mortality. It is generally effective in association with the former mechanism, for a smaller percentage of the eggs develop than are fertilized (Table 88), but may be here an extension of the effect on the male gamete. (It is present in hybrids, both F_1 and F_2, but this phase will be discussed later in sequence.) The best example in this group is the death of most or all of the female zygotes in crosses of *montana* females with *texana* males (Tables 104 to 107). This is the result of the abnormal action of this gene combination in the egg cytoplasm of *montana*, for the same gene combination in *montana/lacicola* hybrid egg cyto-

plasm develops normally (Table 108). In this case we know the approximate locus near the free end of the X chromosome of the major factor in *texana*. The other subspecies, *americana*, does not possess this gene, although nine strains from scattered points in the range of *texana* all had this primary factor. This factor does not exert a noticeably deleterious effect in crosses to *virilis, americana, novamexicana,* and *lacicola*. Here is a case of the fixation of a gene in one of a pair of very closely related subspecies, despite their contact and hybridization, which does, in fact, bring in some modifiers that reduce the lethal effect.

TABLE 103

Egg hatch tests from females mated but once (from Patterson and Stone)

Crosses	Egg Hatch Successive Days after Separation ♀ & ♂									
	1	2	3	4	5	6	7	8	9	10
P_1 b, tb gp, cd, pe ♀ × A_c ♂; F_1 ♂ × A_c ♀	56/250		3/31							
F_1 ♂ × b, tb gp, cd, pe ♀	34/314	6/46	39/202	3/69	1/115	2/49	1/33	2/83	0/46	
F_1 ♀ × b, tb gp, cd, pe ♂	542/1,072	103/228	88/155	37/81				16/100	11/47	
F_1 ♀ × A_c ♂	240/547	91/216	20/46		19/61	12/63	0/20	0/15	0/58	
P_1 b, tb gp, cd, pe ♀ × T_o ♂; F_1 ♀ × T_o ♂	30/313	50/405	76/442	51/340	16/246	1/112	1/102	0/62		
F_1 ♀ × b, tb gp, cd, pe ♂	436/835	116/188	74/176	91/207	38/98	39/67			46/256	
F_1 ♂ × T_o ♀	1/33	16/62	23/133	6/68	2/22	7/73		0/20	0/14	
F_1 ♂ × b, tb gp, cd, pe ♀	29/202	37/244	21/70					0/41		
P_1, b, tb gp, cd, pe ♀ × N ♂	66/466	79/510	57/433	16/222	5/103	5/64	4/60	2/56	1/125	0/368

These mechanisms seem to prevent crosses between these different forms from producing viable F_1 hybrids. Most of the tables of P_1 crosses show the sum of these effects. We can discuss profitably the wide range of variation in this sum of isolating factors. This group shows all kinds and degrees of effectiveness of these mechanisms. We have shown at length that some isolating factors are dominant and others recessive, and that different strains of a species possess

unlike gene systems. As examples of differences between *virilis* strains, Pasadena females cross readily to the males of several other species, while the males are more reluctant to cross-mate; but males and fe-

TABLE 104

P_1 crosses of *montana* and *lacicola* with other species (from Patterson and Griffen)

Crosses	Types of Matings	Females	Males
montana ♀ × *virilis* ♂	Pairs + masses	2	3
virilis ♀ × *montana* ♂	Pairs + masses	891	884
montana ♀ × *texana* ♂	Pairs + masses	0	38
texana ♀ × *montana* ♂	Pairs + masses	172	168
montana ♀ × *americana* ♂	Pairs	42	24
americana ♀ × *montana* ♂	Pairs	11	6
montana ♀ × *lacicola* ♂	Pairs + masses	390	353
lacicola ♀ × *montana* ♂	Pairs + masses	352	350
lacicola ♀ × *texana* ♂	Masses	97	84
texana ♀ × *lacicola* ♂	Masses	130	145

males of Henly are almost completely isolated from other species. The strain from Victoria is nearly as reluctant to cross to *texana* or *americana* as Henly, but the F_1's are quite fertile, unlike the F_1 Henly hybrids. In general, the Henly strain is most thoroughly isolated sexually from other species, for dissection of females in crosses involving this strain rarely demonstrate the presence of sperm in the seminal receptacles. Henly is a vigorous strain and shows no sexual isolation to any other strains of *virilis,* including those from Asia. In fact, we have no evidence for serious sexual isolation within this species, although there is some evidence for differences in sex drive and general vigor (Patterson, McDanald, and Stone 1947). These differences could be extended at length; for example, the eastern and western strains of *americana* are quite unlike in crosses to *novamexicana* (Tables 87 and 109). The strains of *montana* differ genetically in their cross-sterility. In general, the cross-sterility factors reduced crossing between separate species; but some strain isolation may be due to the development of genetic systems affecting isolation between strains of the same species, such as those described by Wharton (1942) in *repleta.*

The fertility of the F_1 hybrids varied from 0 to nearly 100 per cent, depending on the species and strains involved. This has been commented on in general, but we wish to stress the evidence for

genetic variability between strains by commenting on two hybrids with *novamexicana*. The fertility of the F_1 hybrids between *novamexicana* and eastern *americana* (Tables 87 and 96) was low, but with

TABLE 105

Tests of *montana* females with strains of *texana* (from Patterson and Griffen)

Crosses	Mass Matings	Masses Fertile	Females	Males	Percentage Males
M ♀ × T ♂ (841.10, Newton, Tex.)	29	18	0	141	100%
M ♀ × T ♂ (1128.10, New Orleans, La.)	32	5	0	20	100
M ♀ × T ♂ (1148.9, Lake McKethan, Fla.)	41	8	0	20	100
M ♀ × T ♂ (Columbus, Miss.)	62	6	0	11	100
M ♀ × T ♂ (Eva, Tenn.)	30	1	0	2	100
M ♀ × T ♂ (Okefenokee Swamp, Ga.)	17	9	3	39	93
M ♀ × T ♂ (Morrilton, Ark.)	31	23	29	208	88
M ♀ × T ♂ (Hiwassee River, Tenn.)	57	9	4	22	85
M ♀ × T ♂ (Georgetown, Tex., 84.7)	36	13	17	68	80
Totals	335	92	53	531	91%

TABLE 106

Tests of *montana* with *texana* hybrids (from Patterson and Griffen)

Crosses	Type of Matings	Percentage Fertile	Females	Males
M ♀ × TV ♂	Pairs + masses (16)	11%	0	120
TV ♀ × M ♂	Pairs + masses (12)	15	208	193
M ♀ × VT ♂	Pairs + masses (18)	13	9	23
VT ♀ × M ♂	Pairs + masses (12)	6	267	257
M ♀ × TA ♂	Pairs + masses (6)	7	3	36
TA ♀ × M ♂	Pairs + masses (9)	1	30	31
M ♀ × AT ♂	Pairs + masses (72)	1	238	168
AT ♀ × M ♂	Pairs + masses (66)	1	115	97

western *americana* it was high (Table 109). The average percentage of pairs fertile was less than 20 in the first case and more than 80 in the second. A similar difference exists when F_1's of *novamexicana-virilis* Pasadena are compared with those of *novamexicana-virilis* Texmelucan, where the $F_1 \times F_1$ tests in the two cases reveal the decided greater fertility of the F_1 males in the latter cross. In these cases, the fecundity for the tests was about the same. Although the *americana* case may be the result of recent introgressive hybridization, that of *virilis* cannot be of this type, but represents a difference in genotype which determines difference in fertility of the F_1

hybrid males, while having no effect in *virilis*. Only a few of the possible F_2 tests have been run. For example, it seems unnecessary to run extensive F_2 tests where F_1 tests have shown little or no fertility and few hybrids are available, especially when preliminary tests have shown the F_2 to resemble the F_1. This situation holds for many of the crosses and indicates a genic unbalance in the hybrid of such a degree that little more can be said than that the species have evolved different but effective gene systems. Much more convincing evidence on the nature of the genetic systems may be obtained in those cases where there is enough fertility of the hybrids to allow suitable tests for the effect of recombination on F_1 and F_2 hybrids, and their fertility and fecundity.

TABLE 107

Tests of *montana* females with *texana* hybrid males (from Patterson and Griffen)

Crosses	Mass Matings	Masses Fertile	Females	Males	Percentage Males
M ♀ × TV ♂ (Shenking, Manchukuo)	45	33	0	184	100%
M ♀ × TV ♂ (Pasadena, from N. Y.)	28	17	0	120	100
M ♀ × TV ♂ (Tokoyo, Japan)	45	28	0	115	100
M ♀ × TV ♂ (Mexico City, Mexico)	45	33	1	276	100
M ♀ × TV ♂ (New Orleans, La.)	45	39	2	198	99
M ♀ × TV ♂ (Galveston, Tex.)	45	18	1	102	99
M ♀ × TV ♂ (Brooksville, Fla.)	45	43	3	279	99
M ♀ × TV ♂ (Henly, Texas)	38	17	1	76	99
M ♀ × TV ♂ (Memphis, Tenn.)	45	35	2	153	99
M ♀ × TV ♂ (San Antonio, Tex.)	45	31	2	138	99
M ♀ × TV ♂ (Beaumont, Tex.)	45	29	2	129	98
M ♀ × TV ♂ (Mukden, Manchukuo)	42	25	2	126	98
Totals	513	348	16	1,896	99
M ♀ × TL ♂ (Fairbanks, Minn.)	12	11	0	68	100%

Before discussing recombination, it is necessary to point out that the virilis species tested cross over much more than *melanogaster*. Furthermore, crossing over is not seriously reduced, except in the very immediate region of the translocation (Girvin, 1949) or the complex inversions tested (Oshima, 1947). The data, both published and additional tests, do not show the reduction in exchange in inversion or translocation heterozygotes of the virilis series that is normally encountered in *melanogaster*. Therefore we can conclude that there is a great deal of recombination between homologous chromosomes in these hybrids except in the regions bounded by inversions.

Even the *virilis/montana* and *virilis/lacicola* hybrids have not given us much information about the differences between the balanced gene systems in the two species. The few F_2 obtained have not provided much more information, for they were sterile or semisterile. Hybrids between members of this *montana* complex and *texana, americana,* and *novamexicana* have been too sterile to be informative.

TABLE 108

Tests of *montana* hybrids with *virilis* and *texana* (from Patterson and Griffen)

Crosses	Masses Mated	Masses Fertile	Females	Males
ML ♀ × V ♂ (Pasadena)	40	0	—	—
V ♀ × ML ♂	40	26	297	302
ML ♀ × T ♂ (New Orleans)	40	1	46	50
T ♀ × ML ♂	40	2	19	18
ML ♀ × T ♂ (Lake McKethan)	40	6	67	62
T ♀ × ML ♂	28	2	19	16
ML ♀ × A ♂ (Anderson)	40	11	86	35
A ♀ × ML ♂	36	2	24	22
LM ♀ × V ♂ (Pasadena)	13	1	8	10
V ♀ × LM ♂	20	0	—	—
LM ♀ × T ♂ (New Orleans)	20	1	1	2
T ♀ × LM ♂	20	7	73	54
LM ♀ × A ♂ (Anderson)	20	2	2	3
A ♀ × LM ♂	20	10	151	117
VM ♀ × T ♂ (New Orleans)	28	9	12	13
T ♀ × VM ♂	33	0	—	—
VM ♀ × T ♂ (Lake McKethan)	19	9	19	24
T ♀ × VM ♂	19	0	—	—
TM ♀ × T ♂ (New Orleans)	10	3	6	6
M ♀ × TM ♂	10	0	—	—

Patterson's data with the European *littoralis* indicate that few hybrids can be obtained with the americana complex. In fact, no direct hybridization has occurred with *texana* or *novamexicana,* although *virilis/littoralis* female hybrids produce a few offspring with *texana* males. Hybrids between the females of some strains of *virilis* and *littoralis* males can be obtained with difficulty; about as many hybrids as parents have been produced in the tests so far. (The reciprocal cross yields very few offspring.) These *virilis/littoralis* female hybrids are fairly fertile, but the males have not produced offspring. Only

a very few hybrids of *littoralis* and strains of both *montana* and *borealis* have been obtained, so that the two divergent descendants of primitive III are quite different genetically. These tests have provided good evidence for the central position of *virilis* in this group.

The hybrids between *virilis* and the members of the americana complex and those within the complex give us a series grading from hybrid combinations as sterile as those with the *montana* complex to those as viable and fertile as the pure species. There are both general tests and those of particular chromosome combinations in the data. We can obtain a good idea of the comparative effective fecundity from Table 97, which shows that some of the F_1 hybrid combinations produce as many offspring as one parent strain.

The same high effectiveness is true of some F_2 combinations, but low or no fertility is characteristic of others. One special conditioned fertility is the Y-autosome complementary factor balance, for males with the Y chromosome of *texana* and *americana* must have the fused 2-3 and the 5 chromosomes from that species also in order to be fertile (Table 101). In cases where the male fertility with a particular Y chromosome is dependent on autosomal factors that are recessive, the hybrid male will be sterile and no demonstration of this interrelation is possible. The *virilis* Y is not dependent on as many factors. Alexander, Lea and Stone (1952) showed that hybrid males with the *virilis* Y and all autosomes from *novamexicana, americana,* or *texana* except the 5 (marked by *peach*) were fertile. These Y-autosome complementary factors insure the sterility of a large portion of the F_2 recombination males. These are examples where the gene systems of homologous chromosomes differ sufficiently so that they cannot substitute for each other.

The best illustrations of the fact that species are different genetic systems can be had by comparing the egg hatch obtainable in different combinations. These virilis species are excellent forms for careful experiments that can give us repeatable material. The validity of these tests is also reinforced by the consistency in difference that occurs when female and male hybrids with otherwise the same gene combination are tested. The hybrids between the subspecies *texana* and *americana* gave a good egg hatch, although the hatch from those in which western *americana* was used was somewhat below the controls. This would agree with the idea of greater genetic divergence with greater distance. On the other hand, the egg hatch tests of the

hybrids between eastern *americana* or *texana* and *novamexicana* (Table 98) show that a pronounced genetic difference exists between these forms. This opinion is greatly reinforced by the fact that the hybrid females, where the additional recombination due to crossing over would occur, consistently produce a much lower percentage of viable offspring. No such differential reduction in number of viable offspring is observed from *texana/americana* female hybrids. This also affords evidence for a strong intrachromosomal genic balance, as well as for the interchromosomal balance in the sense that a number of genes in several of the chromosomes must be involved. Hybrid egg cytoplasm may also be a contributing factor here, although not through maternal inheritance.

TABLE 109

Tests of *novamexicana* with the western *americana*

Cross ♀ ♂	Number Tested	Percentage Fertile	Average Per Vial	Sex Ratio ♀ D	♀ L	♂ D	♂ L
$A_D \times A_L$	71	42%	29	247	315	283	314
$A_L \times A_D$	90	17	29	115	144	111	153
$A_D \times N$	96	58	34	275	412	195	378
$A_L \times N$	122	34	33	0	1,094	0	929
$N \times A_D$	36	58	—	—	—	—	—
$N \times A_L$	41	54	—	—	—	—	—
$(A_DN)_D \times (A_DN)_D$	63	92	37	637	920	856	889
$(A_DN)_L \times (A_DN)_L$	66	85	56	0	2,846	0	2,880
$(A_DN)_D \times N$	109	90	35	577	1,138	575	1,030
$(A_DN)_L \times N$	73	69	45	0	2,646	0	2,249
$N \times (A_DN)_D$	111	89	15	249	440	333	353
$N \times (A_DN)_L$	109	73	11	0	647	0	634

There does not seem to be the same type of effect in *virilis/americana* or *virilis/texana* female and male hybrids. The egg hatch is ordinarily from 50 to 60 per cent if all chromosomes are heterozygous in male or female. Table 101 shows this better than Table 103, which is further complicated by possible sperm mortality. Hybrid combinations do reduce the viability of their offspring. The results in Table 101 are not as exacting as we would prefer, owing to the reduction in egg hatch in some experiments in column eight, *R cd pe,* which possess no major *texana* or *americana* autosomes. Nevertheless, it is clear that all combinations having the *texana* or *americana* 2-3 fusion chromosome were at a decided disadvantage. This must be a genetic inviability, because the presence of a heterozygous fusion does not lead to the formation of any considerable number of inviable aneu-

ploid gametes (Table 102). With this exception, the *texana* and *americana* chromosomes seem to be able to substitute for *virilis* chromosomes in the heterozygous combinations. When crosses are made back in the other direction, the *virilis* chromosomes are much less effective in producing viable and fertile combinations, for most combinations are sterile.

We have reported in some detail on the genic balance differences in these closely related forms. When forms are so genetically different that hybrids, if produced, are sterile, there is no measure of the number of gene differences that exist. A few genes of drastic effect may prevent the production of hybrid combinations, for example, the factor in the X in *texana* that is lethal in the *montana* hybrids and the similar case in *aldrichi*-2 discussed above.

In these cases we were fortunate to have *americana* and *aldrichi,* and also fortunate that the genes were sex-linked. Such dominant cross-lethals, if autosomal, would lead to complete cross-incompatibility. There is no way of determining how species differ genetically except from these cases where hybrids are fertile. The Y-autosome system shown in *virilis-texana* crosses, and the general disadvantage resulting from recombination agree with the findings of Dobzhansky on the genetic differences determining male sterility of *pseudoobscura/persimilis* hybrids. Dobzhansky (1936, 1941) showed that sterility in the backcross hybrids between these species was caused by several genes in each of the major chromosomes.

It can be stated that, despite the phenotypic similarity between *pseudoobscura* and *persimilis,* they have different gene-balanced systems which are incapable of replacing each other in the heterozygous condition. Sterility in these hybrids is in rough proportion to the amount of the foreign gene systems, for in either backcross the nearly homozygous systems become fertile with normal-sized testes.

The variable genotypes and adaptations found in this group illustrate some very interesting species differences. Stern, Schaeffer, and Spencer (1944) tested the role of the cytoplasm in species differences. They crossed *americana* females to *virilis* males and backcrossed the hybrid line each generation to *virilis* males. A control *virilis* line as nearly genetically alike as possible was carried along for comparison. The original female was *americana;* hence the cytoplasm descended from this line and any self-reproducing cytoplasmic bodies would have been of *americana* origin. The hybrid line and

control were bred thus over twenty-three generations. At the end of this time, a cytological check revealed no residual *americana* chromosomes, in so far as this check is reliable. The species diagnostic characters of the hybrid line were compared with those of the *virilis* control. In all characters the two were alike, so that the differences between *americana* and *virilis* are genic, so far as could be measured, and are not due to any self-reproducing cytoplasmic bodies.

One comment of the similarity and difference between inversions should be made. The 2a and 2c inversions are very nearly at the same place, but differ at each end by a few bands. Heterozygous +/2ac should produce a number of gametes that are aneuploid, either hypoploid or hyperploid when crossing over occurs within the limits of these inversions. This, of course, is the type of situation which can produce duplication repeats and so increase gene number.

It is possible that the X chromosomes of the descendants of primitive III, *littoralis* and the montana complex, which have not been analyzed, might contain X ab. If so, there would be no need for a separate primitive I and II; but the sequence story would otherwise be the same, although *virilis* differs more cytologically from primitive II than from I.

The complex sex chromosome mechanism in *americana* provides us with an interesting problem in chromosome and gene evolution. The *miranda* 3 chromosome incorporated into the Y has lost many of its genes, at least in terms of function. The *americana* free 4 chromosome is also apparently male-limited, but tests have shown that some of them carry lethals while others survive homozygous. Sturtevant (personal communication), who made some of the tests, suggests that an occasional crossover in the male between the free 4 and that attached to the X might account for the normal condition of this male-limited chromosome. This is a very probable explanation, as Patterson and Suche (1934) showed that these crossovers are liable to occur most frequently near the centromere. This would replace the male-limited 4 with most genes which had been subjected to regular selection in the female where the 4 becomes homozygous. An additional mechanism that is important is the hybridization between *americana* and *texana,* and perhaps even between *americana* and *novamexicana.* This would introduce a genetically normal 4 chromosome into *americana* by introgression. The evidence for introgression between these forms can be inferred from the obvious cyto-

logical introgression, and also from the tests of the different strains of *texana* with *montana*. In these last tests, the strains of *texana* most liable to have some *americana* genes from their geographic location gave some female offspring (Table 105). The *miranda* Y-3 complex has lost much of the function of these "Y" genes, which are now taken over by other chromosomes. The *virilis-texana* or *americana* crosses show different necessary complementary gene systems, which are in some respects the same, although in these cases it cannot be proved that the autosomal genes have taken over the functions previously carried out by genes in the Y.

An interesting selective system can be illustrated by the male replacement experiments (Table 90). In this test we can tell the effective sperm by the color of the pupae and the effectiveness of the mating by comparing the number of pupae with the number of eggs. The insemination reaction is not extensive in this group, and the table shows that the last male to mate will determine what type offspring is produced. Cross-mating will reduce the reproductive efficiency of a population. This would provide a mechanism for selecting factors that would decrease the *virilis* females' willingness to mate *texana* or *americana* males. If *virilis* has been in contact with *americana* and *texana* to any appreciable degree, this type of selection has been ineffective. The *virilis* females mate with the other species readily, but *virilis* males are almost completely indifferent to them.

The relationships between the species of the virilis group found in the United States have been tested most extensively. In the Nearctic the populations of these species are small, with isolation by distribution and distance, for they are usually located along streams or around lakes, except the domestic *virilis*. Even so, they occupy a diverse series of habitats, from the desert stream banks of *novamexicana* to the aspens around the lakes of Minnesota frequented by *borealis* and *lacicola*. There is every opportunity in these populations for local adaptation and for the fixation of unusual genotypes in the small populations. These are reflected in some phenotypic variation between strains, but even more in the genetic differences that determine the isolating mechanisms. This last type of physiological difference between strains is most easily measured in the laboratory. Spieth's test on sexual isolation of the different strains and the numerous tests given in the Tables 83 through 109 reveal all grades and variations in combinations of different genes which

affect sexual isolation, sperm mortality (*virilis* × *novamexicana* compared with *virilis* × *americana* or *texana*), or hybrid fertility. The heterosis tests also show that these different local strains are in effect slightly different balanced gene systems. The systems differ in ways that do not cause important differences between strains of the same species, but show in crosses to other species as variations in isolating mechanisms. The extent of the difference in gene systems increases markedly as we go from closely related species to more distantly related ones. There are many well-demonstrated types, combinations, and gradations of differences within this species group.

11 COMPARISONS AND CONCLUSIONS

We have found it necessary to use two definitions of a species. One is Darwin's definition, which places emphasis on the ability and knowledge of the naturalist who decides the form is a separate species. This definition is necessary in studying a large genus such as Drosophila because it is impossible to test all the forms by crossing them, since many of them do not breed in the laboratory or are unavailable. The breeding tests with Drosophila have shown that forms taxonomically described as species usually prove to be separated by isolating barriers in breeding tests. This is due to the fact that Drosophila systematists have usually been conservative in naming species as well as to the relation between morphological changes and separate identity which ordinarily seems to exist. In only a few cases are we convinced that forms called separate species are indeed subspecies. On the other hand, such conservative taxonomic identification of species must necessarily miss sibling species whether these are sympatric or allopatric, for these can be demonstrated only by breeding tests. The biological classification of species, of course, takes precedence where breeding tests are possible. In sexual forms the species is the basic separate unit, for evolution in one species cannot provide the majority of genes that determine evolution in another, except as its ancestor. In fact, the definition of a species as an evolving unit must be based on the concept that the gene systems available in the species as they exist or occur by mutation or recombination are responsible for its further existence and evolution. This does not preclude some hybridization and gene exchange, which may benefit a species, but such donations of genes from another form cannot dominate its evolutionary progress. Our definition of this basic unit is essentially similar to Wright's (1940a):

The ideal has been to apply the specific name to groups within which all subdivisions interbreed sufficiently freely to form intergrading popula-

tions wherever they come in contact, but between which there is so little interbreeding that such populations are not found.[1]

It is also similar to Mayr's (1942):

Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups.[2]

Our own definition is a slightly different statement with similar implications, viz.: In sexual forms, a species consists of the members of a population or group of populations which can exchange genes freely with each other, but which can exchange genes with members of no other form (or population) sufficiently to lose their separate genetic identity. This is to say that the genes and combinations of genes which determine evolution are provided by mutation and recombination within the species, and other species do not furnish sufficient different genes to determine or control their evolution.

Clausen, Keck, and Hiesey (1945) have stressed the importance of genetic relations along with other criteria in their definitions of biosystematic units. Their definitions of terms, which are based on those of Turesson and Danser, are defined and discussed (pages 62 to 69) where they develop their concept of modern biosystematic tests. We include these definitions:

(1) "*Ecotype* (Turesson, 1922a, 1922b), all members of a species that are fitted to survive in a particular kind of environment within the total range of the species." These are genetically and physiologically distinct ecologic races.

(2) "*Ecospecies* (Turesson, 1922b, 1929), all the ecotypes genetically so related that they are able to exchange genes freely without loss of fertility or vigor in the offspring." These correspond in general to the conservative morphological taxonomists species, but of course would detect sibling specific differences. They are the essential units with the genetic balanced systems which largely determine their own evolution, although they may profit by recombination from partly fertile hybrids with other ecospecies. This is essentially equivalent to the biological species definition given above.

(3) "*Cenospecies* (Turesson, 1922b, 1929), all the ecospecies so related that they may exchange genes among themselves to a limited

[1] Wright, S. *The New Systematics,* page 162. Oxford University Press. London. 1940.

[2] Mayr, E. *Systematics and the Origin of Species,* page 120. Columbia University Press. New York. 1942.

extent through hybridization." Partially fertile hybrids provide an avenue of gene exchange between the ecospecies in one cenospecies.

(4) "*Comparium* (Danser, 1929), all the cenospecies between which hybridization is possible either directly or through intermediaries. Gene exchange between different cenospecies in a comparium is impossible."

The authors are emphasizing the importance of a functional gene complex, in this case of a lower order leading to viability but not fertility of the hybrids produced. They point out that hybridization leading to allopolyploids can occur only between forms within a comparium, and on this basis point out certain discrepancies in current taxonomic classifications of certain species. They have used these criteria in developing some interesting relationships in the genera *Madia* and *Layia.*

In plant evolution, it is possible to have parent species and their different descendant species for comparison in the form of polyploids, either autopolyploids or allopolyploids, from hybridization followed by doubling of the chromosome sets. The descendant species may be completely unable to exchange genes with the parent forms, although their origin and relationships may be demonstrated by experimentally repeating the process, as has been done by several investigators, e.g., Clausen, Keck, and Hiesey (1945). We do not have the parent and descendant species to compare in animals, even though some species may seem primitive in many respects. This form of instantaneous species formation is exceedingly rare in animals which have few polyploids, especially among the sexual forms. Nor do we believe that species of animals are ordinarily formed by a master mutation or saltation, although this view is held by Goldschmidt (1940) and also by Willis (1922, 1940). If we refuse to accept the theory of sudden origin of new species through a master mutation, we must provide evidence for other mechanisms, even though we cannot hope, on the basis of our theory of gradual change through mutation and selection, to demonstrate the evolution from one species to another in the laboratory. We must prove our theory, not from the existence of all stages in one form, but rather from the data from many forms. If evolution is occurring, there should be all stages and all degrees and combinations of types of divergence represented in a large evolving group. In our opinion, the data referred to and discussed in the preceding chapters show that there are present in populations the types

of inherited variability that exist between species. Furthermore, there are available various types and combinations of isolating mechanisms that may restrict gene exchange, so that differential and separate adaptations can and will occur in response to the diverse available environments for living forms.

White (1949) has discussed evidence concerning the general relationships in the Diptera. Sturtevant (1942) and Wheeler (1949) have discussed the genera closely related to Drosophila. We have restricted our discussion to those forms now considered members of the genus Drosophila.

This genus as tabulated comprises 613 species. The eight subgenera into which the genus is now divided are justifiably separate categories, according to Sturtevant, Wheeler, and Patterson. Members of the small subgenera have been very slightly studied genetically, except for *busckii,* but most of the major species groups in the subgenera Sophophora and Drosophila have been investigated sufficiently so that we have some test of the validity of our more detailed classification. The species groups were established mainly on morphological similarities. They represent real subdivisions on this basis and are subdivisions in the sense that all hybrids between different species have been produced between members of the same species groups. The only case suggesting that exceptions might occur is in the Sophophora, for *melanogaster* and *pseudoobscura* mate without producing hybrids, although they are members of different but closely related species groups. Species groups must represent the more recent splitting of one or a few very successfully adapted species into descendant forms.

The general systematic phylogeny is based on Sturtevant's (1939, 1942), with a few modifications and additions adapted from Hsu's (1949) study of the male genitalia and Wheeler's (1949) and unpublished studies. Some considerations of the food habit in groups have also been taken into consideration.

The genus centers around *pinicola,* and all the subgenera except Pholadoris tie into the pinicola-virilis primitive stem. We have added species groups to the large subgenus Drosophila, which is modified slightly from Sturtevant to separate the two general ecological groupings, the sap-feeders and the fungus-feeders. The main stem of Drosophila probably gave rise through the virilis stem to two lines; one through the repleta stem remains sap-feeders, the other through the quinaria stem includes the fungus-feeders. In each stem there are

species which are much more generalized (*repleta, hydei, funebris*) and others which are specialized. The most interesting case of specialization is that of the repleta group. These forms have made a new major adaptation for the genus—desert adaptation. The single closely related species *carbonaria* remains a sap-feeder, but is adapted to the sap of the desert mesquite tree. The repleta group is a tremendously interesting group as an example of the explosive speciation that occurs as a new habitat is made available by some major adaptation. It is therefore no accident that this group is the largest known in the genus and, in fact, has been subdivided into closely related subgroups in which hybridization occurs. No other species group in Drosophila is known to be desert-adapted. The Sophophora includes only *pseudoobscura,* which lives in some of the desert regions but is not distributed all through such regions of the United States and Mexico, as are members of the repleta group. Members of the repleta group are very often found associated with cacti, and the distribution of these desert succulents must determine the distribution of many species of this group. In South America, Dobzhansky and Pavan (1950) found five indigenous species in the semi-desert "caatingas," and one of these was a member of the repleta group.

We cannot give much information on the rates of evolution in the genus, nor on the time of origin of a species. The repleta group of some fifty species may be very recent, while *busckii* may be very old, it being a monotypic representative of a subgenus. We are particularly constrained to caution because of the relationships indicated in the virilis group, which is close to the primitive stem of the subgenus Drosophila. The genetic and chromosome analysis indicates that it contains four subgroups: *virilis,* which remains nearly primitive; the American subdivision *texana, novamexicana,* and *americana,* which has developed both genetic and chromosomal differences; the European *littoralis* (and *imeretensis*), which so far is not well analyzed but has evolved considerably from the primitive stem, both in genotype and through chromosomal rearrangements; and the American montana complex, which has evolved from the primitive stem into several species, with very extensive gene sequence changes in the chromosomes.

We can offer a formal explanation for the differences in rates of evolution in this group based on the present distribution of the species. The species *virilis* has a large widespread population in Asia, where it

is one of the successful forms. The populations of this species in America are very localized and are associated with human habitations, so that they may have been recently transported by man. As evidence to justify this opinion, Wheeler found *virilis* on a ship plying between Australia and California. Some American strains do show local genetic differences such as might be fixed in small isolated populations. Although *americana* and *texana* have a wide distribution, they have a sparse population; and *novamexicana* too has a very small, thin population, as far as the collections sample it. The montana complex in the Rocky Mountains is widely distributed, but at very scattered localities. It may well be that the ecological adaptations and the population distributions of these forms will account for the difference in rate of exchange and separation to give diverse strains, subspecies, and species. If the present population distributions are of long standing, we would expect the large *virilis* population to evolve slowly and the broken montana complex to evolve and produce diverse forms much more rapidly. The actual factors which have determined evolution in this group are undoubtedly more complex than indicated in this simple explanation.

The division of a genus into subgenera and these into species groups or somewhat equivalent subdivisions has been practiced in many genera. M. J. D. White is of the opinion that the species groups now recognized on morphological characters in the genus Melanoplus are genuine. Goodspeed (1945a, b, c) divided the genus Nicotiana into three subgenera, which in turn are divided into sections. The taxonomic relations were established on morphological grounds, together with chromosome number and genetic similarity measured as crossability. In some ways the division is equivalent to our treatment of Drosophila, although there is hybridization between different sections, so that the criteria of separation are not wholly similar. Hutchinson, Silow, and Stephens (1947) do not divide Gossypium into subgenera but into eight sections (or species groups) on distribution, genetic tests, cytology, and especially morphology. Here again there is some hybridization between sections. Babcock (1947), in his large monograph on the genus Crepis, stresses the fact that taxonomy must rest on the broadest possible foundation of biological disciplines. He does not divide the genus Crepis into subgenera, but instead into twenty-seven sections corresponding roughly to the species groups of Drosophila, and he also gives a tentative phylogeny of Crepis. It has not

been practical to subdivide this genus on the basis of crossing, as suggested by Clausen, Keck, and Hiesey. The species have been classified using morphological, genetic, chromosomal, and ecological criteria.

The genus Rubus, as characterized by Gustaffson (1943), presents an interesting comparison to these sexual forms in that it is composed of a few diploid sexual species and many polyploid apomictic "circle species." Gustaffson regards as most useful and usable a classification which consists of subsections divided into series. Despite the obvious necessary differences between this type of evolutionary system and the regular diploid system, the classifications possess some interesting similarities to the foregoing types of divisions. Even though the sexual phases are often only temporary, these apomicts still represent a graded series of genetic similarities and differences that allow a reasonable grouping.

The genus Peromyscus has been extensively and intensively studied in recent years by Sumner, Dice, Blair, and others, yet the taxonomic treatment of the genus by Osgood (1909) remains the standard. The genus is divided into subgenera, which in turn are divided into species groups. These categories are similar to those in Drosophila, since no hybridization occurs between species in different species groups (Dice, 1940b). Osgood had a very large collection from all over North America and developed his morphological taxonomy with sufficient understanding of the variation and ecological distributions so that only a few revisions have been necessary.

Clausen, Keck, and Hiesey (1939) discussed their concept of species relations based on various types of evidence. They state: "The writers have performed extensive experiments on several plant groups during a series of years, viewing the problem from morphologic, geographic, ecologic, cytologic, genetic and to some extent also physiologic angles. The largest body of evidence has been obtained from the Madiinae, a West American subtribe of Compositae. Out of some 400 hybrid combinations attempted involving seventy species of six genera, about 200 succeeded, and the others were nonviable or complete failures." These studies lead them to the following species concept: "Plants are organized into groups, the members of each of which are able to interchange their genes freely in all proportions without detriment to the offspring. Such groups are separated from one another by internal barriers that are of a genetic-physiologic nature (including

chromosomal barriers) that prevent such free interchange. These groups correspond fairly closely to the species of the moderately conservative taxonomists working with plants that reproduce sexually." This species concept is essentially the same as that given by Wright, Mayr, and ourselves.

Blair (1943) considers that it should also apply, together with the concepts of ecotype, ecospecies, cenospecies, and comparium, to the vertebrates among animal materials. So far no animal genus has been so subdivided. The situation in Drosophila illustrates some practical difficulties. In this genus the separate species groups are inclusive enough, for all comparia will fall within species groups. There is, in fact, no hybridization between members of separate species groups. As an example, the repleta group is a unit in evolution, even though it would comprise several comparia. We could perhaps call it a subgenus, but this would involve many other difficulties. Another example which presents complications is the willistoni group of the Sophophora, where there are four sibling species now known. These species are so similar morphologically that they are nearly impossible to separate. Cytologically they are sufficiently alike so that homologous chromosomes and chromosome regions can be recognized in the salivary gland nuclei, yet they would fall into four comparia. This type of practical difficulty has caused us to use other criteria in addition to hybridization in establishing groups, but we have given information in Chapter 9 which would allow one to make this type of classification where evidence is available.

One of the remarkable characteristics of the genus Drosophila is the fact that slightly over 90 per cent of the species are endemics, found in only one zoogeographical region. This must be contrasted with the fact that every region has a number of species present (the low number known from the Ethiopian is undoubtedly due to lack of collections from the most favorable areas). To insure that this is not an artifact due to inadequate collections, we can consider the Nearctic and Neotropical regions which have been more extensively collected even in the zone of contact between them. In this case over 83 per cent of the species are endemic. Another evidence of the local development of most of these forms is the fact that only ten of the 613 species are known from more than half the regions, and eight of these are the world-wide forms that can associate and be readily transported by man. The species groups are restricted for the most part to one or

two regions, except for these eight wide-ranging forms. Some, like the virilis, obscura, and melanica groups, are primarily Holarctic; others, like the willistoni and repleta, are primarily Nearctic and Neotropical. The genus may be characterized as being restricted in motility; as being capable of divergent evolution from a few species into large species groups in single regions, with the repleta group as an example of explosive evolution at the species level when a new habitat has been successfully invaded; and as forms which can follow their environmental niche where it is available (for example, *limpiensis* and the quinaria species, which follow the fungus on willow out into the desert along desert streams). The intricate nature of coadaption, enabling related forms to live in the same general habitat, is well illustrated by Wagner's finding that the closely related species *mulleri* and *aldrichi,* although living together in cactus, have different food requirements. This difference extends to other members of this subgroup. In this mulleri subgroup, among the forms associated with cactus and occurring together at the Aldrich Farm outside Austin, Patterson (1943) showed that *longicornis* and *hamatofila* have a spring population peak, while *mulleri, aldrichi,* and *meridiana* have fall population peaks, thus affording seasonal isolation between closely related species which make use of the same general habitat.

In addition to the desert-adapted repleta group, we have several interesting species groups from the standpoint of clines (Huxley, 1939). We might regard the distribution of the forms *novamexicana, texana,* and *americana* as representing a cline (Figure 23), but the cytological relations and possible dual origin of *americana* from the other two make it a special type. Perhaps the *macrospina, limpiensis, trispina,* and *subfunebris* distribution (Figure 25) is a simpler case. Here we have a genetic gradient showing increasing difference with distance east to west. There are various degrees of sharp changes in genetic differences; that involving the subspecies *macrospina* and *limpiensis* is definite but less extensive than that separating *limpiensis* and *trispina,* and isolation is especially strong in the separation of *subfunebris* from the others. These cases resemble those between *Peromyscus maniculatus* and the more peripheral species *polionotus, sitkensis, melanotis,* and *sejugis* (Blair, 1950). In the cases in Drosophila the smaller peripheral species are not necessarily derived from the large species. So far as we can tell, *subfunebris* might have been the original form from an ancestor closer to *funebris,* and the others

derived in turn beginning with *subfunebris*. In the *virilis* series *novamexicana* is not derived from *texana* or *americana,* despite its small peripheral population; indeed the reverse relation for the subspecies *americana* may be the case.

The Drosophila clines and the distributions of isolating mechanisms, sometimes correlating increased isolation with increased separations, in other cases showing an irregular pattern of isolation, in many respects resemble the cline of sex races in *Lymantria dispar* (Goldschmidt, 1934).

We know comparatively little about the ecology and reproduction of Drosophila in the wild; nor do we know very much about their reproductive cycles in relation to their seasonal population peaks. Some interesting examples of reproductive adaptation are known. Buzzati-Traverso (1943) found that the adults of *Drosophila nitens* suspended reproduction from November to March, even in a laboratory held at 25° C. Bertani (1947) was able to break this reproductive diapause by subjecting the adults to temperatures ranging from 2° to 5° C. for ten days. Carson and Stalker (1948) found that *robusta* had a somewhat similar mechanism. Females of these flies collected from their natural habitat near St. Louis, Missouri, from March 5 to September 3 were from 69 to 85 per cent fertile; those collected September 3 to December 10 were from 0 to 4 per cent fertile. Dissection of females caught in the wild in late October showed that about 3 per cent were fertilized. These females contained large fat sheets and had reduced ovaries, presumably as a regular winter diapause mechanism. These authors were unable to break the diapause by cold shock, but laboratory stocks established from breeding females did not go into this winter diapause but bred continuously.

The great number of endemic species in Drosophila raises the question of where and how these species arose. We have some information indicating the effectiveness of certain factors in their formation. It has been pointed out that there exists in Mexico a narrow zone, roughly between 19° and 22° north latitude, which yielded eighty-nine species (Table 19). This is most remarkable, for the region is small and has not been intensively collected by our laboratory crew. This is the region of the transition zone between the Nearctic and Neotropical regions, but the area included is more extensive. As these samples were collected only in the summer, species present in appreciable numbers at different seasons may have been missed. This

region contains about twice as many species as Texas, which has been collected extensively, and even larger ratios to the numbers of species found in California or in Ohio. This is also a much greater diversity of forms than that recorded by Dobzhansky and Pavan (1950) from the most favorable localities in Brazil, the rain forests.

There are two hypotheses that might explain the concentration of species in that region: (1) the inclusion of migrants and relicts; and (2) migrants and new local forms. We believe the latter hypothesis is in general correct. The general distribution and high percentage of endemic species argues for many young species rather than relicts. The area is one that connects the temperate zone with the tropics. However, this is not a region of gradual gradation in habitat; rather it is one of varied and extremely broken and mixed habitats, with many types of ecological niches close to each other in all sorts of mixtures. This is not just a hybrid habitat, to borrow a term used by Anderson (1948), but rather a mixture of various size segments of both pure and hybrid habitats. This region is remarkable, not only for the distribution of Drosophila species which are endemic, but also for the many clines of species which connect into it (Figures 20 to 22 and 26 to 33). Another example is the branched chains of species complexes of the Cynipidae studied by Kinsey (1937), which usually tie into this region.

In our opinion, this is a region which has fostered the origin of new species. Wright (1931, 1932, 1940a, b, 1948, 1949) has developed our concept of the interaction of the numerous factors that enter into evolution. Wright (1931) pointed out that the most favorable species population structure was one with a broken distribution connected by migration, with local populations of intermediate size. Two recent statements are quoted to emphasize certain factors. Wright (1948) states:

> Conditions are held to be most favorable for a continuing process where there are certain states of dynamic equilibrium at many loci. This situation is found to the greatest degree in a population in which there is sufficient isolation of many local centers of population growth and emigration to provide the condition for continual trial and error. The errors are the relatively indeterminate elements in the situation: novel mutations at the gene level, and the effects of accidents of sampling, and of variations in the local conditions of selection and migration at the level of the local population. The trials are the orderly reactions of the species to changes

of gene frequencies, due to more determinate factors, of which selection dominates in the long run. Where there are important secular changes in external relations, selection pressure dominates the situation almost completely until a new state of approximate adjustment is reached and trial and error processes again take over.

Wright (1949) further states:

Perhaps the most important conclusion from statistical genetics is that neither the driving factor nor the actual limiting factor in evolution is ordinarily to be sought in the genetic situation (including the process of mutation) once the stage of the interbreeding species of multicellular organisms has been arrived at. The driving force is the universal tendency of life to expand and to seek out all opportunities. The limiting factor in each case is the ecologic pressure from other species and from the nonliving environment, by which the species is ordinarily kept in place. The genetic situation in an interbreeding species with a finely divided population structure provides a virtually infinite field of potential variability which permits ready adaptation on the part of the species to fluctuating environmental conditions, ready phylogenetic adaptation to secular changes in conditions, effective exploration by trial and error processes of possibilities of breaking through the restraining pressures even under static conditions, and the rapid exploitation by similar means of any major ecologic opportunity.

We must remember also that all types of genetic isolating mechanisms occur often in local populations of Drosophila. These relations explain the rapid evolution and extensive origin of species in this local region.

Species in or entering this ecologically varied region will be established as small populations or series of small-to-intermediate populations with migration between them. As genetic divergence occurs in a particular locale, the variant may replace the original as such; or, because of the multiple adjacent environments, it has also the opportunity of finding an ecological niche to which it is suited. In such broken populations, the testing of new combinations within an environment and the migration of successful combinations to the other subpopulations will lead to their continuing evolution. This process may well have afforded the ancestral repleta forms the opportunity to make the major adjustment to desert existence, using the microorganisms and other nourishment available on the succulents. The distribution of the members of this group are in agreement with the

idea that many of the forms radiated out from this region. This process will also tend to improve and stabilize any new isolated form that arose. With the broken distribution of similar habitats and consequent reduction of migration, a Drosophila form could easily acquire mutations which accumulated to isolate and separate this form from subsequent migrants. The high incidence of different sexual isolating factors in the repleta and virilis groups are particularly suitable for this type of isolation. The occurrence of cross-lethal factors, such as those in *aldrichi* 2 and *texana* that depend on coexistence of a different balanced gene system, could also be a factor, for the divergent evolution which occurs in semi-isolated populations would provide such different gene systems. There may also occur changes due to drift in small populations that, in conjunction with the complex variability of habitat, might provide a source of change. As there exist graded changes as well as abrupt ecological differentiation, semi-isolated populations might have made some adjustments that would start them to fit a slightly different environment, and from this they could gradually move across the inadapted valley up the slope to another peak genotype. If a segment of this new population remained isolated for a time, the accumulation of isolating factors could lead to separation even if it came back into contact with parts of the original population. Local and general fluctuations in this complex mixture of environments must exert pressures that sometimes reduce connections between partially divergent forms and at other times eliminate new or old types entirely. One evidence for the effectiveness of adaptive specialization is the fairly large number of diverse species from this region which breed poorly or not at all in the laboratory. Many of them may be restricted to this region because they have been selected to fit some environment in this hybrid habitat. The problem of rare species has been discussed by Stebbins (1942).

This region is one of such broken and diverse environments that it allows semi-isolated populations to cross the valleys to test the many adaptive peaks represented by a certain group of genes, sometimes fixing isolating mechanisms while isolated because of ecological distribution or because segments of populations are wedged apart by competitor species and thus they can diverge into separate species.

In order to properly evaluate the study of chromosome evolution in Drosophila, it is necessary to consider the information available from other genera of animals and plants. Makino (1950) published

a review of the chromosome numbers in animals. This gives us information on the variation in chromosome numbers in genera, and we have selected some of these for comment. We have tried to select genera where enough species have been determined to provide a satisfactory sample. In the Platyhelminthes, the ten species of Dalyellia have N = 2. On the other hand, among the Arthropods, the Copepod genus Cyclops with twenty-three species has a range of from N = 2 to N = 12, with the largest number of species at N = 7. The distribution is interesting: one (N = 2), two (3), two (4), two (5), three (6), seven (7), three (9), two (11), and one (N = 12).

The Arachnida tend to have very constant chromosome numbers. Among the Araneida we can point to eighteen families studied by Painter (1914) and particularly by Hackman (1948) which illustrate this stability. In these families the chromosome number varies from N = 11 to N = 15, with peaks at N = 12, N = 13, and N = 15. Some families are quite stable, for the seven genera represented in the Drassidae all have N = 12. There is an exception in the Argiopidae, for *Aranea diademata* has N = 8, owing to five separate centric autosomal fusions. This is particularly interesting, for, as Hackman pointed out, almost without exception the autosomes are rods (acocentrics) in the sixty-nine species he investigated. In fact, the closely related species *Aranea foliata* has all acrocentrics. The more normal complement illustrated by this latter species is N = 13 (eleven acrocentric autosomes, X_1 and X_2), while *Aranea diademata* has N = 8 (five metacentric fusion autosomes, one acrocentric autosome, X_1, X_2).

The other exception is *Dictyna arundinacea,* with all autosomes V's (metacentrics). The number of chromosomes was not determined to Hackman's satisfaction, but is given as N = 13 (?) = 11 (?) metacentric autosomes, X_1, X_2. The very interesting X_1, X_2 2A ♂ — X_1 X_1 X_2 X_2 2A ♀ system of sex determination has no counterpart in Drosophila among the few X O forms. The X_1 and X_2 are heteropycnotic in the male, but X_1 X_1 and X_2 X_2 form ordinary bivalents with chiasma in the females where they stain normally. As there are no heteropycnotic or obviously heterochromatic arms in *Dictyna arundinacea,* we presume the autosomal metacentrics are due to pericentric inversions. There are a few other families which differ in chromosome number; N = 18 to N = 23 in Agalenidae, and N = 23 in Atypidae, as well as N = 40 in Liphistiidae. The chromosome

numbers are capable of modification in the Araneida, but this seldom occurs.

Numerous types of insects have been investigated, but we will refer to only a few examples beginning with the Orthoptera. White (1951) has recently reviewed the cytology of many of these forms, and we shall use a few of his examples. The type of variation resembles that in Drosophila, although genera and even families may be much more stable. For example, the common species of six genera of the grouse locusts Tetrigidae all have N = 7, for 2N = 14 ♀, 13 ♂ (XO); all chromosomes are rods (acrocentrics). This includes *Paratettix texana,* where Nebours has demonstrated some very interesting genetic situations.

The family Acrididae is divided into two sections, the Chasmosacci, with the basic chromosome number of its subfamilies 2N = 19 ♂, 20 ♀, and the Cryptosacci, with the basic chromosome number of its subfamilies 2N = 23 ♂, 24 ♀. Since all the chromosomes are rods (acrocentrics) in the majority of the species in each section, and since there are no higher numbers (supernumeraries are not counted) within the sections, White regards these as the primitive configurations. Reduction in numbers within sections has occurred, ordinarily by fusion, as suggested by Robertson (1916), so that some subfamilies (genera) have one fusion, others two, others three (for example, six genera of the subfamily Truxalinae), and others five (for example, *Philocleon anomalus,* which has 2N = 12 ♂, 12 ♀ because one fusion is X-autosome, with the homologous autosome acting as a Y).

Since a number of these forms have reduced the chromosome number below the basic number, yet have all rods, we must assume that such acrocentrics have a selective advantage and that the compound chromosomes formed when such reductions of chromosome numbers occur are similar to the double-length rods of *Drosophila tranquilla* and *Drosophila spinofemora.* The situation in spiders suggests that the situation there is similar.

The orthopteroid insects are not all alike in cytological flexibility. Thus almost or all the species of the large genus Melanoplus have a nearly identical chromosome complement; the cricket genus Nemobius ranges from N = 11 to N = 19; and three species of the genus Isagoras had N = 27, N = 34, and N = 47. In fact, there are several chromosomal races (or sibling species) in the taxonomically single species *Gryllotalpa gryllotalpa* L, which varies with forms having

2N = 12, 2N = 14, 2N = 15, 2N = 18, 2N = 19, and 2N = 23. Another species showed variation in chromosome number of an analyzable kind. *Hesperotettix viridis viridis* ordinarily has a free X chromosome, but populations from Marathon, Texas, showed an X-autosome fusion and may have one or two autosomal fusions present in the heterozygous or homozygous condition (2N in ♂ varies from 18 to 23, with occasional supernumeraries).

White has been particularly interested in the three trimerotropine grasshopper genera, Trimerotropis (with forty-six species), Circotettix (with seven species), and Aerochoreutes (with one). For these grasshoppers there has been recorded one mutual translocation by Carothers. In addition, there have been fusions, numerous species with supernumeraries and added supernumerary (heterochromatin) regions, and "centromere shifts." The mode of origin of these last abnormalities has not yet been determined satisfactorily. They may be centromere transpositions due to three-break rearrangements, since it is just possible that these grasshopper forms have a mechanism related to the odd and remarkable breakage-inducing system present in maize, as described by McClintock (1950). However, they may also be pericentric inversions, such as those in Drosophila, either simple or in compound with paracentric inversions. White is of the opinion that paracentric inversions are uncommon in grasshoppers, but certainly short paracentric inversions might be one cause of the marked localization of chiasmata often encountered in the Orthoptera.

Thirty-three species in the genus Trimerotropis have been studied. White divides the genus into section A, with twenty species having 2N = 23 ♂, all acrocentrics, and section B, with thirteen species, all of which possess some metacentrics. In section B ten species have 2N = 23 ♂, but three species have two races which differ by a fusion, the odd race having 2N = 21 ♂ in each case (White, personal communication). The species of Aerochoreutes and Circotettix are B type, as they possess some metacentrics. The four species studied of the latter genus have 2N = 21 ♂, except one population of *Circotettix undulatus,* which has 2N = 23 ♂ as well as heterozygotes.

Supernumeraries, both ordinary rods and V-shaped isochromosomes with duplicate arms occur in both sections of Trimerotropis. The orthopteroid insects are interesting in that there are several parthenogenetic forms. In fact, one of them, *Saga pedo,* is also a tetraploid, without a regular meiosis.

Among the Heteroptera twenty-eight species of five genera of Corixidae are restricted to N = 12 or N = 13, and among the Neuroptera the twenty species of the genus Chrysopa have the range N = 5, 6, or 7. In the Lepidoptera, fifteen species of five genera of the Lycaenidae have a range of N = 23, 24, 25. The twelve species in the genus *Argynnis* of the Nymphalidae are interesting in that eleven have N = 28, 29, 30, or 31, but one has N = 12, 13 ♂; 13, 14 ♀. Among the Coleoptera in the Curculionidae, there are thirteen sexual diploid species in the genus Otiorrhynchus, N = 11, and seven triploid parthenogenetic species, 2N = 33.

A situation that is of considerable interest in animals exists in the Diprionidae, where one species of the genus of *Diprion* has N = 14 (N = 14 ♂, 2N = 28 ♀), while the other six species have N = 6 or N = 7 (Smith, 1941). In the Chironomidae, with over twenty species, only N = 3 or N = 4 are found, while in the Culicidae the six species of *Aedes* and the seven species *Culex* all have N = 3. The genus *Sciara* of the Sciaridae has six species listed. Despite the odd chromosome mechanisms found in this genus, the only chromosome numbers are 2N = 8 or 2N = 10 (N = 5 or 6 in ♂). The examples given above show considerable stability in the chromosome numbers within these genera of insects.

The chromosomes of vertebrates have been reviewed by Matthey (1949), who has discussed the evolutionary relationships at some length. Both authorities have been employed as sources for the following information. Among the fish, two families of the Teleostomi have been investigated in some detail. The Ciprinodontidae include the related genera Platypoecilus (4 species) and Xiphophorus (three species) which have chromosome numbers of N = 24 or N = 25. In the Salmonidae, the genus Salmo includes eight species which have a fairly wide range of numbers, N = 30, N = 35, N = 40, N = 42, and N = 48. As *Salmo salar* (N = 30) crosses with *Salvelinus fontinalis* (N = 40) to produce hybrids (2N = 70), the differences in number probably are reorganizations rather than added chromosomes.

Among the amphibia several genera of the Urodeles have been investigated. These include thirteen species of Hynobius, where ten species have N = 28, one is given as N = 28 or N = 30, one has N = 29, and one has N = 20. In the family Salamandridae, two related genera Triturus (10 species) and Triton (10 species) all have

N = 12. It is interesting to note that polyploids have occurred with sufficient frequency to afford opportunity for considerable study in both of these latter genera. In the Anura, all fourteen species of Bufo have N = 11, while twelve species of the genus Rana have N = 13, but one additional form has N = 12. Two additional genera with one species each in the family Ranidae also have N = 13.

The reptiles seem to be fairly conservative in changing chromosome number; at least most genera reported by Matthey consist of species which do not differ markedly, although the number found in different genera in the same family may differ rather widely. As an example of constancy, the species in the several genera of Lacertidae have N = 18 or N = 19. Several interesting features exist in the reptiles. Matthey points out that chromosomes can often be divided into macrochromosomes and microchromosomes. Any family or genus may be characterized by a certain number of macrochromosomes and microchromosomes; for example, *Agama stellio* has 2N = 36 (12M + 24m), while *Lacerta muralis* has 2N = 38 (36M + 2m). The microchromosomes may or may not be equivalent to the dot chromosome found in most species of Drosophila, which is a rod in metaphase resulting from added heterochromatin in some species. Matthey showed that an interesting situation existed in *Gerrhonotus.* The chromosome formula of 20M + 24m might exist in three combinations: (a) 18R + 2V + 24m; (b) 20R + V + 24m; (c) 22R + 24m. This would imply a fusion difference as the more probable explanation.

The high chromosome number in birds, as well as the great number of microchromosomes, makes a reliable determination of their chromosome numbers difficult. The more recent counts making use of the better techniques give chromosome numbers in the family Columbidae with ranges from N = 38 to N = 41. This group is interesting in view of the gene differences in inherited antigenic characters demonstrated by Irwin and his coworkers. In the Anatidae, the more reliable recent work indicates that most species of Anas and several related genera have numbers ranging from N = 38 to N = 42, and it may well be that many genera in the Phasianidae have the same range.

The mammals possess a considerable range of chromosome numbers. In the marsupials, four species included in two genera of the Didelphyidae have N = 11. The rodents include genera with several

diverse chromosome numbers. In the Cricentinae of the Muridae, evolution in the genus Peromyscus has been investigated in a number of other ways which increase our interest in the evolution of the chromosomes. Seventeen species or subspecies of the genus Peromyscus have N = 24 to N = 29. Another species in this family, *Tscherkia triton,* has N = 15. This latter figure is also found in the Microtinae. The genus Microtus has a chromosome number range from N = 15 to N = 28. This genus seems to fall into two groups, one at N = 15, a second around N = 25. The genus Apodemus of the Murinae has numbers from N = 23 to N = 25. Microtus shows the major variation encountered here. Several species of the Muridae have an X-O type sex chromosome in the male.

We will comment only briefly on chromosome evolution in plants, except for certain special cases. Darlington and Janaki Ammal (1945) published a very extensive list of plant chromosome numbers. In his introduction of their volume, Darlington has commented on the types of variation encountered. Myers (1947) reviewed the cytology and genetics of forage grasses and discussed in detail the relations in this group.

The vast majority of genera of plants do not show the type of variable numbers found in Drosophila, but very often have diploid and polyploid series which are multiples of the basic number. In these polyploid series, we often find some deviation from the multiple of the base number. Usually this is due to loss or fusion, and so the chromosome number is slightly lower than expected; occasionally it is due to addition and is higher.

A few examples of the types of variation found in gymnosperms are tribe Abieteae, genus Pinus, twenty-two species, N = 12, and no other numbers are recorded. In this tribe there is one species, *Pseudoloxix amabilis,* with forty-four chromosomes, a tetraploid based on N = 11, and one species *Pseudotsuga taxifolia* with N = 13, which represents a gain of one chromosome. Five other genera each with several species analyzed have N = 12.

In the Dicotyledons are found similar stable situations, but there are exceptions, and in the following examples we give a great disproportion of the variable forms to show the comparisons to Drosophila. Among the Ranunculaceae the genus Paeonia has a regular series based on N = 5 with twenty-three diploid, nine tetraploid, and four species which have both diploid and tetraploid strains. The

genera Clematis (thirty-four species) N = 8 and Thalictrum (forty-three species) N = 7 are essentially similar, except that the latter has higher polyploid forms also. Ranunculus is interesting in having forms with base numbers 7 and 8. Furthermore, four species have strains that are 2N = 14 and others that are 2N = 16. One species has strains with the somatic numbers 16 + 2 fragments, 24, 32, 40, and 48.

In the Cruciferae, the genus Brassica has several species with base numbers 9, 10, 11, and 12, as well as some allopolyploids of these and an N = 8 form. The Violaceae is represented by the genus Viola, which has a large number of species based on each of the following haploid numbers 6, 10, 11, 13, and in addition has some compound allopolyploids. A more stable series, showing no reorganizations except polyploidy, is found in the genus Dianthus of the Caryophyllaceae, which has only the base number N = 15. There are fifty diploid (2N = 30), three with diploid or tetraploid (2N = 30, 4N = 60 strains), two diploid and hexaploid (2N = 30, 6N = 90), twenty-two tetraploid (4N = 60), five tetraploid and hexaploid (4N = 60, 6N = 90), and twenty-four hexaploid species (6N = 90). Among the Geraniaceae, Geranium, with only twenty separate species listed, is an example of a variable form with a series of numbers, N = 9, 10, 11, 12, 13, 14, as well as some allopolyploids. In contrast, the genus Oenothera of the Onagraceae has only N = 7 forms together with polyploids, and Gossypium in the Malvaceae has forty-two species based on N = 13 and only one with N = 12. Twenty-three are diploid, and the others polyploid. The Oenothera are very interesting as the best example of species which (usually) are structural heterozygotes; Cleland (1949) has discussed the phylogenetic relationships and pointed out that most species and races within species are heterozygous for mutual translocations, which cause ring formation during meiosis. A few homozygous species have seven pairs, but the majority are permanent heterozygotes with balanced lethals and form a ring of four or more chromosomes, depending on the translocations present. The whole-arm exchanges allow chiasmata to form regularly, and disjunction is normal, a parallel to the normal disjunction from a heterozygous fusion in the virilis group of Drosophila. Cleland (1950) has reviewed work by him and his students on Oenothera and related forms. They have demonstrated how several often detrimental traits (translocations, lethals, and self-fertilization)

have been combined so that, by occasional crossing, the group has been kept vigorous and flexible in evolution.

As an example of the Euphorbiaceae, Euphorbia has many diploid and polyploid species based on N = 6, 7, 8, 9, 10. In contrast, among the Rosaceae all the species in the large genera Rosa, Potentilla, and Rubus, as well as several other smaller genera, are based on N = 7, while the species of the large genus Prunus are all based on N = 8. All the species of the two extensive genera Populus and Salix of the Salicaceae are based on N = 19, but in the Compositae the tribe Heliantheae has several genera with various base numbers, the Madia with 6, 7, 8, 9 and the Layia with 7 and 8. The Primulaceae are represented by the extensive genus Primula, which has many species based on the several numbers 8, 9, 10, 11, 12, 13. The genera in families or tribes may be consistent or may differ. Solanaceae has a variable genus in Nicotiana with numbers 8, 9, 10, 11, 12, while the Lycopersicum and large genus Solanum have only N = 12.

The Monocotyledons are represented by some stable and some variable genera. Tradescantia, although a small genus of the Commelinaceae, is variable with N = 6, 8, 12, 13, 15, 18. In the Liliaceae, the genus Scilla of the tribe Scilleae is variable with N = 6, 7, 8, 9, 10, and 11, but Muscari of equal size has only N = 9. Allium (Allieae) is variable with N = 7, 8, 9. Calochortus (Trilipeae) also varies with N = 6, 7, 8, 9, 10, but in Fritillaria most species have N = 12, with only a few N = 9 and 13 (by fusion and fragmentation), and the large genera Lilium and Tulipa have N = 12.

In the Iridaceae, the large genera Crocus (N = 3, 4, 5, 6, 7) and Iris (N = 7, 8, 9, 10, 11, 12, 13, 14) are quite variable, but Gladiolus is stable (N = 15). An example of heteroploids in polyploids is found in Dioscorea (Dioscoreaceae), which has N = 10. The genus includes some high polyploid species with somatic numbers of 61, 64, 81, and 144.

The Gramineae have been reviewed in greater detail by Myers (1947); hence both his review and that of Darlington and Janaki Ammal are used. This group is one that shows a marked stability of chromosome number within most of the genera. To quote from Myers (1947):

> Chromosome numbers have been recorded for 805 species in 142 genera, exclusive of species of Triticum, Aegilops, Secale, Avena and Zea. In more than half of the species, somatic chromosome numbers are

multiples of 7. Multiples of 5 (or 10), 6 (or 12), 8, 9, 11, 13 and 17 occur also. Variations in basic number occur among genera within tribes, among species within some genera and, in a few cases, among collections within species. An extensive aneuploid series has been found in Stipa.

In another place, he states:

More than two-thirds of the species of Gramineae are polyploid or have one or more polyploid races. In 99 species, races differing in chromosome number have been found. Polyploidy has been an important factor in formation of new species and in extending the range of geographical and ecological adaptation in most genera of the grasses. It has not been, however, important in the origin of major groups, even of genera. Some species are presumed, on the basis of cytogenetical behavior, to be allopolyploids, while a few species behave cytologically like autopolyploids.

The sixty-four species of Triticum, Aegilops, Secale, and Avena listed by Darlington and Janaki Ammal (1945) are all based on $N = 7$, while *Zea mays* has $N = 10$ (base5?). If we ignore Stipa, the largest group of genera have one base number, a small group has two numbers, and only a few genera have species based on three or four different numbers.

Myers points out that supernumerary chromosomes and centric fragments occur in several species and that "inversion hybridity has been found commonly in the species that have been investigated extensively." Although this whole family might be considered to vary as much as the genus Drosophila, no single genus shows an equivalent chromosomal flexibility.

Even though we have presented a distorted picture by listing more of the variable forms, the general impression that one gets in such a brief review of variation in chromosome number in the animal and plant kingdoms is one of stability. Change in number is ordinarily slow, other than polyploidy in plants. The fact that polyploids often form functional aneuploids which differ from a simple multiple of the basic N numbers may cause certain plant genera to seem more variable than they really are (e.g., Stipa, quoted above). The stability of chromosome numbers in most genera implies that evolution is largely through gene changes, often with inversions, and in a few forms, such as Oenothera, with whole-arm translocations.

We find further that several genera or families may be very stable; yet one or a few species (as in spiders), or a section, such as B of

Trimerotropis or *Hesperotettix viridis viridis* among grasshoppers, may show considerable variation. There are but few genera in which change in chromosome number seems comparable to that in the genus Drosophila, where variation in chromosome numbers occurs in almost all species groups that are not reduced to N = 3. In the plant genera Vicia, Euphorbia, Primula, Crocus, and Crepis, and in the animals Cyclops, Salmo, Microtus, and certain Orthoptera, such as Gryllotalpa, there are marked differences in chromosome numbers or series similar to those in Drosophila.

A consideration of the evolutionary processes in certain plant genera makes an informative comparison to Drosophila. R. E. Clausen, Goodspeed, and others have studied Nicotiana taxonomically, cytologically, and genetically by crosses. Goodspeed (1945a, b, c) divides Nicotiana into three subgenera, and these in turn into sections. Thus the division is roughly equivalent to the subgenera and species groups of Drosophila. The hybridization between members of different sections, even in different subgenera, illustrates a fundamental difference between evolution in these plants and in animals. As Goodspeed constitutes the sections, five have only diploids based on N = 12, two are tetraploids also based on N = 12, two have both diploid and tetraploid species (N = 12), and two have both diploids and tetraploids but include forms with 8, 9, 10, 12, 16, 18, 19, 20, 21, 22, and 24 pairs of chromosomes.

Goodspeed describes the chromosome morphology, which may vary considerably between species. The changes may be due in part to pericentric inversions and to fusions, but translocations also occur, even within a species (Mallah, 1943). The systematic relations have been determined by morphology, crossing, and karyotype differences. A great deal of information is available on meiosis in hybrids, which allows a measure of genetic similarity and difference. Goodspeed (1945c) lists 210 F_1 hybrids (involving fifty-three species) which have been studied for the extent of meiotic pairing and other factors. In Nicotiana, the karyotypes of closely related species in a subgenus, and especially in sections, are much alike and in some cases are nearly identical. In fact, each subgenus has a characteristic ratio between median and submedian to subterminal chromosomes: Rustica 9 : 1, Tabacum 5 : 1, and Petunioides 4 : 3.

Goodspeed goes on to comment on degrees of divergence reflected in hybrids:

From the point of view that chromosome homology reflected in the formation of bivalents (or higher valencies) at MI is indicative of the extent to which genes and their arrangements in the conjugating chromosomes are common or similar, the amount of pairing in F_1 interspecific hybrids has been employed to provide evidence concerning the relationships of the species in each case involved: (a) approximately 90 per cent of the F_1 intrasectional hybrids indicate close relationship of the species concerned by exhibiting complete or almost complete pairing of their genoms [1]; (b) by contrast, in only 10 per cent of the hybrids between species of different sections of a given subgenus does appreciable pairing occur; (c) corresponding to the more distant relationship of the species concerned which is postulated in the taxonomic arrangement, none of the F_1 hybrids which involve species of different subgenera shows significant pairing, except certain of those in which *N. glauca* or a species of the Alatae is a parent; (d) in all intra- and intersubgeneric F_1 hybrids between any of the nine amphidiploid species and the descendants of their putative progenitors, "Drosera scheme" pairing or an approximation thereof occurs; (e) on the other hand, when these same amphidiploid species are crossed with species other than those related to their parentage, 85 per cent of the F_1 hybrids exhibit little or no pairing.

An interesting point in plants is that basic relationships can be demonstrated between amphidiploids and the modern diploid representatives of the ancestral species which formed them. For example, the following combinations were either the parent species or very close to them genetically and cytologically: (1) *N. rustica* = *N. paniculata* + *undulata;* (2) *N. tobacum* = *tomentosa* + *N. sylvestris;* (3) *N. arentsii* = *N. undulata* + *N. wigandioides* (Goodspeed and Clausen, 1928; Goodspeed, 1945a, b, c; Clausen, 1928; Clausen and Cameron, 1944, etc.). In the first two of these cases, both parent species were in different subgenera. This illustrates very nicely the difference between gene system compatibility in plant material and that in Drosophila. In the former, gene systems of species in different subgenera have combined, by complete addition of diploid sets of genes, to give a functional species. In Drosophila, the only species hybrids produced are within species groups, which are roughly comparable to the sections of Nicotiana.

Other plant genera illustrate this difference in gene system tolerance. Sears (1948) reviewed the cytology and genetics of the wheats

[1] Hybrids involving amphidiploid species are obviously not included in points (a), (b), and (c).

and their relatives. All these forms are based on N = 7, although tetraploids and higher polyploids are common. In Secale, the seven pairs of chromosomes are homologous in the five species. In Triticum, there are two diploid species, genome A; six allotetraploids, genome AB and two = AG; and six allohexaploids, genomes ABD.

In Aegilops, there occurs the genome D (and possibly B) that are present in the polyploid wheats. Aegilops is more complex in origin. There are at least nine different genomes that occur as such in the nine diploid species, as well as seven additional genomes that occur among the polyploids, nine tetraploids and three hexaploids. The relation and extent of difference between all these genomes has not been completely analyzed. There are eleven different allopolyploid combinations that are successful. This series represents a type of evolution through allopolyploidy that is not generally available to animals where no amphidiploid sexual or even asexual successful species has been demonstrated.

Cotton has been studied genetically for a long period, and a very interesting series of relations have been demonstrated. Hutchinson, Silow, and Stephens (1947) have reviewed the relationships in this genus, which has some interesting parallels to and differences from Drosophila. There is no change in chromosome number comparable to that in Drosophila, for the species of Gossypium are diploid (N = 13) or amphidiploid [N = 26 or ($N_A + N_D$) = 26].

Gossypium, like Drosophila, occurs in all regions of the world, with a heavy concentration in the tropics. Furthermore, most species of cotton are endemics, except as the cultivated forms have been intentionally transported by man. The wild species and some of the cultivated forms are perennials.

The genus is divided into sections corresponding in many respects to the species group. Beasley (1942) devised a nomenclature for the several genomes based on his cytological studies, as well as earlier ones by Skovsted (1937) and Webber (1939). These Gossypium genomes are the Asiatic thirteen-chromosome species *herbaceum* (A_1) and *arboreum* (A_2); the African thirteen-chromosome *anomalum* (B_1); the Australian thirteen-chromosome species *sturtii* (C_1); the American thirteen-chromosome species *thurberi* (D_1) *armourianum* (D_2), *harknessii* (D_2), *davidsonii* (D_3), *klotzschianum* (D_3), *aridum* (D_4), *raimondii* (D_5); the Arabia-India thirteen-chromosome *stocksii* (E_1); and the American amphidiploid twenty-six-chromo-

some *hirsutum* $(AD)_1$ and *barbadense* $(AD)_2$. To this last pair of amphidiploids Hutchinson, Silow, and Stephens (1947) add *G. tomentosum,* found in Hawaii (also AD). They conclude that *raimondii* (D_5) is the New World diploid ancestor of these amphidiploids, together with an A genome from the Asiatic cultivated cottons.

Brown and Menzel (1950) state that, cytologically, the D genomes of *thurberi* (D_1) and *harknessii* (D_2) are about equally similar to that in *hirsutum* (AD). As it seems probable that *hirsutum* and *barbadense* are of common origin, this leaves some disagreement as to the ancestors of the American amphidiploids. It is noteworthy that both these amphidiploids have greater survival value in more extended regions than the endemic or even cultivated diploids.

Gossypium is satisfactory material to study residual similarity between the chromosomes of different species at meiosis in their hybrids, since the chromosomes are small enough to form few chiasmata but large enough to form bridges and fragments when inversion differences exist, and to form multivalents some of the time if several chromosomes are sufficiently homologous. The variation between chromosome systems does not involve fusions, although translocations have been fixed infrequently, as indicated by the *arboreum* and *herbaceum* hybrids discussed by Beasley (1942). However, paracentric inversions are relatively common, for a number of crosses have given up to seven or eight bridges and fragments in meiosis. The number of rearrangements is probably larger, for small or complex paracentric and no pericentric inversions would be detected. The extent of the variation measured roughly in this way shows that within genomes pairing is good, although a few inversions may be present; while crosses between genomes give regular indication of inversion differences, some translocations, and often such extensive changes that synapsis is absent or infrequent (Beasley, 1942).

Babcock (1947) monographed the genus Crepis. The very extensive studies by Babcock and his collaborators using genetic, cytological, ecological, and distributional information, as well as intensive taxonomic studies, have provided a very worthy analysis of a genus. It is particularly interesting to us because it provides so many parallels to the genus Drosophila.

Crepis is a world-wide genus with many endemic species. In fact, 133 of the 196 species listed are regarded as endemic. This is not quite as high as the 90 per cent found in Drosophila, but it represents

a comparable situation. Babcock has illustrated the known distribution of the majority of these forms in a number of maps which often help to clarify descent and relationships. The evolution of Crepis has taken place largely in the Palaearctic, Oriental, and Ethiopian regions, although a rather specialized series of species based on N = 11 and polyploid (apomictic) derivatives are present in the Nearctic. The place of origin of the genus is placed in the Altai and Tien Shan in central Asia, from whence it spread and diverged (Babcock, 1947, Figure 11, page 152). No comparable historical analysis of Drosophila is possible. We shall refer mostly to information from Babcock's monograph, which provides a bibliography for the basic publications on Crepis. This genus is primarily a diploid complex, with only a few polyploid species to illustrate this type of greater genetic flexibility of plant material. Among the species whose chromosome complement is known, only nine American allopolyploids are apomicts out of the 113 species listed (Babcock, 1947, Table 2). If we omit the polypoid species, including the apomicts, there remain ninety-seven diploid sexual species, which form a most interesting comparison to Drosophila. We will disregard the supernumerary chromosomes, such as occur in *Crepis syriaca.*

Babcock divides the genus Crepis into twenty-seven sections, which fall into three groups. In so far as practicable, these are arranged in phyletic groups from the most primitive to most advanced: group 1, the rhizomatous species, includes sections 1 to 5, 13, 21; group 2, the more primitive taprooted species, sections 6 to 12 and 14 to 18; group 3, the more advanced taprooted species, sections 19, 20, and 22 to 27 (see Table 4, Babcock, 1947).

The ninety-seven diploid sexual species have the following distribution of chromosome numbers (cf. to Drosophila, Figure 47): the haploid number 7 = 3, 6 = 14, 5 = 19, 4 = 58, 3 = 3. The same chromosome number spread is present in Drosophila. We would judge that it is much more difficult to increase than it is to decrease chromosome number in Crepis. However, the distribution of frequencies is quite different. This is a notable point, in view of the fact that Babcock and his coworkers believe that the chromosome numbers in most species of Crepis have been progressively decreased.

The three groups in Crepis correspond roughly to subgenera of Drosophila, while the sections are the plant equivalent of the species groups. The chromosome numbers, and in many cases chromosome

size as well, have decreased with evolutionary advancement. The most primitive group has fourteen species N = 6, three species N = 5, and six species N = 4; the intermediate group 2 has three species N = 7, ten species N = 5, and eighteen species N = 4; the most advanced group 3 has six species N = 5, thirty-four species N = 4, and three species N = 3. This may be compared to Drosophila, where the chromosome numbers in the subgenus Drosophila are much more frequently primitive (N = 6) and that of the Sophophora are more frequently derived (N = 3). There is a difference in that, while most species groups in Drosophila have members with the primitive five rods and a dot (N = 6), according to Babcock (1947, Figure 4) most sections in Crepis have only one chromosome number, six sections have two numbers, only one section has three numbers, and no section has four numbers. Apparently changes in chromosome number have been less frequent, but have often given rise to one or several descendent sections with the reduced number. The reduction to N = 3 has occurred at least twice, or else the species in two different sections had the same ancestor in their common parental section. At any rate, this final reduction has not been easily accomplished when compared to Drosophila. In fact, reduction in number does not seem to have occurred independently very often, as judged from Babcock's phylogeny (Figure 4, 1947).

The reduction in chromosome number by fusion in Drosophila with the loss of the very small centromeric fragment has occurred comparatively more frequently than the equivalent change in Crepis, which often involves loss of heterochromatic material with the centromere. Tobgy (1943) analyzed the distribution of heterochromatin in *Crepis neglecta* and investigated the pairing in the hybrid between this species and *Crepis fuliginosa*. The latter is presumed to be a descendant of the former, or of a closely related common ancestor. Chromosome C_N of *neglecta* is in part heterochromatic, and only part of it is retained in combination with part of the B_N chromosome to form the more complex B_F chromosome of *fuliginosa*. There exists a difference as a result of translocation between the A and D chromosomes of the two species, and at least three paracentric inversions, for three bridges with fragments may be present in meiosis of hybrids. The production of a B_F chromosome of reduced size from the hybrid presumably indicates crossing over with a heterozygous pericentric inversion, although Tobgy does not use this explanation. It

may be that the reduction in size of the Crepis chromosomes, due presumably to loss of heterochromatin, may sometimes be due to this mechanism.

The chromosomes in Crepis are very often visibly metacentric, and Tobgy's Figure 6 shows that there is an appreciable body of heterochromatin adjacent to each side of the centromere in *neglecta.* If this is the general situation in the larger Crepis chromosomes, suitable pericentric inversions would give crossover classes with both larger and smaller chromosomes. In Crepis, the latter seems to be generally the direction of change. A similar mechanism may reduce the size of the chromosomes in some Drosophila species as compared with their ancestors. However, here the increase in size seems often to have occurred, especially illustrated by the forms in the melanopalpa subgroup. Translocations of heterochromatic material have also occurred in Drosophila, for example *ananassae,* and presumably often also in Crepis. Translocation has undoubtedly been much more important in Crepis than in Drosophila, if we omit fusions, which are a special type. These are less common in Crepis, where rods are less characteristic of the genus. For example, Sherman (1946) has shown that the component segments of the missing E chromosome of *Crepis Kotschyana,* a four-chromosome species closely related to several five-chromosome species, are present in chromosomes A, C, and D. No comparable system of translocations has been proved for Drosophila.

The net effect of the reduction of chromosome number, due to the comparative ease of loss of a centromere and comparative difficulty in gaining a centromere by duplicate use of one present, has led to the similar spread in chromosome numbers in these two genera.

The genus Sciara is a member of the lower Diptera, and so falls in the same order with Drosophila, which differs in being a member of the higher Diptera. Sciara species show some very remarkable differences from Drosophila and other regular diploid sexual forms, but we will confine our discussion at this point to chromosome system differences, as worked out chiefly by Metz (1938a, b) and his students.

There are present in the germ line limited chromosomes that are transmitted in both male and female gametes, and that are eliminated from the somatic nuclei in the early cleavage divisions (Dubois, 1933).

The X chromosome system and sex determination are aberrant. The sperm bring in duplicate (sister) X chromosomes, which are added to the single X chromosome from the egg. This triple X condition is retained until the seventh or eighth cleavage divisions, when one of the sister X's from the male parent is eliminated from the soma of eggs that will develop into females, while both sister X's are eliminated in those which will become males. As a further complication to providing both sister strands of an X chromosome in the sperm, the first maturation division in the male is unipolar, and the paternal chromosomes are eliminated while the maternal chromosomes go to the pole to produce a functional cell. At second division the X is precocious and both daughter chromatids move to one pole, although the other chromosomes show the usual equational division. The cell with the twin X chromosomes forms a sperm, and the other does not function. This system allows the males to transmit only the maternal chromosome system and so provide the basis for an unusual type of selection. Although this unique system is perhaps not so complex as that of the closely related Cecidomyidae worked on by White (1950), it presents a complex series of specializations unlike anything known in Drosophila.

McCarthy (1945) reported the chromosomes of eight species of Sciara and demonstrated that different species had one V-shaped or two or more V- or rod-shaped limited chromosomes, which are characteristic of the species. Nevertheless, the basic necessary residual set of chromosomes in the soma is four pairs. This is also true of *Sciara impatiens* (Carson, 1944), *Sciara reynoldsi, Sciara ocellaris* (Crouse, 1939), *Sciara pauciseta,* and *Sciara coprophila* (Metz, 1938b). As the males are XO, there are eight chromosomes in the soma of a female and seven in the soma of a male. The position of the centromere cannot be determined from an examination of the chromosomes in the salivary gland nuclei. Therefore, it is not known for sure whether the change from rods to V's or the reverse is due to added heterochromatin or to a shift in the position of the centromere. Other than the changes in shape of the chromosomes and variation in number of limited chromosomes, there are no changes in basic chromosome number in the thirteen species studied.

Although Crouse (1939) studied the problem of the modification in chromosome shape, the Sciara material has not proved completely satisfactory, chiefly because change in the position of the centromere

cannot be tested critically by using the chromosomes seen in salivary gland nuclei. The material she used was quite interesting and suggestive. *Sciara ocellaris* has both unisexual strains, where the adults which emerge from the eggs of some fertilized females (XX) are regularly male while those from other females (X^1X) are regularly female, and bisexual forms that regularly produce both sexes. The unisexual strains may produce a few individuals of the unexpected sex. The bisexual strain possesses alternately a pair of V-shaped autosomes or a pair of rods or a rod and a V heterozygote. Since these chromosomes differ in metaphase configuration and may occur heterozygous, it was presumed that a typical inversion loop would be present if the centromere shift were due to a pericentric inversion. No inversion loop was present, so Crouse decided that the centromere as such had been shifted, a change which would require three breaks to delete and insert the centromere in another position. The absence of any of the several available additional proofs, such as Carson's (1946) cytological demonstration of bridge-fragment formation by heterozygous inversions in *Sciara impatiens* or differential staining tests for heterochromatin, still leaves the question undecided. In the absence of these evidences, the shifting of a centromere by a triple-break rearrangement, when the location of the centromere in the salivary gland chromosomes cannot be demonstrated, must remain a tentative hypothesis, particularly in view of their absence in Drosophila, where the centromeres can be located in most species.

The remarkable factors in Sciara species are the absence of mutations (Metz, 1938a), such as have been obtained readily in Drosophila, and the presence of many minor cytological reorganizations, which have very few counterparts in Drosophila. Metz (1938a), Metz and Lawrence (1938a), Crouse (1939, 1943), Carson (1944), McCarthy (1945), and Rohm (1947) have all stressed the frequency with which very small duplications, deletions, and changes in visible cytological structure are demonstrated by differences in banding of homologous chromosomes, both between strains of the same species and between different species. Carson (1944) made a detailed study of the natural chromosome variations in *Sciara impatiens*. In addition to twelve large inversions, which allowed him to make several chromosome phylogenies, he found thirteen small differences of the duplication or deficiency type. Some seem definitely duplications, others deficiencies, while still others might be either. All live homozygous

and are of wide distribution, and therefore represent changes which are of presumably alternate selective value rather than detrimental modifications.

Metz (1947) has discussed the importance of duplication as a factor in evolution, particularly reviewing the evidence from Sciara. Bridges (1936), Muller, Prokofyeva-Belgovskaya, and Kossikov (1936) demonstrated that *Bar* was due to the presence of a tandem repeat in agreement with Sturtevant's (1925) interpretation. Bridges (1935) had shown that there were repeats of small sectors, as judged by the synapsis of short regions with identical banding at different positions in the chromosomes. All of these authors point out the importance of such small duplications as the origin of available genes for novel mutations. Such duplications are fairly common in Sciara, but rare as alternate variations within species of Drosophila. In fact, only the small duplication found by Warters (1944) is known which is not species-wide. The distribution is interesting, for although it is generally frequent in western North America, it is also present in the Black Hills of South Dakota and eastward in Tennessee and Alabama. It is sufficiently favorable in natural selection to be retained widely in *melanogaster* populations.

There are a few comments on the analysis possible by using chromosome configurations from both the salivary gland nuclei and ordinary somatic or germinal nuclei. The size, shape, and pairing properties seem to be characteristic of the chromosome, as judged by F_1 and F_2 hybrids. The chromosomes in the salivary gland nuclei may pair poorly, even though the banded pattern is the same (e.g., *virilis-texana* hybrids). Since this holds for particular chromosomes in F_2 recombinations, it is a property of the special chemistry of such chromosomes, just as size differences in hybrids of Crepis or cotton show that the chromosome size is determined by the chemistry of the chromosomes and not by the chemistry of the cytoplasm. Buzzati-Traverso (1950) finds that the chromosomes in *ambigua/pseudoobscura* hybrids did not pair to any noticeable degree. Intimate pairing seems to demand both chemical homology and a marked degree of similarity in gene sequence.

The genus Drosophila shows the effect of its meiotic peculiarities in the types of rearrangements tolerated and utilized. The lack of crossing over in the male creates a tolerance for heterozygous paracentric inversions, although it does not insure their presence. If such

inversions are present, they may be concentrated in one of the chromosomes—e.g., the C element in *pseudoobscura,* the E element of *nebulosa,* or the E element of *melanica,* where Ward (1952) has found sixteen inversions—or they may be scattered among the chromosomes, as in *macrospina,* in the montana complex, and in *willistoni.*

On the other hand, there are but few pericentric inversions. These are often protected from selective disadvantage by the presence of accompanying paracentrics, either in the same or in the homologous chromosome, in such a position that they will prevent crossing over or eliminate crossover chromatids through bridge formation. We have only to consider that there have been discovered but a few pericentrics heterozygous in populations, as compared with the paracentrics, which number hundreds. In fact, the frequency of paracentrics may be roughly proportional to the age of the species in large populations. Even though we know a number of pericentrics have occurred in the evolution of the genus, these must have been protected by paracentric inversions, or have been short inversions to survive enough for fixation in new species formation.

Dubinin (1934, 1936) used translocations to reduce and increase the chromosome number of *melanogaster.* He did not use fusions, the type of rearrangement commonly found to account for reduction in the chromosome number between related species. Patterson, Stone, Bedichek, and Suche (1934) and Stone (1934) presented genetic and cytological evidence for two mechanisms which reduce chromosome number, namely, fusion and total translocation. Further details of these cases were published by Painter and Stone (1935) and by Stone and Griffen (1940). Although many fusions are present in the genus Drosophila, only three cases of double-length rods of necessarily independent origin are known (*testacea, tranquilla, spinofemora;* Wharton, 1943). Of the original fourteen X-4 translocations found by Patterson, Stone, Bedicheck, and Suche (1934), eight were fusions and one was a total translocation of the long arm of the 4 chromosome to the tip of the X chromosome beyond the *yellow* locus. This ratio of total translocation to fusion (1 : 8) may account in part for the rarity of such double-length rods, which may have originated in this manner. The abnormalities of disjunction following total translocation probably more nearly resemble those from a heterozygous translocation. Since these latter are so rapidly elimi-

nated from populations, their fixation must be a most exceptional event through very small isolated populations. It seems more probable that double-length rods are protected pericentric inversions from V's, for here disjunction would seem more likely of success. Even fixation of fusions must be rare. If we exclude hybrids, none has been recovered heterozygous in natural populations, despite the fact that the heterozygote is selected against very slightly or not at all (Stone, 1949).

Increase in chromosome number is very difficult in Drosophila. The one proven case of *trispina* involves a small extra heterochromatic chromosome (Ward, 1949). It might be used for further shift of genes by translocation and so become comparable to the other rods, except that translocation is difficult to fix because of its selective disadvantage, especially as a translocation that would increase chromosome number above six could not be a whole-arm exchange. We would expect few $N = 2$ species of Drosophila, as this would probably involve fusion of a rod and double-length rod, or fusion of two double-length rods. Although *miranda* indicates that the number of male haploid genes can be increased element by element, yet the sex-determining gene-balanced system will also make a reduction from $N = 3$ to $N = 2$ more difficult. The frequency of paracentric inversions in populations of species investigated and the almost complete unidirectional change in chromosome number both tend to support the idea that many of the groups and species of the subgenus Sophophora are perhaps older and more evolved than those of the subgenus Drosophila. One comment should be made regarding the implications of the types of chromosomal rearrangements analyzed in the genus Drosophila, namely, that they very abundantly exemplify and prove the general hypothesis advanced by Navashin (1932) on chromosome organization and reorganization.

We have labored the point that mutations in Drosophila are of all types and include all shades and degrees of change in physiology and morphology. Thus we have mutations showing all types of phenotypic changes that exist as differences between species. Species can be described as differing by reason of the accumulation of a combination of mutants that culminate in the phenotype and physiologic changes which are commonly present between species. This hypothesis, together with the genetic evidence, will explain the accumulation of phenotypic differences between forms descended from a

common ancestor. We cannot rule out a major mutation that would change several of the systems, but we consider that the probability of a successfully adjusted and adapted major mutation is low. Goldschmidt (1940) favors this latter view and believes macroevolution due to major mutation is responsible for new species, and even more for differences between higher categories. He (1934, 1940) presented data on the genetic differences between races of Lymantria, in which he showed that such things as sex factors, time of larval growth, instars, diapause, and larval and adult markings are inherited in the usual Mendelian ways—through multiple alleles, multiple factors, and the like, or as cytoplasmic factors—but he does not believe that an accumulation of these types of microevolution effects species formation.

The different male and female sex factors that cause intersex formation in Lymantria have not been found between races in species of Drosophila, but equivalent genetic differences leading to intersex formation exist in the melanopalpa subgroup. Crosses between some strains of *repleta* and *neorepleta,* and between *repleta* and some strains of *melanopalpa,* produce intersexes (Wharton, 1942; Sturtevant 1946b; Ward and Stone, 1952.) Winge and Ditlevsen (1948) added some additional information to Winge's very important work on sex determination in Lebistes. This fish has a regular XX female and XY male type of sex determination. Winge was able to select strains which were XY female and YY male. This paper reports crosses between such XY females and XX males. The XX male comes from a strain in which both sexes are XX, and sex is determined by an autosomal factor or factors. These substitutions of an X for a Y, or the reverse, are possible because the two chromosomes differ only in a strong male factor that is very closely linked or homologous to the Y gene *Maculatus.* The crosses between XY females and XX males gave normal ratios and normal sex determination in F_1 and F_2, that is, XX females and XY males. The authors explain the aberrant sex races as being due to the selection of additional strong sex genes acting in such a way as to reverse the normal factors, but crossing races with genes selected to act in opposite directions returned the balance to the normal. Such genic rebalancing must explain differences between strains or species such as those leading to *repleta/melanopalpa* intersexes.

Many of our concepts of the working of the genetic system are in

reasonable agreement with those of Goldschmidt (1940). For example, in discussing genic balance he states:

> Soon afterwards it was realized that this balance works through the control of properly adjusted reactions, and this permitted us to apply the same principle to all genic actions (Goldschmidt, 1920). The balance theory thus assumed the form of a balance between closely adjusted developmental reactions steered by the genes. As it was assumed that the speed of these reactions was controlled by the quantity of the genes, it followed that a disturbance of the balance was produced either by a change in the quantity of a single gene or by summation of quantities of different genes of identical action.[1]

He points out the fallacy of considering genic balance as the effect of numerous plus and minus modifiers scattered at random on the chromosomes, as stated in the early development of the theory by Bridges. Although numerous genes affecting any trait such as eye color in Drosophila are present on each chromosome, we now know that these are not multiple factors carrying out the same action in a simple sense, but rather a set of genes which controls a series of different cumulative reactions to produce eye color. This seems to be the proper concept of most biochemical sequence reactions and explains why aneuploidy causes particular upsets in the organism, depending on the genes present in the hyperploid or in the hypoploid condition. This effect of genic unbalance was discussed by Patterson, Brown, and Stone (1940) and has long been generally appreciated by those people working with trisomics, such as Goodspeed and Clausen (1916), Clausen and Cameron (1944), Blakeslee and Avery (1938), and Sears (1948), to mention but a few.

In opposition to our view, Goldschmidt rejects change in balanced-gene systems as the mode of evolution and considers that major structural chromosomal rearrangements may be responsible for master mutations that produce species. We do not believe that the evidence from Drosophila agrees with this point of view. Although many paracentric inversions, to use the least harmful structural reorganization as an example, may be present in the populations of a species, and although many inversion differences may exist between closely related species, it is not necessarily the case. Many species

[1] Goldschmidt, R. *The Material Basis of Evolution,* page 231. Copyright 1940 by Yale University Press. New Haven.

in the repleta group have so few inversions present that none has been demonstrated. Furthermore, there are fewer inversion differences between *mulleri, aldrichi, arizonensis,* and *mojavensis,* or between *repleta, neorepleta, melanopalpa, canapalpa,* and *limensis,* than there are present heterozygous in many populations of *pseudoobscura, melanica,* and particularly *willistoni.* Genic balance changes (aneuploidy) can cause drastic phenotype changes and simulate mutations; either mutation or genic unbalance can interfere with the usual functioning of the genetic system, where their effect on the system may be good or bad. This is true as well for position effect with chromosomal rearrangement. Position effect cannot be regarded as a separate type of mechanism, for its effects on the organism are capable of modification by mutation at other loci, just as with any point mutation. Any one of these changes may contribute to the differences between species. We consider that the balanced-gene system determines whether a mutation or any genetic type change is good or bad in a particular environment.

As an example of a major gene difference contributing to the isolation of a species, let us consider the *texana* cross-lethal factor demonstrated in crosses with *montana* and the *aldrichi* 2 cross-lethal factor demonstrated in crosses with *mulleri.* In the latter case, *aldrichi* and *aldrichi* 2 differ by one or a few dominant sex-linked genes. These have no detectable effect in the *aldrichi* genotype or cellular environment, but cause the death of *mulleri/aldrichi* 2 hybrids that carry the factor. Here the gene acts as a lethal in an alternate genotype that survives if it is absent, for *mulleri/aldrichi* female hybrids are viable. In the crosses of *montana* female and *texana* males, the female hybrids die in the *montana* egg cytoplasm. In reciprocal crosses, the same genotype survives in *texana* egg cytoplasm. Here the internal environment determines whether the hybrid survives. If we substitute the other subspecies *americana* for *texana,* there is not the same cross-lethal effect. The factor in the *texana* X is not effective in hybrids with *virilis, novamexicana,* or even *lacicola.*

Other genera include species in which such cross-lethal factors have been demonstrated. Hollingshead (1926) showed that some strains of *Crepis tectorum* carried a gene, either heterozygous or homozygous, which was cross-lethal with *Crepis capillaris* and two other species, but not with still another pair. Hutchinson (1932) discovered two complimentary genes in Gossypium, now Cp_a and Cp_b, which caused

their heterozygote to be "crumpled," ordinarily leading to early death. Cp_a was found only in *Gossypium arboreum soudanensis,* while Cp_b was found in about half of twenty-nine tested strains of *arboreum* and *herbaceum.* Silow (1941) reports that Cp_a was found in another local strain and that Cp_b has been found to be present in twenty-five of forty-one strains tested. These factors act as a complementary lethal. A situation due to two such complementary cross-lethal factors has been found to cause the death of seedlings in some varietal crosses in hexaploid wheats (Caldwell and Compton, 1943; Heyne, Wiebe and Painter, 1943). Other crosses indicate that similar factors operate between other members of these forms.

Such cross-lethal factors, if autosomal and not restricted to act in relation to the cytoplasm present, can isolate two forms so completely that no gene exchange can occur, and so are a fundamental type of species difference between numerous forms. Nevertheless, they demonstrate that this cross-lethality is the result of a difference in basic genic balance between the species dependent on other genes; else they would not survive in their own strain, and thus support the thesis that different species are different balanced-gene systems or, in less controversial terms, different reaction systems. In well-adapted organisms, the genotype is flexible in its control of the phenotype, which can be modified markedly. This flexibility might tolerate a macromutation in Goldschmidt's sense. It seems to us that the flexibility, being a function of the selected genotype, would more readily tolerate changes which, though individually small, could combine to give the same or even much greater final magnitude change from the original system.

As was long ago pointed out, such systems as sterile castes in social insects refute a Lamarckian explanation. Since they require a complex adaptive evolution and yet have evolved, it seems that factors are present to insure evolution in systems which could not be Lamarckian. The flexibility of the genotype is such that it can function to develop modified or alternate phenotypes. If the environment is exercising pressure in a direction which modifies the phenotype of such a flexible genotype, mutations which change the phenotype under most or all circumstances in the direction of the environmental pressure modification will seem to be a directed response, for the more common mutations that change the organism in the many other ways will be selected against and eliminated. Thus selection for those random mutations

which adjust the organism to environmental pressure will simulate Lamarckian adaptation.

In Drosophila species which can be bred in the laboratory for study, we are fortunate in that we can detect most chromosomal rearrangements, except for the very small ones, which may be overlooked. Rearrangements are unique, in that a particular one probably occurs only once, whereas gene mutations occur repeatedly with a comparatively high rate. We sometimes lose sight of this differential when we find several rearrangements present in a number of strains of a species. With proper checks, the distribution of any rearrangement may be detected, whereas it is difficult or impossible to determine how often the same mutant in different populations may have had the same or independent origin. This complicates the analysis of species-wide mutations of low frequency, for it is possible that they are the result of independent mutation part of the time. A properly conducted survey of the metaphase and salivary gland chromosomes will reveal almost all rearrangements in a species, but there is no possibility of determining all the mutations that are present in any species of Drosophila. Species-wide mutants are a form of genetic polymorphism which seems common in Drosophila, although major phenotypic polymorphism such as that described by da Cunha (1949) and by Freire-Maia (1949) is rare, while no such demonstrably adaptive phenotypic polymorphism such as that occurring in Lepidoptera (Fisher, 1930; Ford, 1946) is found in Drosophila. Inversion polymorphism in Drosophila, adaptive or not, does not show a correlated phenotypic polymorphism.

In Drosophila both visible and lethal or sterile types of mutations are common, but in Sciara visible mutations are rare, perhaps even rarer than chromosomal changes (Metz, 1938, 1947; Carson, 1944). *Habrobracon juglandis* resembles Drosophila in having a considerable number of mutations (A. R. Whiting, 1934, 1939; P. W. Whiting, 1938; Martin, 1947); hence, the low rate in Sciara is a peculiarity in those forms, just as are the large number of body-pattern mutants found by Nabours and Stebbins (1950) in *Apotettix eurycephalus*. In the latter, there is a series of one recessive and seventeen dominant color-pattern genes on the same chromosome that fall into six closely linked allele series. The close linkage may be due to a tendency for chiasmata to be localized, but the fact that all are linked on one chromosome is a distinctly nonrandom distribution, not to mention the remarkable

number of dominants. Tan (1946) described a series of twelve alleles at one locus in an autosome which determined color and pattern in the ladybird beetle, *Harmonia axyridis*. Three additional alleles have been described by Hoshino (1940), and a pure black form is known in the variety *corvina* which might be due to an additional allele. As all but one of these alleles are dominants with fixed black patterns, this might be a case of nonallelic dominant factors with localized chiasmata. There is no evidence for this, and it remains a case with at least fifteen known color-factor genes (alleles) on one chromosome. It is clear that mutation rates and patterns differ in various organisms. A low rate such as found in Sciara may be modified, since the mutation rate is under genetic control, as exemplified by the mutators described by Ives (1950) and the more complex unstabilizing modifier described briefly by McClintock (1950). Muller (1942) pointed out that the higher the temperature, the greater the mutation rate, other things being equal. Such a factor may be of importance in the greater diversity found in lower Mexico.

One type of mutation deserves a little more consideration as a factor in evolution, namely, lethal mutations. We refer to mutations rather than losses; early studies such as that of Patterson (1932b) showed that many lethal effects were deficiencies and would very seldom play a part in evolution. One characteristic of crosses between species is the production of lethal zygotic combinations, sometimes demonstrably due to complementary factors, which differ from ordinary lethals in that they do not act in their normal gene milieu but only in crosses, so that a mutation is lethal or beneficial, depending on the gene system present. These resemble the viability factor discovered by Patterson (1932a). The cross-lethal factors discovered are ordinarily dominant and act in F_1, but a number of cases of recombination lethals are known in cotton—for example, the F_2 from crosses of *barbadense* $\times$ *hirsutum;* and in Drosophila, where Dobzhansky (1946a) synthesized recombination lethals and semilethals in *pseudoobscura* from viable strains, an effect which can be compared to F_2 from *pseudoobscura/persimilis* hybrids.

Valencia and Muller (1948) pointed out that the rate of mutation of genes moved near to heterochromatin in *melanogaster* is much increased, even when position effect is omitted. Thus, there are two factors which may explain the loss of function of genes of the originally euchromatic 3 chromosome after it had become attached with the Y

to form the complex Y of *miranda*. The first is position effect of genes moved near the heterochromatin; the second is mutation, perhaps at an accelerated rate because of this association. Part or many of the genes may have mutated to a form which no longer possessed autocatalytic power. Mutation in the 3 fused to the Y will be protected by the normal allele in the free 3 chromosome. Some of the mutations of the normal genes in this chromosome will be to a more active allele, which will be haploid sufficient. Such alleles will allow the accumulation of gene losses from the attached 3. Since they will be effective in both sexes, they have a survival advantage, for retention of normal genes in the 3 attached to the Y will only insure survival in the male. Position effect and a higher rate of mutation of the Y-3 genes will tend to lead to a system where the complex Y is genetically nearly without function. It should be noted that the male-limited free 4 chromosome of *americana* does not seem to have suffered degenerative loss of genes allelic to those in the new complex X-4 element. The following factors may all contribute: *americana* may be a much younger species than *miranda;* crossing over may occur in the *americana* males sufficiently to continue replacing a "degenerating" 4 with a normal selected 4; this 4 is not attached to the Y, and so is not affected by the Y heterochromatin; the introgression with the other subspecies *texana* brings in a 4 chromosome, which was selected to survive homozygous.

Mangelsdorf and Reeves (1939) and Mangelsdorf (1947) have discussed the genetic and cytological relations between *Zea mays* and its relatives. Their studies, and those of many other people interested in maize and its relatives, have presented very detailed evidence of genetic relationships. They have developed theories as to the origin and migration of these forms and their relationships to old world forms. As only a few forms were involved in the analyses, this work is comparable, if more extensive, to the development of gene homologies by Sturtevant and Novitski (1941b).

The existence of different normal alleles, as suggested above, has been proved to be frequent for some genes, at least in maize (Stadler, 1948, 1949) and in different species of cotton—for example, the series of *crinkled* alleles described by Harland and Atteck (1941).

Other than the studies made by Sturtevant and Novitski (1941b) and by Spencer (1947a) on gene homologies between species, there have been no serious attempts to determine whether the same mutants,

and especially the same normal alleles, are present in related species. Tests made by Dobzhansky (1936) show that *pseudoobscura* and *persimilis* differ for many genes; and those made by Patterson, Stone, and their students and coworkers show several gene differences between members of the virilis group but do not demonstrate specific alleles. They prove differences and relations, but not how many alleles differ.

Harland (1936), Harland and Atteck (1941), Silow (1941), and Stephens (1948, 1950) have discussed the similarities and differences between alleles of different species of Gossypium. Harland demonstrated that both the main normal *crinkled* alleles differ and also that the modifier system differs. Silow compared the genetic constitution of three diploid forms and showed not only that different alleles were present, but sometimes that different relative frequencies of the same alleles characterized the species. Stephens considered particularly pseudoalleles, where duplicate loci in each species had diverged in function. In the latter case, two adjacent loci are involved: *Red Spot* (*GS*), *Ghost Spot* (*GO*), *Spotless* (*OS*), and *Basic Spotless* (the double mutant, *OO*). *OS/GO* gives a phenotype equivalent to *GS*. As these loci can be recombined by crossing over, the case is somewhat similar to *Bar* and to *Star-asteroid* (Lewis, 1945).

The dove-pigeon hybrids investigated by Cole, Irwin, Cumley, and their associates give us considerable further information on basic gene difference. In this case, the differences are associated with synthesis of naturally occurring antigens. In a series of papers, Irwin and Cole (1936), Irwin and Cumley (1940), and Irwin (1947) showed that there are genetically determined antigens in the blood cells and in the serum of doves and pigeons which are inherited as units in a regular Mendelian fashion. In F_1 hybrids between species, most of the antigenic factors showed dominance. In certain crosses there was formed in addition a "hybrid substance," which is an antigen resulting from the interaction of factors from the two parents. Thus they demonstrated that genes determine the presence of specific proteins and that these factors are inherited in hybrids and segregate in the backcross hybrids, as would be expected on unit factors, although close linkage might also enter occasionally. Irwin and Cumley (1943) were able to use the cellular substances of eleven species of the genus Columba to show relationship, for there exist some factors in common to all species, some specific to each, and some that are shared in different

combinations. The inherited antigens allowed them to divide these species into two groups, an Old World and a New World group, which correspond to the divisions set up by Cumley and Cole (1942) on more standard taxonomic criteria.

Osgood (1909), Sumner, Dice, Blair, and others have studied the genetic factors and species relations in the genus Peromyscus. Sumner (1932) reviewed briefly certain color factors and discussed in detail variation in and between races and subspecies. Some factors were dominant in crosses, others intermediate. There was a considerable spread in expression of many factors, such as those controlling colored area, and Sumner showed how this varied within races, in their F_1 and F_2 and backcross hybrids.

Dice (1940a, b) discussed variation and speciation in Peromyscus. He pointed out that certain distributions of subspecies of different colors could not have been retained because of their crossing, except for the fact that selection favored the several colors in particular habitats, even though an intermediate group existed in the interzone between them. According to Dice (1940a), the dark subspecies *Peromyscus maniculatus rufinus* has a polyphyletic origin, in the sense that it originated separately in a number of localities from the more widespread lighter subspecies. This would imply that *rufinus* is primarily a color-phase subspecies with associated characters.

Dobzhansky (1943) demonstrated that particular inversions in *pseudoobscura* had different adaptive values, both singly and in combination. In some populations, there is a regular seasonal cycle, with the frequency of certain inversions high at one season and low at another. In fact, seasonal cycles of Standard, Arrowhead, and Chiricahua occurred each year for a six-year test period at Piñon Flats (Dobzhansky, 1947c). The changes in frequency were cyclical in some cases, a balanced polymorphism, but directional in others. These changes were due to natural selection, and in fact the differential in selective value was appreciable. A series of experiments with population cages confirmed these findings in the laboratory.

Dobzhansky and Levene (1948) proved that there was an excess of adults heterozygous for inversions over that expected on the Hardy-Weinberg rule from the frequency of these inversions in the population. This showed that, under natural conditions in *pseudoobscura,* the third-chromosome inversions gave heterosis with each other, except for a few particular combinations.

The effectiveness of laboratory selection had been demonstrated long ago by Payne (1920) and was repeated with more modern statistical manipulation by Mather and Harrison (1949). A very interesting example of the continuing effectiveness of selection has been presented by Goodale (1938), using white mice. Stebbins (1949) discussed several examples in plants. One distinct contribution to selected adaption made by Dobzhansky (1950) was the evidence that inversions from a particular locality were adapted to give heterosis with each other, but not necessarily with a strain from another locality. Thus *pseudoobscura* is utilizing the advantage of heterotic gene interaction associated with its chromosome polymorphism to gain selective advantage. This type of polymorphism is characteristic of several species of the Sophophora, but is not known to be characteristic of any species with world-wide distribution.

Although heterosis in *pseudoobscura* is established with inversions, the species *hydei, virilis, americana,* and *texana* show decided heterosis for vigor, fertility, and fecundity (Stone, 1942), without a corresponding inversion heterozygosity. Almost all crosses between different strains gave heterosis, so that we can expect vigor increases without inversion heterozygotes. It seems to us that this indicates that each population had achieved an adaptive peak possible with available genes, and that heterosis in these crosses shows that migration between populations continually presents new genes and gene combinations for testing in the possible recombinations. Some of these recombinations may be superior, and so lead to a higher adaptive peak, in the sense of Wright (1949). This conclusion is favored by data from *pseudoobscura.* Dobzhansky (1946a) showed that recombinations of genes not involving inversions gave different adaptive values, although the most striking result was of negative value, that is, a recombination lethal effect.

East (1935) pointed out that *Nicotiana rustica* was an exceedingly variable plant. He obtained genetically homozygous diploids from a haploid by doubling the chromosome complement. All were very strikingly similar in phenotype. But within three or four years their descendants were highly variable, indicating a high mutation rate. On the other hand, Lindstrom (1941) reported that a haploid tomato, *Lycopersicon esculentum,* and the diploid and tetraploid homozygous lines established by doubling this strain remained exceedingly stable over many years, with only a few mutations. Thus plants as well as

the comparable animal material, Drosophila and Sciara, may be quite different in mutation rate, and this characteristic rate may be changed by mutators. Wright (1949) has stressed that there is sufficient genetic variability in sexual forms to provide endless variation, sometimes achieving a new peak if the interacting forces are suitable. It is difficult to prove that genetic variation has provided such opportunity. Nevertheless, the explosive evolution of the repleta group, when desert adaptation had been achieved, seems a case in point.

Isolating mechanisms are the *sine qua non* for evolution of genetically separate strains of sympatric organisms. Sexual reproduction keeps genetic variability in motion and continues testing combinations; but unless the mutations and recombinations are kept channeled into separate aggregates, there is no possibility of originating and retaining different organisms in the same area. Evolution from one species to another through time requires only this factor as an isolating mechanism. Evolution from one species into two or more descendent species requires isolating mechanisms, if these are to be contemporaneous and sympatric.

Plant species are not as often completely isolated in terms of gene exchange. However, they utilize many isolating mechanisms equivalent to those used by animals. Although sexual isolation is not a characteristic of plants, differential attractiveness to different insect pollen vectors is roughly equivalent in effect. Self-sterility and cross-sterility factors are common and some of these cause gamete mortality. Zygote mortality which results from complementary cross-lethal factors has been genetically analyzed in some crosses in Crepis, wheat, and Gossypium. Some of these act between strains and show that the mechanisms are widely distributed within and between species. Many crosses fail to go, and species hybrids are often sterile, just as in animals.

In animals, we find parallels to all types of isolating mechanisms found in Drosophila (Patterson, 1947). Vanderplank described mechanical isolation in the tsetse fly. An accessory part of the male genitalia of one species would usually pierce the abdomen of the female of another species, leading to her death. If Vanderplank clipped off this pointed spike, copulation was normal and hybrids were produced. No such clear case of mechanical isolation is known in Drosophila.

Blair (1950), in reviewing the ecological factors in the evolution

of *Peromyscus,* has discussed his own work, as well as the work of Sumner, Dice, and others. Fortunately, these men have been able to show more about the ecological relations in distribution and habitat selection than has yet been done in Drosophila (Blair, 1951). They find selective adaptation in correlation of pelage color to ground color; of shape and tail length, foot size, and other characteristics to habitat. Such details of adaptation are nowhere equaled in Drosophila, unless it is in Wagner's analysis of the food needs, in terms of yeast, bacteria, and other nutriment of the mulleri subgroup.

The other extensive experiments on isolation in vertebrates has been reviewed by Blair (1951). In Amphibia (Bufo and Hylidae), habitat preference, breeding season, call, sexual dimorphism, and cross-sterility all serve as isolating mechanisms. In Peromyscus, there is mating preference, habitat preference, and slight cross-sterility. In fishes, as reported, there is at least some habitat preference; mating preference has been demonstrated or seems probable; in some forms the courtship pattern is important; sexual dimorphism may exist and cross-sterility does exist.

Birds possess many recognition signals, which are often not only species recognition stimuli but may enter into courtship and mating, including in many species the formation of permanent pairs. Birds possess a very well-developed neural integration for stimulus and response, coupled in many cases with an eye and ear for "beauty and the ballet." They possess very effective stimulus-response systems, so that sexual isolation is highly effective in preventing mating. We have only to consider the very elaborate plumage and display technique of the birds of Paradise to appreciate the effectiveness of their sexual stimulus systems.

We cannot summarize the many studies on isolating mechanisms in Drosophila in a short space, for this genus is favorable for such tests. The theory of evolution by the accumulation of differences, in this case differences that reduce gene transfer, is strengthened and clarified by the multitude of diverse mechanisms found in the genus, combined in many different ways. Isolating mechanisms prevent gene flow and allow two or more relatively similar forms to diverge, and so permit exploration and exploitation of the numerous diverse environments that may be available. As adaptation to one environment may partially or wholly exclude adaptation to another, one population, which would exist without the isolating factors, could not utilize them all. There

are adaptations which are in part equivalent to self-sterility factors. In many species the females, and especially the males, are not fertile for several days after emergence. This not only reduces brother and sister mating, but also insures that the flies which breed have passed the selective test of surviving for several days, which certainly eliminates the less vigorous. A tendency to dispersal, rather than sedentary existence, enhances the opportunity for outcrossing. The tendency to mate soon after emergence of the males and females of some species may in fact be a mechanism to promote inbreeding in large motile populations, where it would be otherwise improbable.

With over 90 per cent of the species of Drosophila endemic to one zoogeographical region, geographical isolation is obviously a very important factor. An interesting point in relation to this isolation is that we do not know of any sympatric species that cross in nature with much gene exchange. Although *mulleri* and *aldrichi* hybridize, their hybrids are sterile; and *texana* and *americana,* which do exchange genes freely at the overlap zone, we regard as subspecies. Where species in a group are tested together, they demonstrate various degrees and types of isolation; species in different groups are so thoroughly isolated that no analysis of the mechanisms involved is possible, other than the existence of sexual isolation in most cases.

In addition to the spatial geographic isolation, which implies a physical barrier that prevents sufficient migration so that local evolution can occur in forms so separated, there is the related ecological isolation. To us this implies prior genetic divergence, usually of whole complexes of factors, so that the organisms can or must utilize different ecological niches. There is an interesting possibility connected with ecological isolation of the seasonal variety, such as demonstrated for different members of the repleta group at the Aldrich Farm by Patterson (1943; see Table 17). If a single mutation occurred which would permit the effective use of a food that grew at a different season of the year from those used by its sibs, it could allow the development of two breeding cycles per year in the population. If this mutation tended to reduce the efficiency of the strain under the previously normal conditions, the two populations might drift and be selected apart by the accumulation of correlated factors. This type of ecological adaptation, and perhaps the occurrence of recessive sexual isolating factors in the progeny of a female which wintered over, so that a considerable related population might be built up locally, may

lead to sympatric speciation. We believe that these will be very uncommon, as compared with allopatric development of the accumulation of differences which can lead to separation into two species from a common ancestor. It is difficult to see how genotypes leading to hybrid sterility or inviability, or an insemination reaction leading to death of alien sperm (Patterson, 1947), could be developed within a population.

It is interesting and instructive to note that, by the time two forms have diverged sufficiently to be classed as separate species, they ordinarily have many barriers between them. For example, it is theoretically possible that there might be species which differ only by recessive sexual isolation factors, so that if this initial isolation were to break down, the F_1 and F_2 and later recombination hybrids could give good viable fertile progeny. We know of no such case. There are subspecies and strain differences with isolating factors, but we have found no forms that seem far enough apart to be regarded as separate species from a conservative morphological taxonomic basis that have not been separated by many cumulative genetic barriers. The converse is not true, for sibling species may be phenotypically nearly identical, but separated by numerous isolating barriers.

We have shown that there are recessive isolating factors (*repleta, virilis*), dominant isolating factors (*aldrichi 2, texana*), and complex multiple-factor systems (the Y-autosome F_2 sterility in the virilis group). We have been particularly intent on demonstrating different degrees of divergence and the correlated difference in factors involved in the several available strains. These range from partial strain isolation (*repleta, peninsularis*) to subspecific divergence (*texana-americana, fulvimacula-flavorepleta*), to closely related species (*virilis-texana, mojavensis-arizonensis*), to distantly related species in the same species group which would not cross, and to members of different species groups and subgenera which do not ordinarily even show interest or attempt to mate in Drosophila. We have been able to show the presence of some isolation between separate subspecies or even strains. We do not believe that each subspecies will necessarily develop into a separate species, but we do believe that evidence favors the view that ordinary species differences have come about through the accumulation of steps, through allopatric strains and subspecies to species.

Dobzhansky has on numerous occasions indicated his belief that

after species diverge from a common ancestor, so that the reproductive efficiency of their hybrids is greatly reduced, there is a positive selection for additional isolating mechanisms, chiefly factors increasing sexual selection against crossing in zones where the two forms overlap until full isolation is achieved. His student Koopman (1950) has presented evidence for such an increase in isolation in a population cage experiment. As stated previously (Patterson, Stone, and Griffen, 1940), we do not believe that complete isolation, to the extent that no gene flow can occur between species, is the optimum condition. Semi-isolation, which allows some gene flow, equivalent to migration as expressed by Wright (1931), and in addition including the introduction of perhaps even more unusual gene complexes, seems more favorable to a continuous progressive evolution.

Muller (1949) believes that isolating factors are usually the result of interaction of several genes and that isolation will usually develop between populations which are geographically isolated. He believes further that even sexual isolation is ordinarily due to factors selected into a population for their value to the genotype, rather than for use in isolating the genotype. In this regard the evidence by Lamy (1943, 1948), indicates that several mutant stocks of *pseudoobscura* produce much more sterile and inviable hybrids with *persimilis* than do several wild strains tested, even those which had been kept in the laboratory for a period of time equal to that of the mutant strains. Certainly the differences in crossability here were not the result of cross-sterility selected in the laboratory for this reason.

In *pseudoobscura* and *persimilis,* the amount of isolation was sometimes high for stocks in contact, but a few stocks of *pseudoobscura* from outside the range of *persimilis* were as thoroughly isolated from the latter. In the virilis and the repleta groups, the amount of isolation may be related to overlap zones, but often it is not. In tests with *americana* and *texana, virilis* from Hanchow, China, crossed readily, while *virilis* from Victoria, Texas, crossed poorly; but *virilis* from Galveston, Texas, crossed just as readily as the stock from Hanchow. *Drosophila littoralis* from Europe is much more isolated from the *americana, texana, novamexicana* complex than is *montana* and its relatives, although both cross about the same to *virilis.*

As a further case, we have a nice example of the accumulation of isolation by distance (Wright, 1943) in the funebris group. In this group *macrospina* and *ohioensis* are color phases without serious iso-

lation. *Drosophila macrospina* is partially isolated from the eastern strains of *limpiensis,* with which it might conceivably cross occasionally; it is much more effectively isolated from the western strains of *limpiensis* (which cross freely with the eastern strains), and it is probably unable to exchange genes with *subfunebris* or even *trispina* from California, except perhaps through the intermediacy of *limpiensis.* The cline of isolation increases with distance in this case and agrees with the expected degree of differentiation correlated with restriction on gene flow with distance.

Patterson (1942b) reviewed the information then available on isolating mechanisms. The broad outlines of the problem were well established even then, but many additional factors and mechanisms have since been added. Even in a genus with as many types and combinations of isolating mechanisms as Drosophila, hybridization is still often possible.

Patterson (1942a) reviewed the background and development of the use of the genus Drosophila to study the problem of evolution. It is an interesting historical verification of the choice of the proper material for experimental work that Patterson began his first collections of species of Drosophila in 1934, when only two cases of hybridization were known, soon after the implications of the salivary gland chromosome analysis became apparent. The extensive collecting of Drosophila material only began in 1938, at a time when five cases of hybridization had been found in the subgenus Sophophora. That same year Spencer discovered the first case of hybridization in the subgenus Drosophila, and most of the 101 known cases have been in this latter subgenus, which seems to be more fluid and more rapidly evolving, at least in the Americas.

One of the early important works on crossing between species is that on natural violet hybrids by Brainerd (1924). These included both F_1 and backcross hybrids. Many of the plants and their progeny were grown in Brainerd's garden at Middleburg, Vermont, so that he could test the segregation of characters among the offspring of the fertile forms. Some character differences between the species segregated with regular Mendelian ratios, but often showed the effects of the different gene systems by some modification in phenotype. A great many of the hybrids were sterile or semi-sterile. Some introgression from the hybridization is suggested, and must occur with the fertile forms. In all, eighty-two hybrids were found, involving thirty species

of violets. There is a very interesting spread in the number of hybrids produced by the several species. One species produced fifteen hybrids; one, fourteen; three, twelve; two, eleven; two, nine; one, eight; one, seven; one, five; four, four; two, three; five, two; and seven, one.

Goldschmidt (1934) reviewed his work on crosses between races of *Lymantria dispar*. Although the races studied all belong in one species, he was able to make a very interesting analysis of the geographic and ecological variation, and to show the adaptation to particular environments. In addition to a number of other genetic systems, he studied the sex races and abnormalities in sex determination in race crosses. European and Japanese races differed as to strength of their sex-determining factors, and there are a number of races with sex determiners of different strength in various localities in Japan and the adjacent mainland. Crosses between races gave intersexes, sex-reversal males or sex-reversal females in F_1 or F_2, depending on the strains tested. This is an excellent study showing local adaptation and giving extensive information on how the inherited systems interact to produce a final character.

Federley (1949) published another in his series of studies of species of Lepidoptera of Finland and their hybrids. He reported that the reciprocal crosses between the two closely related species *Drepana facataris* and *Drepana curvatula* gave male hybrids that showed the same type of incomplete chromosome pairing he previously reported in hybrids between *Pygaera pigra* and *Pygaera curtula*. The result is the production of sperm with aneuploid chromosome complements, so that very few F_2 are produced. In the case of the Drepana species, sexual isolation is not operative, apparently owing to the great sexual drive present. In the species stocks, less than 98 per cent (from 44 to 98 per cent) of the eggs hatched caterpillars, but some crosses in each direction gave 100 per cent hatch. Inbreeding or backcrossing gave from 1 to 3 per cent hatch, for the most part because of the aberrant hybrid meiosis.

Crosses between *Drepana falcataria* females and *Drepana curvatula* males produced both male and female hybrids, but Federley was unable to get adult female hybrids to emerge from pupae in the reciprocal cross, a situation similar to the one he found in crosses of *Deilephila elpenor* females by *Metopsilus porcellus* males, additional cases of sex-chromosome genic unbalance. Both F_1 and F_2 hybrids of *falcataria* females times *curvatula* males were sometimes intersexes. The small

numbers available prevented him from determining whether only intersexes and sex-reversal individuals occurred or whether both sexes were occasionally present.

Seiler (1949) reviewed the extensive work by himself and his colleagues on triploid intersexes in Solenobia. These intersex types have been extensively analyzed in terms of organ-system development, and interaction of the gene systems and the egg cytoplasm in development, parallel to Goldschmidt's studies of Lymantria. In addition, Seiler obtained intersexes from one but not the other of the reciprocal crosses between *Solenobia triquetrella* and *Solenobia fumosella.* It would seem that the Lepidoptera fairly often give intersexual hybrids. This is not true in Drosophila, where the only cases known are the repleta group, involving some strains of *repleta* on the one hand and of *neorepleta* or *melanopalpa* on the other (Wharton, 1942; Sturtevant, 1946b; Ward and Stone, 1952).

The only analysis of the cause of intersexuality in these hybrids is Sturtevant's study of *repleta* and *neorepleta.* The intersexes are due to X-autosome unbalance. There is a dominant autosomal *neorepleta* gene that transforms its F_1 female offspring, carrying two *repleta* X chromosomes into intersexes. The gene works through the cytoplasm and does not have to be present to be effective. A dominant *neorepleta* gene in the X chromosome close to the *white* locus is normally in balance with the dominant autosomal male determiner, to give the normal sexes when both are present. No Y-chromosome gene has been found in Drosophila that affects sex determination, other than male fertility genes; for it does not seem that the genes in the *busckii* Y are sex determiners, although they may have some effect (Krivshenko, 1950). There has been demonstrated no situation comparable to that in *Melandrium album,* where the Y carries the primary male-determining segment, as demonstrated by Westergaard (1940, 1948) and Warmke (1946).

Hubbs (1940) discusses speciation and hybridization in fish. He stressed the fact that, in crosses between species (and genera) of fish, the factors responsible for the differences are multiple, leading to a blending effect in F_1 and backcrosses. He also concludes that, in some isolated waters, subspecies have developed in from a few hundred to a few thousand years. As crosses between genera occur, fish must be regarded as more flexible genetically and less isolated, resembling plants in this respect.

Gordon (1947) discusses ecological and spatial distribution of fish with respect to their evolution. He used certain genetic differences here present in wild populations to study the range of distribution and relationship between forms. One very interesting series of tests, showing that distinct genes giving the simple Mendelian ratios were important in fish as well as multiple factors, was published by Gordon earlier. In transferring genes from *Platypoecilus maculatus* to *Xiphophorus hellerii,* the spotted factor, *Sp,* which is responsible for a lightly marked pattern of macromelanophores in *maculatus,* when transferred into *hellerii* causes a rapid multiplication of macromelanophores, so that the hybrid is melanotic, occasionally producing a true neoplasm, as a result of its interaction with the foreign set of genes. The dorsal-fin-spot gene, *Sd,* produces melanoma of this organ in such hybrids, while the *N* factor produces tumor of the flanks. The expression of the red factors, *R, Rt, Dr,* all increase in intensity and become darker in the hybrid. The genotypes of the two species differ significantly in their interaction with these single dominant factors.

Winge and Ditlevsen (1948b) reported reciprocal hybrids between the salmon, *Salmo salar* (2N = 60), and the brown trout, *Salmo trutta* (2N = 80). They noted that the hybrids were much lower in viability than the parent species. Abnormal development occurred occasionally, and the hybrids were infertile within the limits of the experiment. The authors point out that the difference in chromosome number would usually result in abnormalities in meiosis, such as Federley reported in moth hybrids, leading to the formation of many sperm with aneuploid chromosome combinations. In accord with this hypothesis, crosses of hybrid males to trout females resulted in different embryo abnormalities. The F_1 hybrids were in general intermediate in phenotype.

A very different genetic system is illustrated by the genus Rubus. Gustafsson (1943) published a volume reviewing his and other workers' investigations of the European blackberries. Despite the fact that this genus is for the most part polyploid and most forms are apomicts, usually reproducing by pseudogamy, there are some very interesting comparisons to Drosophila. The few diploid members are sexual species with 2N = 14. Most forms are tetraploid, although a few successful triploids and pentaploids are recorded, and there are a very few aneuploids. Gustafsson believes that hexaploids have exceeded the viability optimum. The genus Rubus is divided into subgenera.

Nine of these have from one to nineteen species, and three have more than a hundred species each. Thus it resembles Drosophila in being composed of several minor and several major subgenera.

The subgenus Eubatus is divided into five subsections, which in turn may be further divided into series that correspond roughly to species groups. There is a complication entirely unlike *Drosophila,* for the first four subsections are the *Moriferi veri,* or true blackberries, while the fifth is the *Rubi Corylifolii,* consisting of *Rubus caesius* and the numerous *Corylifolii* which are primary and secondary cross products of this species and the *Moriferi veri.* Gustafsson gives an incomplete list, including *Rubus caesius* (with the innumerable *Corylifolii*) and seventy-two other "circle-species" of the *Moriferi veri.* These circle-species do not correspond to sexual species, but are perhaps a fairly reliable taxonomic entity of about equal importance among apomictic forms. Those grouped as circle-species by Gustafsson are the most widespread and distinct forms.

The diploids are separate sexual species, while most of the polyploids are apomicts. The polyploid is for the most part allopolyploidy, although some ancestral crosses were between closely related forms, so that they are not amphidiploids.

One of the remarkable features so well illustrated in this genus is the partial cross-fertility of the apomicts and its results. Only *Rubus caesius* is entirely pseudogamous, or nearly so, which explains why this European dewberry could become so common in many parts of Europe without being lost into the complex of hybrids in the process. The pollen of *Rubus caesius* is active in fertilizing other forms to produce the *Corylifolii.* All other microspecies and local forms of any importance produce up to 15 per cent embryos which develop from fertilization. Since these forms are heterozygous, this leads to an extensive array of variable forms. In addition, all species hybrids are sexual, no matter whether they are diploid or polyploid. Sexual reproduction develops as a heterosis phenomenon, and on further breeding the descendants of hybrids fix again the recessive genes which allow pseudogamy. Here we have an excellent example of the best use of the characteristics of a genus, even though these characters are often detrimental *per se.* These forms propagate by means of: (1) sexual seed; (2) pseudogamically arising seed; (3) subterranean root shoots; and (4) rooting stem tips.

The several modes of propagation allow the development and re-

tention of groups of plants which have selective value from heterogeneous recombinations, even when these are poorly fertile sexually. Thus asexual reproduction and polyploidy, which of themselves would lead to rigidity, are combined with some sexual reproduction, including hybridization, to achieve flexibility. This tremendous variable and effective complex depends on hybridization, including introgression for additional variability. Whether or not it could effectively produce the novelty necessary for a new and different genus is unknown, but at present this system is very successful.

Anderson and Sax (1936) discussed the cytology of American species of Tradescantia. These consist of diploids (N = 6) and tetraploids (N = 12). A significant difference between this plant genus and *Rubus* is the fact that, in the former, most polyploids are autopolyploids, with little indication of any strict amphidiploidy. In this case also the polyploids are more successful than the diploids. The average range of diploids is 83,475 square miles, and of tetraploids is 376,300 square miles. The effectivenes of autopolyploidy in producing wider-ranging and more northerly forms is an important characteristic of the genus. Although structural variation, such as fragmentation and chromosomal interchange, are present, the authors do not believe they are the important factors in speciation.

Neither polyploidy nor apomixis is involved in Drosophila species formation. Both Rubus and Tradescantia have a basic haploid chromosome number, with diploid and polyploid species based on this number and its multiples. Drosophila has a range of haploid numbers from 7 to 3, based on a primitive number of 6, and has changed chiefly by fusion. Internal reorganization of the chromosomes is characteristic of Drosophila, but its importance is not known in the other forms, although it may be useful in the chromosome mechanics of allopolyploid Rubus.

Balanced chromosome polymorphism producing heterosis in forms like *pseudoobscura* is present in a number of other members of this genus, but phenotypic polymorphism is rare in Drosophila, causing it in that respect to differ from Lepidoptera and from birds (Mayr and Stresemann, 1950). Chromosome polymorphism is known in Oenothera, where it is associated with balanced lethals, and in some species the orthopteran insects.

The extensive work of Clausen, Keck, and Hiesey (1939, 1940, and 1945) has demonstrated the extreme effectiveness of selection in

establishing different ecotypes suited to their local environment in widespread successful species. They report the production of nearly completely sterile hybrids between two species which produced among its few offspring some fertile amphidiploids. These progeny gave rise to a fertile new species, as judged by isolation, phenotype, and adaptability. Brown (1951) has recorded a somewhat similar case in cotton, where a hybrid produced a new fertile amphidiploid form. These cases represent a type of immediate formation of a new plant species, but which is very uncommon, if it occurs at all, in animals. The division of related forms into ecotypes, ecospecies, cenospecies, and comparia undoubtedly characterizes the evolutionary relations of the systems in terms of gene exchange and shared descent in sexual forms. We have found it too exclusive a system to describe descent in animal forms because of the erratic distribution of isolating factors that may prevent crossing between closely related forms. The repleta and especially the willistoni groups are examples of forms where close relatives may give no hybrids. We have found it necessary to establish species groups and even species on phenotype, especially those that do not breed in the laboratory. We have given the data on hybridization in Chapter 9 in order that the reader can use them to establish cenospecies and comparia if he so desires. The virilis group is a good example of a species group that is also a comparium with each of the species listed equivalent to cenospecies. Although heterosis is commonly the result of crosses between strains of the same species, there have been established no cases of balanced chromosomal polymorphism in this group, but otherwise the numerous evolutionary variables are abundantly exemplified.

These three species groups, virilis, repleta, and willistoni, provide information on almost all types of variation known within the genus that contribute to evolution, including splitting into several species from a common ancestor. They illustrate gene changes, chromosomal rearrangements, heterochromatin changes, and gene-balance shifts that provide for physiological and phenotypic divergence. They also provide examples of all types and combinations of isolating mechanisms, leading to various degrees of separation. The chromosome rearrangements in the virilis group, as demonstrated by Hsu, allows us to establish a phylogeny of these species. If we consider Nearctic and Palaearctic as separate zoogeographical regions, and they are obviously separate as far as this genus is concerned, at least two

different primitive forms crossed the Bering Straits. Furthermore, in view of the fact that *texana* and *americana* are now in contact, we must assume one and probably two or more periods when the *novamexicana, texana,* and *americana* populations were isolated from one another for a considerable period of time. Introgressive hybridization is now occurring between *texana* and *americana,* and undoubtedly has occurred between *novamexicana* and western *americana.* We do not know how important a factor this is in Drosophila and other animal species. It may have occurred in the melanopalpa subgroup, although this subgroup could have arisen from a common heterozygous ancestor. We believe that a small effective gene exchange between species will contribute very effective and useful divergence to the recipients of the alien genes. As foresight is seldom a characteristic of the genetic system, isolating factors are often fixed in species which prevent such exchange because of the immediate advantage to those so isolated.

Mayr (1946c) has discussed the history of American bird fauna, and Simpson (1947a, b; and previous publications) has developed the relations of the genera and families present in the Neotropical, Nearctic, and Palaearctic regions in terms of the possible land connections and separations by water between the continents of the Americas and Asia. We find that the greatest number of species of Drosophila are endemic. Although lack of collecting may account in part for the results, the largest group of shared species are those found both in the Nearctic and Neotropical, using Wallace's (1876) dividing line along the northern edge of the tropical rain-forest belt in Mexico. Mayr gives a map showing four probable water gaps that alternately acted as barriers between North and South America from the middle Eocene to middle Pliocene. Although it is impossible to be sure that these continents were separated all this time, yet the lack of exchange of mammals or Drosophila suggests few temporary connections at most. According to Mayr, the southern part of North America was subtropical much of this time, and the belt across lower Mexico from the 19° to 22° north latitude lying north of the water barriers was much warmer. This area is of importance, not only for Drosophila, but also for Cynips and Peromyscus. Kinsey (1937) showed that most of the species of Cynips connect through chains of species into this region. Osgood (1909) gave maps of the distribution of Peromyscus, which showed this region to be the base of a series of chains of species that extended up through North America. More different Drosophila

species are known from this small area than have been reported from any area of comparable size anywhere in the world. Our series of figures on distribution in Mexico and North America demonstrate that very many of the species groups are connected into this region by chains of species. The eight cosmopolitan species are found here, as is *virilis,* which occurs in regions 1, 2, 3, and 5. Three species, including *mercatorum* (1, 2, 6), have another subspecies in South America, and nine other species occur on both continents. The cosmopolitan species and *virilis* give us no information on origin and movement of species, being too widespread. These three forms with a different subspecies in North and South America, and the nine species present as such in both regions, constitute a very small group, and there are at most only four additional species found in both continents with which we are familiar. Omitting nine forms that are of no analytical value in this problem, there are only sixteen of 288 (5.5 per cent) of the species known from and presumably native to these two continents that are present in both. Most evolution of species has been local, and the water barriers between these two continents have been very effective in preventing the exchange of enough individuals to establish successful colonies across these gaps.

Even though Mexico has had a subtropical history part of the time, we do not believe that this is the critical factor which allows for the present multiplicity of species. It seems more probable that the opportunity for species diversification lies in the multiplicity of diverse, scattered, and discontinuous environments, which have remained diverse in this region even through the several temperature shifts since the Eocene. We have only to remember the insects of Hawaii, as reported by Zimmerman (1948), to see how ecological opportunity in diverse, changing, and fragmented environments has allowed remarkably rapid evolution of species and higher categories of insects. The Hawaiian Drosophila are not well known, but probably some areas there have a larger number and certainly more remarkably different species of Drosophila. These facts are in agreement with our concepts of the population dynamics and environmental factors that promote rapid evolution and the attainment of new and useful novelty.

No single genus can give us an adequate example of all the multitude of variables and their combinations found in the evolution of living forms. Drosophila is a very successful genus, having split into several subgenera and many species, occupied numerous habitats,

and utilized most of the major genetic systems to effect this diversification. Although the many species hybrids and intermediate situations that we have reported are a source of difficulty in taxonomy, it is necessary to explore this type of variability, as it is only through hybridization and its products that we can demonstrate the genetic mechanisms and differences present between evolving forms. Each species consists of organisms with an integrated genetic system that adapts it to some mode of life. It is separated from other species by an array of isolating mechanisms which reduce gene exchange to zero, or to some tolerable amount which provides an additional source of genetic novelty. Darwin's theory of the origin of species through natural selection was inadequate to provide a full picture of evolution. Paleontologists and numerous biological disciplines have provided additional information. Population geneticists have developed theories and integrated systems to illustrate how populations may change. These studies have presented evidence of the genetic systems, including the isolating factors that introduce, retain, and accumulate diverse variation. Lotsy (1916) developed the original concept for evolution through hybridization. Anderson (1949) has presented a much more informed discussion of the role of introgressive hybridization, especially in the evolution of plants. While this may play a much less important role in animals, yet we believe it can provide another source of novelty, to be accepted or rejected by natural selection. The synthesis of these various types of information has allowed us to attain a working approximation of the course of evolution in this genus and a better understanding of the general field of evolution.

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In addition to the abbreviations of periodicals usually employed, the following are also used below.

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P.N.A.S.—Proceedings of the National Academy of Science (U.S.A.)
U.T.P.—University of Texas Publications, Genetics
Z.i.A.V.—Zeitschrift fur inductive Abstammungs- und Vererbungslehre
Z.Z.m.A.—Zeitschrift fur Zellforschung und mikroskopische Anatomie

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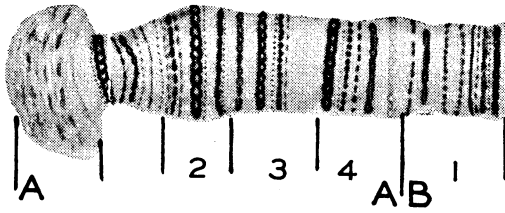
INDEX

D. REPLETA X

2



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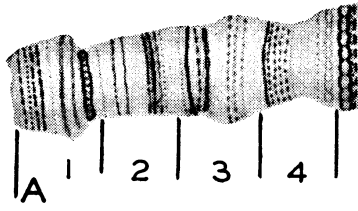

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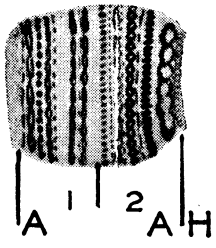

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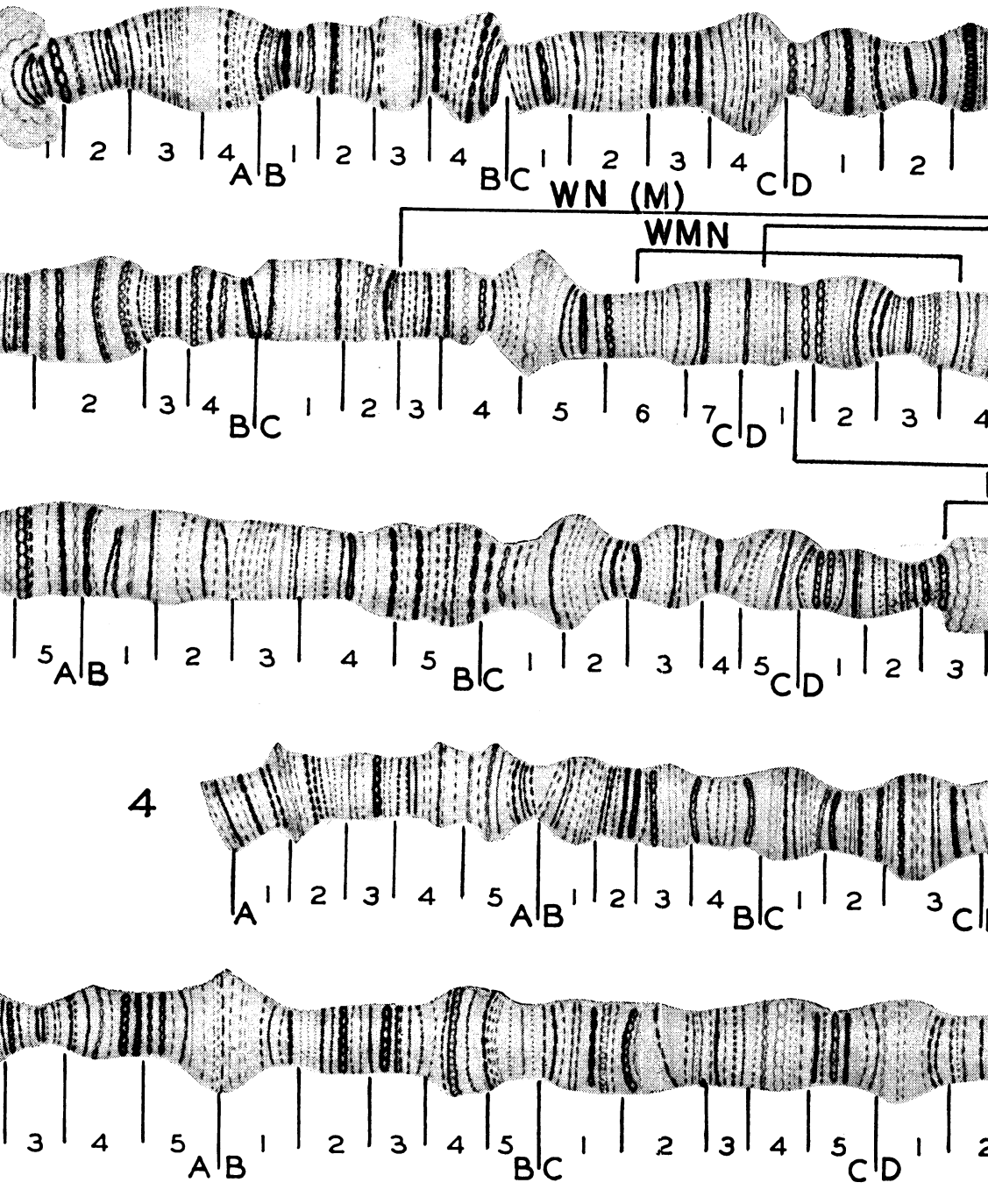
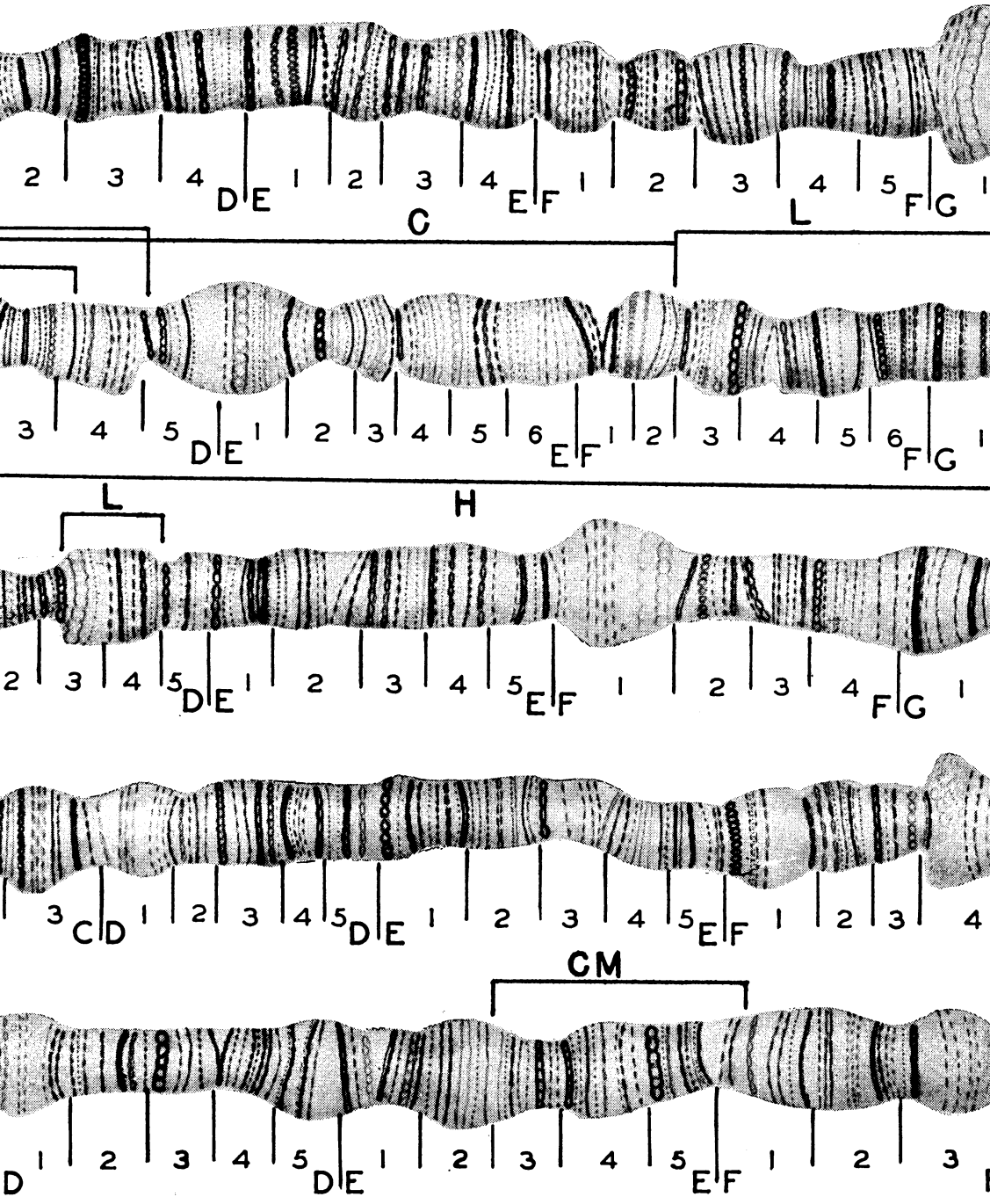
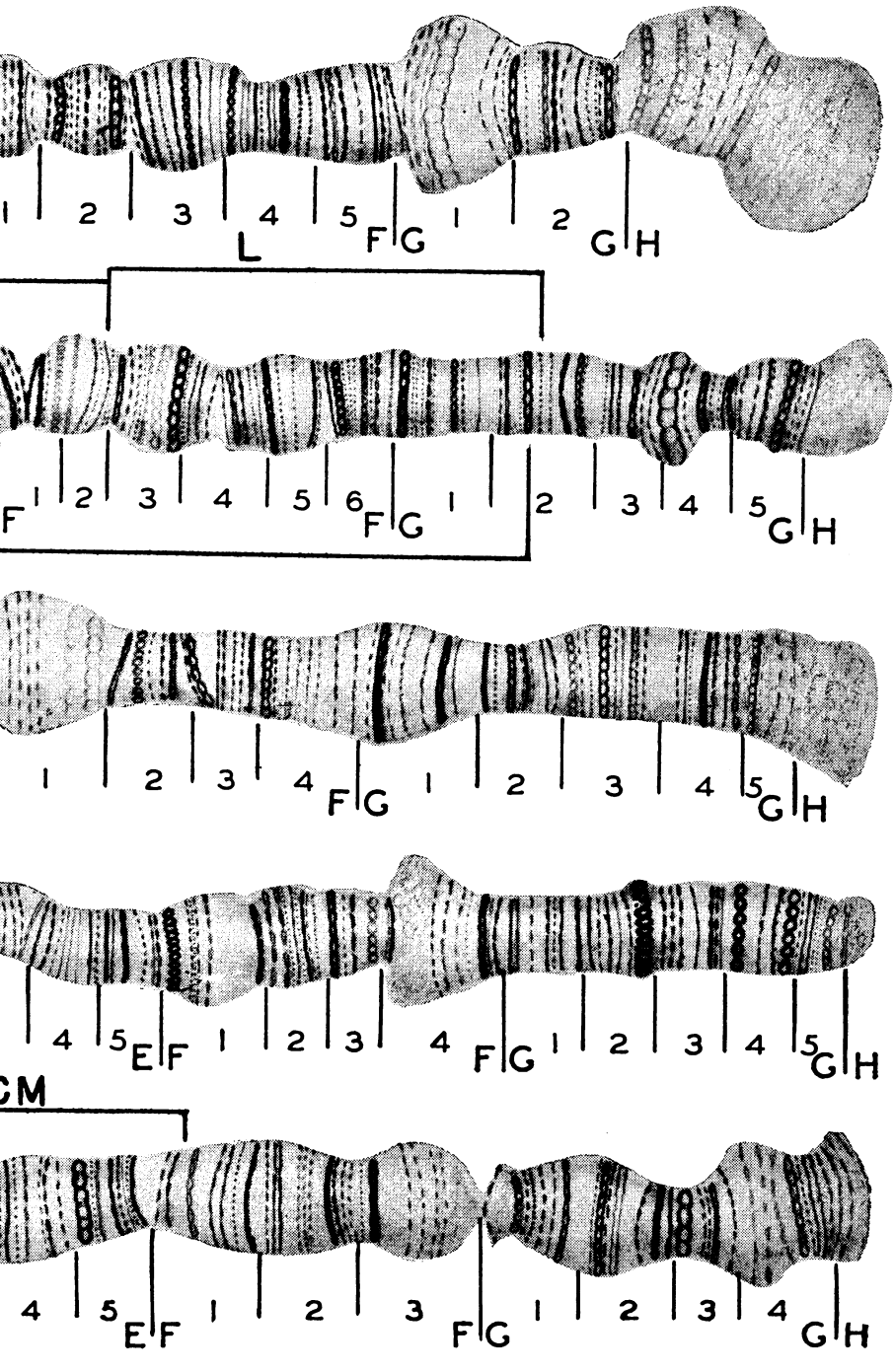


Fig. 67 Salivary gland chromosomes of *repleta* showing position



ng positions of rearrangements. (Modified from Wharton.)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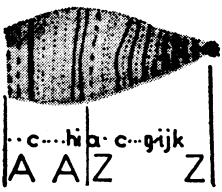
CM
4 5 E F 1 2 3 F G 1 2 3 4 G H

arton.)

6



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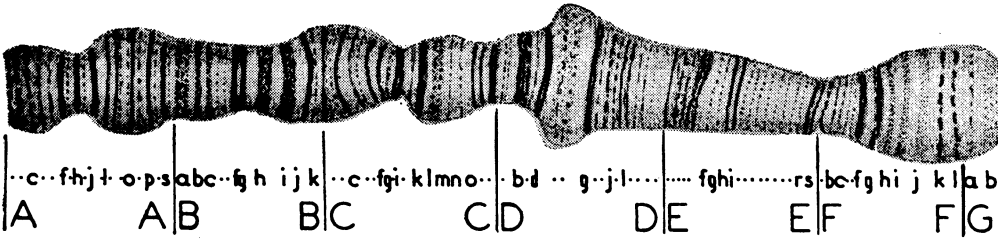

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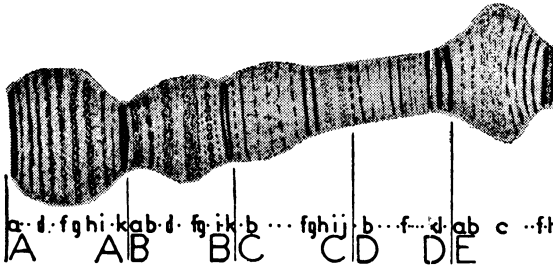

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DROSOPHILA VIRILIS PASADENA

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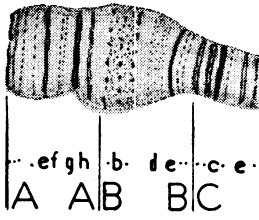


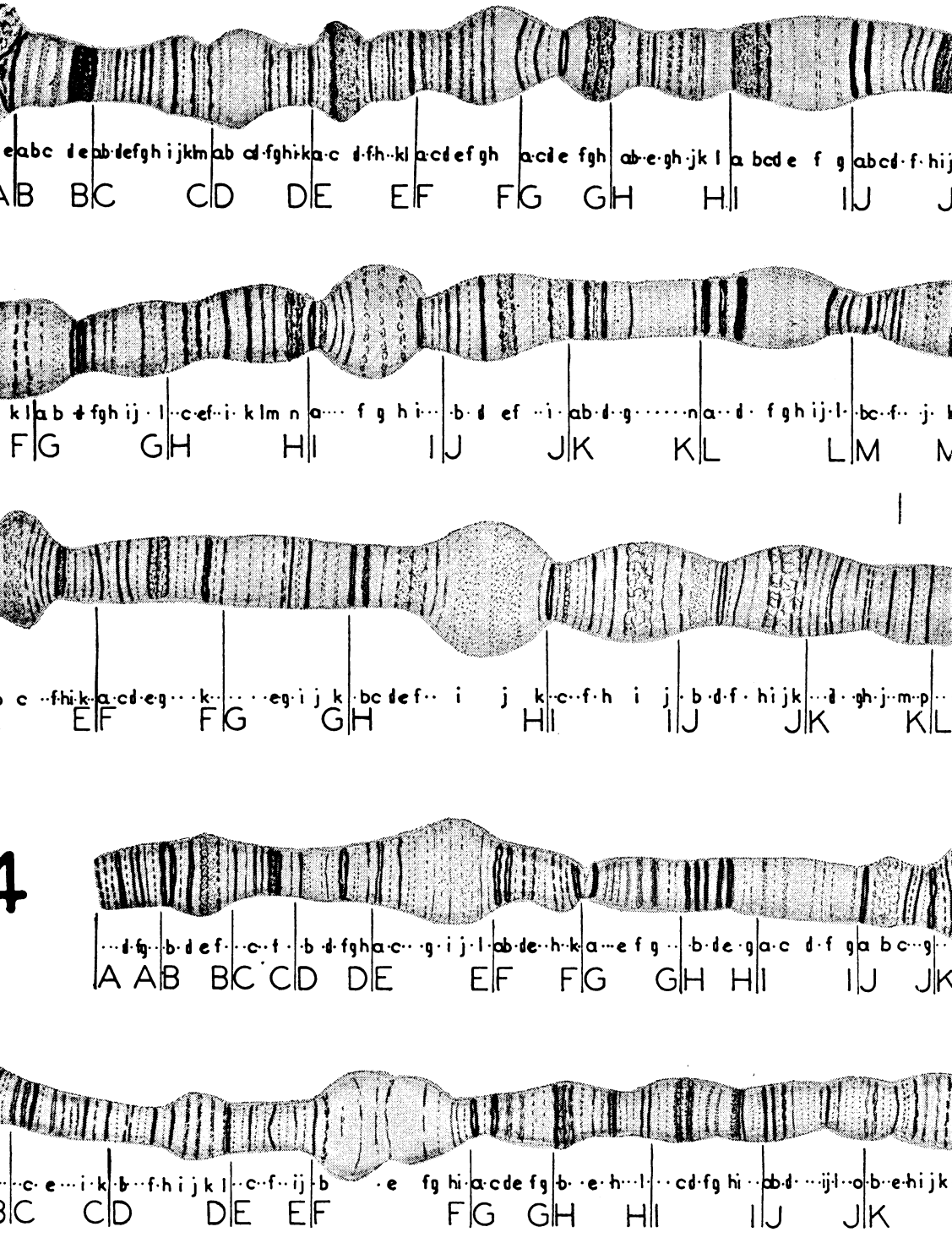
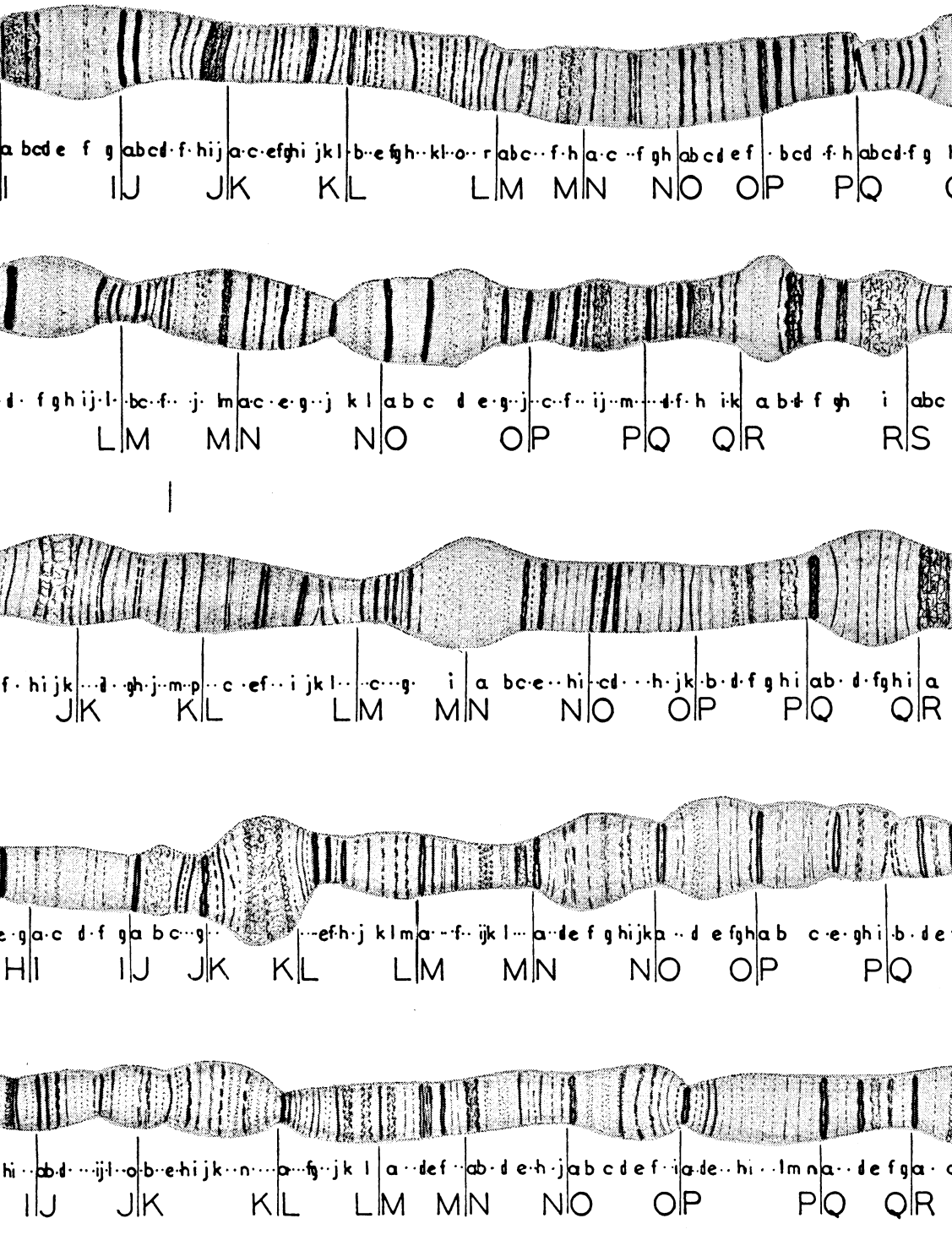
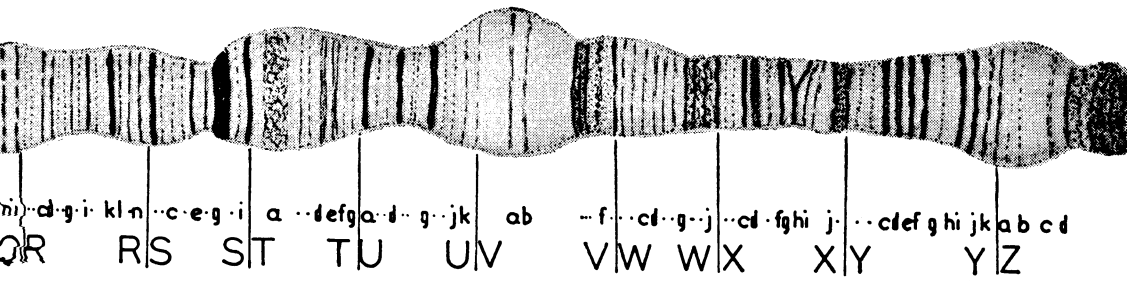


Fig. 68 Map of the salivary chromosomes



salivary chromosomes of *D. virilis*. (From T. C. Hsu.)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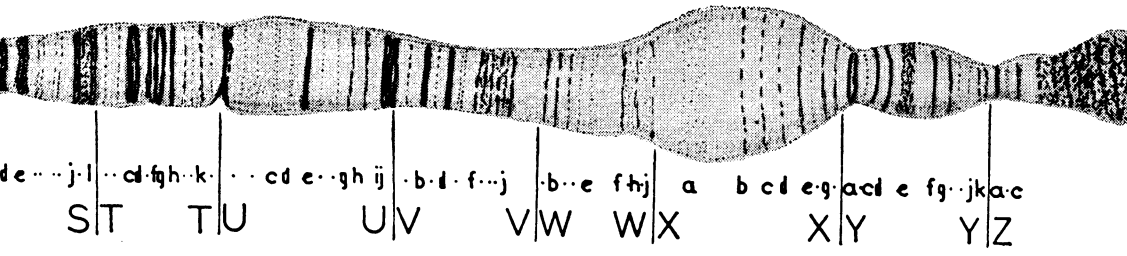
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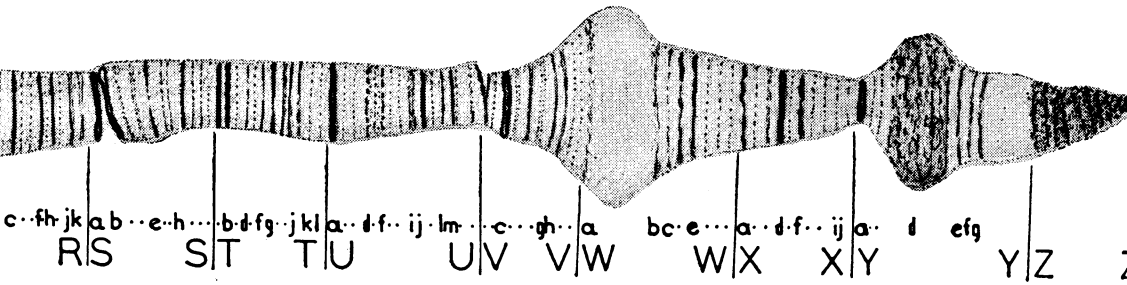
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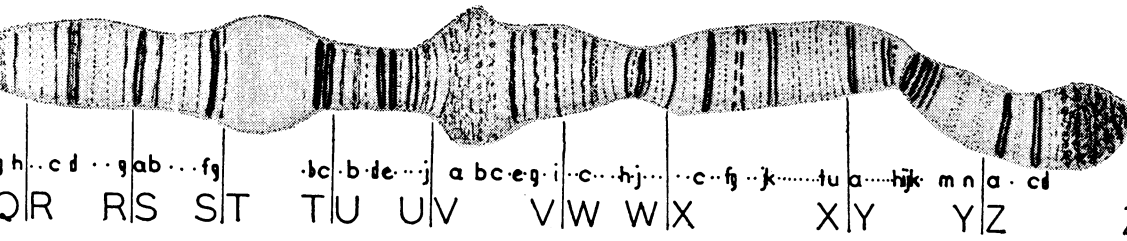
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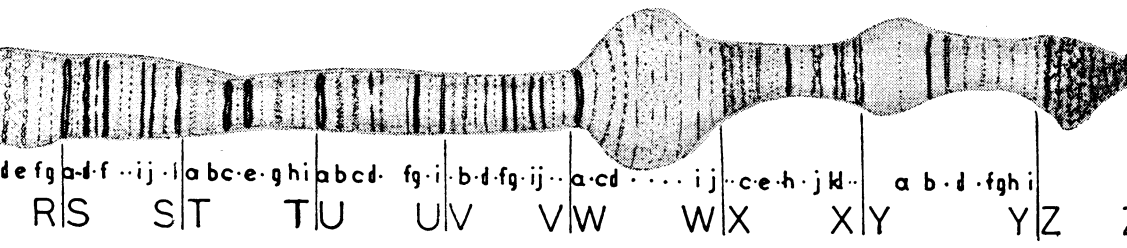
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