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# X. The Problem of Phylogeny In the Genus *Drosophila*[1]

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## INTRODUCTION

Some time ago the writer began investigations directed toward determining the usefulness of biochemical characteristics in Drosophila taxonomy. Before this problem could be resolved satisfactorily it was necessary to determine to what extent biochemical and morphological characteristics followed the same patterns of behavior during evolution, and to what extent these patterns could be interpreted to produce a model for phylogenetic analysis consistent both with morphological and biochemical evidence and with our present concepts of the dynamics of adaptive change. In determining patterns of morphological evolution the central problem is, of necessity, the problem of phylogeny. One cannot follow the behavior of characteristics, either morphological or biochemical, during evolution until phylogenetic relationships have been shown with reasonable accuracy. Several phylogenies for the species involved are available, but they show considerable inconsistency, both as to method of derivation and as to result. It has, therefore, been necessary to re-evaluate phylogenetic relationships within the genus and to develop a conceptual model for phylogenetic analysis. The present paper deals with the distribution and phylogenetic significance of certain morphological features. Biochemical aspects will be covered separately (see Throckmorton, and Throckmorton and Magalhaes, This Bulletin).

In order to evaluate the behavior of morphological characteristics during evolution it is necessary to have available a phylogeny based on characteristics other than morphological ones. Several phylogenies based on cytological evidence have been prepared for a number of species groups in the genus. The most extensive ones are those for species of the virilis group (Stone, *et al.*, 1960) and for species of the repleta group (Wasserman, 1960). It is thus possible to relate distributions of distinct morphological characteristics to the evolutionary sequences which produced them. When this is done, inferences can be drawn regarding the behavior of the genetic systems producing the various phenotypes during the speciation events initiating the phyletic lines under consideration. The results from these analyses indicate useful methods for phylogenetic study, and these methods can be applied to investigate distributions of morphological features throughout the genus.

The morphological characteristics chosen for this study are, for the most part, those used widely in Drosophila taxonomy. Several, however, are described in detail for the first time here. The features investigated are as follows: 1) morphology of paragonia and relationship between paragonia and vasa deferentia, 2) morphology of ejaculatory bulb and of ejaculatory apodeme, 3) morphology of the testes, 4) characteristics of the first and sixth abdominal sternites in the male,

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5) characteristics of the spermathecae, 6) morphology of the ventral receptacle, 7) characteristics of the Malpighian tubules, 8) arrangement of branches of the anterior pupal spiracle, and 9) characteristics of the egg filaments. Several additional features have been noted also, and these will be commented on briefly but not considered in detail.

The characteristics of these structures provide a large amount of anatomical detail, adequate both for correlations with known phylogenies and for determining the main outlines of evolution within the genus. The phylogeny arrived at through their use is broadly consistent with those previously produced by other means (Sturtevant, 1942; Hsu, 1949; Patterson and Stone, 1952; Malogolowkin, 1953; Okada, 1956 and 1958), and, in addition, the conceptual framework upon which it is based allows data from species in closely related genera to be integrated with data from species in the genus *Drosophila*. When this is done, relationships between these genera and certain phyletic lines within the genus are indicated, thus opening the possibility for extending this type of phylogenetic analysis to problems of relationships between genera and between groups at higher taxonomic levels.

One major conclusion indicated by the analysis of this material is that the majority of groups are derived from populations "heterozygous" for the genetic determiners of various alternate forms of a given characteristic. Thus, for any single morphological feature there has been a considerable amount of "parallel" evolution. A consequence of this is that two or more phenotypes, expressing substantially homologous genotypes, may arise separately in phyletic lines, themselves derived from a single ancestral population. This appears to complicate, but really adds precision to, the phylogenetic analysis, and the problems involved will be discussed in detail later. This consideration should, however, be kept in mind during the presentation of the data.

## Materials

The materials used in this investigation have come from several sources. The great majority of species and strains were from those maintained as stocks at the University of Texas Laboratories. Some of the stocks have been provided by other workers, and their sources will be acknowledged elsewhere in this publication. In addition to utilization of laboratory strains, it has been advisable to include other species which cannot be reared in the laboratory. For the most part these have been collected by the author, either in the vicinity of Austin, Texas, or in the vicinity of Riverside, California.

The species and strains used, together with their classification, University of Texas collection numbers (where applicable) and collection localities are listed in the Appendix. The classification followed is that of Wheeler (1949b), Patterson and Stone (1952), Okada (1956) and others. Two changes involving subgeneric reference have been made to conform with the results of the present study. *D. nannoptera*, previously assigned to the subgenus *Sophophora* (Wheeler, 1949b), has been transferred to the subgenus *Drosophila*. Species of the bromeliae group, previously assigned to the subgenus *Sophophora* (Patterson and Stone, 1952) are likewise transferred to the subgenus *Drosophila*. Species of both of

these groups are related most closely to species of the virilis-repleta section of this subgenus. Only two minor changes have been made. *D. guttifera* is here included as a member of the quinaria group, and *D. aureata* has been removed from the repleta group.

## Methods

Individuals to be used for dissection have come from laboratory cultures or from local collections. When laboratory cultures were used flies to be dissected were taken directly from the stock, and no effort was made to obtain flies of uniform age. Age may have an effect upon the appearance of some structures, and these effects will be indicated during the discussion of the individual characteristics. When flies were from local collections, age was also unknown. Individuals from only one strain of a given species were used, and the size of the sample varied, depending on the characteristic being investigated. Therefore, few conclusions regarding intraspecific variability of a trait can be drawn from the present data. From ten to twelve individuals were used to determine the internal characteristics of the male. Approximately five individuals were used when the internal characteristics of the female were recorded. If details were observed in cleared material, as for the ejaculatory bulb in the male and spermatheca in the female, general characteristics were noted from uncleared material and only one specimen was cleared and used to prepare the figures. Additional specimens were cleared when unusual features were noted. Samples for other characteristics varied between five and ten individuals.

The methods used for demonstrating internal structures have been quite simple. The fly was dissected in *Drosophila* Ringer's solution (Ephrussi and Beadle, 1936). The relationships *in situ* of the various organs were noted, and then the structures of particular interest were separated and figures made of their major features. The low powers of a binocular dissection microscope were generally adequate, both for dissection and for observation of structure. Most of the organs were observed without further treatment. To determine the characteristics of the ejaculatory bulb and of the ejaculatory apodeme it was necessary to clear the material. This was done by transferring the bulb, generally with external genitalia and ejaculatory duct attached, to a drop of phenol on a slide. In a short time, often less than one minute, the cellular envelope and the contents of the bulb were dissolved away leaving the transparent, thinly-chitinized lining with the ejaculatory apodeme attached to it. During this treatment the shape of the bulb was retained, except for the shape of very long and fine caecae (ejaculatory sac diverticula of Rosenblad, 1941), when present. In the cleared condition it was a simple matter to rotate the organ to any position desired and to determine its structure in some detail, even when using low magnifications. Only rarely was it necessary to check the bulb at higher magnifications by the use of the compound microscope. The figures of the ejaculatory bulb show the organ after clearing in phenol.

It was also necessary to clear the spermathecae, and this was done by methods similar to those used for the ejaculatory bulb. The female reproductive tract was dissected free from other internal organs. The ovaries, and generally the egg

guides, were removed, and the major portion of the vagina with its attached spermathecae, parovaria and ventral receptacle was transferred to a drop of phenol on a slide. Clearing time varied greatly from species to species, although it generally was considerably less than five minutes. The spermathecae, parovaria and ventral receptacle were observed, first with the binocular dissecting microscope and then with the low power of the compound microscope. Figures for the spermathecae were made from the cleared material as seen through the compound microscope. Figures of ventral receptacles involve observations from both cleared and uncleared material. Where pertinent, the source of certain details, whether from cleared or uncleared material, will be indicated during the description of the individual characteristics.

The morphology of the Malpighian tubules was checked from both males and females. About five individuals of each sex were dissected. The general features of the Malpighian tubules were noted by using the magnifications of the dissecting microscope. The posterior Malpighian tubules were then transferred to a drop of *Drosophila* Ringer's solution on a slide, and they were observed with the compound microscope to determine whether the tips were apposed or fused. Free tips could be identified readily without use of the compound microscope.

Larvae and pupae were taken directly from stock cultures. Only third instar larvae were used. For observation of pupal spiracles the pupae can be of any age, so long as the anterior spiracle is completely everted. Pupae taken from the medium rather than from the walls of the culture vial were preferable for observing spiracle characteristics. The anterior spiracle must always be checked to be sure that the branches are completely everted. This is not hard to do so long as the necessity for the check is recognized. Some previously published figures (e.g., *busckii* in Patterson and Stone, 1952) appear to be from specimens in which the branches of the anterior spiracle had failed to evert, or had everted only partially. In some species a large proportion of the pupae have spiracles which fail to evert completely. Figures were made only from pupae with the branches completely everted, regardless of the proportion of everted and uneverted spiracles in the total sample. Eggs were taken directly from fresh cultures.

At the time of observation free-hand sketches were made from the material, and note was taken of any unusual features. Within each figure relative proportions were, as nearly as possible, as observed. No effort was made to draw the figures to any set scale. Thus, strict size comparisons between individual species cannot be made from these figures. General comparisons can be made between species in the genus *Drosophila*, and in certain cases (the ejaculatory bulb and the spermathecae) the relative sizes compare very closely with those observed. In some cases it has been necessary to exaggerate the size of certain structures, and these cases will be noted individually. All of the figures show semi-diagrammatic representations of the structures involved. The organs, particularly the testes and paragonia, are often twisted around each other in such a way as to make a photographic representation of their relationships quite uninformative.

## The Characteristics

Direction of evolution is an immediate problem in any phylogenetic study.

Most of the characteristics selected for use in this investigation show directional change, and the more primitive form of expression of each trait can generally be inferred, either on theoretical grounds or by analysis of the distribution of the various forms of the trait among related groups. In this section the various characteristics will be described individually, and the data for each characteristic will be presented. Here the major emphasis will be on the description of the different forms of the trait and on the features which indicate its evolutionary status (primitive vs. derived). The emphasis here will also be on broad general similarities which tend to unite major groups. This overemphasizes uniformity, but discussion of types of variation will make up a major part of a later section, and the significance of the variants will be emphasized at that time. The data from each trait will also be summarized in the form of a phylogeny. These initial phylogenies should be interpreted only as working models, although they may also have a certain usefulness as pictorial keys. Integration of all the data for all characteristics will be deferred until the next section, and a general phylogeny will be developed there. Data from other genera and families will be included, where applicable, to support conclusions regarding the evolutionary status of a characteristic.

*Internal Reproductive Structures of the Male*—The terminology to be used follows, for the most part, that of Patterson (1943) or of Okada (1956). Figure 1.1 is labeled to indicate the major features. The male reproductive system of *melanogaster* has been described elsewhere (Miller, 1950), and since other species, from other families of Diptera as well as from the genus *Drosophila*, differ from *melanogaster* only in detail, it is not necessary to discuss the system more fully here. In the case of material from other families there may be some doubt regarding homology of certain structures. For the most part the structures involved are of little significance for the present purpose and are included in the figures primarily to give an indication of the range and type of variation encountered outside of the family Drosophilidae. Figures 1 and 2 show internal structures for males from other families of Diptera and from the family Drosophilidae, respectively.

With reference to terminology, one major departure from usual usage has been made. The common practice has been to refer to the "inner" and "outer" coils of the testes in species where the testes have a spiral form. Stern (1941a, 1941b) has described the growth of the testes in several species of *Drosophila*, and it is clear from his description that the testis proper makes up only the outer coil. The so-called inner coil of the testis is a part of the vas deferens caused to coil mechanically by the coiling of the testis following its attachment to the vas deferens during development. It therefore seems advisable to drop the term inner coil, and to use the term vas deferens to apply to the duct leading from the base of the paragonia to the testis (i.e., to the beginning of the outer coil). This duct may be differentiated in several ways, and its characteristics will be commented on later. The term testis will be used only to refer to the outer coil.

*Morphology of paragonia and relationship between paragonia and vasa deferentia*—Aside from the extensive work of Hori (1960) describing the male internal organs of Calyptrate Muscoid flies, little information is available regarding the types and distributions of the internal reproductive organs of the

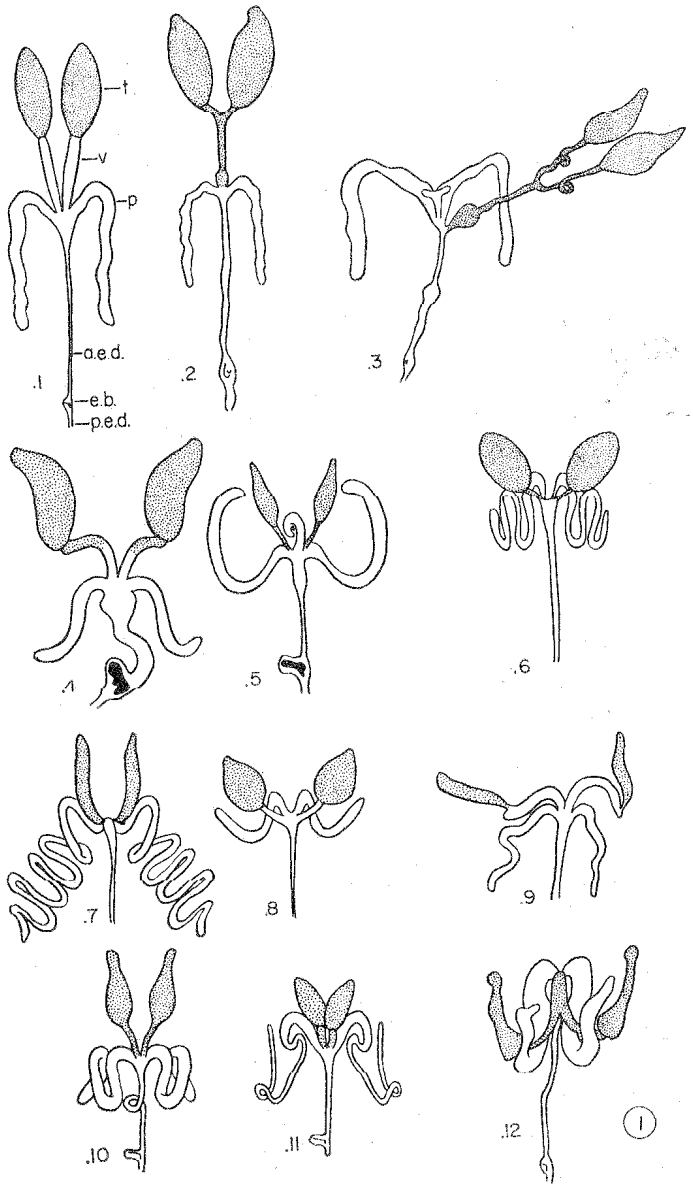


FIG. 1. Internal male reproductive structures from representatives of various families of (Aulacigastridae); .11) *Periscelis annulata* (Periscelidae); .12) *Diastata vagans* (Diastatidae). *obscurella* (Ephydridae); .4) *Phytobia* sp. (Agromyzidae); .5) *Sepsidimorpha secunda* (Sepsidae); .6) *Mumetopia occipitalis* (Anthomyzidae); .7) *Thaumatomyia glabra;* .8) *Oscinella coxendix* (Chloropidae); .9) *Prochyliza xanthostoma* (Piophilidae); .10) *Aulacigaster leucopeza* (Aulacigastridae); .11) *Periscelis annulata* (Periscelidae); .12) *Diastata vagans* (Diastatidae). Symbols in Figure 1.1 indicate: t-testis; v-vas deferens; p-paragonium; a.e.d.-anterior ejaculatory duct; e.b.-ejaculatory bulb; p.e.d.-posterior ejaculatory duct.

male. Although the sample from other Acalypterate families is not large, the general types encountered are consistent enough, and compare closely enough with types seen by Hori (*op. cit.*) among the Muscoids, that they may be used as

a point of departure in discussing the direction of evolution for the different characteristics. As a general rule the paragonia are rather thin, but they may be quite variable in length (Fig. 1). Within the family Drosophilidae, and particularly within the genus *Drosophila*, the paragonia become unusually robust and exhibit highly specific orientation and morphology (Figures 2–14). The vas deferens in other families is almost always a short, rather heavy duct which may or may not be pigmented. Aside from the Drosophilidae and the Diastatidae (Figure 1.12),

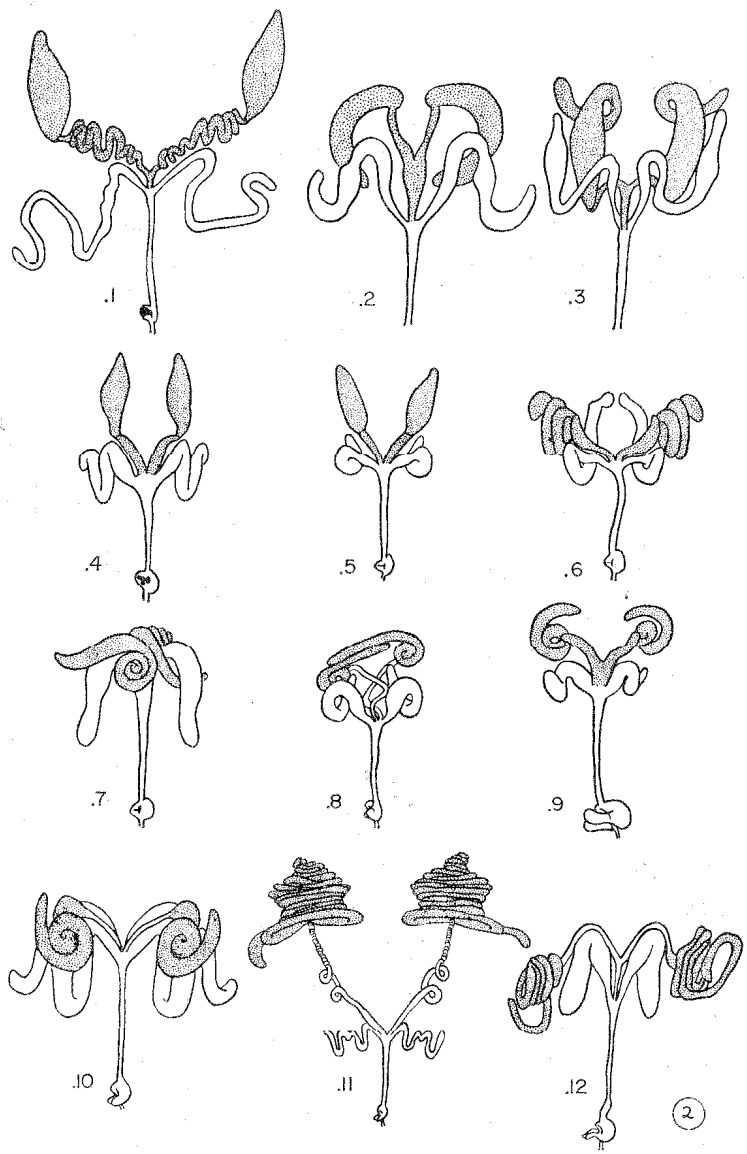


FIG. 2. Internal male reproductive structures from representatives of the family Drosophilidae. .1) *Zapriothrica dispar;* .2) *Gitona americana;* .3) *Rhinoleucophenga obesa;* .4) *Drosophila victoria;* .5) *D. pseudoobscura;* .6) *D. tolteca;* .7) *D. yakuba;* .8) *D. equinoxialis;* .9) *D. busckii;* .10) *D. aldrichi;* .11) *D. bifurca;* .12) *D. subbadia.*

both closely related, a basal fusion of the vasa has been seen only in the Ephydridae. In at least one of the Ephydrids figured (Figure 1.3) it is probable that the apparent vas deferens is not homologous with that seen in other families. In this instance the testes appear to have established a new connection with the ejaculatory duct, and the old vasa remain as a Y-shaped remnant (still showing basal fusion, however). Among the Chloropids at least two genera (an example of only one, Figure 1.7, shown) show no recognizable vasa and the testes attach directly to the bases of the paragonia. Other genera of Chloropids (e.g., Figure 1.8) show the more usual configuration. These variants, however, do not seriously detract from the impression that short, unfused vasa are the general condition. This is the type seen in such species as *victoria* (Figure 3.1), and, since the other types seen in the genus *Drosophila* are highly distinctive (e.g., Figures 2.8, .12) the assumption that this is a primitive type for the group seems most reasonable. The basal fusion seen in *busckii* (Figure 2.9) apparently represents a modification of this type, but since similar fusions of the vasa are evident in other, more primitive genera (Figures 2.2–.3), it cannot be considered as being derived within the genus.

Aside from the development of specific relationships between the vasa and the paragonia, which will be discussed shortly, the major change of the vasa with evolution has involved lengthening and regional differentiation. The differentiation generally takes the form of an expansion of the region of the vas just proximal to the testis proper, and it is seen in almost all species in the genus. In forms with spiral testes it is this region which is coiled mechanically by the asymmetrical growth of the testis. In no cases does the number of coils in this region exceed the number of coils in the testis, and it is generally considerably less. This region is pigmented if the testes are pigmented, apparently due to the migration of pigment cells from the testes (Stern and Hadorn, 1940). This differentiated region has been named the inner coil of the testis by some, and the seminal vesicle by others (Miller, 1950; Okada, 1956). In species where the vasa remain short, differentiation involves the whole structure (e.g., Figure 13.7). In others, the proximal portion appears as a thin, hyalin duct while the distal portion, generally one-fourth to one-third the total length, is expanded and has an opaque, granular appearance. Presumably, it is this portion which represents the original vas, with the proximal, hyalin duct representing the modification added during evolution. This last assumption can, however, only be verified by more detailed, comparative histological and developmental studies. Until such studies are made, application of a particular name such as seminal vesicle, to the differentiated region seems premature. If the term seminal vesicle were generally used, it would have to be applied in some cases to the entire duct between the bases of the paragonia and the testis. In others it would apply only to a part of this duct. It seems best to defer naming this region until its functional and evolutionary significance throughout the family is known.

Within the genus *Drosophila* the most extreme development of this region is seen in species from the immigrans group and in species belonging to the quinaria section of the subgenus *Drosophila* (see Patterson and Stone, 1952, p. 81, for general phylogeny). Many other species in the subgenus *Drosophila*, particularly within the repleta group, also have this region strongly developed. Using

the methods of simple dissection, no sharp distinctions based on this feature can be made within the genus, although it seems probable that more detailed investigations, and particularly a histological study, would show significant patterns of distribution. One apparent exception to this general statement involves the type of vas development seen in some members of the hydei subgroup of the repleta group. The most extreme example is seen in *bifurca* (Figure 2.11) where there is no distal swelling of the vas, and instead the vas in this region is thrown into fine, regular coils. One other species in the genus, *nannoptera*, shows this same type of vas development. *D. neohydei* and *eohydei* show this to a limited degree, and *hydei* does not show it at all, although its vasa show little differentiation and are almost uniform in diameter throughout. *D. castanea* strongly resembles *hydei* in this respect. *D. nigrohydei* has the usual, moderately differentiated vasa.

The most conspicuous and most useful details of structure for the vasa and the paragonia are shown in Figures 3 to 12 for the genus *Drosophila* and in Figure 13 for species from related genera. Although changes in the major components, i.e., the vasa and the paragonia, have, in the evolutionary sense, developed independently of each other, it is most convenient to treat them both at the same time. As has been said earlier, the type of vas development seen in such species as *victoria* (Figure 3.1) represents a primitive form, and the abbreviated phylogeny shown in Figure 14 is based on this assumption.

The members of the subgenus *Pholadoris* (Figures 3.1–.7) show either the primitive, short, pigmented form of the vas or a simple modification of this type. The paragonia are rather variable, but almost all are relatively long and slender. They are usually folded at least twice, and most often are folded three or more times. In this case it is not possible to specify a distinct primitive type. Paragonia in other families are too variable, as are those from other genera in the family. The great majority of types within the genus *Drosophila* are themselves distinctive. Those seen among the species of the subgenus *Pholadoris* resemble types seen in other families and in more primitive members of the family. The assumption most consistent with all available data is that the types seen in *victoria* and *pattersoni* are near the primitive, although types seen in some species of the virilis group (Figures 4.25–.28) and in *Phloridosa* species (Figures 6.10–.12) may be equally primitive if not more so. When both the vasa and the paragonia are considered, the subgenus *Pholadoris* is placed at the base of the phylogeny of the genus (Figure 14).

Several types are seen in the subgenus *Sophophora*. In none of these is there an association between the vasa and paragonia such as is seen in the other major phylogenetic branch of the genus. The first type is that of *populi* (Figure 14.15), showing the short, pigmented vasa. It has paragonia which are, at present, distinctive in the genus, unless those of *busckii* (Figure 3.11) represent a variant of this form. The paragonia are quite robust, more so than any others in this subgenus, and are bent so that their ends point in opposite directions. It is of interest to note that Okada (1956, Figure 34, p. 62) shows a figure of *Chymomyza nigrimana* in which the paragonia appear to be of this general type.

In species of the obscura group (Figures 3.13–.22) the vasa are all short and pigmented, and the paragonia have a characteristic twist which usually results in their ends being directed anteriorly. This same type is seen in species from

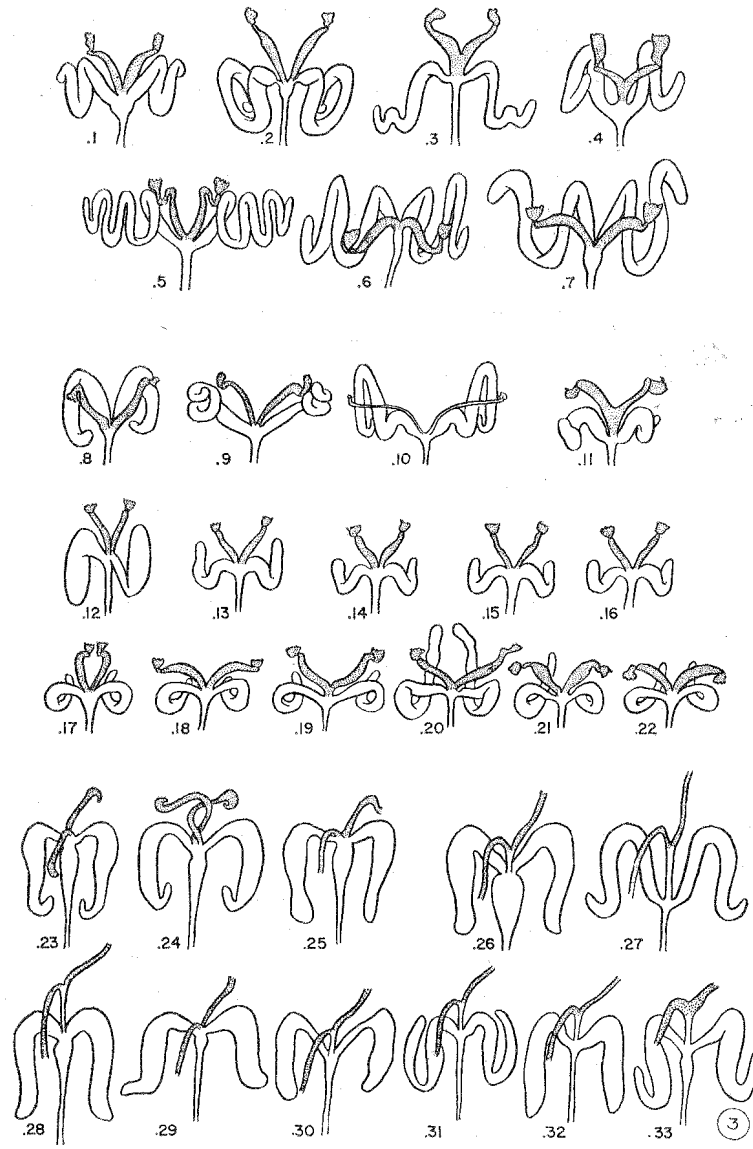


FIG. 3. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

Subgenus: PHOLADORIS

**victoria group**
.1 victoria
.2 pattersoni

**bryani group**
.3 bryani

**latifasciaeformis group**
.4 latifasciaeformis

**coracina group**
.5 cancellata
.6 lativittata
.7 novopaca

Subgenus: HIRTODROSOPHILA
.8 duncani
.9 pictiventris
.10 thoracis

Subgenus: DORSILOPHA
.11 busckii

Subgenus: SOPHOPHORA
.12 populi

**obscura group**
*obscura subgroup*
.13 miranda
.14 pseudoobscura
.15 persimilis
.16 ambigua

the saltans and willistoni groups (Figures 4.1–.24; Figures 14.10–.11). In some members of the obscura, saltans and willistoni groups the paragonia are reduced in size. In the sturtevanti subgroup of the saltans group the paragonia may appear almost as vestiges.

Most species of the melanogaster group lack the twisted paragonia seen elsewhere in the subgenus, although both *ananassae* and *bipectinata* approach this condition, or are intermediate between this and the type seen in *populi*. The paragonia are generally large and show a second fold distal to the major arch (Figures 14.12–.13; 3.23–.33; *bipectinata* has not been included in the figures and is substantially as in *ananassae*). All species of the melanogaster group have the testes located anterio-posteriorly rather than laterally (Figure 2.7). In cases where the vasa are not fused to form a common duct basally there is generally a crossing of the vasa. For *melanogaster* this cross is clearly shown by Miller (1950, Figure 38A). Stern (1941b) describes this cross as being due to asymmetric growth of the testes. The left testis unites with the left vas, its continued growth causes the vas to be swung to the right, and the testis comes to lie in the anterior part of the abdomen. The opposite is true for the right testis. Even in *melanogaster*, however, the cross is not always seen since the two vasa occasionally appear to arise dorso-ventrally rather than laterally, or, in other species, they may be fused basally. The anterio-posterior orientation of the testes is therefore more diagnostic for the group than are the crossed vasa.

Among species in the saltans and willistoni groups the testes are located laterally, but their free ends either cross or lie parallel to each other and point in opposite directions (e.g., Figure 2.8). Basally the vasa cross at least once and often twice. This cross is shown for *nebulosa* by Patterson (1943). In these species the mass of testes and paragonia is tightly packed and strongly bound together with tracheae. This makes the demonstration of the details of the system very difficult, and dissections were directed only toward determining the presence of crossed vasa. It should be possible to determine the cause and significance of the double cross of the vasa, but no attempt was made to do that at this time. Superficially, it would appear that the species of the saltans and willistoni groups exhibit the same general phenomenon seen in species of the melanogaster group. They would differ mainly in having "completed" the cross, so that the larval right and left testes are seen as left and right testes respectively in the adult. A more detailed investigation will be required to show whether or not this is actually the case.

In the saltans, willistoni and melanogaster groups the vasa may or may not be fused basally to form a common duct. In the saltans and willistoni groups the

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*affinis subgroup*
.17 affinis
.18 algonquin
.19 narragansett
.20 tolteca
.21 athabasca
.22 azteca

**melanogaster group**
*melanogaster subgroup*
.23 simulans
.24 melanogaster
.25 yakuba
*takahashii subgroup*
.26 takahashii

*ananassae subgroup*
.27 ananassae
*montium subgroup*
.28 rufa
.29 nikananu
.30 serrata
.31 auraria
.32 seguyi
.33 kikkawai

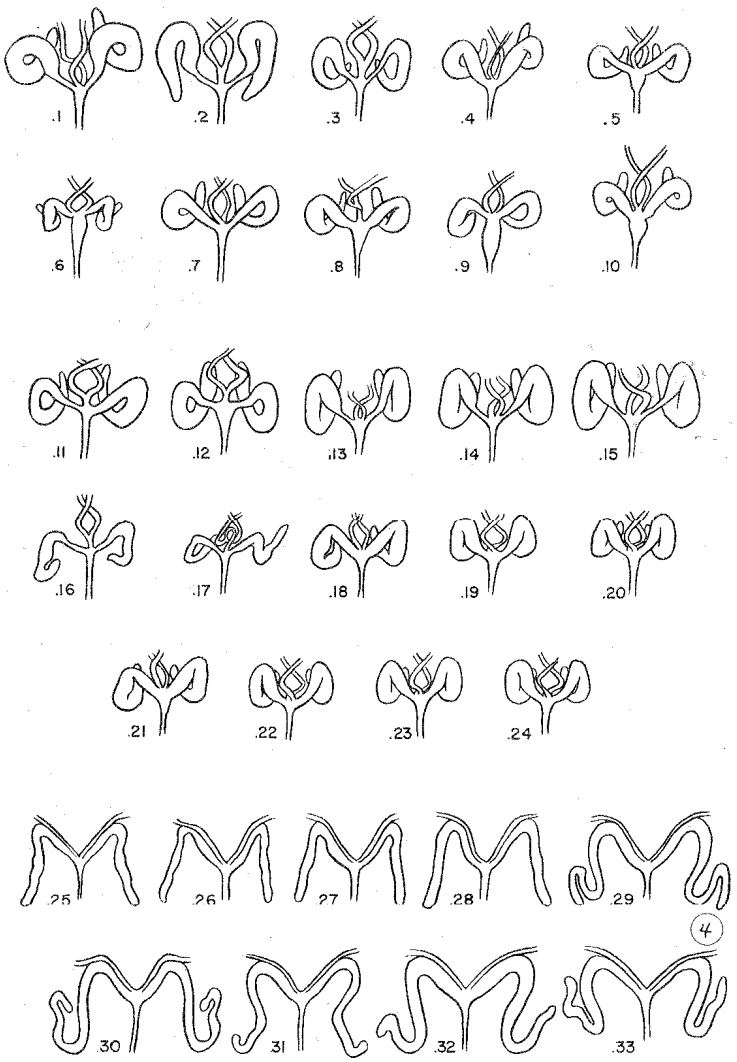


FIG. 4. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

Subgenus: SOPHOPHORA
**willistoni group**
.1 equinoxialis
.2 paulistorum
.3 tropicalis
.4 willistoni
.5 fumipennis
.6 nebulosa
.7 sucinea
.8 capricorni
.9 changuinolae
.10 pseudobocainensis
**saltans group**
*parasaltans subgroup*
.11 subsaltans
.12 parasaltans
*cordata subgroup*
.13 neocordata
*elliptica subgroup*
.14 neoelliptica
.15 emarginata
*sturtevanti subgroup*
.16 sturtevanti
.17 milleri
*saltans subgroup*
.18 lusaltans
.19. nigrosaltans
.20 pseudosaltans
.21 austrosaltans
.22 prosaltans
.23 septentriosaltans
.24 saltans
Subgenus: DROSOPHILA
**virilis group**
.25 virilis
.26 americana
.27 novamexicana
.28 littoralis
.29 ezoana
.30 montana
.31 flavomontana
.32 lacicola
.33 borealis

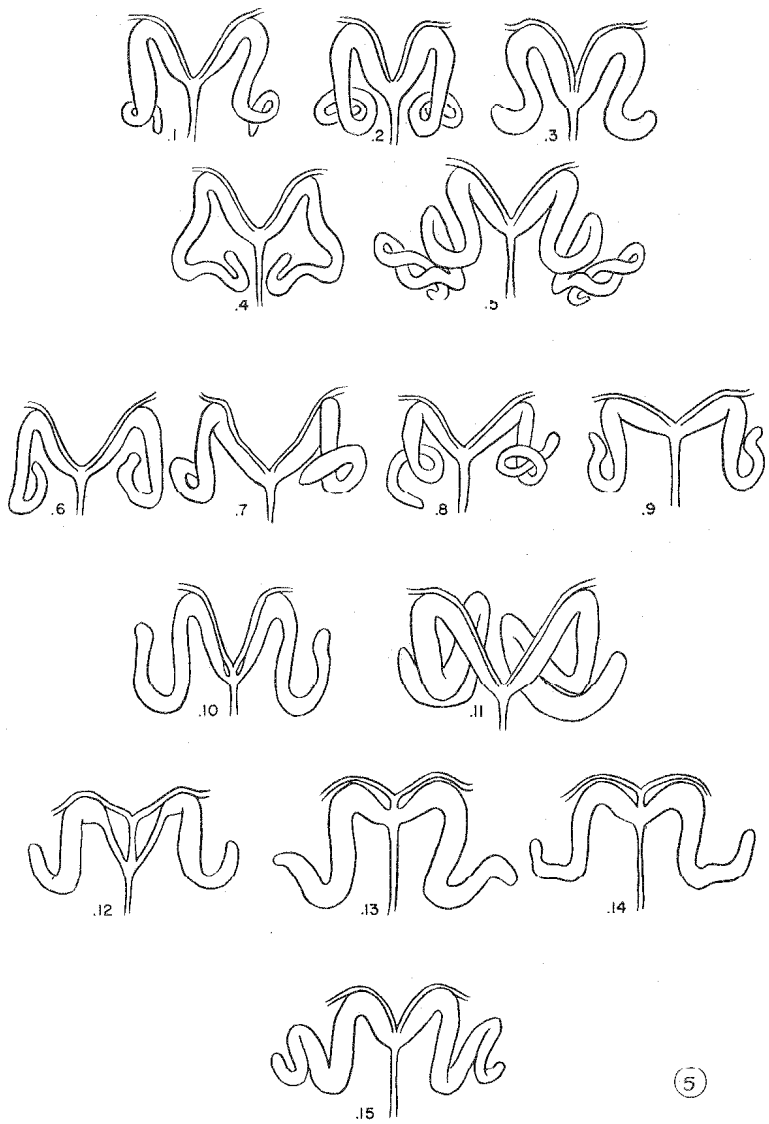


FIG. 5. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

Subgenus: DROSOPHILA

**melanica group**
.1 micromelanica
.2 melanica
.3 paramelanica
.4 euronotus
.5 nigromelanica

**robusta group**
.6 lacertosa
.7 colorata
.8 sordidula
.9 robusta

**annulimana group**
.10 gibberosa
.11 species D

**immigrans group**
.12 hypocausta
.13 spinofemora
.14 immigrans

Subgenus: HIRTODROSOPHILA
.15 histrioides

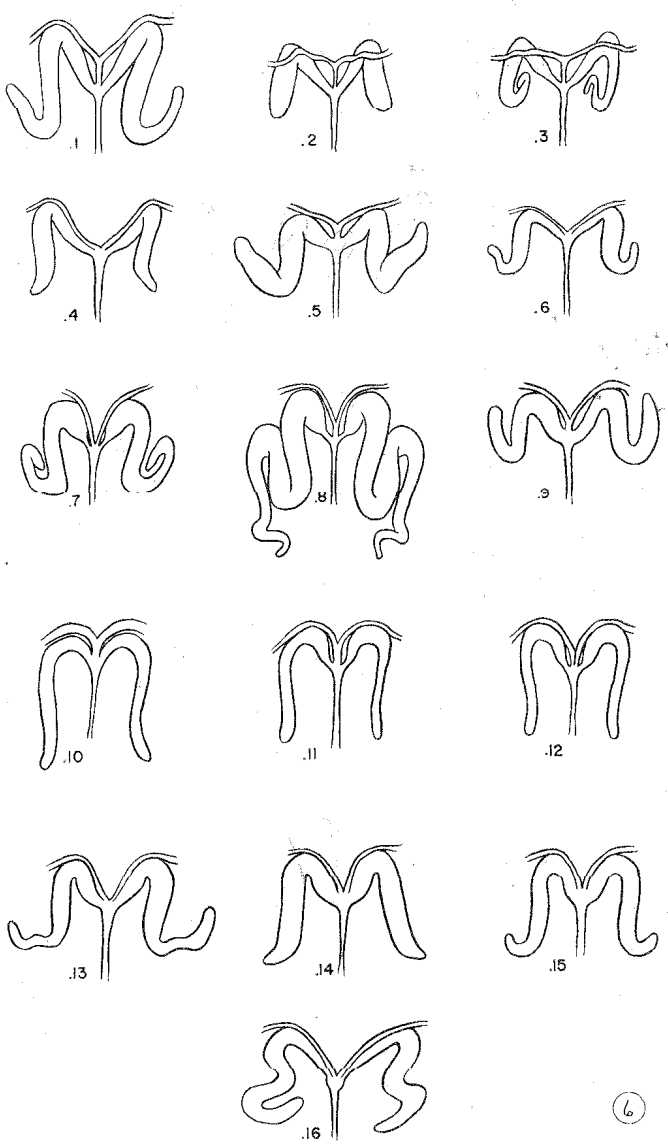


FIG. 6. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

Subgenus: DROSOPHILA

**funebris group**
.1 macrospina
.2 subfunebris
.3 funebris

**carbonaria group**
.5 carbonaria

**nannoptera group**
.6 nannoptera

**bromeliae group**
.7 species I

**polychaeta group**
.9 polychaeta

**miscellaneous species**
.4 carsoni
.8 aracea
.13 peruviana
.14 species H
.15 species G
.16 tumiditarsus

Subgenus: PHLORIDOSA
.10 species O
.11 species Q
.12 species P

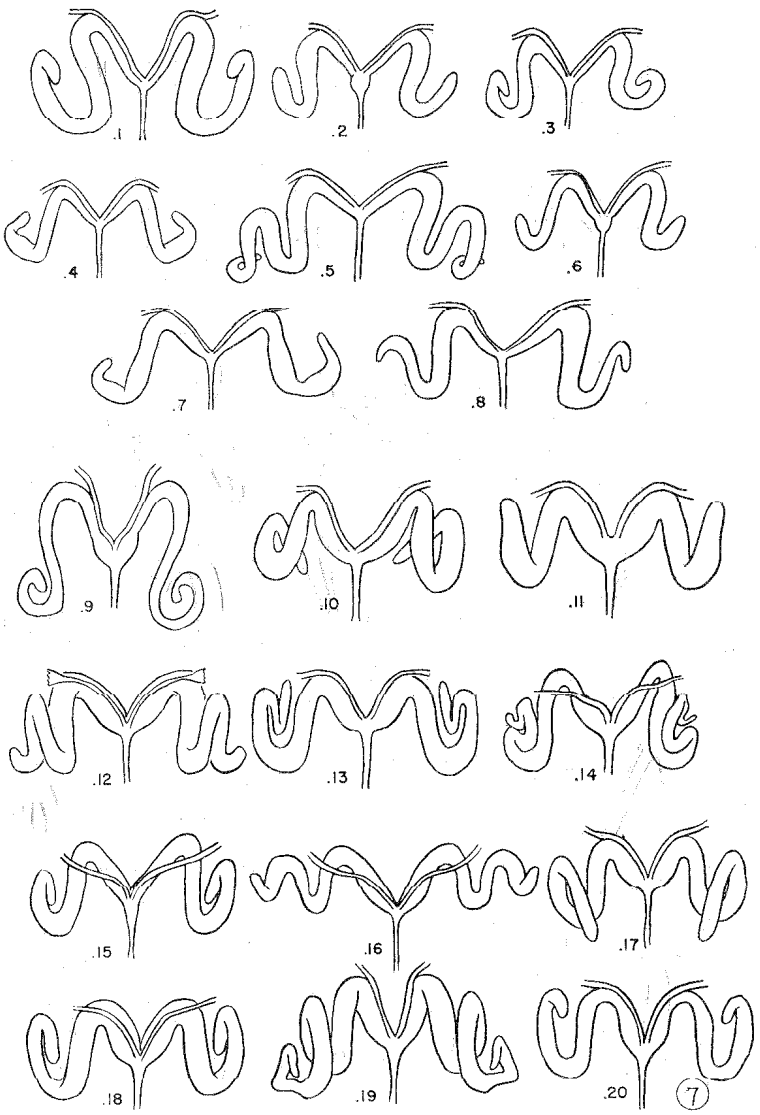


FIG. 7. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**repleta group**

*fasciola subgroup*
.1 fulvalineata
.2 fasciola
.3 coroica
.4 pictura
.5 pictilis
.6 fascioloides
.7 moju
.8 mojuoides

*mulleri subgroup*
.9 tira
.10 pachuca
.11 nigricruria
.12 aldrichi
.13 mulleri
.14 longicornis
.15 arizonensis
.16 mojavensis
.17 martensis
.18 stalkeri
.19 hamatofila
.20 eremophila

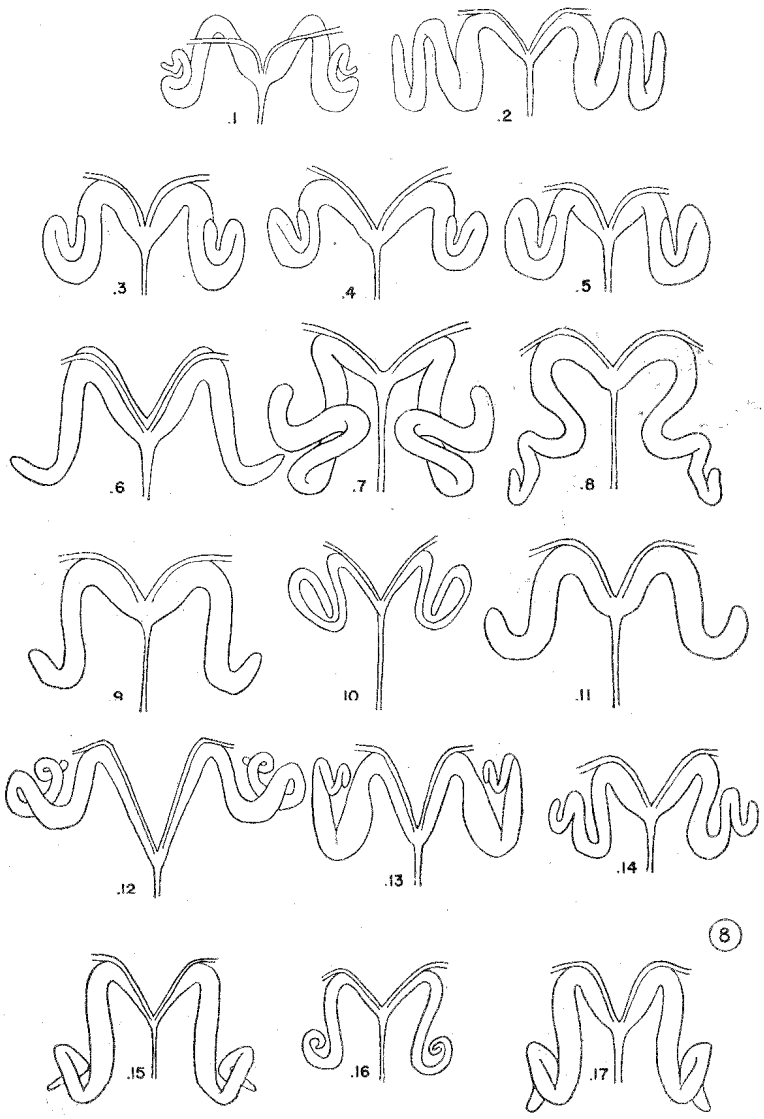


FIG. 8. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**repleta group**

*mulleri subgroup*
.1 buzzatii
.2 pegasa
.3 meridionalis
.4 promeridiana
.5 meridiana
.7 anceps
.9 peninsularis

*mercatorum subgroup*
.10 paranaensis
.11 mercatorum

*melanopalpa subgroup*
.12 fulvimacula
.13 fulvimaculoides
.14 limensis
.15 repleta
.16 canapalpa
.17 melanopalpa

*not assigned to subgroup*
.8 serenensis

*ungrouped species*
.6 aureata

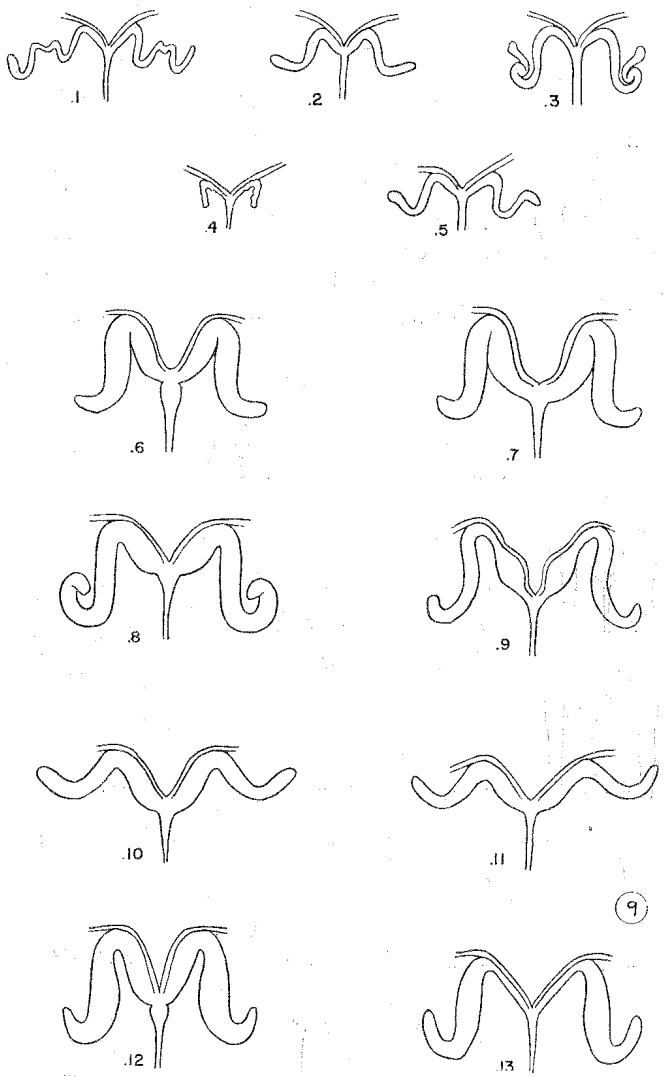


FIG. 9. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**repleta group**

*hydei subgroup*
.1 bifurca
.2 nigrohydei
.3 eohydei
.4 neohydei
.5 hydei

**canalinea group**
.6 canalinea
.7 paracanalinea

**dreyfusi group**
.8 camargoi
.9 briegeri

**mesophragmatica group**
.10 gaucha
.11 pavani

**ungrouped species near repleta group**
.12 castanea
.13 species F

fused section is short when present (*e.g.*, Figures 4.3–.11). In the melanogaster group the common duct may reach considerable length. It is longest in *ananassae* and *rufa* (Figures 3.27–.28). A similar fusion has already been mentioned for *busckii* and for two *Pholadoris* species. Since these species have the

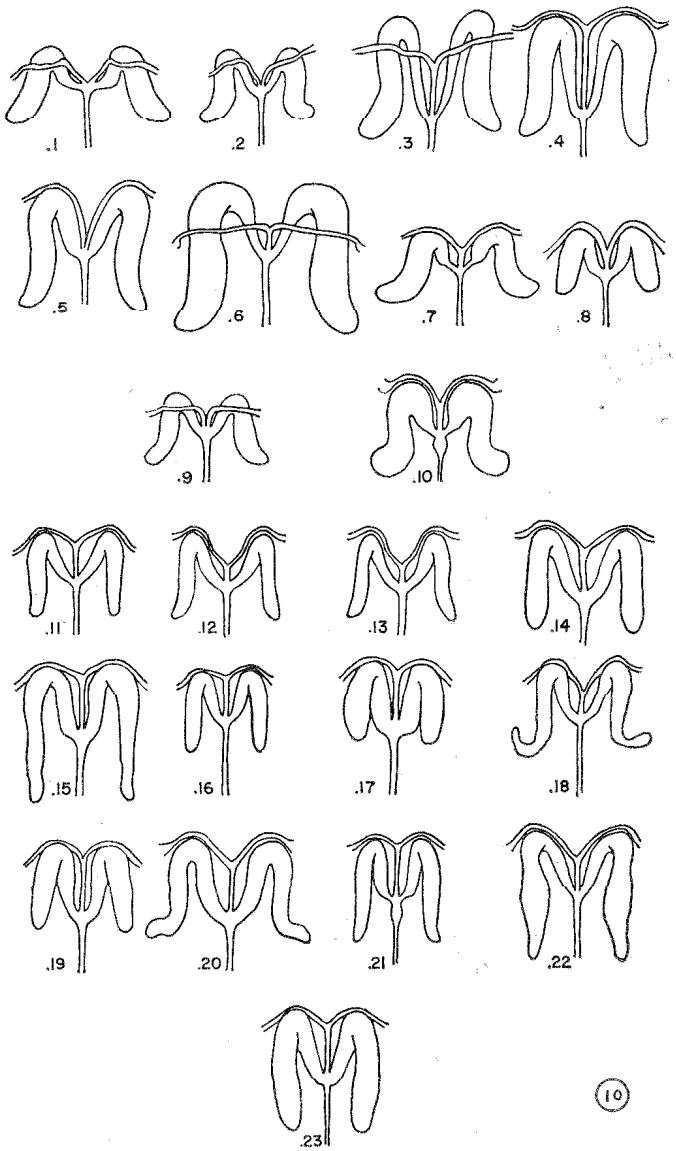


FIG. 10. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**ungrouped species near cardini group**

.1 species K
.2 species L

**rubrifrons group**

.3 parachrogaster
.4 uninubes

**ungrouped species**

.5 sticta

**pallidipennis group**

.6 pallidipennis

**testacea group**

.7 putrida
.8 testacea

**macroptera group**

.9 submacroptera
.10 macroptera

**quinaria group**

.11 innubila
.12 quinaria
.13 rellima
.14 falleni
.15 phalerata
.16 species J
.17 occidentalis
.18 tenebrosa
.19 subquinaria
.20 transversa
.21 palustris
.22 subpalustris
.23 guttifera

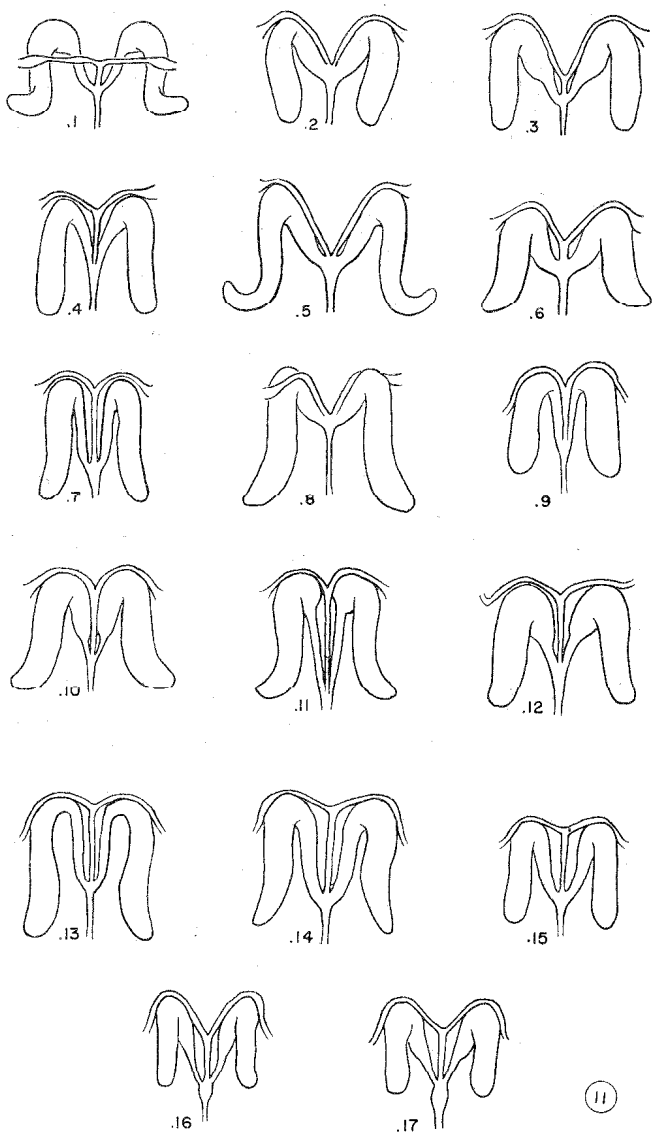


FIG. 11. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**tripunctata group**

.1 mediodiffusa
.2 albicans
.3 albirostris
.4 tripunctata
.5 mediopunctata
.6 unipunctata
.7 trapeza
.8 bandeirantorum
.9 paramediostriata
.10 mediostriata
.11 mediopictoides
.12 crocina

**guarani group**

.13 guaramunu
.14 guaraja
.15 griseolineata
.16 subbadia
.17 guarani

primitive, short vasa, the fusion here may only be analogous to that in Sophophoran species. As will be seen shortly, many species in the subgenus *Drosophila* also have the vasa fused basally. The fusion of the vasa seen in species of the

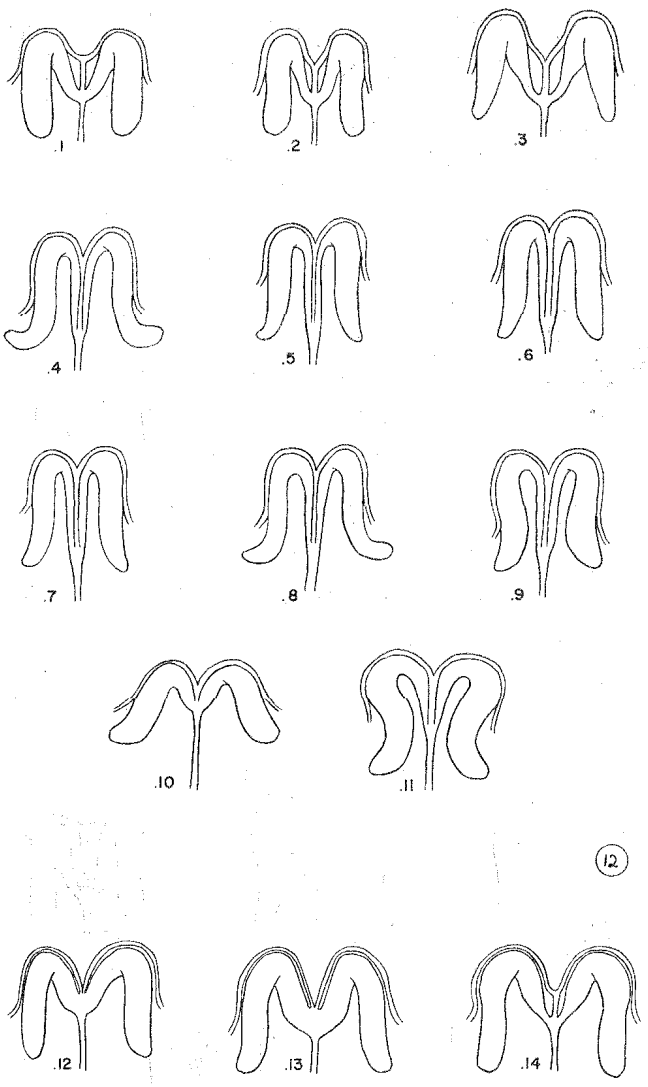


FIG. 12. Morphology of paragonia and relationship between paragonia and vasa deferentia in *Drosophila* species.

**cardini group**
.1 dunni
.2 belladunni
.3 nigrodunni
.4 polymorpha
.5 neomorpha
.6 neocardini
.7 parthenogenetica
.8 acutilabella
.9 procardinoides
.10 cardinoides
.11 cardini

**calloptera group**
.12 ornatipennis
.13 calloptera
.14 schildi

melanogaster group, however, may be of a type distinct from that seen in species of the subgenus *Drosophila*. In at least some of the melanogaster group species the two vasa only tightly adhere to each other. In such cases they can be separated and then they are seen to be truly fused only in a very short basal section.

In some species of the melanogaster group there is an extreme expansion of

the anterior end of the ejaculatory duct. This is most prominent in *takahashii* (Figure 3.26), moderate in members of the melanogaster subgroup, and slight or absent in members of the montium and ananassae subgroups. This feature is also seen in some members of the willistoni group (Figures 4.5–.6; 4.8–.10). It is, at best, only slightly developed in members of the saltans group. This feature is present in some members of the subgenus *Pholadoris* (Figures 3.1–.2, .7) and in a few scattered species in the subgenus *Drosophila*. In these cases, however, its expression is not as extreme as in some species of the melanogaster group.

In the subgenus *Drosophila* the number of types is too great to allow inclusion of all of them in the space available for a pictorial phylogeny. Figure 14 shows only some of the major types. Others will be pointed out below. In this subgenus, none of the vasa are of the primitive type, and evolution has been toward the acquisition of a specific relationship between the vasa and the paragonia. The final configuration arrived at has been determined, in part, by the presence or absence of basal fusion of the vasa. Two major phyletic lines are indicated within the subgenus. One line leading to such forms as *mediostriata* (Figure 14.6) has involved increasing length of the fused section of the vasa and an increasingly regular association between the vasa and the paragonia. There has also been a shortening and thickening of each paragonium to form a high arch with slight or no indication of a second fold. In the other section (Figures 14.2–.3) there has been almost no basal fusion of the vasa, and there has been an increasingly regular association between the vasa and paragonia. In this section shortening of paragonia is not pronounced. Indeed, sine a primitive type cannot be surely specified, it is not improbable that an increase in length may have been involved. Some forms from this section also show a reduction in the

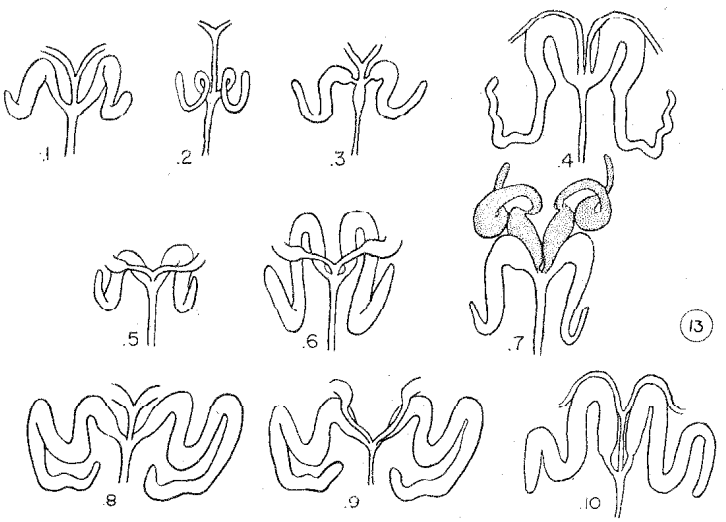


FIG. 13. Morphology of paragonia and relationship between paragonia and vasa deferentia in species from other genera of Drosophilids. .1) *Chymomyza amoena*; .2) *C. aldrichi*; .3) *C. procnemis*; .4) *Mycodrosophila dimidiata*; .5) *Scaptomyza adusta*; .6) *S. pallida*; .7) *S. hsui*; .8) *Zaprionus ghesquierei* (3/12 individuals); .9) *Z. ghesquierei* (9/12 individuals); .10) *Z. vittiger*.

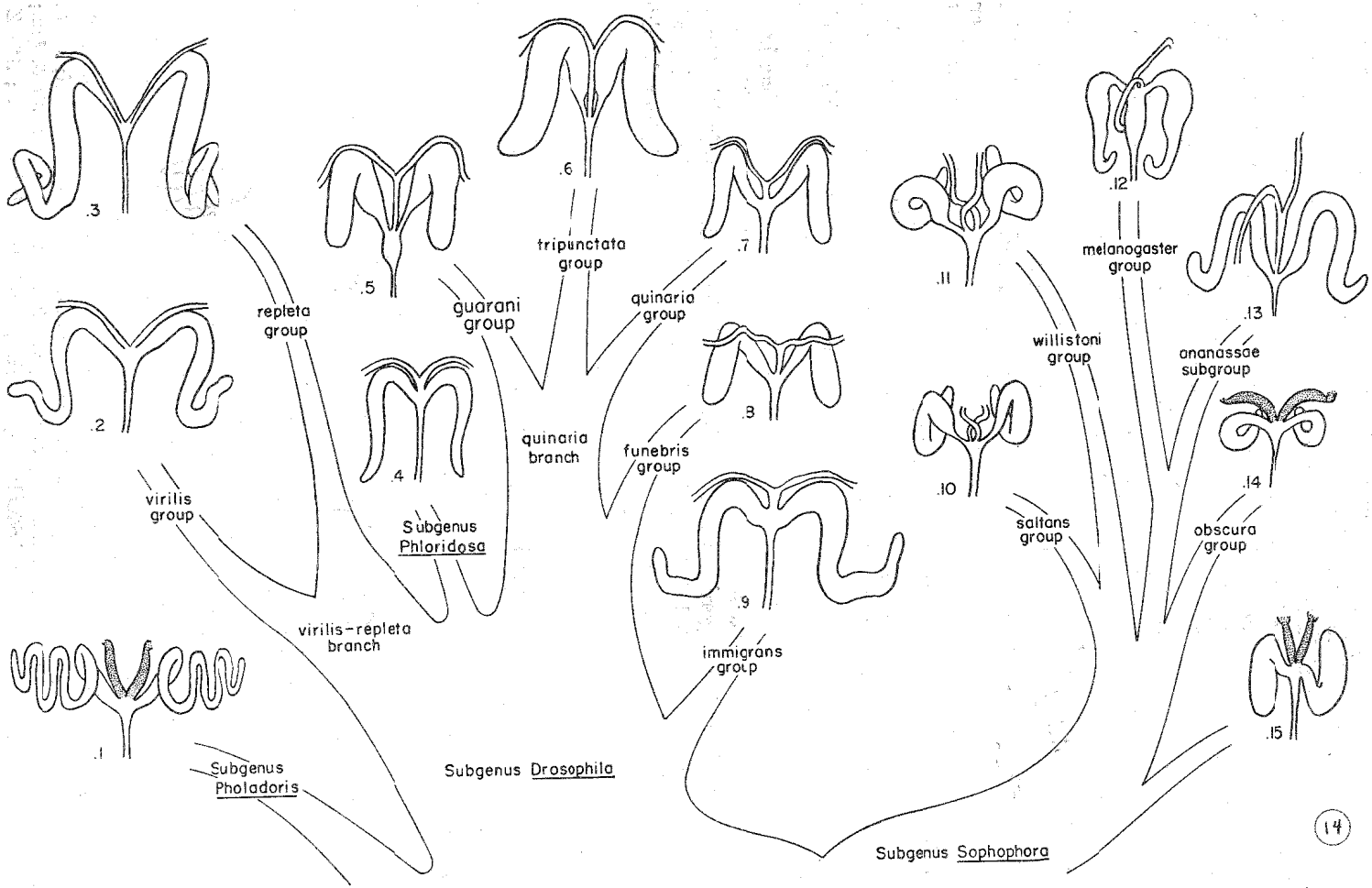


FIG. 14. Pictorial phylogeny of paragonia and vasa deferentia. .1) cancellata; .2) lacicola; .3) repleta; .4) species 0; .5) guarani; .6) mediostriata; .7) quinaria; .8) subfunebris; .9) immigrans; .10) neocordata; .11) equinoxialis; .12) simulans; .13) ananassae; .14) algonquin; .15) populi.

diameter of the paragonia, followed apparently by a reduction in length also, so that only vestiges remain. This is most conspicuous in the members of the hydei subgroup of the repleta group where the paragonia are very small (Figures 9.1–.5). In these figures the size of the paragonia is somewhat exaggerated. An apparent reduction is also seen in some members of the virilis group (Figures 4.25–.33) and in members of some other subgroups of the repleta group (Figures 8.10, .16). These latter cases, however, probably represent retention of an ancestral, less robust form of paragonia rather than an actual reduction.

The association between the vasa and the paragonia is one of the most characteristic features of species in the subgenus *Drosophila*. Although no sharp distinction can be made, species from both sections of this subgenus fall into two major categories. In one the vasa are in close association with the paragonia but not actually adhering to them. In the other the vasa adhere so closely to the walls of the paragonia that they can be separated from them only with difficulty, if at all. Between these two extremes are many species in which the vasa adhere to the paragonia, but so loosely that careless dissection will disrupt the association. These categories can be further subdivided into forms in which the vasa are rather irregularly associated with the paragonia, generally crossing their ventral surface, and forms in which the association is very regular and the vas adheres to, and follows, the major curvature of the arch of the paragonium. This association generally ends at the point where the differentiation of the vas begins, although in species where the vas is unusually long a considerable portion of its distal end may be free. Generally, forms having the most irregular association between the vasa and the paragonia also have a more loose attachment between the two. Forms having a very regular association between the two may occupy either extreme and have the vas strongly adhering or completely free (but still following the major curvature of the paragonia very closely). Presumably, the forms in which the vasa are free but associated with the paragonia approach the primitive type for the subgenus.

In the figures a common line serving both to delineate the outer surface of the paragonium and the inner surface of the vas indicates that the vas adheres to the paragonium in that region (e.g., 14.2–.3, .5–.6). Where separate lines are used the association is loose and generally the vas does not adhere (Figures 14.4, .7, .9).

The great majority of species belonging to the virilis-repleta section (Figures 4.25–.33; 5.1–.11; 7; 8; 9; as well as others in Figure 6 which can be picked out by inspection) show a regular association between the vasa and paragonia and a relatively tight adherence between the two. An irregular association is seen only in some species of the repleta group, and even here the association is not highly irregular. The examples of this type are seen in Figure 7 (.12, .14–.16, .18) and in Figure 8.6. In most of these cases the irregularity lies in the vas crossing a surface of the paragonium rather than following the major curvature. In some, however, the association is very brief, as in *longicornis* (Figure 7.14). Generally speaking, none of the species of the virilis-repleta section appear to be primitive with respect to the characteristics just discussed, although the repleta species just mentioned appear to have retained some features of more primitive forms.

As was indicated earlier, in members of the virilis-repleta section the paragonia

generally are large and robust, and exceptions to this have already been noted. Since most of the individuals involved are themselves rather large, it is difficult to say, without careful measurements, whether there actually has been a relative increase in size of the paragonia during the evolution of this branch. The general impression is that the paragonia are distinctly more robust than in presumed primitive forms, but this will need to be verified. It is pertinent in this respect to note that there is some difference with age in the characteristics of the paragonia. In newly emerged and very young adult males of some species, the paragonia are not fully developed. That is, they appear to have their normal length and characteristic folding, but they are not completely filled out and may have a somewhat shriveled appearance. Paragonia in very old males may also have this appearance. This condition generally changes within a day or two of emergence, and, during dissection, an effort was made to use only fully mature males. However, the possibility remains that an entire sample may have been of relatively young, or of very old, males. This seems somewhat improbable, but it may explain why species such as *paranaensis* (Figure 8.10) differ in this respect from their close relatives. It is highly improbable that age differences are responsible for the small paragonia seen in virilis group species, and age definitely is not responsible for the previously mentioned characteristics of hydei subgroup species.

In other respects the morphology of the paragonia in species from the virilis-repleta section is relatively constant. Generally the paragonia are folded at least three times, and often more. Some species of the virilis group are not of this type (Figures 4.25–.28) and resemble *Phloridosa* species (Figures 6.10–.12) to a limited extent. A few species from this section have the paragonia only twice folded, or folded only once, and these can be picked out by inspection of Figures 5 through 9.

The next cluster of species to be considered is, in many ways, intermediate between the two major branches. Species of the immigrans group (Figures 5.2–.14), for example, show the basal fusion of the vasa generally associated with species of the quinaria section, but the paragonia are twice folded, and they may show a reduced (or incipient) third fold. In two of the species, *immigrans* and *spinofemora*, the vasa closely follow the major curvatures of the paragonia but do not adhere to them. As was said earlier, this presumably approaches the primitive condition for the subgenus *Drosophila*. In the third species, *hypocausta*, the vasa adhere to the paragonia and follow their curvature. Except for the presence of a second fold of the paragonia, this configuration is very similar to that seen in species from the quinaria branch. In the funebris group (Figures 6.1–.3) a somewhat similar pattern is seen. The paragonia vary from three folds in *funebris* to one fold in *subfunebris*. The association between the vasa and the paragonia is loose and irregular in two species but regular and of the quinaria type in the third. The pattern seen in *carbonaria* (Figure 6.5) is also generally of the type seen in *subfunebris*, although the vasa more nearly follow the major curvature of the paragonia.

Among the species which most distinctly belong to the quinaria section there is one group, and another cluster of species from various groups, which closely resemble the types seen in the funebris and immigrans groups. Species from the

calloptera group closely resemble immigrans group species (Figure 12.12–.14), except that they lack the second fold of the paragonia. The vasa uniformly follow the major curvatures of the paragonia, but they do not adhere to them. In only one of the three species available, *schildi*, are the vasa fused basally, and this only for a short distance. The other group of species (Figures 10.1–.3, .6, .9; 11.1) may or may not have the vasa fused basally, and the vasa are generally short and cross the surfaces of the paragonia rather than follow the major curvatures. A comparison between Figure 10.1 and Figure 6.2 will indicate the general similarities between these two types.

Other species belonging to this section (Figures 10, 11 and 12) are very similar in general appearance of the vasa and paragonia. A few species (Figures 10.5; 11.2, .5, .8), mostly belonging to the tripunctata group, show the virilis-repleta pattern with the vasa unfused basally and closely adhering to the paragonia. The paragonia, however, are typical of those seen in other members of the quinaria section. The remaining species vary chiefly in the closeness of the association between the vasa and paragonia. In some (e.g., *quinaria*, Figure 14.7) the association is very loose. In others (all species of the cardini and guarani groups, most species of the tripunctata group, and about half of the species in the quinaria group), the association is strong and the vasa adhere closely to the paragonia.

The three species available from the subgenus *Phloridosa* are all very similar and differ among themselves primarily in that the association between the vasa and paragonia is loose in one but stronger in the other two (Figures 6.10–.12). The paragonia are only slightly arched and have only one fold. In general aspect they resemble types seen in virilis group species (Figures 4.25–.28) more closely than any others, and thus they are placed in the phylogeny as shown in Figure 14.4.

Only four species were available from the subgenus *Hirtodrosophila*, and these are quite variable (Figures 3.8–.10 and 5.5). Even from such a small sample this subgenus appears to be very heterogeneous. Thus far, *pictiventris* is the only species seen to have spirally coiled paragonia. *D. duncani* has the primitive vasa and has paragonia which probably are not too different from the primitive type. *D. thoracis* and *histrioides* are somewhat similar to species of the virilis-repleta section, although neither resembles them closely. Perhaps *histrioides* is closer to the immigrans group (and was placed on the same figure with these species for comparison), but such a relationship is highly improbable for *pictiventris* and *duncani*. The relationships of the *Hirtodrosophila* to other members of the genus will be discussed later. They have not been included in the initial phylogenies.

Paragonia and vasa of species from other genera are shown in Figure 13. In all *Chymomyza* species available for dissection (Figures 13.1–.3) the vasa are completely free of association with the paragonia and generally resemble Sophophoran types. The single available species of *Mycodrosophila* (Figure 13.4) is similar in general respects to the immigrans type, although it lacks the basal fusion of the vasa. The paragonia are strongly arched, as are those of most species from the quinaria section of the subgenus *Drosophila*, but they have a long, strongly attenuated section distal to the major arch. Species from the genus *Scaptomyza* are of two general types. The paragonia and vasa of *S. adusta* and *S. pallida* (Figures 13.5–.6) are similar to the type seen in the funebris group,

although the vasa are much shorter. *S. hsui* belongs to a type of its own, although others similar to it are figured by Okada (1956). Here the vasa are greatly expanded, and the figure (13.7) includes the testes. Species from the genus *Zaprionus* resemble species from the subgenus *Drosophila* but have characteristics of both major branches. The paragonia are very robust and are folded at least three times. In *Z ghesquierei* the individuals were variable, showing two major types with only slight intergradation between them. Of twelve males dissected, three were as in Figure 13.8 and nine were as in Figure 13.9. Association between the vasa and paragonia was almost absent in the first type since the vasa are short and almost immediately expand to form the strongly differentiated region typical of so many *Drosophila* species. In the second type the vasa are somewhat longer and associate closely with the ventral surface of the paragonia, somewhat after the fashion of some species of the repleta group. Except for the extra folds of the paragonia, *Z. vittiger* resembles species from the quinaria section.

*The ejaculatory bulb and the ejaculatory apodeme*—There is considerable variation in the characteristics of the ejaculatory duct among the various Acalypterate families. In the Piophilidae, Chloropidae and Anthomyzidae the duct is undifferentiated and shows neither ejaculatory bulb nor ejaculatory apodeme (Figures 1.6–.9). In the family Ephydridae (Figures 1.2–.3) there may be one or several enlargements of the ejaculatory duct, but these bear very little resemblance to those seen in other families. In one case, *Scatella stagnalis*, there is a dark-pigmented, chitinized structure *within* an enlargement. In the other, *Discocerina obscurella*, the enlargement of this region is very slight and there is a very small chitinized structure resembling a limpet shell in shape and attached to the outside of the chitinized lining of the duct. In position at least, this resembles the condition seen in other families. This "ejaculatory bulb" has been shown, relatively *much* enlarged, in Figure 22.16. Figures 22.11–.15 show ejaculatory bulbs and ejaculatory apodemes from several other families of Diptera. Although varying in detail, the ejaculatory bulbs and ejaculatory apodemes seen in these families (Diastatidae, Periscelidae, Aulacigastridae, Sepsidae, Sphaeroceratidae) appear to be similar in their basic characteristics. The ejaculatory apodeme seen in one member of the family Agromyzidae is rather complex and has not been figured in detail. In its general features, however, it appears to resemble the types just mentioned.

Many types of variation are seen within the family Drosophilidae. In *Gitona bivisualis* and *Rhinoleucophenga obesa* the ejaculatory duct is undifferentiated, with neither ejaculatory bulb nor ejaculatory apodeme. At the other extreme are seen such species as *Zaprionus vittiger* (Figure 22.8) where the ejaculatory bulb is large and itself further differentiated, with paired and bifurcated posterior caecae.

Okada (1958) has made an extensive survey of the characteristics of ejaculatory bulbs within the family Drosophilidae, and he places them in seven types, as follows:

Type O: no bulb
Type A: bulb, no caecae
Type B: bulb, a single posterior caecum

Type C: bulb, paired posterior caecae
Type D: bulb, paired and bifurcated posterior caecae
Type E: bulb, paired anterior and posterior caecae
Type F: bulb, three anterior, and paired posterior caecae.

All of these types, except type B and type F, were present among the species available for this study. Okada (*op. cit.*) concludes that type A or type O represents the primitive type in the family, and he recognizes the possibility that type O may be a degenerate form of type A. Considering the types of bulbs seen among the Acalypteratae, it seems more probable that type O is primitive among the acalypterates and that type A *and* type O represent primitives for the family Drosophilidae. Type A would be primitive for the genus *Drosophila*.

The type classification of Okada is apparently based on uncleared material. A brief inspection of Figures 15–19 will show that there is some overlapping of types, and there are many more types apparent when cleared material is used. In addition there are some useful differences in the morphology of the caecae which complicate any simple system of types. While reference to types is useful for generalization, it is not adequate for the present purpose, and Okada's types will be referred to only occasionally in the following discussion. Even a moderately complete division into types, based on the data presented in Figures 15–22, would more than quadruple Okada's number of seven. Such a system would probably be intelligible only to its author, and the multiplication of types would serve no useful purpose in the present discussion.

Nater (1950) made a survey of the ejaculatory apodeme (Sammenpumpensklerit) in *Drosophila* and related species, and he also summarizes its major features in a later paper (Nater, 1953). All of his types were seen in the present study, although some differences of interpretation are apparent. He does not indicate a primitive type, but since the spade type (e.g., Figure 15.6) is found in species from all the major subgenera, it will be considered as near the primitive.

If the absence of an ejaculatory bulb is considered primitive among the Acalypteratae, then the most primitive type in the genus *Drosophila* should be that showing the least differentiation of the bulb relative to the ejaculatory duct, and the apodeme should be only slightly developed. As indicated by Okada (1958), such types might be degenerate, and this must be recognized as a possibility. On the whole, however, interpretation of these types as primitive seems more reasonable, and the phylogeny (Figures 20 and 21) will be based on this assumption. Ejaculatory apodemes are arranged in a phylogeny in Figure 24.

When the characteristics of the ejaculatory bulb are considered, the type seen in coracina group species (Figures 15.3–.5) of the subgenus *Pholadoris* (Figures 15.1–.7) appears to be nearest the primitive. (Note: *in situ* the apodeme is directed ventrally. In the figures, the bulb has been inverted so that ventral is toward the top. Anterior is toward the right.) In these species the bulb is very small and only slightly more enlarged than the ejaculatory duct. In Figures 15.3–.5 the size of the bulb is exaggerated. In relative proportion, the bulb of *victoria* (Figure 15.1) is at least six times as large as that of *cancellata* (Figure 15.3). The ejaculatory apodeme in these species is small, almost unpigmented, and weakly chitinized. The ejaculatory bulb and apodeme in *latifasciaeformis*

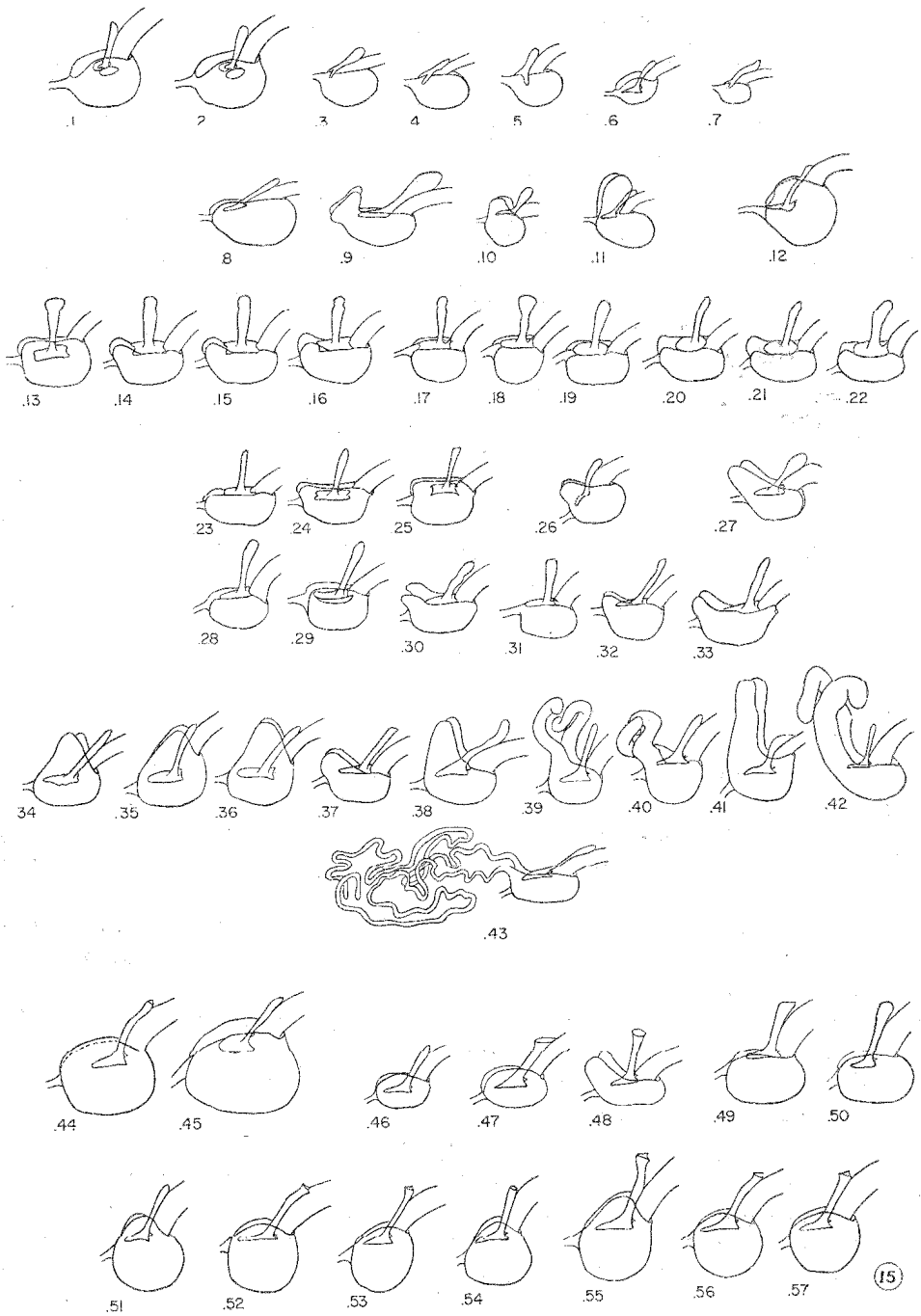


FIG. 15. Ejaculatory bulbs and ejaculatory apodemes from *Drosophila* species.

Subgenus: PHOLADORIS

**victoria group**
.1 victoria
.2 pattersoni

**coracina group**
.3 cancellata
.4 lativittata
.5 novopaca

**bryani group**
.6 bryani

**latifasciaeformis group**
.7 latifasciaeformis

are almost identical with those seen in coracina group species. In *bryani* the apodeme is of the spade type and the bulb has been modified through the addition of lateral folds, or lobes. Simiar lobes are seen in bulbs from victoria group species (Figures 15.1–.2) but the bulb itself is much enlarged and is comparable in size to those seen elsewhere in the genus. The apodeme in species of the victoria group is of a distinctly derived type and is seen nowhere else in the genus, although it is almost certainly a modification of the type seen in species of the coracina group. In *pattersoni* and *victoria* the apodeme is large and heavily pigmented. It is forked basally, as was the apodeme in species of the coracina group. The two branches turn downward (upward in the figure) at their tips and expand to form rather large, oval plates which lie against the inner surfaces of the lateral lobes of the bulb.

All species in the subgenus *Sophophora* have moderate to large ejaculatory bulbs. In *populi* (Figure 15.12) the apodeme is slightly modified from the spade type. The plate (the part of the apodeme in contact with the ventral surface of the bulb, as apposed to the handle, which is either associated with the anterior ejaculatory duct or free) has the triangular shape but the handle arises from the surface of the plate rather than from its anterior edge. The bulb is relatively large and has lateral lobes which meet and fuse in the mid-ventral line. This fusion is represented by a dashed line in the figure.

Species of the obscura group (Figures 15.13–.22) all have ejaculatory bulbs of the same general type. Bulb shapes vary from subspherical to quadrate, and several have small but distinct posterior caecae. The apodemes are all of the same general type, the obscura type. The plate is either quadrate (e.g., Figure 15.13) or roughly oval (e.g., Figure 15.19) in shape. The handle is relatively heavy

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Subgenus: HIRTODROSOPHILA
.8 duncani
.9 pictiventris
.10 thoracis
Subgenus: DORSILOPHA
.11 busckii
Subgenus: SOPHOPHORA
.12 populi
**obscura group**
*obscura subgroup*
.13 miranda
.14 pseudoobscura
.15 persimilis
.16 ambigua
*affinis subgroup*
.17 affinis
.18 algonquin
.19 narragansett
.20 tolteca
.21 athabasca
.22 azteca
**melanogaster group**
*melanogaster subgroup*
.23 simulans
.24 melanogaster
.25 yakuba
*takahashii subgroup*
.26 takahashii
*ananassae subgroup*
.27 ananassae
*montium subgroup*
.28 rufa
.29 nikananu
.30 serrata
.31 auraria
.32 seguyi
.33 kikkawai
**willistoni group**
.34 equinoxialis
.35 tropicalis
.36 willistoni
.37 paulistorum
.38 pseudobocainensis
.39 sucinea
.40 nebulosa
.41 changuinolae
.42 capricorni
.43 fumipennis
**saltans group**
*parasaltans subgroup*
.44 subsaltans
.45 parasaltans
*cordata subgroup*
.46 neocordata
*elliptica subgroup*
.47 neoelliptica
.48 emarginata
*sturtevanti subgroup*
.49 sturtevanti
.50 milleri
*saltans subgroup*
.51 lusaltans
.52 nigrosaltans
.53 pseudosaltans
.54 austrosaltans
.55 prosaltans
.56 septentriosaltans
.57 saltans

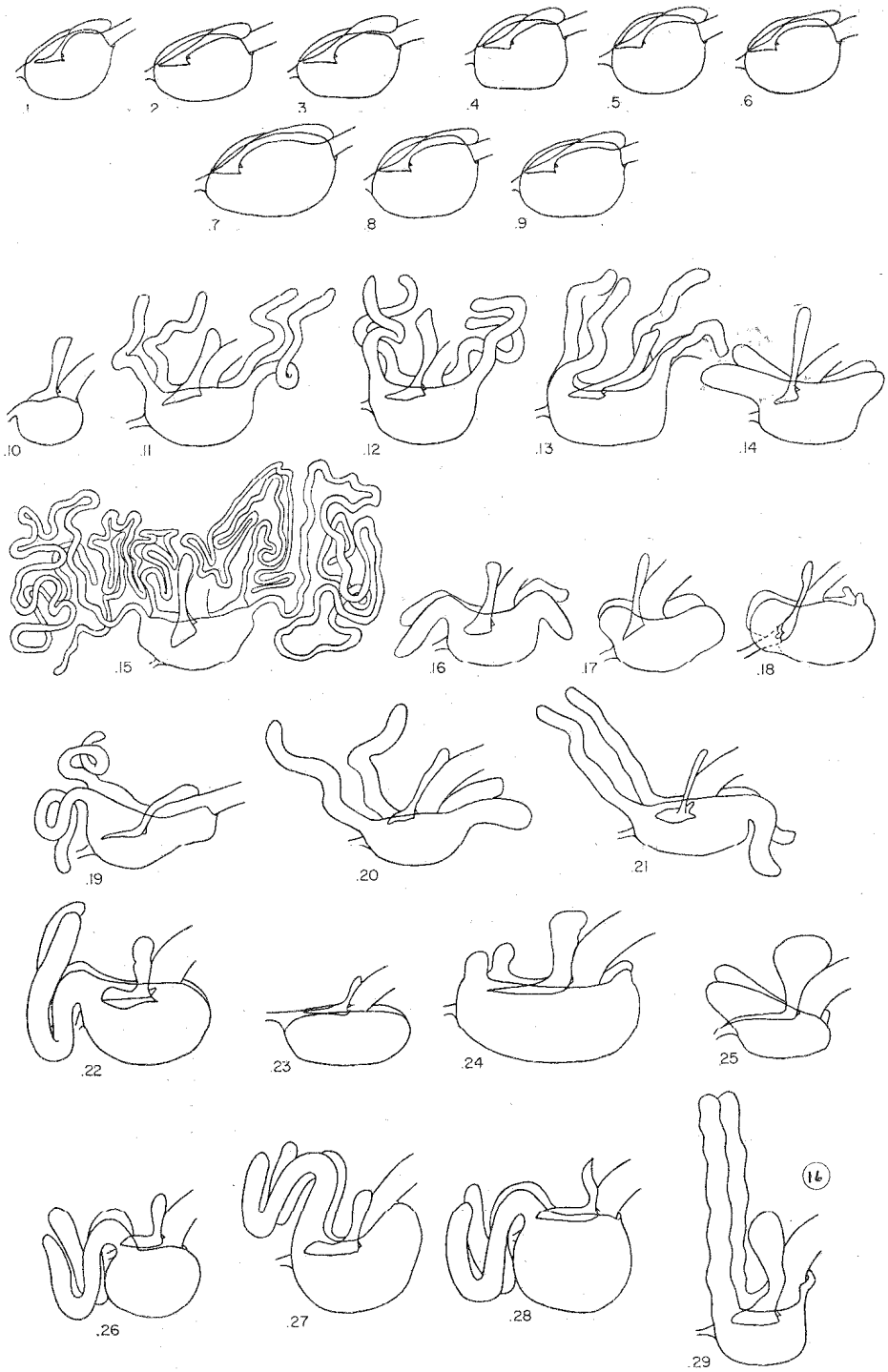


FIG. 16. Ejaculatory bulbs and ejaculatory apodemes from *Drosophila* species.

and projects almost directly ventrally from near the center of the plate. The ejaculatory bulbs of species in the melanogaster group are quite varied (Figures 15.25–.33). In general they are of types seen in species of the obscura group. Some appear to have both anterior and posterior caecae. The posterior caecae of *ananassae* and *bipectinata* are quite large. The ejaculatory apodemes of melanogaster group species are also varied. Those of species in the melanogaster subgroup are of the obscura type. The remainder in the group are of a modified spade type, with the plate triangular and the handle arising from its surface rather than from the anterior edge.

The ejaculatory bulb in species from the willistoni group varies as shown in Figures 15.34–.43. Some have distinct literal lobes, others have strong posterior caecae, and *fumipennis* has extremely long and slender caecae. It might be well to point out that the distinction between lateral lobes and blunt or short caecae is not a sharp one. If, for example, Figure 15.38 were tilted so that the handle of the apodeme were horizontal, the caecae would be seen as lateral lobes. Much the same thing is seen in *ananassae* (Figure 15.27). The distinction used here is an arbitrary one. If the major axis of the lobe (or caecum) crosses the long axis of the apodeme handle at nearly a right angle it is considered to be a caecum. If it parallels the long axis of the handle it is called a lateral lobe. There is no difficulty in terminology when the lobe extends the length of the bulb or when the caecae are strongly differentiated from the rest of the bulb. There are many intermediates between these two types, and it is possible that posterior caecae and lateral lobes represent the same basic phenomenon. Comparative histological and developmental studies may clarify this point.

In species of the willistoni group the ejaculatory apodemes are of two general types. The first is the standard spade type (Figures 15.38–.43). The second (Figures 15.34–.37) is the modified spade type seen in many species of the melanogaster group (e.g., Figure 15.32).

Ejaculatory bulbs in species of the saltans group are of four general types: elliptical without lobes or caecae (Figure 15.49), elliptical with lateral lobes (Figures 15.44, .46–.47, .50), elliptical without lobes but with posterior caecae (Figure 15.48), and spherical with lateral lobes (Figures 15.51–.57). With the exception of *parasaltans* (Figure 15.45), the apodemes are of the spade type. That

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Subgenus: DROSOPHILA
**virilis group**
.1 virilis
.2 americana
.3 novamexicana
.4 littoralis
.5 ezoana
.6 montana
.7 flavomontana
.8 lacicola
.9 borealis
**melanica group**
.10 micromelanica
.11 melanica
.12 paramelanica
.13 euronotus
.14 nigromelanica
**robusta group**
.15 lacertosa
.16 colorata
.17 sordidula
.18 robusta
**annulimana group**
.19 gibberosa
.20 species E
.21 species D
**immigrans group**
.22 hypocausta
.23 spinofemora
.24 immigrans
**funebris group**
.26 macrospina
.27 subfunebris
.28 funebris
**ungrouped species**
.29 carsoni
Subgenus: HIRTODROSOPHILA
.25 histrioides

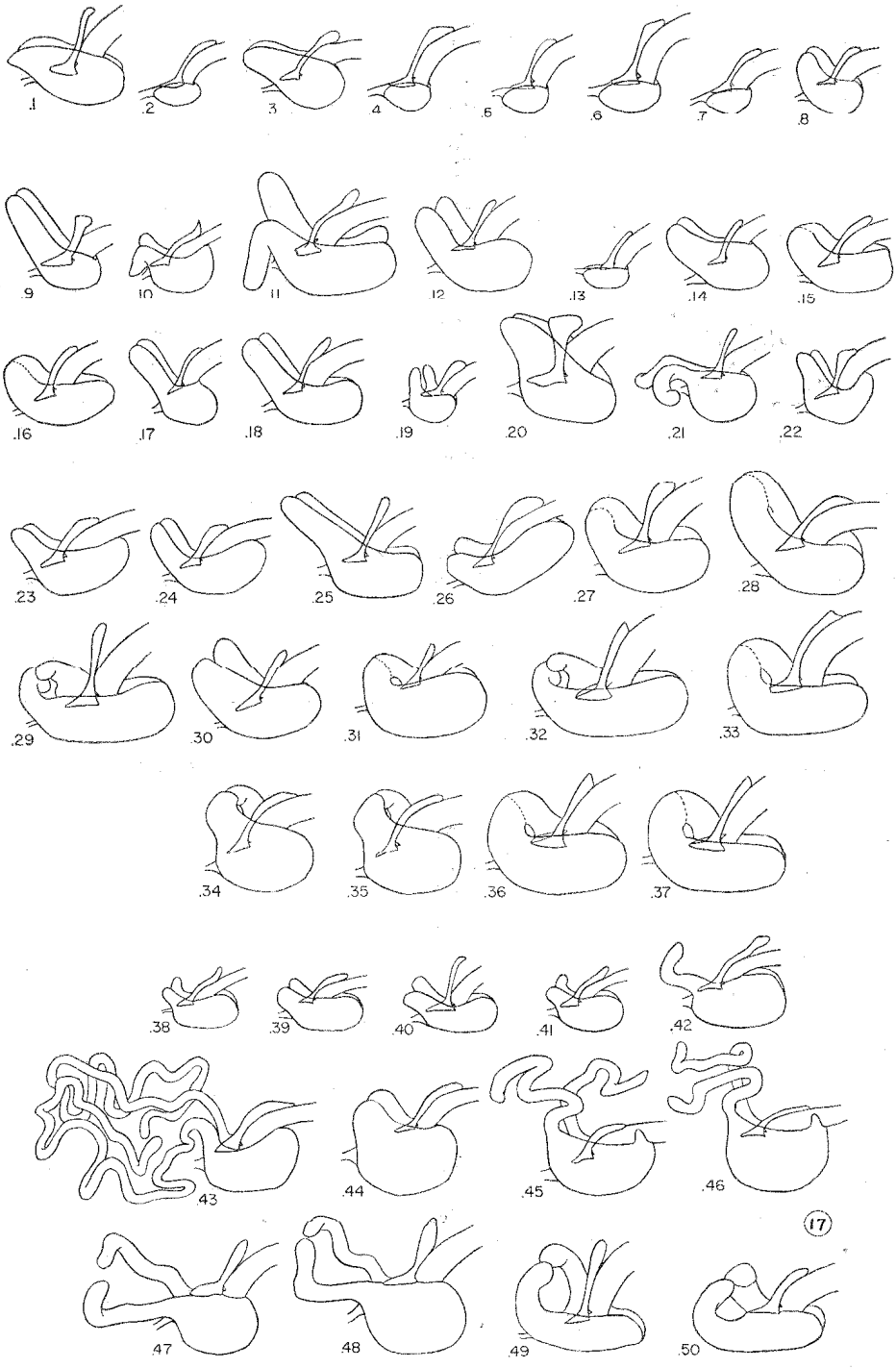


FIG. 17. Ejaculatory bulbs and ejaculatory apodemes from *Drosophila* species.

of *parasaltans* is more nearly like the modified spade type seen in some willistoni and melanogaster group species (e.g., Figures 15.32, .34).

In the genus as a whole the handle of the apodeme shows few distinctive features of use in phylogeny. Often it is compressed laterally to form a thin blade, although in most Sophophorans the handle is thick and heavy. Some species of the saltans group depart from the general pattern. In species of the elliptica subgroup (Figures 15.47–.48) the handle is roughly cylindrical. It is flared apically and has a conical depression at the tip. In most species of the saltans subgroup (Figures 15.51–.57) the handle is roughly triangular in section, and the tip may be slightly flared but it generally is not noticeably concave. In *lusaltans* the handle is rather irregular, but nearly cylindrical. In *austrosaltans* the handle is a simple cylinder, slightly, if at all, flared at the tip. In *neocordata* and in species of the sturtevanti subgroup the handle is a simple blade. In *subsaltans* the handle is triangular in section, almost exactly as is seen for most saltans subgroup species, and in *parasaltans* the handle is short and stubby, and roughly cylindrical in section.

Ejaculatory bulbs from species in the subgenus *Drosophila* are shown in Figures 16 through 19. As can be seen from an inspection of these figures, the types shown in the phylogeny (Figure 21) only inadequately represent this very diverse group. Most of the important types from the quinaria section have been included in the phylogeny, but many of the major types from the virilis-repleta section could not be shown. Members of the virilis group (Figures 16.1–.9) are almost uniform with respect to the ejaculatory bulb and ejaculatory apodeme. Basically, the ejaculatory bulb is of the type seen in *bryani*, except that it is proportionally much larger, and the ejaculatory apodeme is somewhat more massive. Except for the shape of the bulb, this type resembles that seen in many species of the saltans group (e.g., Figure 15.57). The only other species in the subgenus *Drosophila* having a similar type of bulb is *polychaeta* (Figure 18.8).

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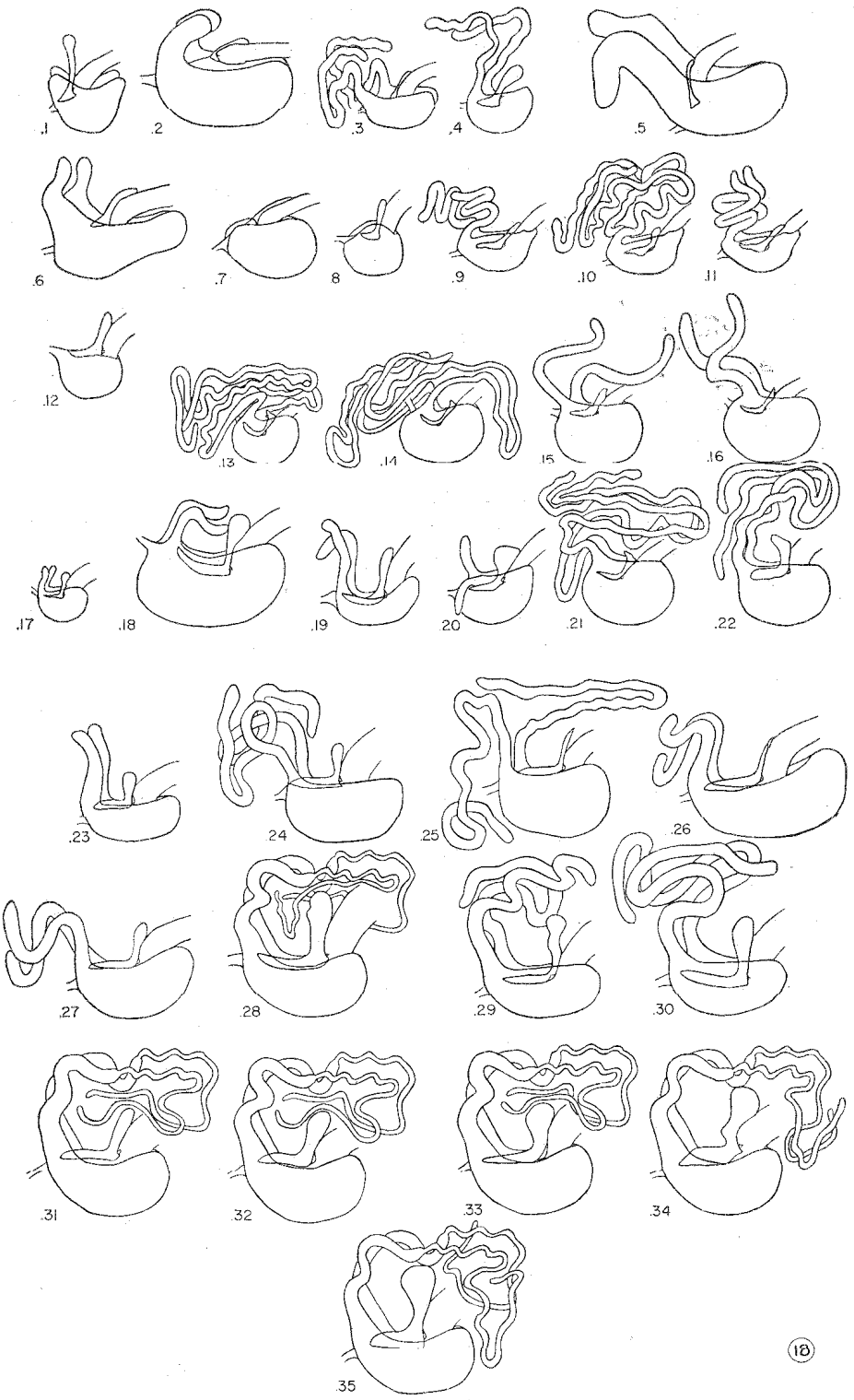
Subgenus: DROSOPHILA
**repleta group**
*fasciola subgroup*
.1 fulvalineata
.2 fasciola
.3 coroica
.4 pictura
.5 pictilis
.6 fascioloides
.7 moju
.8 mojuoides
*mulleri subgroup*
.9 tira
.10 pachuca
.11 nigricruria
.12 aldrichi
.13 mulleri
.14 longicornis
.15 arizonensis
.16 mojavensis
.17 martensis
.18 stalkeri
.19 hamatofila
.20 eremophila
.21 buzzatii
.22 pegasa
.23 meridionalis
.24 promeridiana
.25 meridiana
.27 anceps
.29 peninsularis
*mercatorum subgroup*
.30 mercatorum
.31 paranaensis
*melanopalpa subgroup*
.32 fulvimacula
.33 fulvimaculoides
.34 limensis
.35 repleta
.36 canapalpa
.37 melanopalpa
*hydei subgroup*
.38 bifurca
.39 nigrohydei
.40 eohydei
.41 neohydei
.42 hydei
**canalinea group**
.43 canalinea
.44 paracanalinea
**dreyfusi group**
.45 camargoi
.46 briegeri
**not assigned to subgroup**
.28 serenensis
**not assigned to group**
.26 aureata
.49 castanea
.50 species F
**mesophragmatica group**
.47 gaucha
.48 pavani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However, in *polychaeta* the bulb is relatively very small and the ejaculatory apodeme is distinctly different from the virilis type.

Species in the melanica group (Figures 16.10–.14) vary from the simple, nearly primitive type seen in *micromelanica* to the derived types seen in such species as *melanica*. In *micromelanica* the bulb lacks either lateral lobes or caecae, and the apodeme is rather large and heavy. The remainder of the species in the group would fall in Okada's type E, with paired anterior and posterior caecae. The bulbs are quadrangular and have rather inconspicuous lateral lobes. In *nigromelanica* the caecae are short, heavy and blunt. In the others they are longer and more slender. In *melanura* (not figured) caecae are very long and slender. Except for the ejaculatory apodeme, the bulb is identical with that figured for *lacertosa* (Figure 16.15) of the robusta group. The ejaculatory apodemes are relatively large, and the handle may be expanded as a rather large, flat blade. All of the apodemes are of the spade type, but that of *melanura* is small and weakly chitinized. In most respects it resembles that figured for *C. procnemis* (Figure 22.3).

All species of the robusta group (Figures 16.15–.18) have paired anterior and posterior caecae, although in some the caecae are rather short and blunt. *D. robusta* is rather variable. The posterior caecae are always short and blunt. The very small anterior caecae may be present (as shown in Figure 16.18) or absent, or a caecum may be present only on one side. In *sordidula* the caecae are blunt lobes, and in *colorata* the caecae are somewhat longer and curve dorsally (down, in the figure). *D. lacertosa* has very long and slender anterior and posterior caecae. The posterior ones arise from blunt lobes. In this group the apodemes are quite massive, but they are of the spade type. The apodeme of *robusta* is distinctive. It may represent a modification of the spade type by reduced chitinization at the tip of the blade, or it may represent a modification of the forked type seen in coracina group species.

In addition to the general features of the ejaculatory bulbs of species in the robusta group, one other characteristic needs to be noted. In all of these species except *robusta*, the posterior part of the bulb is laterally expanded. Several other

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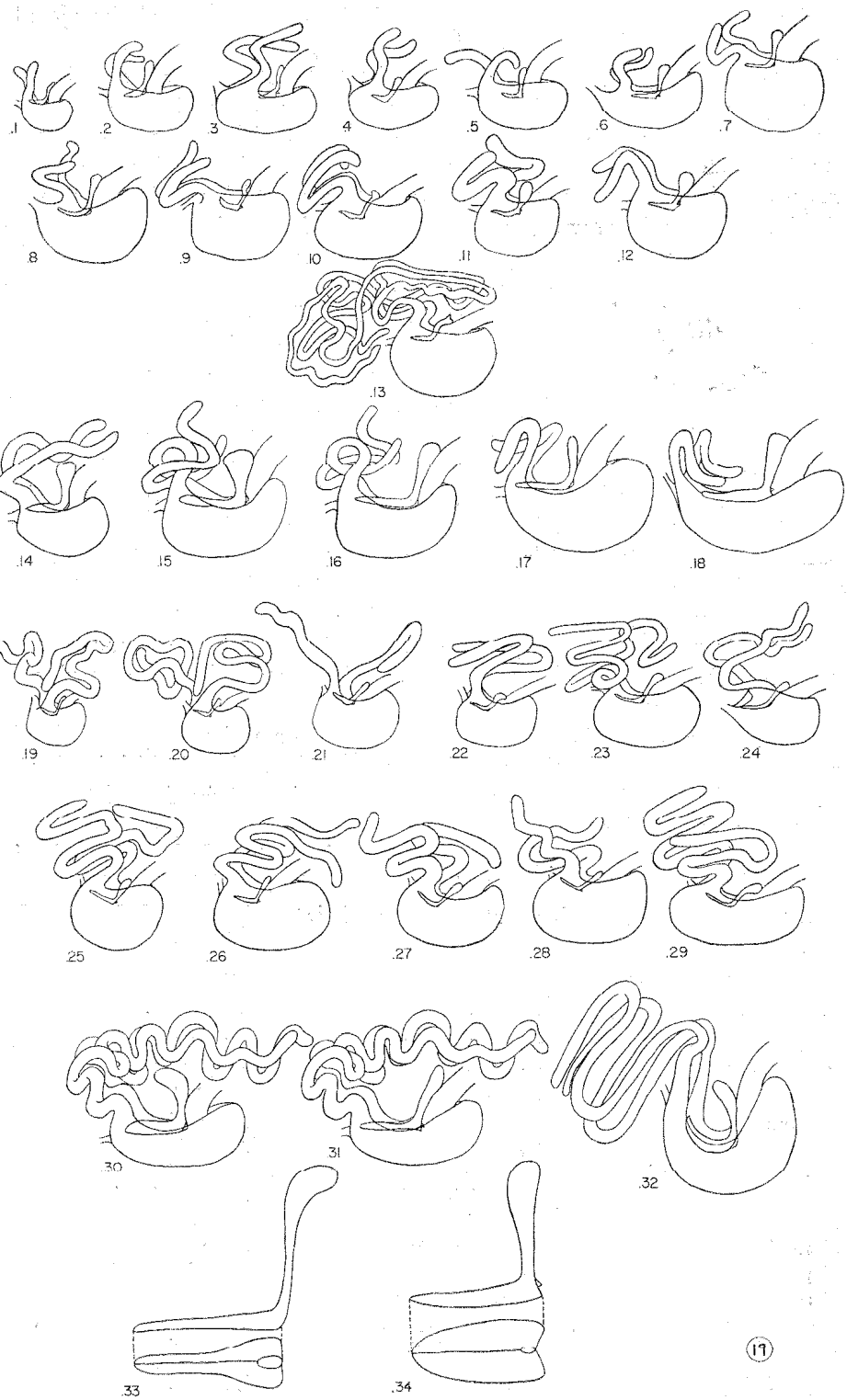
FIG. 18. Ejaculatory bulbs and ejaculatory apodemes of *Drosophila* species.

Subgenus: DROSOPHILA
**carbonaria group**
.1 carbonaria
**nannoptera group**
.2 nannoptera
**bromeliae group**
.3 species I
**ungrouped species**
.4 aracea
.5 species G
.6 peruviana
.7 species H
**polychaeta group**
.8 polychaeta
**ungrouped species**
.12 tumiditarsus
.13 species K
.14 species L
.17 sticta
**rubrifrons group**
.15 parachrogaster
.16 uninubes
**pallidipennis group**
.18 pallidipennis
.19 putrida
**testacea group**
.20 testacea
**macroptera group**
.21 submacroptera
.22 macroptera
**quinaria group**
.23 innubila
.24 quinaria
.25 rellima
.26 falleni
.27 phalerata
.28 species J
.29 occidentalis
.30 tenebrosa
.31 subquinaria
.32 transversa
.33 palustris
.34 subpalustris
.35 guttifera
Subgenus: PHLORIDOSA
.9 species P
.10 species Q
.11 species O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species from this section of the subgenus show various modifications of this type of bulb, and some representatives of this type are shown in Figure 23, together with figures of two "normal" bulbs for comparison. Figures 23.1–.2 show lateral and ventral views of the ejaculatory bulb for *castanea*. Figures 23.3–.4 show ventral views of the bulbs of *lacertosa* and *sordidula* respectively. Only a part of the long caecae of *lacertosa* has been shown in the diagram, and the anterior ejaculatory duct and ejaculatory apodeme have been omitted. Figures 23.7–.10 show lateral and ventral views of species having the more normal type of ejaculatory bulb.

Species belonging to the annulimana group (Figures 16.19–.21) have two major types of ejaculatory bulbs. *D. gibberosa* has posterior but no anterior caecae. The others have both anterior and posterior caecae. Except for the length of these caecae, these latter types resemble that seen in *colorata* (compare Figures 16.16 and .20). Ejaculatory apodemes in this group are also of two types. Two species have the spade type. The third has a modified type similar to that seen in some Sophophorans (compare Figures 15.45 and 16.21).

The ejaculatory bulb in species of the repleta group varies from very small and simple to very large. The simplest types are seen in the fasciola subgroup (Figures 17.1–.8). In most of these species the bulb is very small and has neither lateral lobes nor caecae (e.g., Figure 17.7). In others (e.g., Figure 17.3) the bulb is somewhat larger and there are short, blunt, posterior caecae (lateral lobes tilted posteriorly?). Both in size and in extent of differentiation, ejaculatory bulbs in this subgroup are among the simplest in the genus. The bulb itself is only a slight enlargement of the ejaculatory duct. The ejaculatory apodeme is of the spade type. Species of the mulleri subgroup (Figures 17.9–.25) show almost all of the types of variation seen in the repleta group as a whole. The ejaculatory bulb in *mulleri* is virtually identical with those seen in some fasciola subgroup

FIG. 19. Ejaculatory bulbs and ejaculatory apodemes of *Drosophila* species.

**tripunctata group**
.1 mediodiffusa
.2 albicans
.3 metzii
.4 albirostris
.5 tripunctata
.6 mediopunctata
.7 unipunctata
.8 trapeza
.9 bandierantorum
.10 paramediostriata
.11 mediostriata
.12 mediopictoides
.13 crocina

**guarani group**
.14 guaramunu
.15 guaraja
.16 griseolineata
.17 subbadia
.18 guarani

**cardini group**
.19 dunni
.20 belladunni
.21 nigrodunni
.22 polymorpha
.23 neomorpha
.24 neocardini
.25 parthenogenetica
.26 acutilabella
.27 procardinoides
.28 cardinoides
.29 cardini

**calloptera group**
.30 ornatipennis
.31 calloptera
.32 schildi
.33 detail of ejaculatory apodeme, generalized cardini type
.34 detail of ejaculatory apodeme, generalized tripunctata type

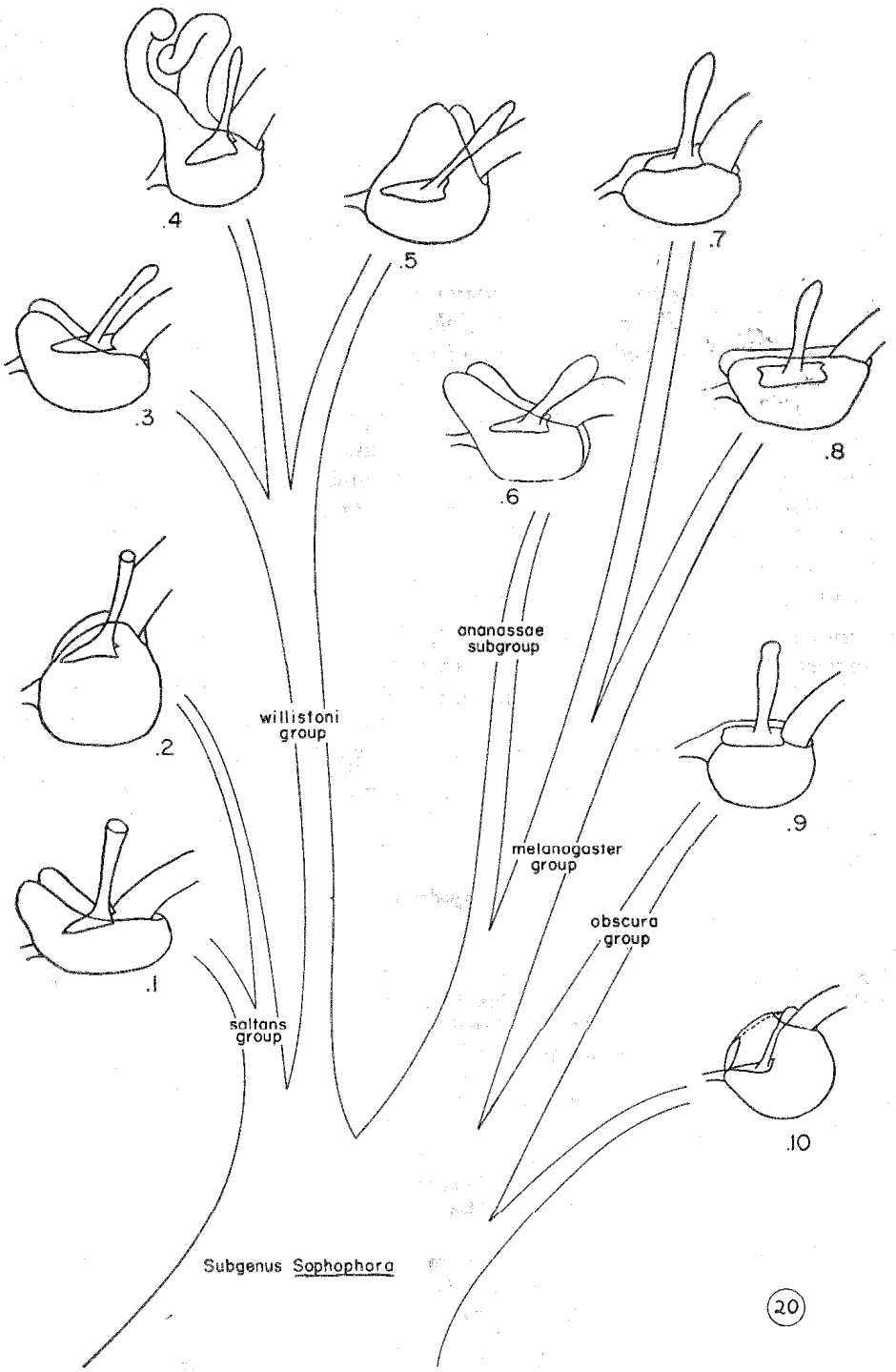


FIG. 20. Pictorial phylogeny of ejaculatory bulbs (*Sophophora*). .1) emarginata; .2) austrosaltans; .3) paulistorum; .4) sucinea; .5) equinoxialis; .6) ananassae; .7) rufa; .8) melanogaster; .9) affinis; .10) populi.

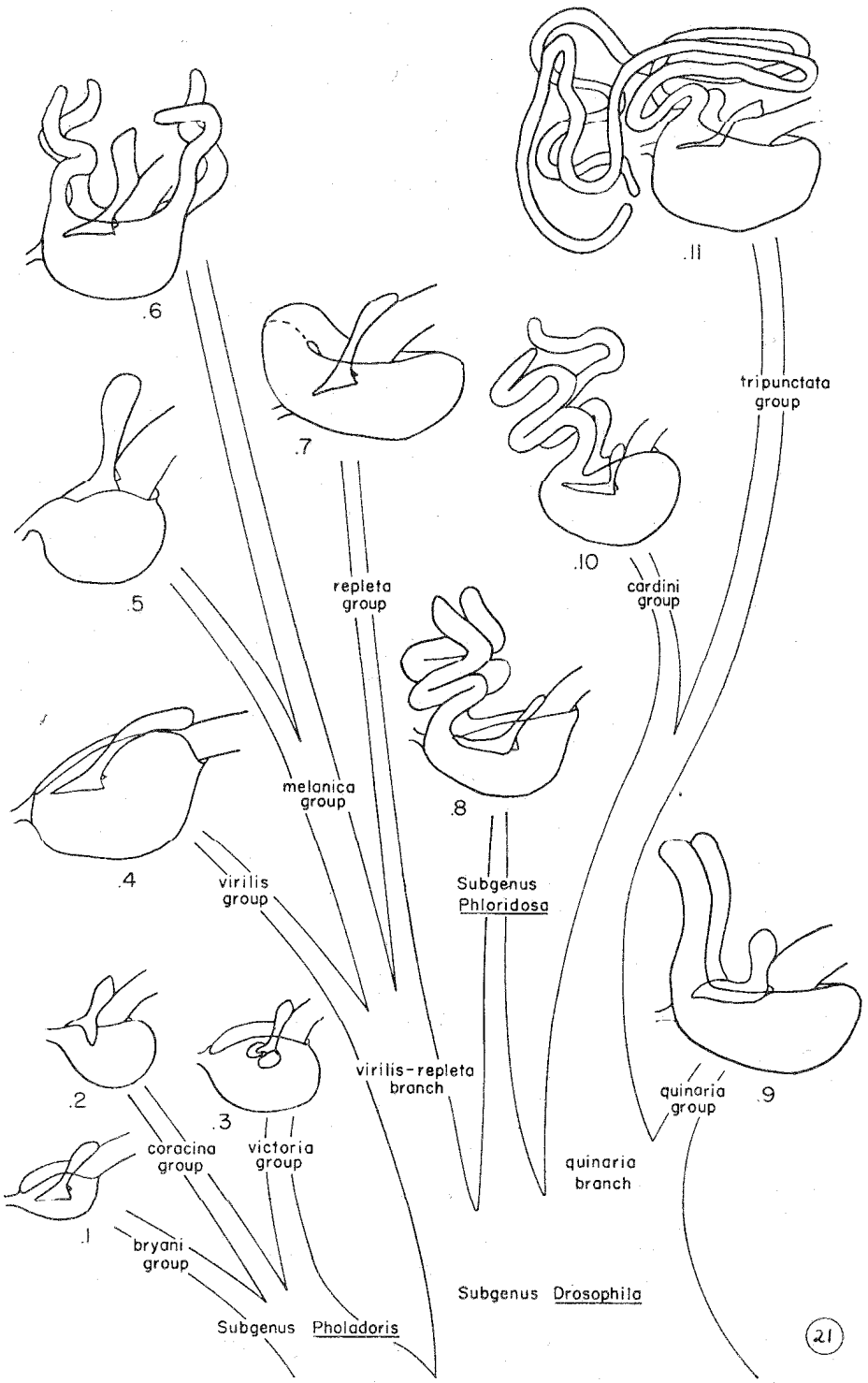


FIG. 21. Pictorial phylogeny of ejaculatory bulbs (*Drosophila* and others). .1) bryani; .2) cancellata; .3) victoria; .4) virilis; .5) micromelanica; .6) paramelanica; .7) mojavensis; .8) species P; .9) innubila; .10) parthenogenetica; .11) crocina.

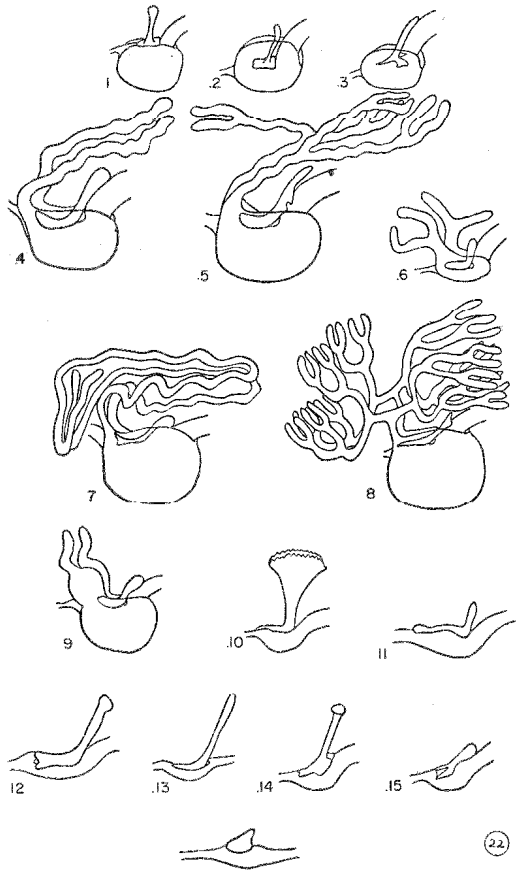


FIG. 22. Ejaculatory bulbs and ejaculatory apodemes from other Drosophilid genera, and from other families of Acalpyteratae. .1) *Chymomyza amoena*; .2) *C. aldrichi*; .3) *C. procnemis*; .4) *Scaptomyza adusta*; .5) *S. pallida*; .6) *S. hsui*; .7) *Zaprionus ghesquierei*; .8) *Z. vittiger*; .9) *Mycodrosophila dimidiata*; .10) *Zapriothrica dispar*; .11) *Diastata vagans* (Diastatidae); .12) *Periscelis annulata* (Periscelidae); .13) *Aulacigaster leucopeza* (Aulacigastridae); .14) *Sepsidimorpha secunda* (Sepsidae); .15) *Leptocera* sp. (Sphaeoceratidae); .16) *Discocerina obscurella* (Ephydridae).

species. The bulb is very small and simple. Most species in this subgroup (e.g., Figure 17.12) have moderately large bulbs with short posterior caecae. Three species (Figures 17.15–.16, .27) have a bulb derived from a type seen in species from the melanopalpa and mercatorum subgroups. The ejaculatory apodemes are all of the spade type. According to Wasserman (1960) species of the mulleri and fasciola subgroups are related cytologically as shown in Figure 25. Correlation of cytological and morphological details will be deferred until a later section, but it is helpful to refer to the cytological phylogeny at this time. As can be seen from Figure 25, there are three major phyletic lines within the group. All of these derive from the repleta standard gene sequence. There are several species, chiefly members of the mulleri subgroup, which are derived independently from the standard.

The next cluster of species to be considered makes up the second major branch

within the repleta group. The species involved belong to the melanopalpa and mercatorum subgroups. In addition, *peninsularis*, generally considered a member of the mulleri subgroup, belongs cytologically to this branch. Ejaculatory bulbs of these species are of three types. The first, seen in *mercatorum* (Figure 17.30), is moderate in size and has short posterior caecae. This closely resembles types seen in the mulleri and fasciola subgroups. The second, seen in *peninsularis* (Figure 17.29) and several other species, is rather elongate and has short posterior caecae which curve inward and generally touch at the midline. The third type (Figure 17.31 and others), almost certainly a modification of the type just mentioned, is the same in all details except that the caecae fuse at their tips and form a continuous tube around the posterior ejaculatory duct. It is this type which was seen in some of the members of the mulleri subgroup mentioned previously (e.g., Figures 17.15–.16). Two types of apodemes are seen in members of this branch. Most are of the usual spade type. In some (e.g., Figure 17.36) the plate is much flattened.

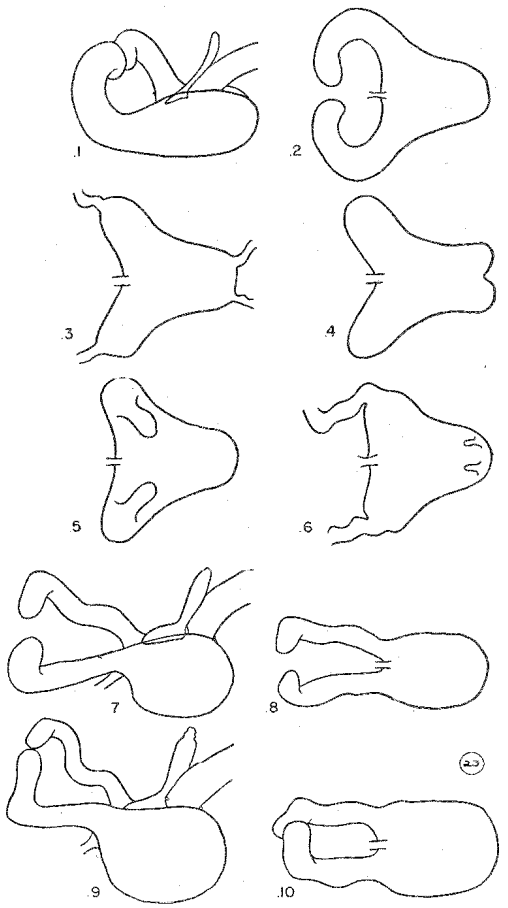


FIG. 23. Lateral and ventral view of certain ejaculatory bulbs. Anterior ejaculatory duct and ejaculatory apodeme have been omitted from ventral views. .1) castanea (lat.); .2) castanea (vent.); .3) lacertosa (vent.); .4) sordidula (vent.); .5) peruviana (vent.); .6) camargoi (vent.); .7) gaucha (lat.); .8) gaucha (vent.); .9) pavani (lat.); .10) pavani (vent.).

The third major branch of the group consists of hydei subgroup species (Figures 17.38–.42). Here the ejaculatory bulbs are very small, and they are much alike in general morphology. Posterior caecae are small and generally outcurved. All have the spade type of apodeme. The ejaculatory bulb of *castanea* is shown in Figure 17.49. This species is derived, cytologically, from the repleta standard. Species F is, on the basis of internal morphology, very close to *castanea* and it has an ejaculatory bulb identical with that figured for *castanea*. These two species have the posterior part of the ejaculatory bulb expanded, and they resemble some species of the robusta group in this respect.

Aside from the repleta group species and *castanea*, two other groups, canalinea and dreyfusi, are derived cytologically from the repleta standard. The cytological evidence indicates the relationship shown in Figure 25. The ejaculatory bulb of *paracanalinea* (Figure 17.44) is small and has two short, blunt posterior caecae. The bulb of *canalinea* (Figure 17.43) is larger and has long, slender posterior caecae. Both species have the spade type of apodeme, although that of *paracanalinea* is rather small and weakly chitinized. The ejculatory bulbs of species from the dreyfusi group (Figures 17.45–.46) have moderately long posterior caecae. There are also very small anterior caecae resembling those seen in *robusta* (Figure 16.18). In these two species, the anterior caecae are in close contact with the anterior ejaculatory duct and appear to clasp it basally. In ventral view (Figure 23.6) the bulb is seen to be of the type mentioned earlier for *castanea* and many robusta group species. The general similarities between the ejaculatory bulbs of dreyfusi and robusta group species can be seen by comparing Figures 23.3, .4 and .6. Ejaculatory apodemes are of the spade type.

Species of the mesophragmatica group are also cytologically related to the repleta complex, but not as closely as are the species just covered (Wasserman, pers. comm.). They have the normal type of bulb with two moderately long posterior caecae. The ejaculatory apodemes are different in the two species. That in *pavani* (Figure 17.48) is of the usual spade type. That of *gaucha* (Figure 17.47) has the plate shaped more like a spoon, and in this respect resembles somewhat the types which will be seen shortly in species from the quinaria section. The plate, however, is much shorter than that of the quinaria type.

On the basis of external morphology the next group of species is heterogeneous. Some features, particularly of the ejaculatory apodeme, suggest a relationship between them. The simplest type is that seen in an undescribed form (species H, Figure 18.7). Here the bulb is moderately large but lacks both lateral lobes and posterior caecae. The ejaculatory apodeme is minute, and its size is exaggerated three to four times in the figure. The size of the bulb is as it should be in proportion to other bulbs in the genus. If the ejaculatory apodeme were drawn to the same scale it would appear as a fine line in the figure. The apodeme plate is almost non-existent. The base of the handle simply flares slightly and curves around the base of the ejaculatory duct. *D. peruviana* is, on the basis of external morphology, related to this species, but it has a quite different ejaculatory bulb and apodeme. This is figured in lateral view in Figure 18.6 and in ventral view in Figure 23.5. Another undescribed form (species G, Figure 18.5) has an ejaculatory apodeme very similar to that seen in species H. The apodeme is minute, and the plate is only slightly more developed than in the previously

mentioned species. The ejaculatory bulb is moderately large with stout posterior caecae. A species of the bromeliae group (Figure 18.3) has an ejaculatory bulb and apodeme very similar to that seen in species G. The major difference between the two is in the length of the posterior caecae. The last species having this type of apodeme is *nannoptera* (Figure 18.2). The ejaculatory bulb has short posterior caecae. The apodeme is minute and almost identical with that seen in species H.

The ejaculatory bulb of *carbonaria* (Figure 18.1) shows few features which clearly associate it with any group in the genus. The bulb is small with lateral lobes and short anterior and posterior caecae. The ejaculatory apodeme is of the spade type and, on the whole, the ejaculatory bulb most nearly resembles a type seen in the melanica group (compare Figures 16.14 and 18.1).

Species of the immigrans group (Figures 16.22–.24) have large ejaculatory bulbs. That of *spinofemora* is undifferentiated. That of *hypocausta* has slightly developed lateral lobes and folded posterior caecae of a type to be seen among species from the quinaria section of the subgenus. *D. immigrans* has both anterior and posterior caecae, although all are very short. In this respect the bulb somewhat resembles that of *robusta* (Figure 16.18), although the position of the anterior ejaculatory duct and the ejaculatory apodeme is quite different in the two species. The ejaculatory apodeme is very large in two species of the immigrans group. That of *spinofemora* is, relative to the others in the group, rather small, but this appearance is due mainly to the short handle. The plate is of the spade type, but it is about twice as long as those previously seen of this type. In length it approaches types seen in the quinaria section. The apodeme of *immigrans* is large and heavy. The plate is long and its anterior angles are rounded, a feature which is also characteristic of many species of the quinaria section. The apodeme of *hypocausta* is unique. The handle is large, but very thin and flat. It is continuous with a thin keel which extends down the mid-ventral line of the plate. The plate is also thin and flat.

In species of the funebris group the ejaculatory bulb is rather uniform (Figures 16.26–.28). In two species the bulb is almost spherical. Lateral lobes are inconspicuous and the posterior caecae are folded. In the third species the bulb is elongate and somewhat expanded posteriorly. Ejaculatory apodemes are of the spoon type and have short handles. In general aspect they most closely resemble types seen in species from the quinaria and calloptera groups.

Ejaculatory bulbs of species in the quinaria group fall into four types. In *innubila* (Figure 18.23) the bulb is moderate in size and has two short posterior caecae. In *falleni* and *phalerata* (Figures 18.26–.27) the bulb is elongate and has short, folded posterior caecae. In *quinaria*, *rellima*, *tenebrosa* and *occidentalis* (Figures 18.24–.25, .29–.30) the bulb is elongate and the posterior caecae are moderately long. The caecae in these four species are almost of uniform diameter throughout. In the remainder of the species in the group (Figures 18.28, .31–.35) the caecae are longer and have two distinct sections. The proximal section has the same diameter as that of caecae seen elsewhere in the group. Somewhat before the midpoint of the caecum there is a constriction, and the distal section has a diameter roughly half that of the proximal section. In most individuals the constriction is sharp and the two sections are distinct. In a few the diameter tapers gradually, but over a distance of less than one-tenth the total length of the cae-

cum, to the smaller size. Although the apodemes vary in detail, they are all of the spoon type.

Species of the calloptera group (Figures 19.30–.32) have very elongate ejaculatory bulbs. That of *schildi* is flexed near its midpoint and has moderately long, and precisely folded, posterior caecae. The other two species have elongate bulbs, but they are not flexed. The posterior caecae are long and much folded, but they do not show the regular long folds seen in *schildi*. Ejaculatory apodemes are all of the spoon type.

Species of the testacea group (Figures 18.19–.20) have small ejaculatory bulbs with short posterior caecae. In *testacea* the posterior caecae are somewhat displaced anteriorly, a condition seen often and more conspicuously in many of the species remaining to be covered. Ejaculatory apodemes are of the spoon type. Species of the rubrifrons group (Figures 18.15–.16) have ejaculatory bulbs of moderate size with short posterior caecae. The bulb of one (Figure 18.16) is slightly flexed and the caecae are displaced somewhat anteriorly. Apodemes are of the spoon type. Ejaculatory bulbs from species in the macroptera group (Figures 18.21–.22) have long posterior caecae. Both in respect to characteristics of the bulb and of the apodeme, they resemble some species of the tripunctata group. Most species of the tripunctata group (Figures 19.1–.13) have moderate sized ejaculatory bulbs which are elongate to very elongate. Caecae may arise almost at the posterior end of the bulb (e.g., Figure 19.2), but they are generally displaced anteriorly (e.g., Figure 19.3) and appear to arise from the apex of a lateral fold. This appearance is due, in part, to a flexure of the bulb similar to that seen in *schildi* (Figure 19.32). The posterior caecae are generally short (and folded when long enough). In *crocina* the caecae are very long. Ejaculatory apodemes are rather variable. Some (e.g., Figure 19.4) have an elongate spade type. Most (e.g., Figure 19.5) are of the spoon type.

Species of the cardini group (Figures 19.19–.29) have ejaculatory bulbs of moderate size. The posterior caecae are rather long and generally are folded. Often they are displaced anteriorly, sometimes conspicuously so (Figure 19.24). In this last case, *neocardini*, the bulb is not flexed, and the caecae arise at the apices of distinct lateral folds. In this case also, the caecae cross over each other and are often intertwined. This crossing of the caecae is seen in some individuals from all the species in the group but it cannot be considered characteristic of any species (with *neocardini* a possible exception). The ejaculatory apodemes of species in the cardini group are quite distinctive, although they show obvious resemblances to the spoon type seen in other species from this branch of the genus. In most cases the plate is extremely long and slender, generally equaling the length of the handle. The plate is of the usual width across the anterior angles, but it immediately constricts sharply and becomes very narrow and pointed. Figures 19.33 and 19.34 show details of the apodeme for generalized cardini and tripunctata types. Only *polymorpha* has an apodeme which approaches the tripunctata type.

Ejaculatory bulbs of guarani group species are generally large. Posterior caecae are rather short and generally are folded. Anterior displacement of the caecae is not pronounced, and lateral folds are not conspicuous. The ejaculatory apodemes are large and of the spoon type. Ejaculatory bulbs and apodemes of

miscellaneous species from this branch fall into one or another of the types already discussed. *D. pallidipennis* (Figure 18.18) is similar to *trapeza* (Figure 19.8); *sticta* (Figure 18.17) to *mediodiffusa* (Figure 19.1), etc.

In species from the subgenus *Phloridosa* (Figures 18.9–.11) the ejaculatory bulb is small with lateral lobes and folded posterior caecae. In one species the caecae are relatively long. The apodeme is of the spoon type similar to those seen in species from the quinaria section or in *polychaeta*. Species from the subgenus *Hirtodrosophila* are quite variable (Figures 15.8–.10; 16.25). The ejaculatory bulbs in *duncani* and *thoracis* have short, blunt posterior caecae. The posterior caecae in *pictiventris* (Figure 15.9) are peculiar to that species. In *histrioides* (Figure 16.25) the ejaculatory bulb is small with blunt posterior caecae. The bulb itself resembles types seen in the repleta group (e.g., Figure 17.20), but the ejaculatory apodeme is quite different. The plate is rather broad, flat and long. In general shape it resembles types seen in species of the quinaria branch, but it is too flat to appear very similar to them. The ejaculatory apodemes of *duncani*, *pictiventris* and *thoracis* are slight modifications of the spade type. The ejaculatory bulb of *busckii* is moderately large and has lobose posterior caecae (Figure 15.11). The apodeme is of the standard spade type.

The ejaculatory bulbs of species in the genus *Chymomyza* (Figure 22.1–.3) most nearly resemble those of species in the obscura group (compare Figures 22.1 and 15.17 or 22.2 and 15.13). The apodemes are of two types. The first (Figures 22.1–.2) resembles the obscura type but is much more delicate. The apodeme of *C. procnemis* (Figure 22.3) approaches the standard spade type. The lateral borders of the plate are weakly chitinized and not pigmented. Nater (1953) gives a figure of the apodeme of *C. procnemis* which differs from this. His figure shows an apodeme of the type seen in *C. amoena* and *C. aldrichi*.

The ejaculatory bulbs of *Scaptomyza* species vary (Figures 22.4–.6). That of *S. adusta* is moderate sized. In the individuals dissected the caecae were unbranched. However, Rosenblad (1941) describes this species as having branched posterior caecae (as figured for *S. hsui*, except that the caecae are longer), and Patterson (1943) gives a figure for this species in which one caecum is branched and the other not. Apparently this species is variable in this respect. The caecae of *S. hsui* are short and branch once. Those of *S. pallida* are longer and branch twice. The apodemes are of the spoon type. Ejaculatory bulbs of *Zaprionus* species are moderately large and almost spherical. One (Figure 22.7) has long, unbranched posterior caecae. The other (Figure 22.8) has long caecae, each of which branches four times. The apodemes are modifications of the spoon type (compare Figures 22.8 and 18.21). The ejaculatory bulb of *Mycodrosophila dimidiata* (Figure 22.9) is distinctive, although one might force a resemblance between it and that of *pictiventris* (Figure 15.9). The ejaculatory apodeme is of the spoon type.

*Morphology of the testes*—In the majority of the Acalypteratae the testes are elliptical or nearly so (Figure 1). Spiral testes have been seen only in the Drosophilidae, and in this family there is great variability in testis form (Figure 2). Investigations by Stern (1940, 1941a, b) indicate the mechanisms involved in the formation of the spiral testes in several species from both the subgenus *Drosophila* and the subgenus *Sophophora*. During early developmental stages

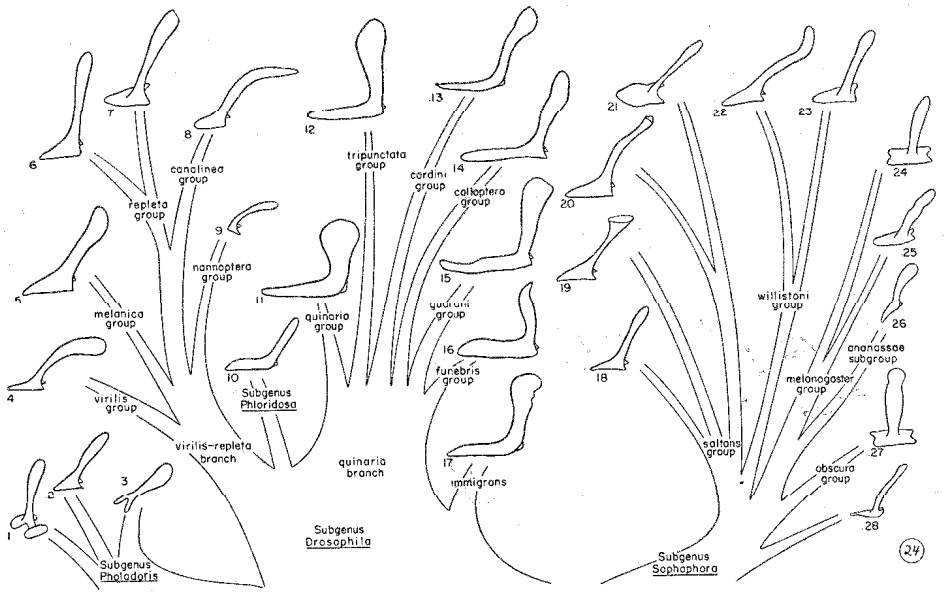


FIG. 24. Pictorial phylogeny for ejaculatory apodemes. .1) victoria; .2) bryani; .3) cancellata; .4) virilis; .5) melanica; .6) peninsularis; .7) melanopalpa; .8) canalinea; .9) nannoptera; .10) species 0; .11) palustris; .12) tripunctata; .13) cardini; .14) calloptera; .15) guarani; .16) funebris; .17) immigrans; .18) neocordata; .19) neoelliptica; .20) subsaltans; .21) parasaltans; .22) pseudobocainensis; .23) willistoni; .24) melanogaster; .25) serrata; .26) ananassae; .27) pseudoobscura; .28) populi.

the testes are completely free, and it is only during later ontogeny (pupal) that they become attached to ducts which arise from the genital imaginal disc. Stern (1941a) finds that the form of the testes is determined by the thin inner membrane of the testis capsule, and growth occurs by terminal addition to this membrane. Differential elongation is explained as being due to the release of the growth promoting substance from the vas in different amounts to opposite sides of the testis. The membrane elongates most near the point of attachment and curvature of the testis is thus *away* from this point (see Figures 2.9, 13.7 and 26.2). This is important, since it provides the criterion by which true spiral coiling is differentiated from pseudo-coils or curvatures produced primarily by crowding of an elongate testis in the body cavity (e.g., Figures 2.2–.3).

There can be little doubt that the elliptical form of the testis is primitive and that the spiral form is derived within the family Drosophilidae. To some extent the number of testis coils is an index of the evolutionary status of a species, in that the more primitive forms tend to have a lower number of coils. Within the family testis form varies from true elliptical (e.g., Figures 2.1, .4–.5), to elongate elliptical (e.g., Figures 2.2–.3), to true spiral (e.g., Figures 2.6–.13). Other forms are figured by Okada (1956), but in many cases these cannot be evaluated as to whether they belong to the true spiral form or to some other type. This can only be decided by use of the criterion mentioned earlier, i.e., direction of testis curvature relative to its point of attachment to the vas. In some cases the presence of counter-coiling of the vas deferens (inner coil of testis) can be taken

as indicative of true spiral coiling in the testes, since the counter-coil of the vas results mechanically from asymmetric growth of the testis. However, not all species having true spiral testes exhibit counter-coiling of the vasa (e.g., Figures 2.6, .10 and 13.7). In these cases, curvature of the testes relative to the point of attachment is the only available criterion. Since the need for this type of distinction has not been recognized previously, earlier descriptions and figures of testes are of limited value. In most cases it seems probable that those described as spiral exhibit true spiral coiling, but some doubt must remain until these species have been re-examined with appropriate criteria in mind. Within the genus *Drosophila* there is little difficulty on this point. All species examined fall into two categories, true elliptical and true spiral. The second category can be arbitrarily subdivided according to the number of coils in the spiral.

Since only a few (10–12) individuals have been examined for any one species, and since there is some individual variation in number of coils (generally differences are in fractions of a coil over or under a modal value), the data for all the species will not be tabulated. Table 1 summarizes the data on testis coils by groups. Most of the miscellaneous species have been omitted. As can be seen by inspection of Table 1, the number of testis coils ranges from zero (elliptical testis) to approximately twenty. Probably several distinct factors influence the number of coils in the adult testis. Two obvious factors will be the amount of growth stimulation substance produced by the vas and the duration of the period during which this substance is provided to the testis (and/or the duration of the period during which the testis is capable of responding to this substance). The amount of growth substance appears to be the major factor in determining the number of testis coils in some affinis subgroup species, and differences between the number of coils in *melanogaster* (three) and in *virilis* (six) are due to difference in rate of testis growth relative to the pupal period (Stern 1941b). At the present time it is not possible to determine which of these factors are operating, singly or in combination, to produce the various numbers of coils and other variations in testis form seen among *Drosophila* species. Thus, the major distinction which can safely be made is between elliptical and spiral testes, and, as was said previously, the higher number of coils generally indicates more derived forms. Inferences regarding types of spiral testes must be tentative, but conclusions drawn from certain closely related species will probably be valid. Figure 26 gives a much abbreviated phylogeny of testes forms. Representatives chosen as examples of forms from the two major branches of the subgenus *Drosophila* indicate the more extreme types. Intergradations between these two types do exist, but generally the two types are recognizably distinct.

Species in three of the four groups in the subgenus *Pholadoris* have elliptical testes similar in type to that shown in Figure 26.1. The testes of *bryani* (Figure 26.2) have about one-half coil. Of the *Hirtodrosophila*, one, *duncani*, has elliptical testes. The others vary from approximately one, to approximately three coils. *D. busckii* (*Dorsilopha*) has about two coils (Figure 2.9). In the subgenus *Sophophora*, *populi* has elliptical testes. Species of the obscura group fall into two types. Those of the obscura subgroup have elliptical testes (e.g., Figure 26.12) and those of the affinis subgroup have spiral testes varying from one-half coil (*affinis*) to three coils (*azteca*). Details will not be presented for most of the

TABLE 1

Testes coils of *Drosophila* and other species. The denominator indicates the number of species examined in each group. The numerator indicates the number of species in the group which have the indicated number of coils in the testes. Zero (no coils) indicates elliptical testes.

| | Number of coils | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0–1 | 1–3 | 3–6 | 6–9 | 9–12 | 12–15 | 15–20 |
| *Pholadoris* | | | | | | | | |
| victoria gp. | 2/2 | .... | .... | .... | .... | .... | .... | .... |
| coracina gp. | 3/3 | .... | .... | .... | .... | .... | .... | .... |
| latifasciaeformis gp. | 1/1 | .... | .... | .... | .... | .... | .... | .... |
| bryani gp. | .... | 1/1 | .... | .... | .... | .... | .... | .... |
| *Hirtodrosophila* | 1/4 | .... | 3/4 | .... | .... | .... | .... | .... |
| *Dorsilopha* | .... | .... | 1/1 | .... | .... | .... | .... | .... |
| *Sophophora* | | | | | | | | |
| populi | 1/1 | .... | .... | .... | .... | .... | .... | .... |
| obscura gp. | 4/10 | 1/10 | 5/10 | | | | | |
| melanogaster gp. | .... | .... | 5/12 | 6/12 | 1/12 | .... | .... | .... |
| willistoni gp. | .... | .... | 10/10 | .... | .... | .... | .... | .... |
| saltans gp. | .... | .... | .... | .... | 10/14 | 4/14 | .... | .... |
| *Drosophila* | | | | | | | | |
| virilis-repleta section | | | | | | | | |
| virilis gp. | .... | .... | 1/9 | 5/9 | 2/9 | 1/9 | .... | .... |
| melanica gp. | .... | .... | 1/6 | 4/6 | 1/6 | .... | .... | .... |
| robusta gp. | .... | .... | .... | 3/4 | 1/4 | .... | .... | .... |
| annulimana gp. | .... | .... | .... | .... | 4/4 | .... | .... | .... |
| canalinea gp. | .... | .... | .... | 1/2 | 1/2 | .... | .... | .... |
| dreyfusi gp. | .... | .... | .... | 2/2 | .... | .... | .... | .... |
| mesophragmatica gp. | .... | .... | .... | 2/2 | .... | .... | .... | .... |
| repleta gp. | | | | | | | | |
| mulleri sbgp. | .... | .... | 12/19 | 7/19 | .... | .... | .... | .... |
| fasciola sbgp. | .... | .... | .... | 3/8 | 5/8 | .... | .... | .... |
| mercatorum sbgp. | .... | .... | 1/2 | 1/2 | .... | .... | .... | .... |
| melanopalpa sbgp. | .... | .... | .... | 3/6 | 3/6 | .... | .... | .... |
| hydei sbgp. | .... | .... | .... | .... | .... | 1/5 | 2/5 | 2/5 |
| polychaeta gp. | .... | .... | 1/1 | .... | .... | .... | .... | .... |
| nannoptera gp. | .... | .... | .... | .... | .... | .... | .... | 1/1 |
| bromeliae gp. | .... | .... | .... | 1/1 | .... | .... | .... | .... |

| | Number of coils | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0–1 | 1–3 | 3–6 | 6–9 | 9–12 | 12–15 | 15–20 |
| quinaria section | | | | | | | | |
| immigrans gp. | .... | .... | .... | 3/3 | .... | .... | .... | .... |
| funebris gp. | .... | .... | .... | 3/3 | .... | .... | .... | .... |
| testacea gp. | .... | .... | .... | 2/2 | .... | .... | .... | .... |
| calloptera gp. | .... | .... | .... | 3/3 | .... | .... | .... | .... |
| quinaria gp. | .... | .... | .... | 2/13 | 6/13 | 5/13 | .... | .... |
| guarani gp. | .... | .... | .... | 1/5 | 1/5 | 3/5 | .... | .... |
| cardini gp. | .... | .... | .... | 6/11 | 5/11 | .... | .... | .... |
| tripunctata gp. | .... | .... | .... | 9/13 | 4/13 | .... | .... | .... |
| *Phloridosa* | .... | .... | .... | .... | .... | .... | .... | 3/3 |
| *Scaptomyza* | .... | 1/3 | 2/3 | .... | .... | .... | .... | .... |
| *Zaprionus* | .... | .... | 1/2 | .... | 1/2 | .... | .... | .... |
| *Mycodrosophila* | .... | .... | .... | .... | .... | 1/1 | .... | .... |
| *Chymomyza* | .... | .... | 1/3 | .... | 1/3 | 1/3 | .... | .... |

remainder of the groups in the genus or they will be given later with the pertinent discussion.

Morphological differences, aside from differences in coil number, are of several types. In the majority of groups in the genus, and particularly where the number of testes coils is low (below six), the testes are thick and heavy (e.g., Figures 26.3–.4, .8–.11). Where the number of coils is relatively high, the coils are thin (e.g., Figure 2.11), although the thickness may be somewhat variable throughout the length of the testis. In the majority of species from the quinaria section of the subgenus *Drosophila* the testes coils are relatively thin, regardless of the number of coils. It is also in these species that the distal region of the vas is strongly differentiated, and in many species from this section the counter-coils of the vasa are larger and more bulky than the coils of the testes (e.g., Figures 26.5–.6). Taken together, the thin testes and the pronounced counter coils of the vasa produce a pattern which is almost constant for the more derived species belonging to the quinaria section. As was mentioned previously, species from other sections of the genus may have strongly differentiated vasa, but almost invariably these are associated with thick, heavy testes (e.g., Figures 26.3–.4). It should be noted that the counter-coils of the vasa are often pigmented. In Figures 26.3 and 26.5–.6 the counter-coils have been left unshaded to provide a contrast between them and the testes.

Data for testes coils seen in other genera are included in Table 1. The testes of *Chymomyza* species are relatively heavy. In general they resemble the spiral types seen in Sophophoran species. Okada (1956) figures one species (*C. caudatula*) having elliptical testes. *Scaptomyza* species also have thick, heavy testes, as do species of the genus *Zaprionus*. *Mycodrosophila dimidiata* has around ten thin coils in the testes, and these somewhat resemble the testes seen in species of the quinaria branch. The vasa, however, are only slightly differentiated, and in this respect the pattern does not follow that mentioned for species of the quinaria section.

*Internal Reproductive Structures of the Female*—Characteristics of the sper-

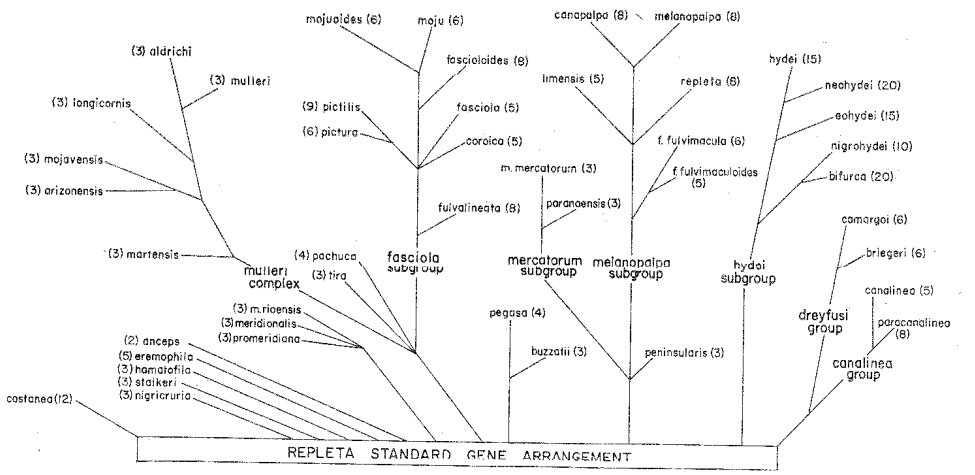


FIG. 25 Cytological phylogeny for species in the repleta group (after Wasserman, 1960 and Wasserman, personal communication).

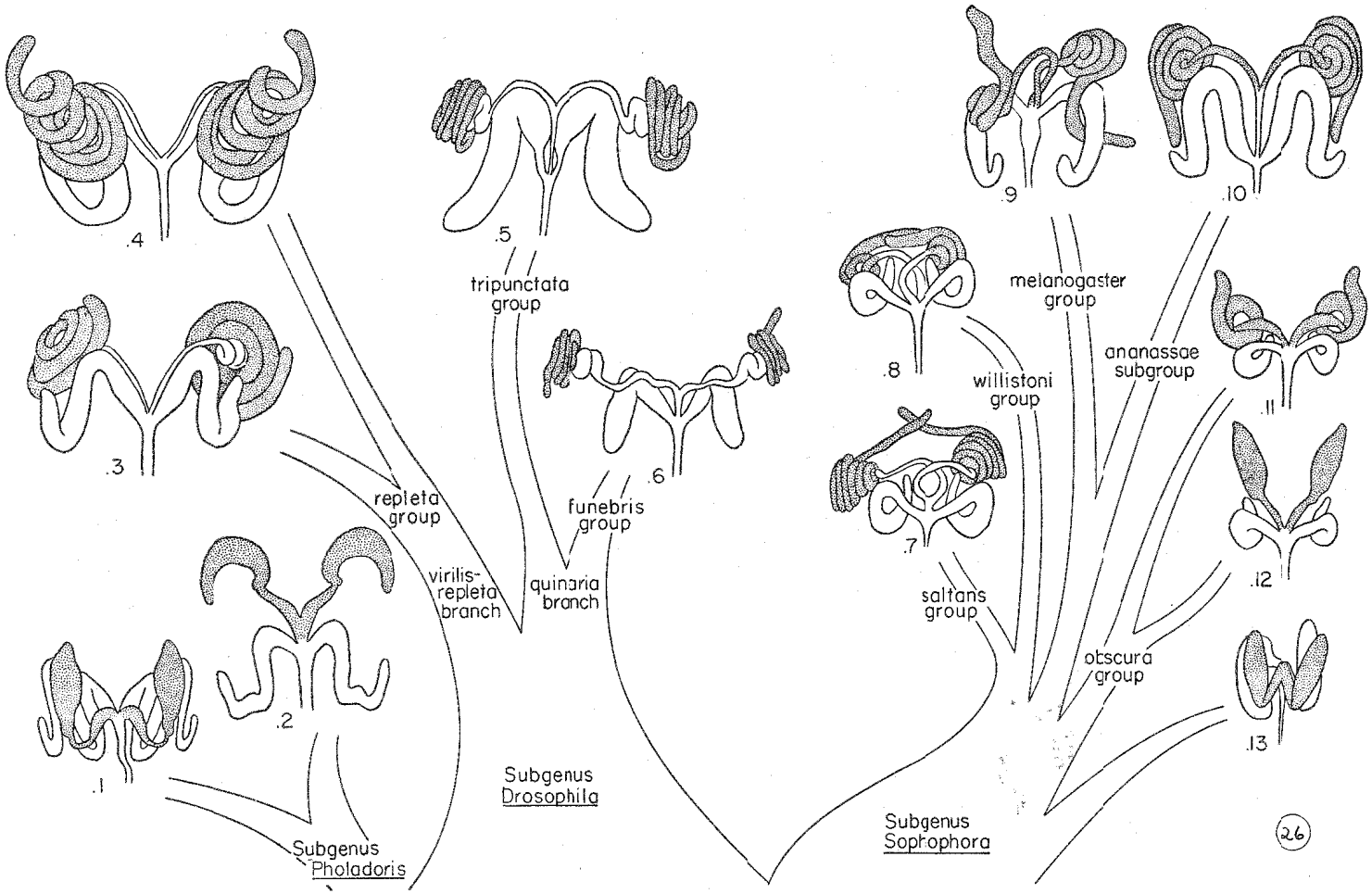


FIG. 26. Pictorial phylogeny of testes forms. .1) lativittata; .2) bryani; .3) nigricruria; .4) fulvalineata; .5) mediostriata; .6) subfunebris; .7) subsaltans; .8) sucinea; .9) melanogaster; .10) ananassae; .11) algonquin; .12) pseudoobscura; .13) populi.

mathecae, ventral receptacle and parovaria have been widely used by *Drosophila* taxonomists and they require little introduction. Only the characteristics of the spermathecae will be considered in detail. Those of the ventral receptacle will be summarized briefly. Some additional, general features can be noted before turning to detailed descriptions.

Both the spermathecae and the parovaria arise from a small, chitinized plate in the antero-dorsal vaginal wall. In all species investigated there are four stalks originating from this plate, the two anterior being the spermathecal ducts, the two posterior the parovarial ducts. There is considerable variation in the shape of this plate and in the relative positions of the stalks which arise from it. Characteristics of this plate are rather difficult to determine and they were not routinely recorded. The few notes available suggest that its characteristics will prove useful, particularly for classification at the higher taxonomic levels.

Characteristics of the parovaria and their ducts have been recorded, but they will not be reported in detail. In most species the length of the parovarium plus its duct is equal to or less than the length of the spermathecal duct (to the base of the spermatheca), and the parovaria are markedly smaller than the spermathecae. In a few species, mainly some more derived forms from both the virilis-repleta and quinaria sections of the subgenus *Drosophila*, the length of the parovarial duct equals that of the spermathecal duct. In some species, again generally more derived forms from the subgenus *Drosophila*, the parovaria and spermathecae are almost equal in size. There is no apparent correlation between length of duct and size of parovarium. Among Sophophorans, the montium subgroup species of the melanogaster group have spermathecae and parovaria which are almost equal in size and whose ducts are almost equal in length. The length of the parovarial duct cannot be determined accurately unless the material is cleared. In many cases a duct may be of usual length but appear shorter due to coiling which is not apparent before clearing. Or the duct may have a surprisingly long section folded and imbedded within the muscular wall of the vagina. In some species a thinly chitinized and crumpled lining of a parovarium may remain after clearing. In these cases, the cleared parovaria strikingly resemble the weakly chitinized and non-telescoped spermathecae seen in some species (e.g., montium subgroup species of the melanogaster group, etc.—see Figures 27.29–.33).

Pigmentation of spermathecae varies within the genus. In the more primitive forms (*Pholadoris*, virilis group, obscura group, etc.) the spermathecae are black. More derived forms vary from brown, to golden, to pale yellow in color. Some species of the tripunctata group have spermathecae which are yellow basally but dark apically (see Frota-Pessoa, 1954). Weakly chitinized spermathecae are also weakly pigmented. At the present time judgment of color would necessarily be subjective, and variation is gradual in some groups. Also, changes seen during clearing suggest pigmentation differences not evident by simple inspection. Two forms having identical color may vary greatly in clearing time and in final appearance. This may reflect heavier chitinization, but it seems probable that other factors (qualitative differences in pigment, differences in dispersion, etc.) contribute. For these reasons it seems advisable to defer treatment of spermathecal pigmentation until more reliable criteria are available for its evaluation.

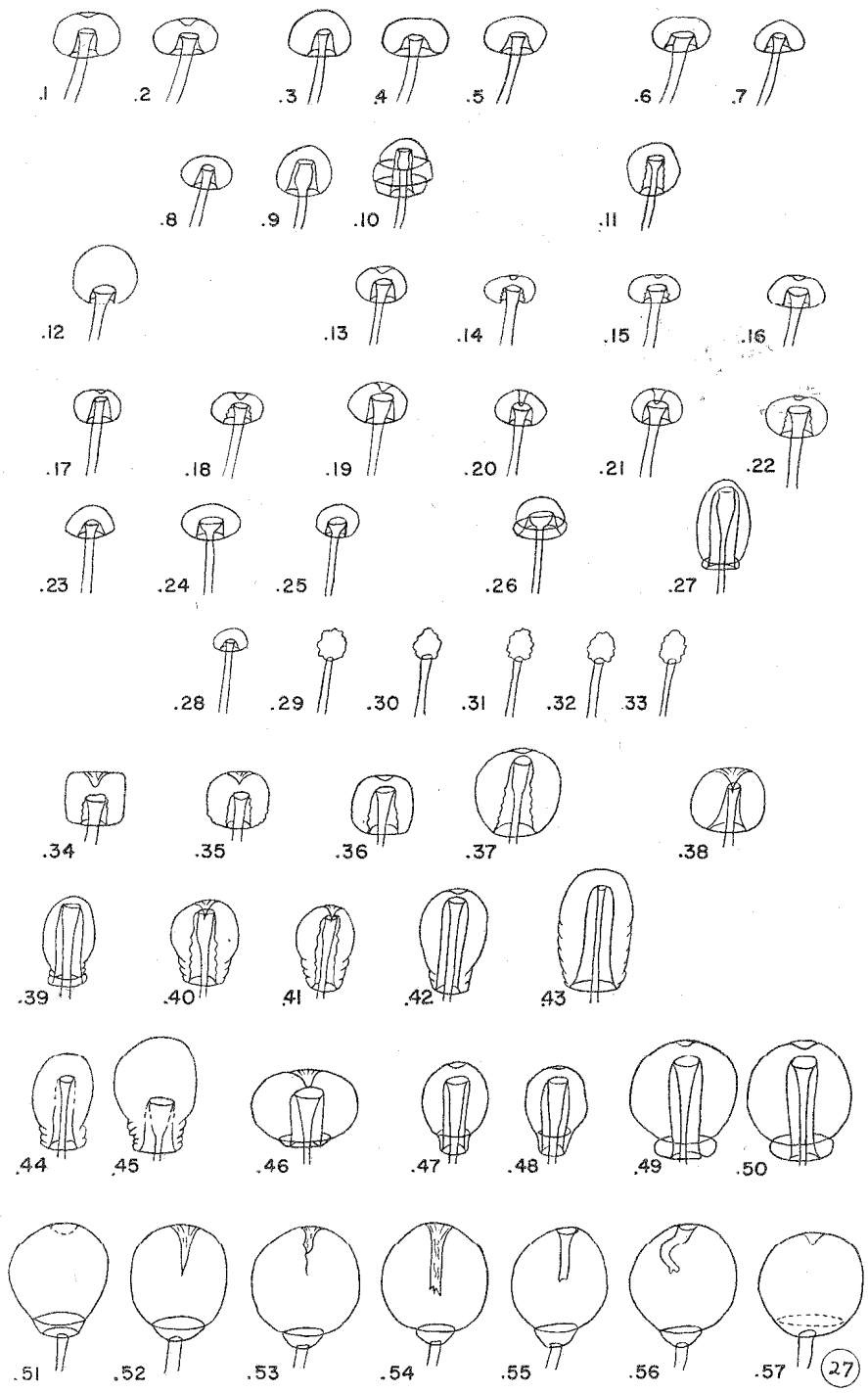


FIG. 27. Spermathecae of *Drosophila* species.

The cellular envelopes surrounding the spermathecae vary considerably, both in thickness and in distribution. Characteristics of the envelope will best be investigated histologically, so they will not be covered in detail. However, Figures 33.13–.26 show spermathecae before clearing to give an indication of the range in types of spermathecal envelopes. In these figures the spermathecae have been shaded and Figure 13.14 has been labeled to show the major parts. In most species in the genus the envelope is relatively thin. In species of the subgenus *Pholadoris* (*bryani* an exception) the envelope is much thickened apically (Figure 33.14). If only the envelope characteristics are considered, the spermathecae shown in Figures 33.15–.17, .21, .23 and .26 are more nearly typical for the genus. The *Pholadoris* type of envelope is seen in *populi*, *nigromelanica* and *R. obesa*. Modifications of this type *may* be present in several species of the repleta complex (e.g., Figure 33.20) and in many species of the quinaria section (e.g., Figure 33.24). As will be seen shortly, species of the repleta complex have unusually variable spermathecae and their spermathecal envelopes are likewise variable. Only the more distinctive types are shown in Figures 33.18–.22.

Species from the quinaria section show spermathecal envelopes of two major types, with some intergradation between them. In the one type (Figures 33.23, .26) the envelope is relatively thin and uniformly distributed (the more usual type for the genus). In the other type the envelope is very thin over the basal part of the spermatheca but expands to form a heavy crown over the apex (Figures 33.24–.25). As a general rule species from this section having spherical spermathecae (see Figure 30) have envelopes which are thin and uniformly dis-

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Subgenus: PHOLADORIS
**victoria group**
.1 victoria
.2 pattersoni
**coracina group**
.3 cancellata
.4 lativittata
.5 novopaca
**bryani group**
.6 bryani
**latifasciaeformis group**
.7 latifasciaeformis
Subgenus: HIRTODROSOPHILA
.8 duncani
.9 pictiventris
.10 thoracis
Subgenus: DORSILOPHA
.11 busckii
Subgenus: SOPHOPHORA
.12 populi
**obscura group**
*obscura subgroup*
.13 miranda
.14 pseudoobscura
.15 persimilis
.16 ambigua
*affinis subgroup*
.17 affinis
.18 algonquin
.19 narragansett
.20 tolteca
.21 athabasca
.22 azteca
**melanogaster group**
*melanogaster subgroup*
.23 simulans
.24 melanogaster
.25 yakuba
*takahashii subgroup*
.26 takahashii
*ananassae subgroup*
.27 ananassae
*montium subgroup*
.28 rufa
.29 nikananu
.30 serrata
.31 auraria
.32 seguyi
.33 kikkawai
**willistoni group**
.34 equinoxialis
.35 paulistorum
.36 tropicalis
.37 willistoni
.38 fumipennis
.39 nebulosa
.40 sucinea
.41 capricorni
.42 changuinolae
.43 pseudobocainensis
**saltans group**
*parasaltans subgroup*
.44 subsaltans
.45 parasaltans
*cordata subgroup*
.46 neocordata
*elliptica subgroup*
.47 neoelliptica
.48 emarginata
*sturtevanti subgroup*
.49 sturtevanti
.50 milleri
*saltans subgroup*
.51 lusaltans
.52 nigrosaltans
.53 pseudosaltans
.54 austrosaltans
.55 prosaltans
.56 septentriosaltans
.57 saltans

tributed. Exceptions to this are species from the calloptera and cardini groups, where the spermathecae are not spherical but have a thin uniform envelope. Aside from these exceptions, elongate spermathecae generally have the envelope in the form of an apical crown. The apical crown is thus seen primarily in species from the quinaria, tripunctata and guarani groups. Members of the guarani group (Figure 33.25) are most extreme in this respect.

The spermathecae of *M. dimidiata* (Figures 33.12–.13) are unusual in that the envelope is concentrated basally, with only a thin membrane covering the apical portion. Uncleared, these spermathecae have a conspicuous acorn shape. Figures 33.12 and 33.13 show these spermathecae cleared and uncleared for comparison. In *C. procnemis* the spermathecal envelope is very thin and inconspicuous.

*Spermathecae*—The term spermatheca should be applied to the entire organ. However, for convenience of reference the term will here be used to refer to the inner capsule only. A portion of the capsule may be telescoped within the base, and this will be called the introvert. Most of the taxonomically useful characteristics of the spermathecal duct are seen in that portion of the duct within the introvert. The major features of the spermatheca, introvert and spermathecal duct are shown in Figures 27–30 for the genus *Drosophila* and in Figure 33 for other genera.

Sturtevant (1925–1926) described a broad array of spermathecae from many families of Diptera. This organ is quite variable among and within these families, and it is difficult to arrive at any firm conclusion regarding primitive types. The writer has looked at cleared spermathecae from several families, but the series is not yet broad enough to be useful. Very tentatively, weakly chitinized and non-telescoped spermathecae (e.g., Figure 28.35) appear as the most probable primitive type for the family Drosophilidae (and possibly also for the Acalyptera-tae). Weakly chitinized and non-telescoped spermathecae resembling types seen within the genus *Drosophila* (e.g., Figure 28.35 and many others) have been seen in the Ephydridae (*Scatella*), and Sturtevant (*op. cit.*) describes others from other families which are probably of this type. This also appears to be a type from which most other types could be derived, and there are evolutionary series within the genus *Drosophila* which suggest derivation of heavily chitinized and telescoped spermathecae from non-telescoped and weakly chitinized types. The phylogeny for spermathecae (Figures 31–32) is based on those phylogenies previously used for characteristics of the male. Characteristics of the spermathecae suggest the division of the virilis-repleta section shown in Figure 32.

Spermathecae from species in the subgenus *Pholadoris* are uniformly small and oval (Figures 27.1–.7). In members of the victoria group the apex is indented. In members of the other groups this indentation is absent. Spermathecae of Sophophoran species are varied (Figures 27.12–.57). Those of *populi* are spherical with an unusually short introvert. Those of species in the obscura group are uniformly small and oval with an apical indentation similar to that seen in species of the victoria group. Members of the montium subgroup of the melanogaster group have weakly chitinized spermathecae (Figures 27.28–.33). Except for *rufa*, these spermathecae are both weakly chitinized and non-telescoped. Spermathecae of *rufa* are somewhat more heavily chitinized and are telescoped basally.

Members of the melanogaster subgroup (Figures 27.23–.25) have spermathecae which are well chitinized and generally oval in outline. The distal end of the spermathecal duct may expand rather sharply as is shown for *melanogaster* and *yakuba*. There are a few individuals from these species, however, in which the spermathecal duct resembles that figured for *simulans*. In *takahashii* (Figure 27.26) the spermathecae resemble those of *melanogaster*, except that they are more expanded basally and have a conspicuous flange around the base. In *ananassae* (Figure 27.27) and *bipectinata* the spermathecae are elongate with a basal flange, and the spermathecal duct expands to form a small bulb just before its union with the introvert.

Most members of the melanogaster group have both the spermathecae and parovaria embedded in fat body. For species of the montium and takahashii subgroups there is a single fat body on each side. The left parovarium and left spermatheca are embedded in one fat body, the right in the other. In the remainder of species there is a separate fat body capping each spermatheca and parovarium (i.e., four separate fat bodies). Since the presence or absence of such fat bodies may be dependent on the state of nutrition of the individual, this feature cannot be considered a distinctive characteristic at this time. However, it was not noted in any of the other species dissected for this study, and future investigation may show it to be a useful group and subgroup characteristic. (A fat body similar to that seen in montium subgroup species has been seen in *reticulata*. Since only a single female of this species was available for dissection, it has not been included in the present study.)

Spérmathecae from members of the willistoni group are of two major types. Those of *willistoni* and its closest relatives (Figures 27.34–.37) are almost quadrate in outline and have a strong apical indentation. Those of *fumipennis* are also of this type. The remainder of the species in the group (Figures 27.39–.43) have elongate spermathecae. Those of *sucinea*, *capricorni* and *changuinolae* are constricted basally, those of *nebulosa* have a basal flange and resemble spermathecae of *ananassae*. Those of *pseudobocainensis* are very large and not markedly constricted basally. Except for *nebulosa* and *pseudobocainensis*, all spermathecae have a marked apical indentation.

Spermathecae of species from the saltans group include some very unusual types. In members of the cordata, elliptica, sturtevanti and parasaltans subgroups the spermathecae are telescoped basally (Figures 27.44–.50). In members of the parasaltans subgroup the apical part of the introvert is very weakly chitinized (shown by dashed line in the figure). Of these species, only those of the parasaltans subgroup lack an apical indentation. Members of the saltans subgroup have spermathecae which appear to lack an introvert, and in most cases there is an inner structure resembling an extreme condition of the apical indentation seen in other species. The exact nature of this structure is not known, since in most species it is very weakly chitinized and it collapses during clearing. In some cases more extensive structure is faintly visible during the early stages of clearing. In *austrosaltans*, for example, the inner column appeared to continue to the base of the spermatheca and to flare out and attach to the base. It is possible that the telescoping of spermathecae is normally brought about by contraction of an inner sheath attaching the tip of the spermathecal duct with the

apex of the spermatheca. Alternately, such a sheath might be established and fixed at an early stage, and subsequent growth might cause a part of the capsule to turn inward and form the introvert. If such a sheath were unusually long, or if it failed to contract, telescoping would not occur. If it became weakly chitinized figures such as those seen in saltans subgroup species might appear. Regardless of the mechanism, it seems probable that study of sectioned material from developmental series would give considerable insight into the problem of form development in these spermathecae, and it might well provide a basis for more detailed analyses of spermathecae throughout the genus.

Spermathecae from species in the subgenus *Drosophila* are shown in Figures 28–30. Those from species in the virilis group (Figures 28.1–.9) are all well chitinized and vary in shape from subspherical to elongate oval. There is a small apical indentation in the spermathecae from *ezoana* and *flavomontana*, and in several species (Figures 28.5–.9) the spermathecal duct expands to form a small bulb just prior to its union with the introvert. Spermathecae of species from the melanica group (Figures 28.10 .14) are rather small, and those of *micromelanica* are very small and rather weakly chitinized. Shape varies as shown in the figures, and only the spermathecae of *paramelanica* show an apical indentation. The spermathecae of *melanura* are as shown in Figure 33.7 for *S. adusta*. The spermathecae of species from the robusta group are elongate oval. In two species there is an apical indentation. That of *sordidula* is broad and saucer-shaped. That of *robusta* is broad and deep and surrounded by a cylindrical rim. The spermathecal ducts of these last two species are very similar, although the shapes of the spermathecae are different. One aberrant female of *robusta* was found having four spermathecae, three of which were of the type figured for the species, and the fourth resembled types seen in virilis or melanica group species (e.g., Figure 28.12). There was also a fifth structure, in appearance

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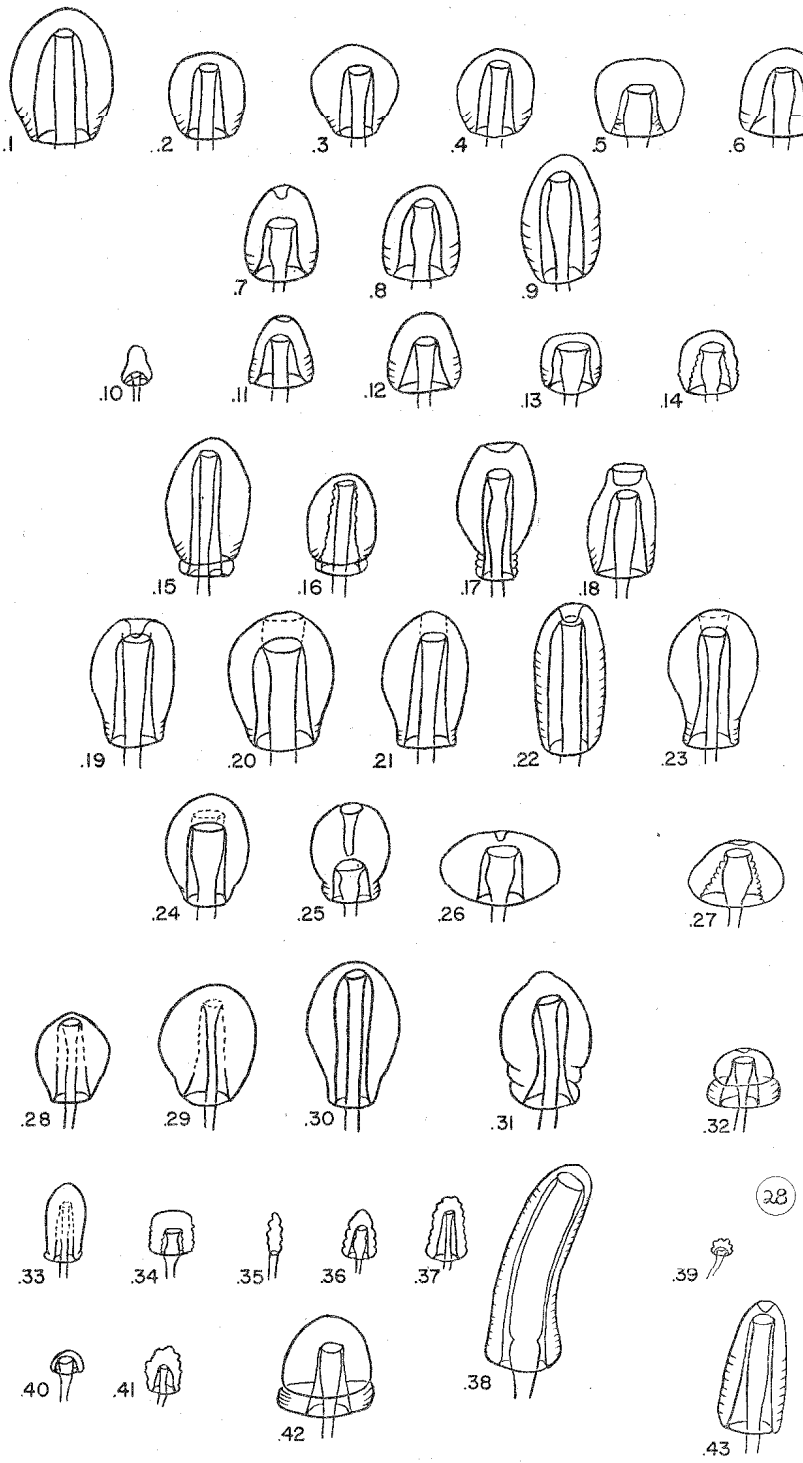
FIG. 28. Spermathecae of *Drosophila* species.

Subgenus: DROSOPHILA
**virilis group**
.1 virilis
.2 americana
.3 novamexicana
.4 littoralis
.5 ezoana
.6 montana
.7 flavomontana
.8 lacicola
9. borealis
**melanica group**
.10 micromelanica
.11 melanica
.12 paramelanica
.13 euronotus
.14 nigromelanica
**robusta group**
.15 lacertosa
.16 colorata
.17 sordidula
.18 robusta
**annulimana group**
.19 gibberosa
.20 species B
.21 species C
.22 species D
.23 species E
**immigrans group**
.24 hypocausta
.25 spinofemora
.26 immigrans
**funebris group**
.28 macrospina
.29 subfunebris
.30 funebris
**carbonaria group**
.32 carbonaria
**nannoptera group**
.33 nannoptera
**bromeliae group**
.34 species I
**polychaeta group**
.39 polychaeta
Subgenus: PHLORIDOSA
.36 species O
.37 species Q
.38 species P
**ungrouped species**
.31 carsoni
.35 aracea
.40 peruviana
.41 species H
.42 tumiditarsus
.43 species G
Subgenus: HIRTODROSOPHILA
.27 histrioides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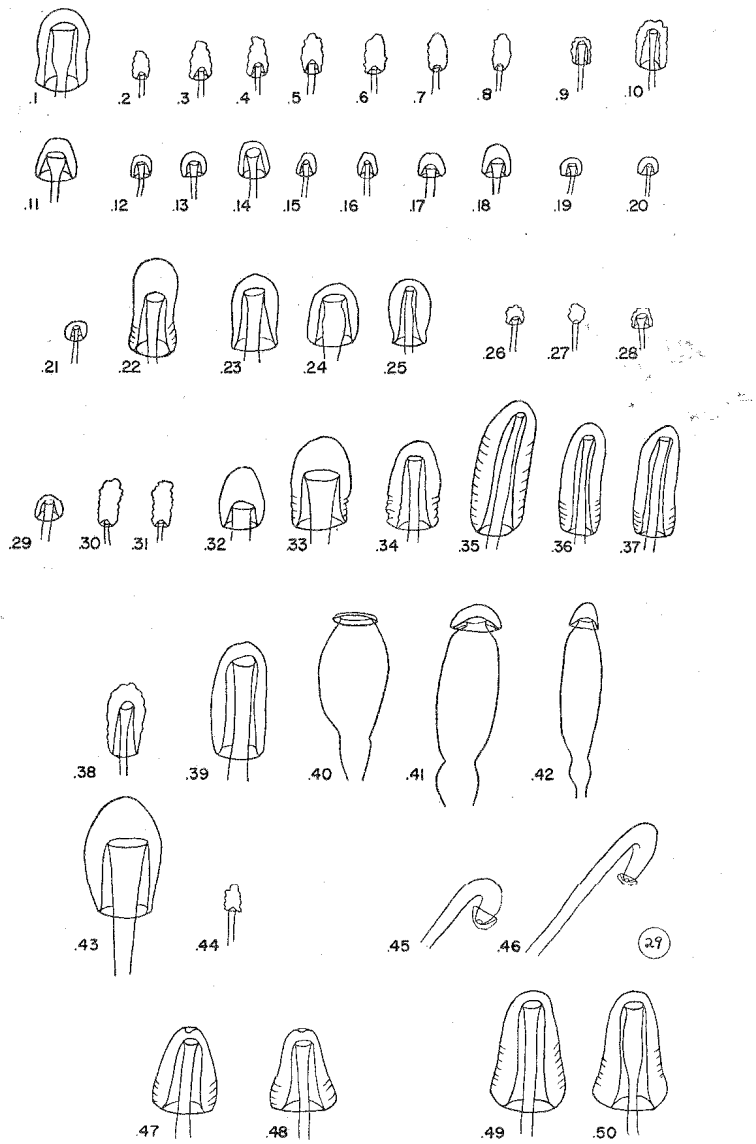


FIG. 29. Spermathecae of *Drosophila* species.

Subgenus: DROSOPHILA
**repleta group**
*fasciola subgroup*
.1 fulvalineata
.2 fasciola
.3 coroica
.4 pictura
.5 pictilis
.6 fascioloides
.7 moju
.8 mojuoides
*mulleri subgroup*
.9 tira
.10 pachuca
.11 nigricruria
.12 aldrichi
.13 mulleri
.14 longicornis
.15 arizonensis
.16 mojavensis
.17 martensis
.18 stalkeri
.19 hamatofila
.20 eremophila
.21 buzzatii
.22 pegasa
.23 meridionalis
.24 promeridiana
.25 meridiana
.27 anceps
.29 peninsularis
*mercatorum subgroup*
.30 mercatorum

intermediate between a spermatheca and a parovarium. No normal parovaria were present.

Spermathecae from species in the annulimana group are also elongate oval in outline. Two species (Figures 28.19, .22) have apical indentations. The others show a peculiar feature which may be an inner sheath attaching the tip of the spermathecal duct with the apex of the spermatheca. This is generally visible only during the early stages of clearing. Occasionally a remnant of it remains as a very thin cylindrical extension from the tip of the spermathecal duct. A similar feature has been noted in one species of the immigrans group (*hypocausta*). Both position and appearance suggest a possible relationship between this structure and that noted earlier for saltans subgroup species.

Spermathecae from the repleta complex of species are shown in Figure 29. Members of the fasciola subgroup of the repleta group have spermathecae of two general types (Figures 29.1–.8). Except for *fulvalineata*, the spermathecae are both weakly chitinized and weakly telescoped. Members of the mulleri subgroup (Figures 29.9–.25, .27, .29) show several types. In most of these species the spermathecae are very small but are strongly telescoped and rather well chitinized (e.g., Figure 29.11). In one species, *anceps* (Figure 29.27), the spermathecae are weakly chitinized and not telescoped. In *pachuca* and *tira* (Figures 29.9–10) they are strongly telescoped but weakly chitinized. In *meridiana* and its close relatives (Figures 29.23–.25) the spermathecae are larger, well chitinized and somewhat resemble the type of spermathecae seen in *fulvalineata*. *D. pegasa* has rather large, elongate spermathecae. Members of the mercatorum subgroup (Figures 29.30–.31) have spermathecae virtually identical with those seen in most fasciola subgroup species. As will be recalled from earlier discussions, these two subgroups belong to separate evolutionary lines within the group (see Figure 25). Spermathecae in members of the melanopalpa subgroup vary from a rather short type with very wide spermathecal duct (Figures 29.32–.33) to a very elongate type with narrow spermathecal duct. Members of the hydei subgroup have three types of spermathecae. That of *bifurca* (Figure 29.38) resembles the type seen in *pachuca* in that it is strongly telescoped but weakly chitinized. It is, however, somewhat larger than that of *pachuca*. Although somewhat more elongate, the type seen in *nigrohydei* (Figure 29.39) resembles that seen in *meridiana*, *fulvalineata*, etc. The remaining three species (Figures 29.40–.42) have very unusual spermathecae. The spermathecae proper are very small and form a flat shield at the tip of the much expanded and elongated spermathecal duct. A vari-

.31 paranaensis
*melanopalpa subgroup*
.32 fulvimacula
.33 fulvimaculoides
.34 limensis
.35 repleta
.36 canapalpa
.37 melanopalpa
*hydei subgroup*
.38 bifurca
.39 nigrohydei
.40 eohydei
.41 neohydei
.42 hydei
**canalinea group**
.43 canalinea
.44 paracanalinea
**dreyfusi group**
.45 camargoi
.46 briegeri
**mesophragmatica group**
.47 gaucha
.48 pavani
**species not placed in subgroup**
.28 serenensis
**ungrouped species**
.26 aureata
.49 castanea
.50 species F

ation of this type of spermathecae is seen in members of the dreyfusi group (Figures 29.45–.46), except that here the spermathecal duct is not as greatly expanded. The spermathecal ducts of species from the dreyfusi group are flexed just proximal to the spermathecae. A somewhat similar flexure is seen in most species of the annulimana group and in *fulvimacula* and *fulvimaculoides* (see below for further description). The spermathecal ducts of these last two species rather strongly resemble those of dreyfusi group species. The spermathecae of the two species from the canalinea group are sharply different (Figures 29.43–.44). Those of *paracanalinea* are very small, weakly chitinized and not telescoped. They resemble the type seen in *anceps* (Figure 29.27). Those of *canalinea* are much larger, well chitinized and strongly telescoped. In shape they resemble the type seen in *promeridiana* (Figure 29.24). The spermathecae of members of the mesophragmatica group, of *castanea* and of species F (Figures 29.47–.50) are well chitinized, strongly telescoped and bell-shaped. Those of mesophragmatica group species have a slight apical indentation.

The spermathecae from the remainder of the species in this section of the subgenus need not be considered in detail. Those of *peruviana* and species H (Figures 28.40–.41) resemble types seen in species of the mulleri subgroup of the repleta group (compare with Figures 29.10–.11). Those seen in species G resemble spermathecae of mesophragmatica group species (compare Figures 28.43 and 29.48). The spermathecae of *polychaeta* (Figure 28.39) resemble those of *aureata* (Figure 29.26).

Spermathecae from species in the immigrans group (Figures 28.24–.26) vary from flat oval to an elongate oval form similar to those seen in members of the robusta and annulimana groups. Two species show an apical indentation, that of *spinofemora* being unusually long. The spermathecae from species in the funebris group all have the same general shape and characteristics (Figures 28.28–.30). The apical part of the introvert in *macrospina* and in *subfunebris* is very weakly chitinized.

The spermathecae of the remainder of the species from the quinaria section fall into three major types: spherical, bell-shaped, and pear-shaped. These types can be further subdivided according to the shape of the spermathecal duct, presence of apical indentation, etc. (Figure 30). Members of the quinaria group (Figures 30.13–.25) have two general types of spermathecae. In two species, *quinaria* and *rellima*, they are spherical. In one, *innubila*, they are slightly elongate. In most of the remaining species the spermathecae are elongate and bell-shaped. In many of the species the spermathecal duct expands to form a small bulb just prior to its union with the introvert, although in some it just expands gradually in this region.

In members of the tripunctata group (Figures 30.26–.38) spermathecal shapes vary from spherical to pear-shaped. Perhaps the most conspicuous trend in the group involves the spermathecal duct. In some species, mostly those having more nearly spherical spermathecae (e.g., Figure 30.26), the duct has only a slight terminal expansion. In other forms (e.g., Figure 30.38) the expansion is very marked. In some species the duct may expand to form a small bulb just prior to the terminal expansion, but this characteristic may be rather variable. In at least one species, *crocina*, one strain showed a distinct bulb, another showed none

The widely flared cup at the end of the spermathecal duct remained the same in both strains.

All species of the guarani group have pear-shaped spermathecae with the end of the spermathecal duct flared strongly (Figures 30.39–.43). Species of the cardini group (Figures 30.44–.54) show somewhat the same variation as was seen in the tripunctata group. Spermathecae vary from spherical (Figure 30.44) to pear-shaped (e.g., Figure 30.48), and spermathecal ducts are moderately to extremely expanded or flared. Three species (Figures 30.44–.46) have very slight apical indentations of the spermathecae. Species of the calloptera group (Figures 30.55–.57) have pear-shaped spermathecae and the spermathecal ducts are moderately to strongly flared. The spermathecae of *schildi* have a very slight apical indentation.

The remainder of the species belonging to this section (Figures 30.1–.12) have generally spherical spermathecae with the spermathecal duct slightly to moderately expanded terminally. The spermathecae of *pallidipennis* (Figure 30.8) approach the pear-shape seen in other members of this section. Most members of the rubrifrons group (Figures 30.3–.6) have pronounced apical indentations of the spermathecae. One member of the testacea group (Figures 30.9–.10), *putrida*, has the apex of the spermathecae slightly indented.

Spermathecae from members of the subgenus *Phloridosa* (Figures 28.36–.38) generally resemble types seen among species of the repleta complex. One species (Figure 28.37) has spermathecae which resemble those of *pachuca* (Figure 29.10). Spermathecae of species 0 are similar in shape but are somewhat shorter and more strongly chitinized. Spermathecae of species P are well chitinized and extremely elongate. In general shape they resemble spermathecae seen in members of the melanopalpa subgroup of the repleta group (compare Figures 28.38 and 29.35). However, the spermathecal duct of species P is wide, whereas that in *repleta* is narrow. Species P appears to combine the wide spermathecal duct seen in *fulvimacula* (Figure 29.32) with the elongate spermathecae seen in *repleta*, and the resemblance between *Phloridosa* and the repleta complex is further enhanced by the fact that the spermathecal duct in species P is bent sharply at the base of the spermathecae, just as it is in *fulvimacula*.

To summarize the data with reference to the flexure of the spermathecal duct, species can be grouped in four general categories: no flexure, weak flexure, moderate flexure, and strong flexure. Species with strongly flexed ducts are: species P (*Phloridosa*); *serenensis*, *fulvimacula*, *fulvimaculoides* (repleta group); species of the dreyfusi group. Species with moderate flexure are: *repleta*; species of the annulimana group. Species with weak flexure are: *pegasa*, *bifurca*, *limensis* (repleta group); species of the mesophragmatica group, and *tumiditarsus*. Species with no flexure include the remainder of the species in the genus. With the exception of *tumiditarsus* and species P, all of these species belong to the virilis-repleta section of the subgenus *Drosophila*.

Species from the subgenus *Hirtodrosophila* (Figures 27.8–.10 and 28.27) have spermathecae which are generally spherical in shape. Those of *thoracis* are unusual in that they have a thickened band around the middle. Spermathecae of *busckii* (Figure 27.11) are spherical.

Spermathecae from species in other genera are shown in Figures 33.1–.12.

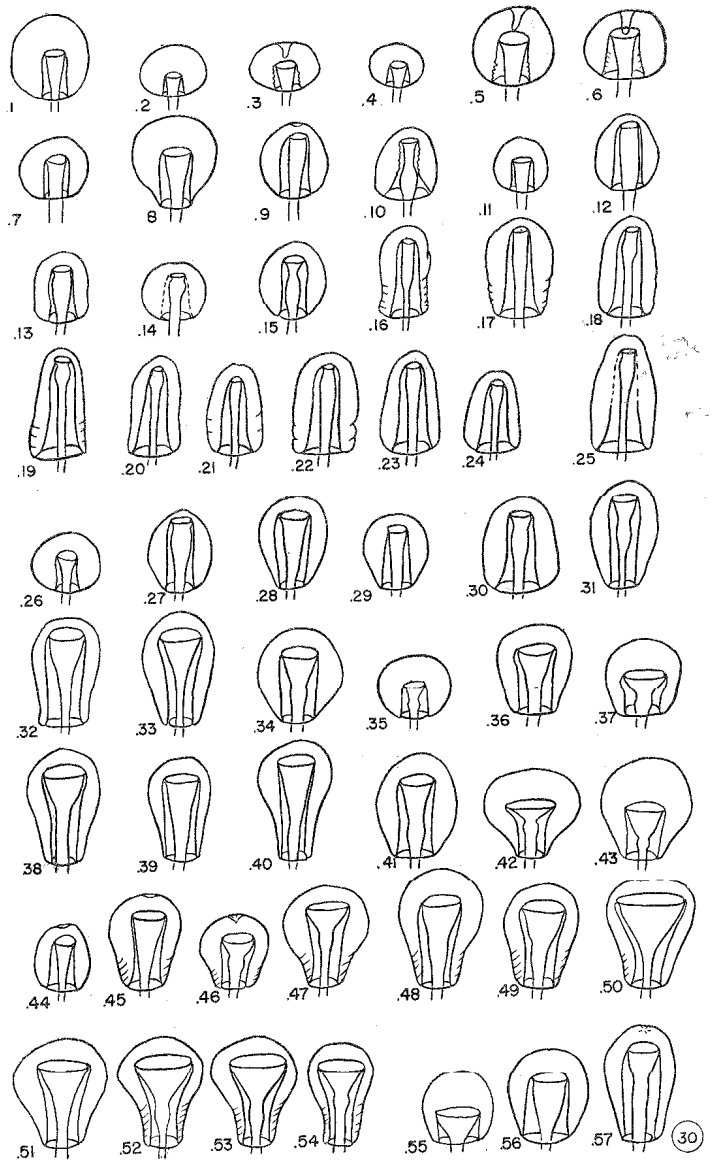


FIG. 30 Spermathecae of *Drosophila* species.

Subgenus: DROSOPHILA

**ungrouped species**
- .1 species K
- .2 species L
- .7 sticta

**rubrifrons group**
- .3 parachrogaster
- .4 species M
- .5 uninubes
- .6 species N

**pallidipennis group**
- .8 pallidipennis

**testacea group**
- .9 putrida
- .10 testacea

**macroptera group**
- .11 submacroptera
- .12 macroptera

**quinaria group**
- .13 innubila
- .14 quinaria
- .15 rellima
- .16 falleni
- .17 phalerata
- .18 species J
- .19 occidentalis
- .20 tenebrosa
- .21 subquinaria
- .22 transversa
- .23 palustris
- .24 subpalustris
- .25 guttifera

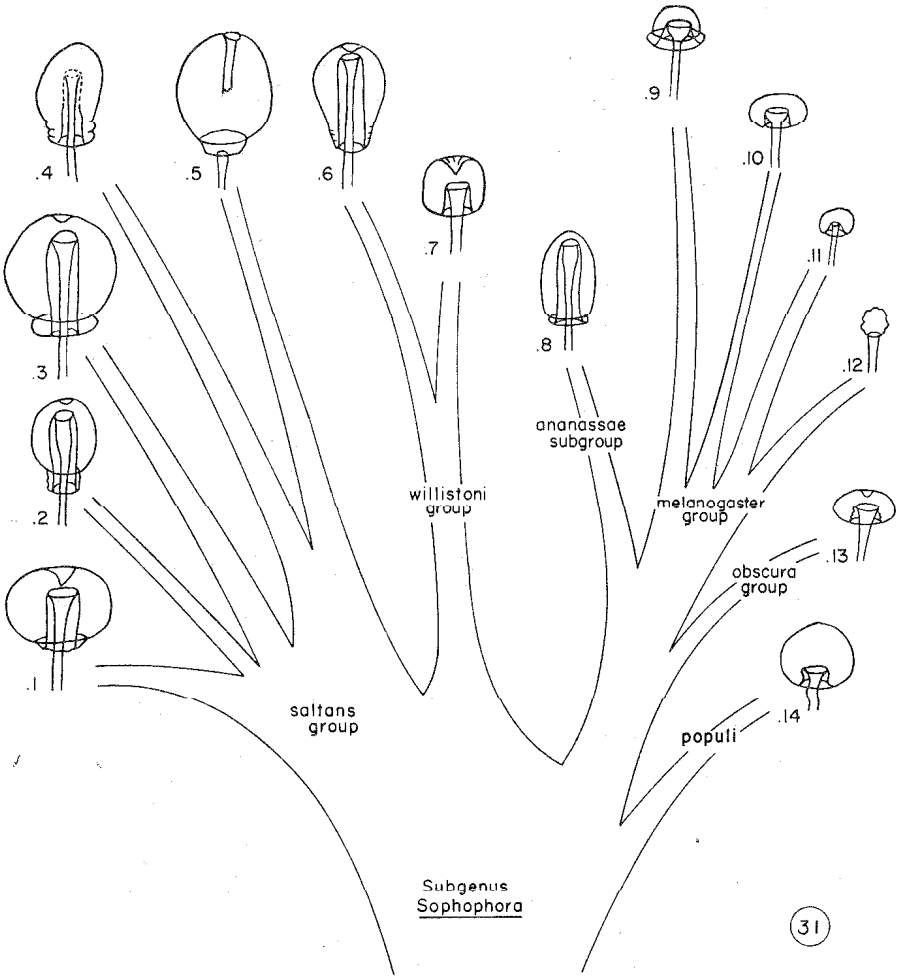


FIG. 31. Pictorial phylogeny of spermathecae from the subgenus *Sophophora*. .1) neocordata; .2) emarginata; .3) sturtevanti; .4) subsaltans; .5) prosaltans; .6) changuinolae; .7) equinoxialis; .8) ananassae; .9) takahashii; .10) melanogaster; .11) rufa; .12) serrata; .13) miranda; .14) populi.

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**tripunctata group**
.26 mediodiffusa
.27 albicans
.28 metzii
.29 albirostris
.30 tripunctata
.31 mediopunctata
.32 unipunctata
.33 trapeza
.34 bandeirantorum
.35 paramediostriata
.36 mediostriata
.37 mediopictoides
.38 crocina
**guarani group**
.39 guaramunu
.40 guaraja
.41 griseolineata
.42 subbadia
.43 guarani
**cardini group**
.44 dunni
.45 belladunni
.46 nigrodunni
.47 polymorpha
.48 neomorpha
.49 neocardini
.50 parthenogenetica
.51 acutilabella
.52 procardinoides
.53 cardinoides
.54 cardini
**calloptera group**
.55 ornatipennis
.56 calloptera
.57 schildi

Those of *R. obesa* and *G. bivisualis* (Figures 33.1–.2) are well chitinized, telescoped, and subspherical to quadrate in outline. Those of *R. obesa* have a broad apical indentation. Spermathecae of *Zapriothrica dispar* (Figure 33.3) are weakly chitinized and not telescoped. They resemble types seen in some *Drosophila* (e.g., Figures 27.30, 29.27, etc.). Spermathecae of *Chymomyza* species are spherical (Figure 33.4) or elongate (Figures 33.5–.6). The introvert and the terminal portion of the spermathecal duct are very weakly chitinized in *C. amoena*. Spermathecae of *Scaptomyza* and *Zaprionus* species (Figures 33.7–.11) are quite similar. All are well chitinized, and they resemble spermathecae seen in species from the virilis-repleta section of the subgenus *Drosophila* (compare Figures 28.10 and 33.10, 29.18 and 33.11, etc.). Spermathecae of *M. dimidiata* are constricted basally as shown in Figure 33.12.

Figures 31 and 32 summarize the data from the spermathecae. As can be seen in these figures, spermathecal types, while quite varied, tend to delimit major groups and distinct phylogenetic lines.

*The ventral receptacle* Sturtevant (1942) first showed the usefulness of the ventral receptacle as a subgeneric and group characteristic. Its characteristics are among the best available for separating the various subgenera. The major types from species in the genus *Drosophila* are shown in Figures 34–38. Those from other genera are shown in Figure 39. Inspection of these types and of types shown by Okada (1956) suggests that short (or short and folded) ventral receptacles are primitive for the family Drosophilidae. The phylogeny shown in Figure 40 is consistent with this assumption.

The figures of the ventral receptacle combine information from observations both before and after treatment with phenol. The general morphology is as seen before clearing. Specific details, such as attenuation of the terminal segment of the ventral receptacle as shown in Figure 36.5, shape as is shown in Figure 34.2, etc., are from observations made after clearing.

Species from the subgenus *Pholadoris* (Figures 34.1–.3) have three types of ventral receptacle. In species of the victoria group it is a simple bent pocket of almost uniform diameter throughout. In species from the coracina group it is a simple tube, sometimes somewhat bent or folded, which flares and is conically indented at the tip. This terminal depression is seen best after clearing in phenol. In species from the bryani and latifasciaeformis groups the ventral receptacle is somewhat longer and more folded, and it is strongly attenuated.

Species from the obscura and melanogaster groups of the subgenus *Sophophora* have folded ventral receptacles of the same general type. The major differences (Figures 34.7–.10) are in length, and therefore the number of folds varies. *D.*

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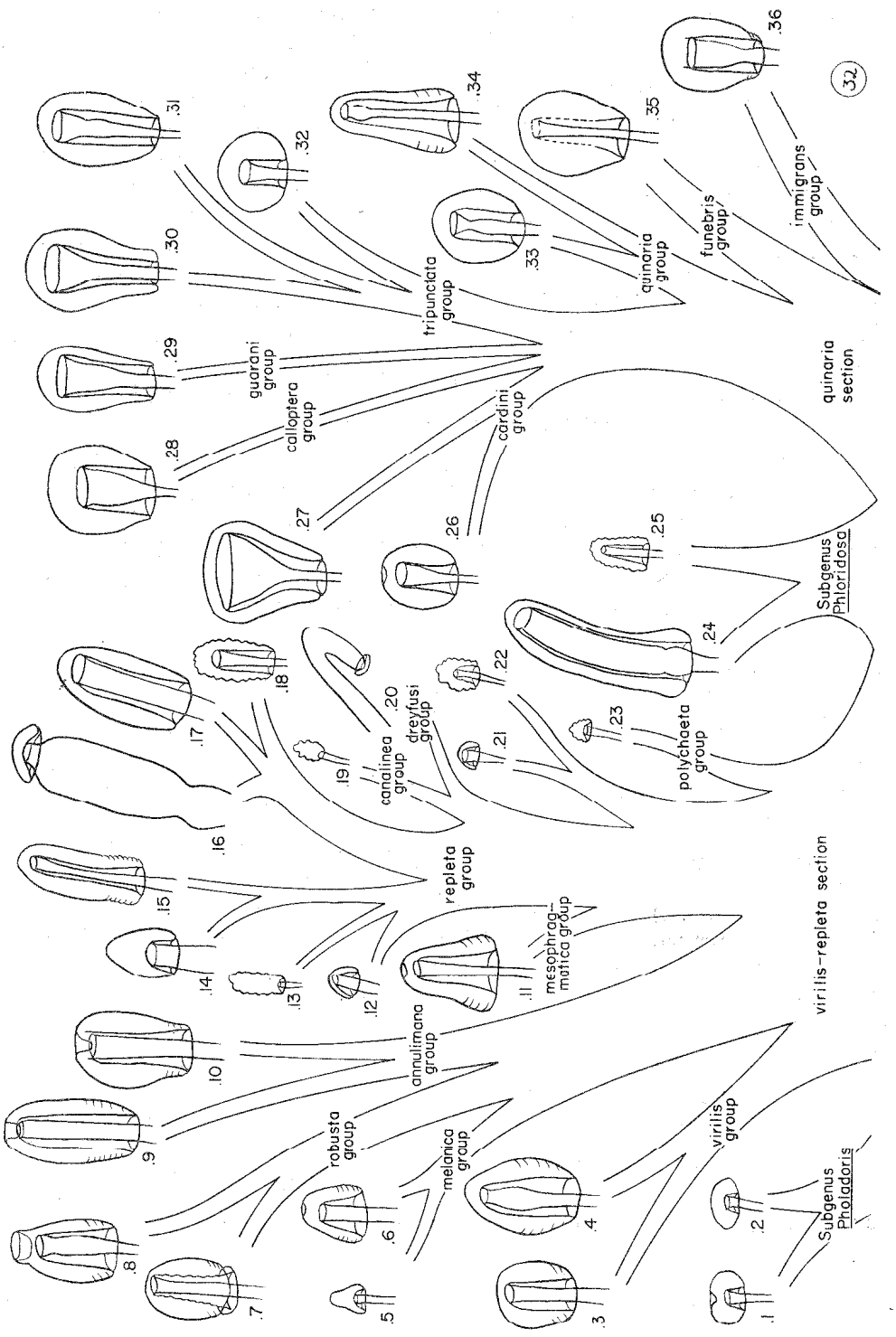
FIG. 32. Pictorial phylogeny of spermathecae from *Drosophila*, *Phloridosa* and *Pholadoris*. .1) victoria; .2) novopaca; .3) littoralis; .4) borealis; .5) micromelanica; .6) melanica; .7) colorata; .8) robusta; .9) species D; .10) gibberosa; .11) pavani; .12) peninsularis; .13) mercatorum; .14) fulvimacula; .15) melanopalpa; .16) neohydei; .17) nigrohydei; .18) bifurca; .19) paracanalinea; .20) briegeri; .21) peruviana; .22) species H; .23) polychaeta; .24) species P; .25) species Q; .26) dunni; .27) parthenogenetica; .28) calloptera; .29) guaraja; .30) crocina; .31) mediopunctata; .32) mediodiffusa; .33) rellima; .34) occidentalis; .35) subfunebris; .36) hypocausta.


Subgenus Phloridosa
Subgenus Pholadoris
virilis-repleta section
quinaria section
virilis group
repleta group
robusta group
melanica group
annulimana group
mesophragmatica group
canalinea group
dreyfusi group
polychaeta group
cardini group
guarani group
calloptera group
tripunctata group
quinaria group
funebris group
immigrans group
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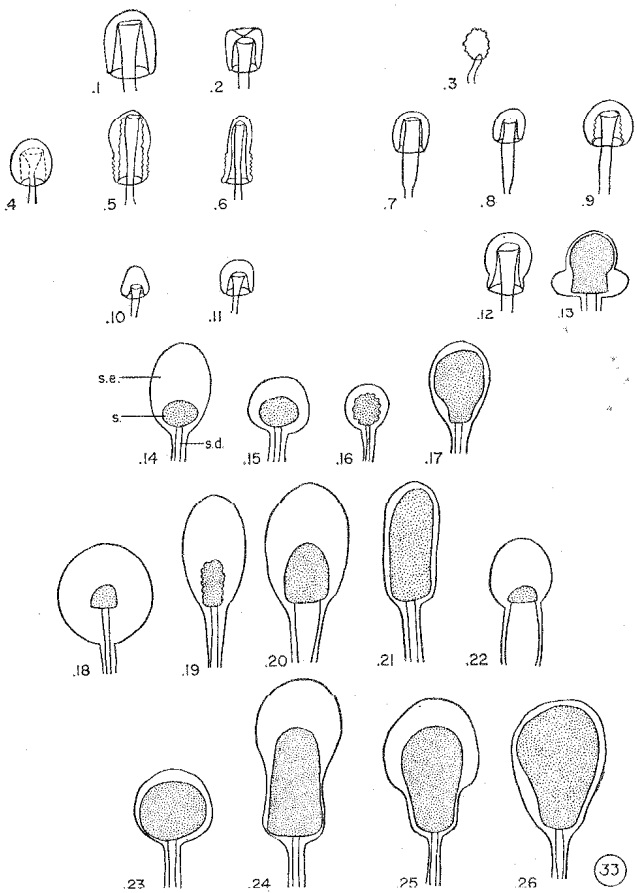


FIG. 33. Spermathecae from species in other genera, and types of spermathecal envelopes. .1) *G. bivisualis*; .2) *R. obesa*; .3) *Zapriothrica dispar*; .4) *C. amoena*; .5) *C. aldrichi*; .6) *C. procnemis*; .7) *S. adusta*; .8) *S. pallida*; .9) *S. hsui*; .10) *Zaprionus ghesquierei*; .11) *Z. vittiger*; .12) *M. dimidiata*; .13) *M. dimidiata* (with spermathecal envelope); .14) lativittata; .15) miranda; .16) nikananu; .17) subsaltans; .18) mulleri; .19) mercatorum; .20) fulvimacula; .21) melanopalpa; .22) hydei; .23) quinaria; .24) occidentalis; .25) guaramunu; .26) neomorpha. s.e.—spermathecal envelope; s.—spermatheca; s.d.—spermathecal duct.

*populi* (Figure 34.6) has a ventral receptacle of this type also. For all of these species clearing in phenol shows the ventral receptacle to be distally attenuated. Species from the saltans and willistoni groups have extremely long and attenuated ventral receptacles. The folds may be rather precise, as in Figures 34.11 and 34.13, or they may be somewhat more irregular as in Figure 34.12. In all species from both groups the base of the ventral receptacle is corrugated if observed after clearing. In the figures the base of the ventral receptacle is shown in lateral view (anterior toward the left) while the remainder is shown in dorsal view. The ventral receptacle shown in Figure 34.12 has rather irregular folds basally, then a straighter section, and finally a region of regular folds. The basal section is shown in lateral view, the other section has been rotated 180° and is shown in dorsal view. The ventral receptacles from all species of *Pholadoris* and *Sopho-*

*phora* are tightly appressed to the surface of the vagina, at least in their basal sections.

Ventral receptacles from species in the subgenus *Drosophila* are variously coiled or kinked but seldom regularly folded. None are appressed to the surface of the vagina. Although some of them appear to be rather disorganized and irregular (e.g., Figure 34.15, etc.) dissection of many individuals shows a surprising consistency of form, both within species and between related species. As can be seen from inspection of the figures, the usual descriptions of ventral receptacles (spiral, of about twenty coils; short, irregularly coiled; etc.) only inadequately indicate the important features of this organ, and they ignore some

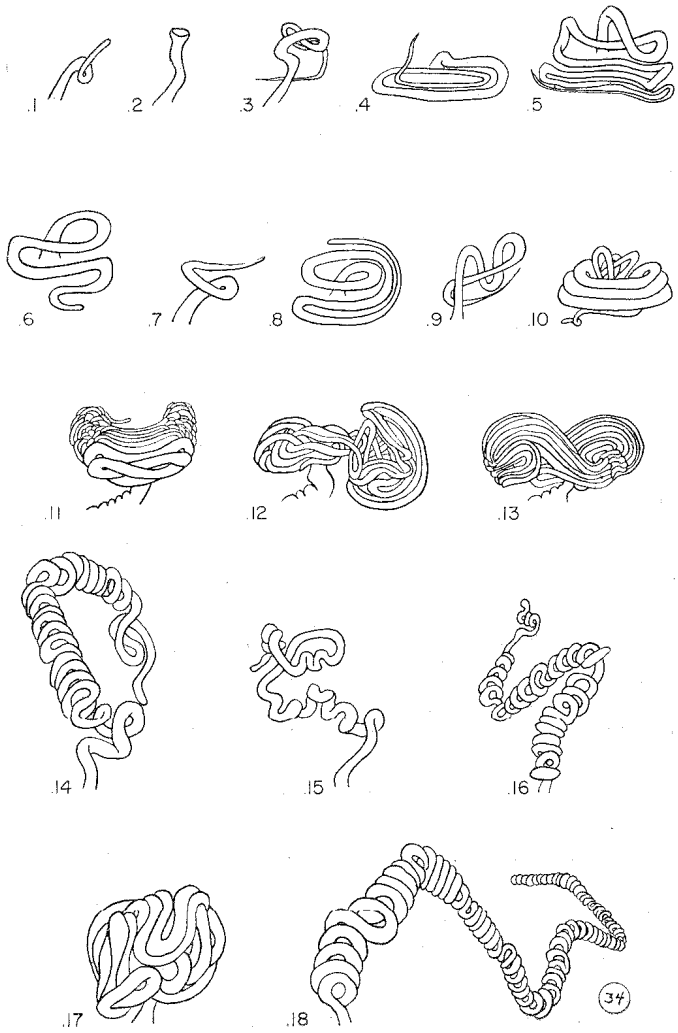


FIG. 34. Ventral receptacle types. .1) victoria; .2) cancellata; .3) bryani; .4) thoracis; .5) buskii; .6) populi; .7) miranda; .8) affinis; .9) nikananu; .10) serrata; .11) equinoxialis; .12) neoelliptica; .13) austrosaltans; .14) lacicola; .15) nigromelanica; .16) melanica; .17) colorata; .18) lacertosa.

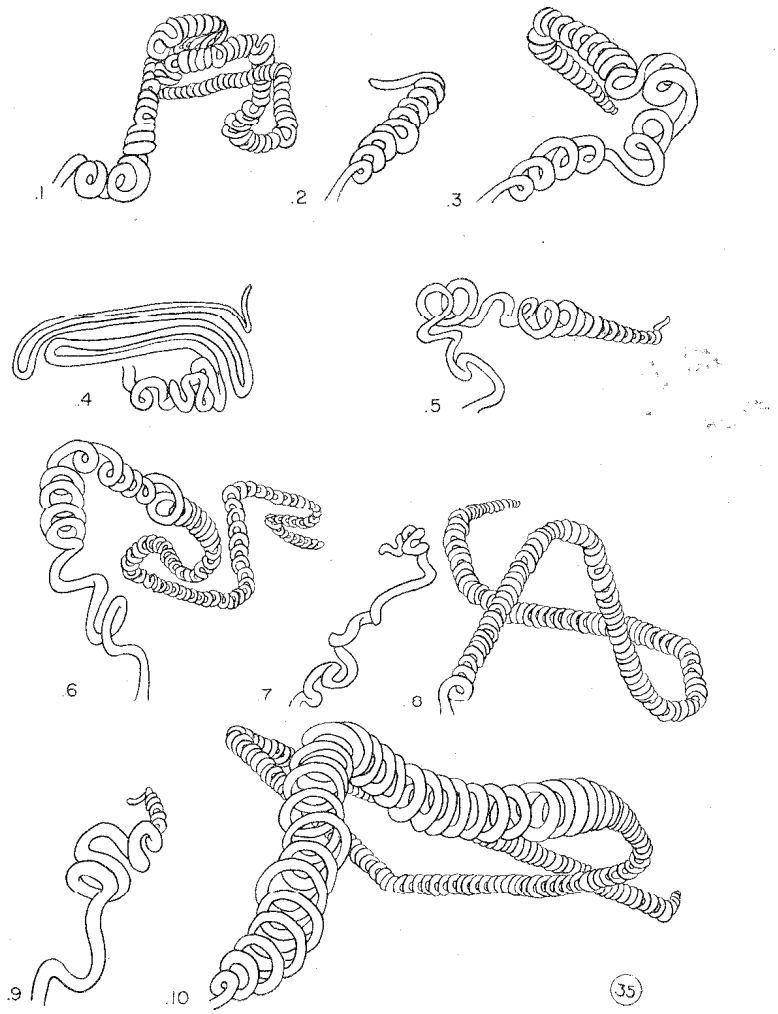


FIG. 35. Ventral receptacle types. .1) gibberosa; .2) immigrans; .3) macrospina; .4) histrioides; .5) carbonaria; .6) nannoptera; .7) species I; .8) aracea; .9) polychaeta; .10) species O.

of its most useful characteristics.

The ventral receptacles shown in the figures indicate the major types seen. In the phylogeny (Figure 40) the types shown for species from the quinaria section of the subgenus *Drosophila* include all the major types from that branch. All of these types are also seen in many species from the virilis-repleta section. With the exception of Figure 40.2, the types shown for the virilis-repleta section have not been seen in species from the quinaria section.

All species of the virilis group, most melanica group species, all species of the annulimana group, and most species of the robusta group have the same general type of ventral receptacle (Figures 34.14, .16, .18; 35.1). This type is also seen in many members of the repleta complex (Figure 37.1, .3 and perhaps also 37.2) and in several other members of the virilis-repleta section (Figures 36.1–.2). It is also seen in a majority of the species from the quinaria section. It is seen in all

species of the guarani and cardini groups, most tripunctata group species, most quinaria group species and in one species of the calloptera group (Figures 35.2–.3; 37.7, .10–.11; 38.3–.7, .9). All species from subgenus *Phloridosa* (Figure 35.10) also have this type. The type shown in Figure 34.15 is seen in two members of the melanica group (*micromelanica* and *nigromelanica*), in many members of the mulleri subgroup of the repleta group (Figure 36.4), in species I of the bromeliae group and (perhaps) in *polychaeta* (Figures 35.7, .9). Among species from the quinaria section it is seen in species from the testacea group

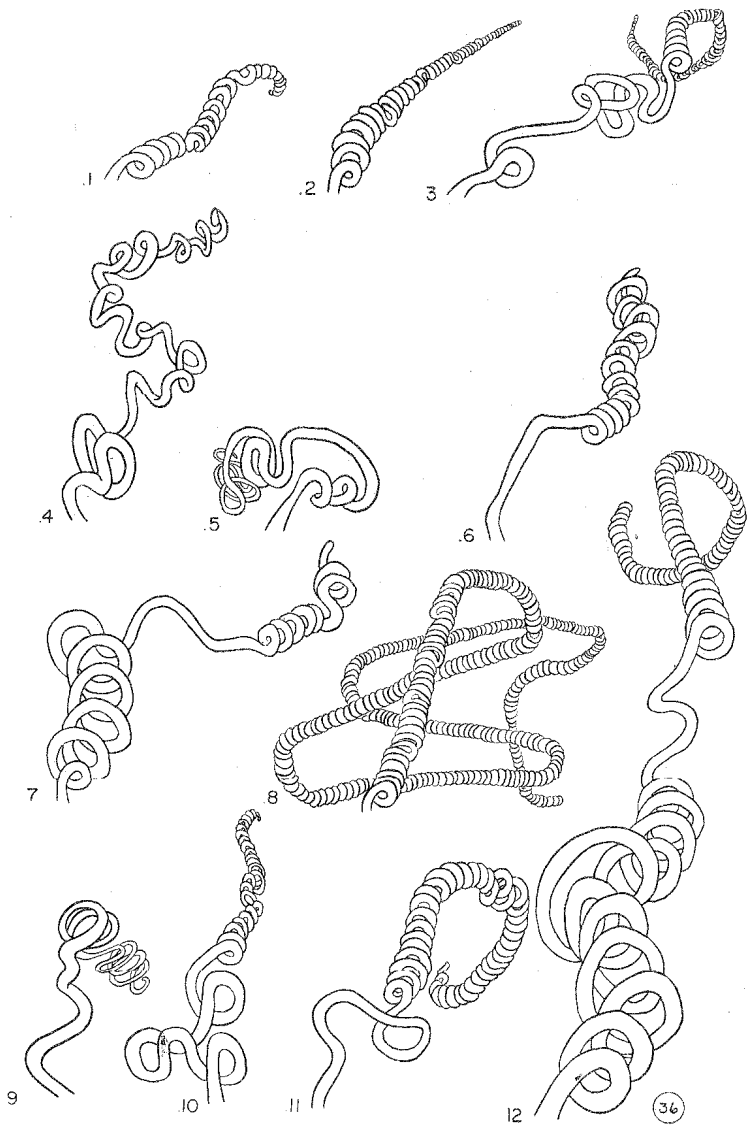


FIG. 36. Ventral receptacle types. .1) peruviana; .2) species G; .3) pictura; .4) meridionalis; .5) aldrichi; .6) longicornis; .7) nigricruria; .8) nigrohydei; .9) mercatorum; .10) limensis; .11) fulvimaculoides; .12) melanopalpa.

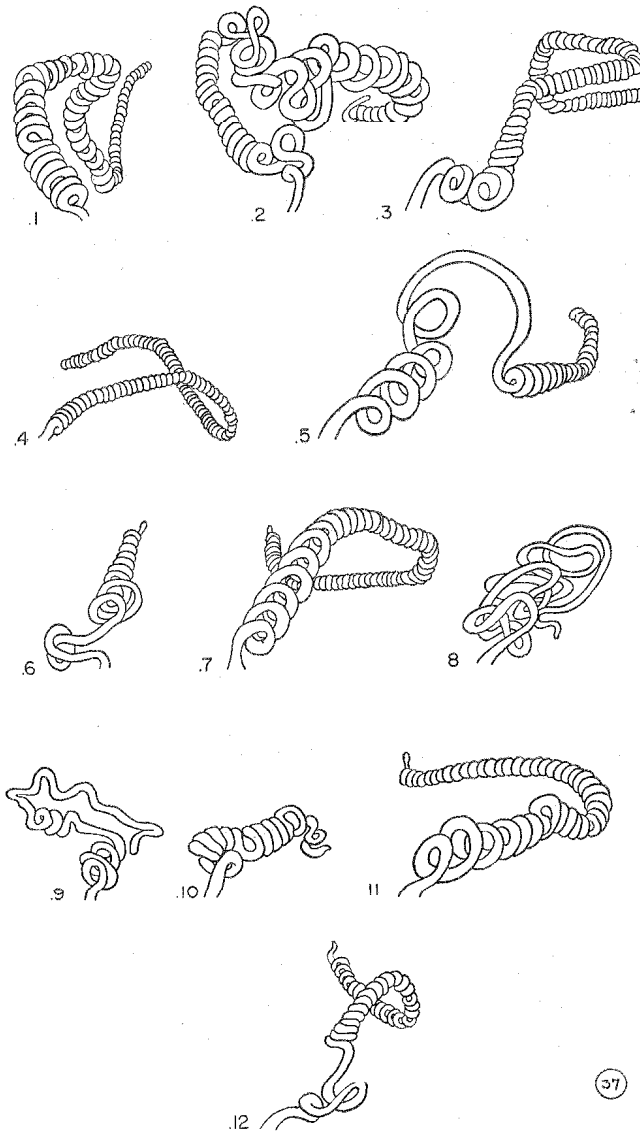


FIG. 37. Ventral receptacle types. .1) castanea; .2) gaucha; .3) camargoi; .4) canalinea; .5) paracanalinea; .6) species K; .7) parachrogaster; .8) testacea; .9) submacroptera; .10) rellima; .11) occidentalis; .12) guttifera.

(Figure 37.8; that of *putrida* is virtually identical with that shown in Figure 35.7) and several species from the tripunctata group (Figure 38.1). Those shown in Figure 37.9 (*submacroptera*) and 38.2 (*albicans*) are probably only slight modifications of this type. In all of these species the ventral receptacle is not markedly attenuated distally. Before clearing, this type closely resembles that shown in Figures 36.5 and 36.9. After clearing these types are seen to be quite distinct, and this last type is found only among members of the mulleri and mercatorum subgroups of the repleta group. The type shown in Figure 36.3 is

found in most members of the fasciola subgroup (Figure 36.3), some members of the mulleri subgroup (Figure 36.6) and in some members of the melanopalpa subgroup (Figures 36.10–.11) of the repleta group. It is also seen in some members of the quinaria section (e.g., Figures 37.12; 38.8), in *carbonaria* (Figure 35.5), and perhaps *polychaeta* (Figure 35.9) would better be placed in this type than in the type mentioned earlier. The type seen in Figure 36.7 is found in several species of the mulleri (Figure 36.7) and melanopalpa (Figure 36.12) subgroups of the repleta group. It is also seen in one species of the canalinea group (Figure 37.5) and the mesophragmatica type (Figure 37.2) may represent a variation of this. The type shown in Figure 36.8 is generally typical of species from the hydei subgroup of the repleta group, although some of these species (e.g., *bifurca*) have four or five larger coils basally. *D. aracea* (Figure

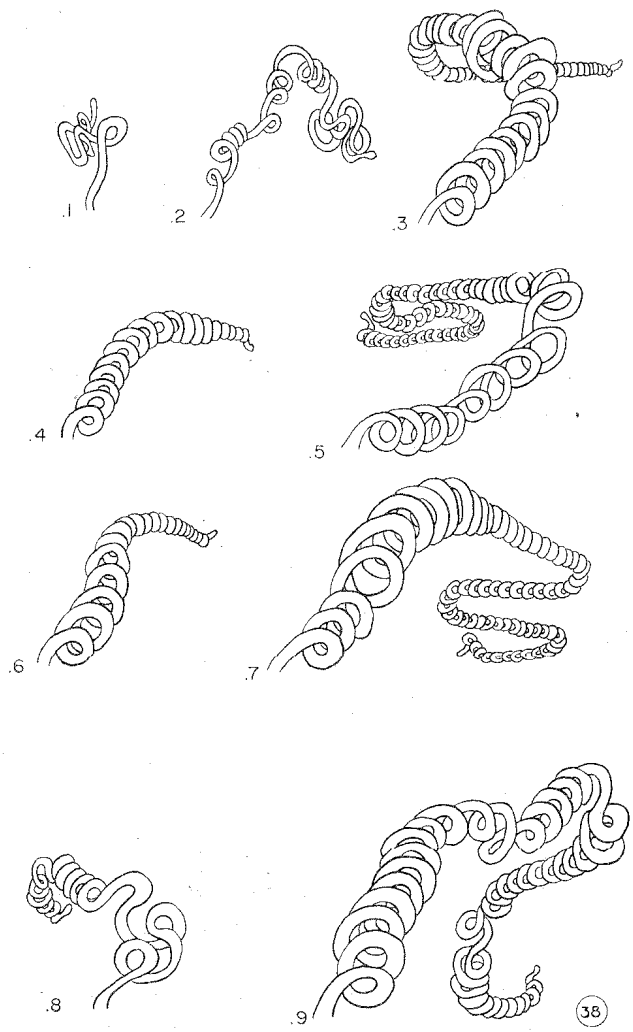


FIG. 38. Ventral receptacle types. .1) mediodiffusa; .2) albicans; .3) unipunctata; .4) tripunctata; .5) guaramunu; .6) dunni; .7) cardini; .8) ornatipennis; .9) schildi.

35.8) and *canalinea* (Figure 37.4) also have a ventral receptacle of this type, and that of *fulvalineata* (fasciola subgroup of the repleta group) somewhat resembles it.

As can be seen from this brief survey, ventral receptacle types are quite varied, and in several cases the types are restricted to closely related species. At the present time sharp distinctions between some types cannot be made, and the above assignment to types must be considered tentative. The present data do serve to show that evolution of ventral receptacles has followed the same general pattern as was seen for characteristics covered earlier. The primitive type for the subgenus *Drosophila* was probably similar to that shown in Figure 35.7 or 36.5 (i.e., short, not appressed to the vagina, either weakly or strongly attenuated distally, and it may or may not have been folded). The type shown in Figure 35.4 (*histrioides*, *Hirtodrosophila*) suggests derivation of a coiled type from a folded type. Also many of the coiled types show a reversal of coiling at approximately every fifth coil, suggesting that coils have been superimposed on a folded structure, with the points of reversal representing the original folds. It was not possible to check all ventral receptacles for this feature, but all of those which were checked showed the reversal. In the figures (e.g., Figure 34.14) the reversal has been shown only for cases which have been specifically checked for it. If it is not shown in the figure, reversal may or may not be present, but it probably is. Regardless of the origin of coiling, subsequent evolution in both major branches of the subgenus *Drosophila* has involved increasing length and addition of specific coiling relationships. There is no general correlation between length of the ventral receptacle and number of coils in the testes.

Ventral receptacles of the *Hirtodrosophila* (Figures 34.4; 35.4) and of *busckii* (Figure 34.5) are of the obscura type (folded and appressed to the vagina). That of *histrioides* (Figure 35.4), previously mentioned, is distinctive, but it is still basically a folded type appressed to the vagina. Okada (1956) figures several other species having the same type of ventral receptable.

Figure 39 shows ventral receptacles from other genera. Members of more primitive genera (Figures 39.1–.3) have short and folded ventral receptacles

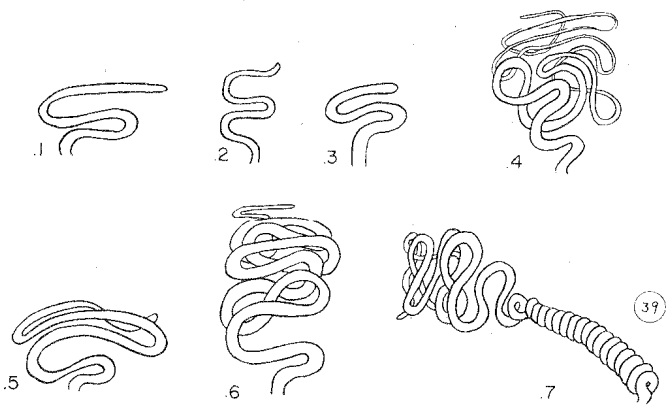


FIG. 39. Ventral receptacles from other genera: .1) *G. bivisualis;* .2) *R. obesa;* .3) *Zaprionthrica dispar*; .4) *C. aldrichi*; .5) *S. adusta*; .6) *Z. ghesquierei*; .7) *M. dimidiata.*

which are appressed to the surface of the vagina. Those from the genus *Chymomyza* vary in length, but all are strongly attenuated distally. They are rather irregularly folded, there is no indication of coiling in those seen (Okada, 1956, shows both coiled and folded types), and they tend to be appressed to the surface of the vagina. The ventral receptacles from *Scaptomyza* species resemble the obscura type in being folded and generally appressed to the vagina. Ventral receptacles of *Zaprionus* species resemble those of *Scaptomyza* species. They are folded and appressed to the vagina in *Z. ghesquierei*, folded and rather loosely associated or free of the vagina in *Z. vittiger*. *M. dimidiata* (Figure 39.7) has a ventral receptacle which is free of the vagina, coiled basally but folded distally. It somewhat resembles the type seen in *histrioides*, except that the latter type is appressed to the surface of the vagina.

*Malpighian Tubules*—The various types of Malpighian tubules are shown phylogenetically in Figure 41. Table 2 summarizes data relating to the condition (free, apposed, fused) of the tips of the posterior tubules. Only the major groups of species have been included in the table.

In related Acalypterate families, Malpighian tubules have the posterior tips free (Sturtevant, 1942), and this is also the condition in the more primitive genera in the family Drosophilidae. Okada (1955), after a survey of Malpighian tubules within the family, concluded that the type seen in most Sophophoran species (posterior tips free) represents the primitive.

Where tips are free the posterior tubules pass from their common stalk back toward the posterior end of the abdomen, then turn forward and their tips lie lateral and adjacent to the gut a short distance posterior to the origin of the stalk.

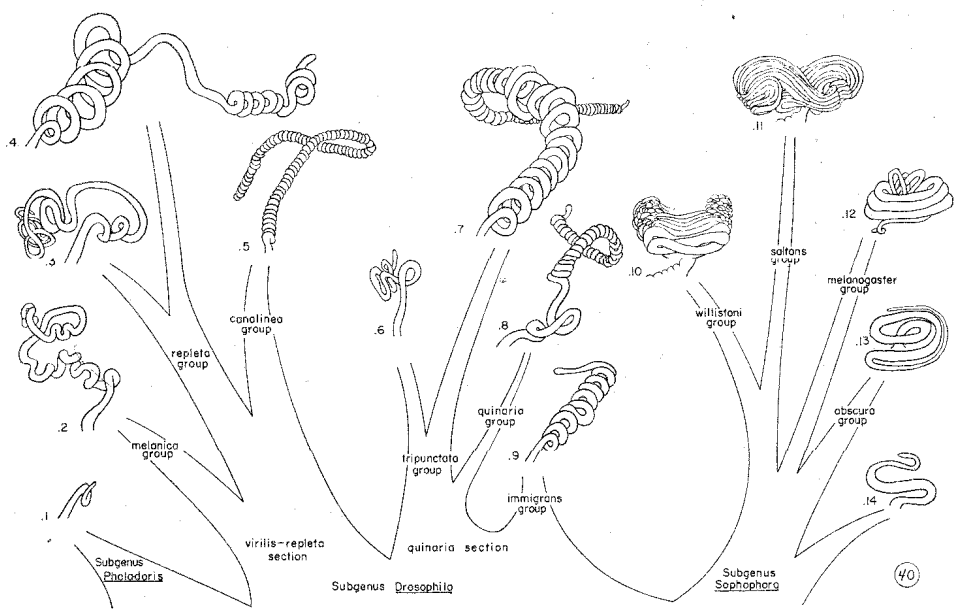


FIG. 40. Pictorial phylogeny of ventral receptacles. .1) victoria; .2) nigromelanica; .3) aldrichi; .4) longicornis; .5 canalinea; .6) mediodiffusa; .7) mediopunctata; .8) subpalustris; .9) immigrans; .10) equinoxialis; .11) austrosaltans; .12) serrata; .13) affinis; .14) populi.

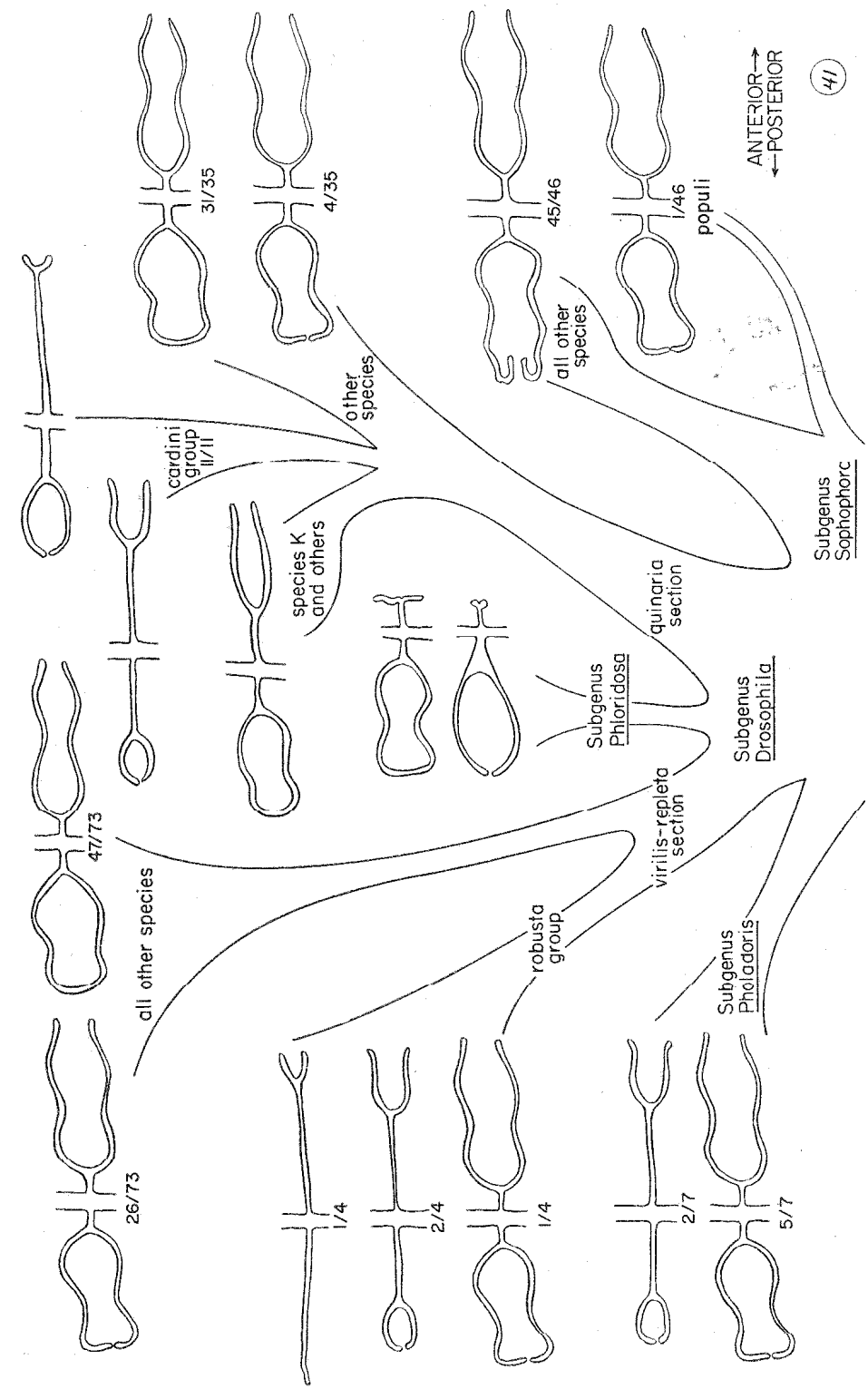


FIG. 41. Pictorial phylogeny of Malpighian tubules. For explanation see text.

TABLE 2

Characteristics of posterior Malpighian tubules

| | Free | Apposed | Fused |
|---|---|---|---|
| *Pholadoris* | | | |
| victoria group | ... | 2/2 | ... |
| coracina group | ... | 3/3 | ... |
| latifasciaeformis group | ... | 1/1 | ... |
| bryani group | ... | ... | 1/1 |
| *Hirtodrosophila* | ... | ... | 4/4 |
| *Dorsilopha* | ... | ... | 1/1 |
| *Sophophora* | | | |
| populi | ... | 1/1 | ... |
| obscura group | 10/10 | ... | ... |
| melanogaster group | 12/12 | ... | ... |
| willistoni group | 10/10 | ... | ... |
| saltans group | 14/14 | ... | ... |
| *Drosophila* | ... | | ... |
| virilis-repleta section | | | |
| virilis group | ... | ... | 9/9 |
| melanica group | ... | ... | 6/6 |
| robusta group | ... | 3/4* | ... |
| annulimana group | ... | ... | 4/4 |
| canalinea group | ... | 1/2 | 1/2 |
| dreyfusi group | ... | ... | 2/2 |
| mesophragmatica group | ... | ... | 2/2 |
| repleta group | | | |
| mulleri subgroup | ... | 11/19 | 8/19 |
| fasciola subgroup | ... | 5/8 | 3/8 |
| mercatorum subgroup | ... | ... | 2/2 |
| melanopalpa subgroup | ... | ... | 6/6 |
| hydei subgroup | ... | 1/5 | 4/5 |
| polychaeta group | ... | ... | 1/1 |
| nannoptera group | ... | 1/1 | ... |
| bromeliae group | ... | 1/1 | ... |

| | Free | Apposed | Fused |
|---|---|---|---|
| quinaria section | | | |
| immigrans group | ... | ... | 3/3 |
| fimebris group | ... | ... | 3/3 |
| testacea group | ... | ... | 2/2 |
| calloptera group | ... | 2/3 | 1/3 |
| quinaria group | ... | ... | 13/13 |
| guarani group | ... | 3/5 | 2/5 |
| cardini group | ... | 11/11 | ... |
| tripunctata group | ... | 3/13 | 10/13 |
| *Phloridosa* | ... | 2/3 | 1/3 |
| *Scaptomyza* | ... | 2/3 | 1/3 |
| *Zaprionus* | ... | ... | 2/2 |
| *Mycodrosophila* | ... | ... | 1/1 |
| *Chymomyza* | ... | ... | 3/3 |
| *Zapriothrica* | ... | ... | 1/1 |
| *Gitona* | 2/2 | ... | ... |

* Posterior Malpighian tubule in *colorata* unbranched.

In these forms the tips of the posterior tubules are lightly attached to the gut by tracheae. In these forms also, both the anterior and posterior tubules are differentiated, with the proximal portion of the tube being yellow in color, the distal portion being somewhat irregular in shape and white in color. During the course of evolution a change in orientation appears to have occurred, with the tips of the posterior tubules curving around the gut and meeting in the midline. The tips retain their close association with the gut and are lightly held together by tracheae. This is, presumably, the most primitive type within the category of apposed tips. In most species, however, the walls of the tips have fused, but the inner lining has not broken down and the lumen is not continuous. In these cases also, the tips of the tubules are often much less closely associated with the gut and may be quite independent of it. In most species having the posterior tips of the Malpighian tubules apposed, the individual tubules are differentiated as were those where the tips are free (i.e., a proximal yellow, and thin, white, distal section). In some, however, the posterior tubules lack the distal white section and are of uniform color and diameter all the way to the tips. In species having the posterior tips fused, the inner walls have broken down and the lumen is continuous between the right and left tubules. In many of these forms, association between the gut and the tips of the tubules is absent, but in some the association with the gut has been retained. Here, as in the apposed category, the posterior tubules are generally differentiated, but in some the distal white section is absent and the ring formed by the fusion of the tips is of uniform appearance and diameter throughout. For brevity, only the three major categories (free, apposed, fused) will be used in the following discussion.

As was noted earlier, Figure 41 shows the major types of Malpighian tubules seen in the various groups. A fraction is also included with most figures. The numerator of this fraction indicates the proportion of the group having that type of Malpighian tubule. The denominator indicates the total number of species involved. For example, the data from seventy-seven species from the virilis-repleta section of the subgenus *Drosophila* have been summarized in the figure. Seventy-three species ("all other species," upper left) have the usual type of Malpighian tubules. Twenty-six of these have the tips apposed and forty-seven have the tips fused. Where needed, further explanation will be given below.

In most respects species from the subgenus *Pholadoris* show primitive characteristics. Their Malpighian tubules, however, are of derived types. In species from the victoria and coracina groups the posterior tips are apposed, in *victoria* very loosely so and almost free. In *bryani* and *latifasciaeformis* the stalks of both the anterior and posterior tubules are relatively very long (Figure 41). In *latifasciaeformis* the posterior tips are apposed, in *bryani* they are fused. Only one of these types is shown in the figure, and the fraction (2/7) in this case indicates the proportion of *Pholadoris* species having Malpighian tubules with long stalks. With the exception of *populi*, all Sophophoran species have Malpighian tubules with the posterior tips free. In *populi* they are apposed. In some species of the melanogaster group the stalks of both the anterior and posterior tubules are longer than usual, being about twice as long as in other species in the subgenus. This is particularly noticeable in *ananassae* and *takahashii*. Species in the montium subgroup vary from *auraria* with short, to *seguyi* with longer (about 1½X)

stalks. Members of the melanogaster subgroup and *bipectinata* have the usual short stalks. Only the usual type is shown in Figure 41.

In the subgenus *Drosophila* species from both sections have the same general types of Malpighian tubules. In the majority of species the posterior tips are fused (see Table 2). Many species from both sections have the apposed type. None have tips which are completely free, although some species from the tripunctata group approach this type. In these species (e.g., *crocina*) the tips curve toward each other around the gut and are *very* loosely tied together with tracheae. In this respect they differ from the typical Sophophoran type and so were classified as apposed. Frota-Pessoa (1954) describes other species from the tripunctata group as having free tips, and presumably these are all of this same general type.

Only two major groups of species in the subgenus *Drosophila* have Malpighian tubules which depart from the usual type. These species are in the robusta group from the virilis-repleta section and in the cardini group of the quinaria section. Some species of other groups from the quinaria section vary in the direction of cardini types, as do some of the undescribed forms (e.g., species K, see Figure 41).

Species in the robusta group have three types of Malpighian tubules. Those of *lacertosa* are of the usual type with posterior tips apposed. Those of *robusta* and *sordidula* resemble each other in that both the anterior and posterior stalks are very long. In *sordidula* the stalks are about three-fourths the total length of the tube, in *robusta* they are about one-half the length of the tube. The posterior tips are apposed in both species. This type of Malpighian tubule is the same as that seen in *latifasciaeformis*, and it is seen also in species of the cardini group. In *colorata* the stalk of the anterior tubule is about four-fifths the total length and the posterior tubule is long and unbranched.

The types of Malpighian tubules shown in Figure 41 for species of the cardini group represent the two extremes of types seen within the group. Both the anterior and posterior stalks are very long and the posterior tips are apposed. The Malpighian tubules of species K have stalks markedly longer than the usual type, but they are not as long as those seen in species from the cardini group. In this species the posterior tips are fused. Several other species (*griseolineata* and *subbadia* of the guarani group; *uninubes* of the rubrifrons group) have Malpighian tubules of this type, and the posterior tips are fused in some, apposed in others. Another cluster of species have Malphighian tubules intermediate between this last type and the usual type with short stalks. These are: *putrida* (testacea group), *calloptera* and *ornatipennis* (calloptera group), *guaramunu, guaraja, guarani* (guarani group), *submacroptera* (macroptera group), *parachrogaster* (rubrifrons group), and *albicans, metzii, mediopictoides* (tripunctata group). The intermediate types between that of the cardini group and normal were omitted when the fraction of apposed *vs.* fused was calculated for "other species" in Figure 41. The only groups from the quinaria section in which all species have the usual short stalks are the funebris, immigrans, and quinaria groups. However, only in species K, in two species of the guarani group, and in one species of the rubrifrons group does the type approach that of the cardini group.

Within the genus *Drosophila* the type of Malpighian tubules seen in members

of the subgenus *Phloridosa* is distinctive. All of these species have very short anterior tubules. In species P and species Q the anterior tubules are reduced to small lobes at the tip of the stalk. In species O they are somewhat longer (see Figure 41). The posterior tips in species P are fused. In the other two they are apposed. In species Q the posterior stalk is somewhat longer than usual and it expands greatly toward its point of union with the posterior tubules. *Zapriothrica dispar* has Malpighian tubules identical with those described for species O, except that the posterior tips are fused.

Malpighian tubules from species in other genera (see Table 2) are of the usual types. Anterior and posterior stalks are short in all species examined.

*Other Features of Internal Anatomy*—Wheeler (1947) described a structure, apparently attached to the base of the ovipositer, in females of species belonging to the willistoni group. During dissection of all species this feature was checked, and it was found in species of the willistoni group but nowhere else. It varies from species to species in this group, generally being a rather short, corrugated and indistinctly bilobed pouch. In *fumipennis* it has a long, narrow stalk and a long, fusiform bulb. It is very conspicuous when fully distended and, as indicated by Wheeler (*op. cit.*), it appears to arise from the base of the ovipositer. When the vagina is punctured this pouch collapses also, suggesting a connection between them. A similar structure is present in *Chymomyza coxata* (Wheeler, 1952).

Another interesting structure was noted in *peruviana*. In this species the rectum appears only as a slight enlargement of the posterior digestive tract. It lacks the usual four rectal papillae. Instead, there are two large lateral pouches arising on narrow stalks from the posterior end of the rectum, and each of these has two typical rectal papillae. The position of these papillae varies somewhat, but generally they are apical in each pouch. These pouches are present in both males and females.

*Anterior Spiracles of the Pupa*—For the most part characteristics of the pupa have been little used in *Drosophila* classification, although horn indices have been useful as species characteristics. The horn index will not be covered at the present time. In species of the subgenus *Pholadoris* the anterior horn is very short (almost non-existent) relative to the length of the puparium. It is also short in all Sophophorans. In species of the subgenus *Drosophila* the anterior horn varies from short to very long.

The characteristics of the branches of the anterior spiracle are occasionally noted in species descriptions, but no extensive systematic study has been made of them. They provide one of the best indications of subgeneric relationships available, and it is unfortunate that they have not been reported in greater detail. Representatives of spiracle types for the genus *Drosophila* are shown in Figures 42 and 43. They are summarized phylogenetically in Figure 44. Types from other genera are shown in Figure 45. Figures are not drawn to scale, so no size comparisons can be made. The type shown for *pattersoni* (Figure 42.1) is presumably near the primitive for the genus. The branches are short, generally recurved, and arranged in a simple whorl around the spiracle opening. The ring of branches does not make a complete circle. It is interrupted and there is a distinct gap (basal gap) directed dorso-laterally. The branches adjacent to the gap

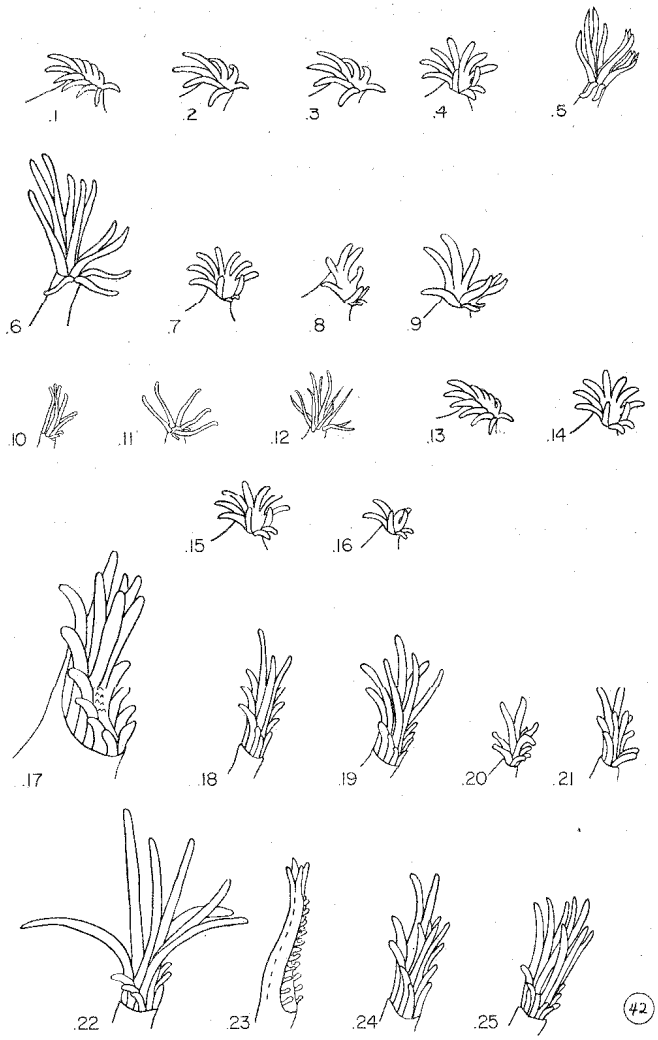


FIG. 42. Characteristics of the anterior pupal spiracle.

.1 pattersoni
.2 novopaca
.3 bryani
.4 duncani
.5 thoracis
.6 busckii
.7 pseudoobscura
.8 athabasca
.9 tolteca
.10 melanogaster
.11 takahashii
.12 serrata
.13 willistoni
.14 fumipennis
.15 parasaltans
.16 neoelliptica
.17 ezoana
.18 melanica
.19 robusta
.20 gibberosa
.21 mojuoides
.22 longicornis
.23 aureata
.24 canalinea
.25 camargoi

(basals) are short and the following ones increase in size regularly, with the ones opposite the gap (antibasals) being longest. For descriptive purposes the branches can be divided into three categories: basals, laterals, and antibasals. In some species, particularly from the subgenus *Drosophila*, there are branches within the ring and these will be referred to as centrals. For discussing certain

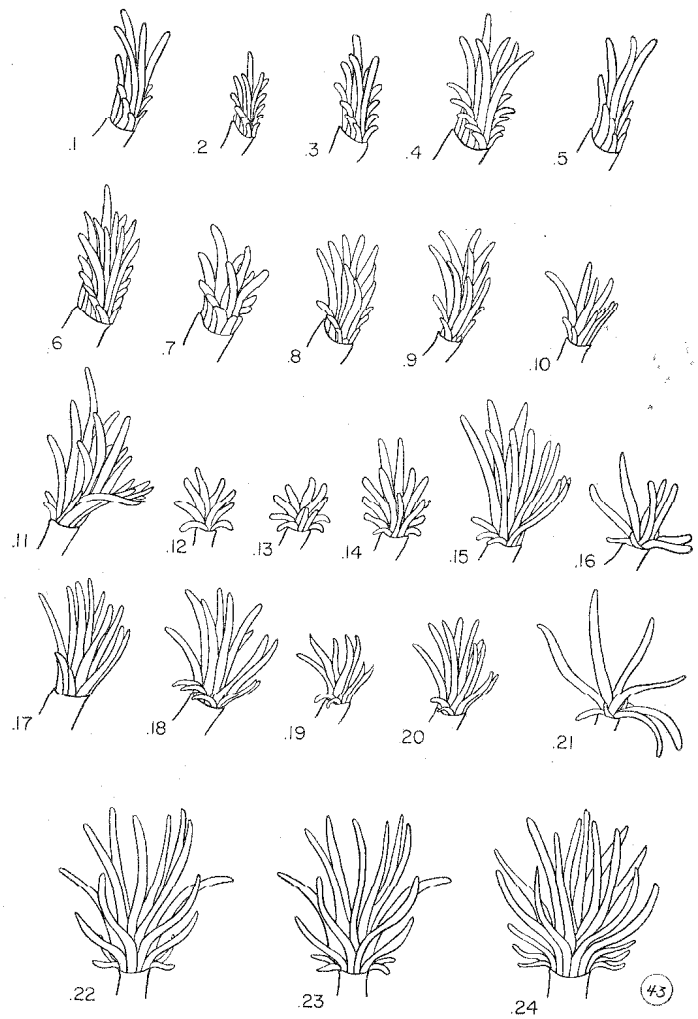


FIG. 43. Characteristics of the anterior pupal spiracle.

| | | |
|---|---|---|
| .1 gaucha | .9 subfunebris | .17 parachrogaster |
| .2 nannoptera | .10 macrospina | .18 pallidipennis |
| .3 carbonaria | .11 hypocausta | .19 testacea |
| .4 polychaeta | .12 innubila | .20 macroptera |
| .5 species I (bromeliae gp.) | .13 species J | .21 neocardini |
| .6 species G | .14 tenebrosa | .22 griseolineata |
| .7 peruviana | .15 palustris | .23 tripunctata |
| .8 funebris | .16 species K | .24 ornatipennis |

types it is convenient to designate the lateral branch adjacent to the basal as the sub-basal. The sub-basals often differ in position from other branches in the ring, being displaced inward and standing erect rather than being recurved. A spiracle with sub-basals of this type is shown in Figure 42.4. The ring is always symmetrical (in fully everted spiracles), and the two halves are mirror images of each other. All figures except 43.22–.24 show dorsal views of the right spiracle.

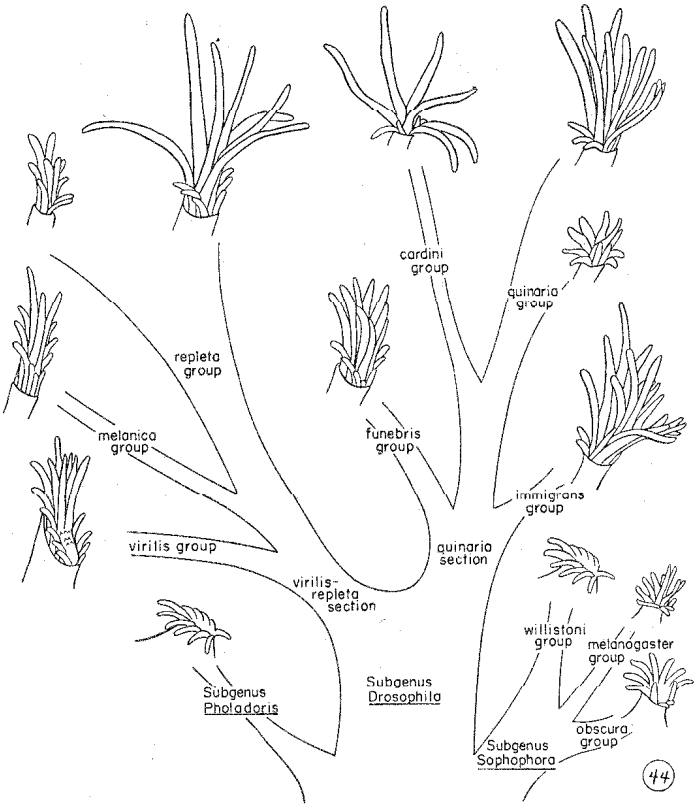


FIG. 44. Pictorial phylogeny of anterior pupal spiracles. For further explanation see text.

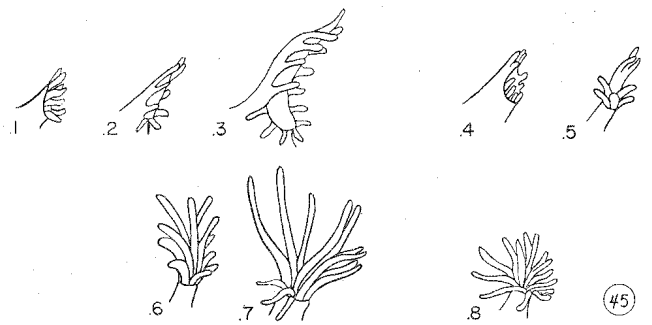


FIG. 45. Anterior pupal spiracles from members of other genera.

.1 *C. amoena*
.2 *C. aldrichi*
.3 *C. procnemis*
.4 *S. adusta*
.5 *S. pallida*
.6 *Z. vittiger*
.7 *Z. ghesquierei*
.8 *M. dimidiata*

All species of the subgenus *Pholadoris* have the same basic type of spiracle (Figures 42.1–.3). Branches are arranged in a simple whorl and they increase slightly in size from basals to antibasals. The chief variation within the group is in number of branches. Species of the victoria group have eleven, those in other groups have five to eight. The spiracle of *victoria* differs from the others

in having sub-basals erect and slightly displaced inward. Branches are uniform in color and are of the same color as the puparium.

Species from the obscura, saltans and willistoni groups of the subgenus *Sophophora* have the same general type of spiracle as was seen in *Pholadoris* species. Branch number varies from seven to eleven in species of the obscura group, and all members of this group have the sub-basals erect and displaced inward (Figures 42.7–.9). In some members of the group the sub-basals are rather strong and almost completely displaced inward so that they appear to be within the ring (Figure 42.9). In most of these species the branches form a flat whorl, although in some they are more nearly erect (this varies with age and dryness of pupa). In *narragansett* the branches are shorter and strongly recurved. In most cases the length of the branches increases regularly and markedly from basals to antibasals and the color of the branches is brown with tips slightly darkened.

The number of branches varies from nine to eleven in members of the willistoni group. The type shown in Figure 42.13 represents *willistoni, equinoxialis, tropicalis* and *paulistorum*. That shown in Figure 42.14 represents *sucinea, capricorni, nebulosa* and *fumipennis*. Pupae of *changuinolae* and *pseudobocainensis* were not seen. In most species of this group the branches are uniform brown, although in some the tips are dark. The number of branches in species from the saltans subgroup varies from eleven (*parasaltans*) to seven (*neoelliptica* and *emarginata*), and all have erect sub-basals (Figures 42.15–.16). Color of branches is variable.

Spiracles from species in the melanogaster group are difficult to interpret. In most species the branches, particularly the antibasals, are much longer and more erect than in the previous type. The number of branches in the outer whorl varies from seven (*yakuba*) to eleven (*ananassae*). Members of the montium and ananassae subgroups have from two to four distinct long central branches (Figure 42.12). It seems probable that at least two of these represent sub-basals displaced inward. In *melanogaster* there appear to be two branches within the ring but their position and appearance suggests that they are enlarged sub-basals. In *takahashii* (Figure 42.11) and *yakuba* the true basals appear to be almost missing (only vestiges seen, and these were not shown in the figure) and the enlarged sub-basals now occupy the position of the basals in the ring. This interpretation is, however, quite tentative. The branches in montium (except *serrata*) and takahashii subgroups are black with light tips. Those of the melanogaster subgroup are a uniform brown.

Spiracles from species in the subgenus *Drosophila* are of several types (Figures 42.17–.25; 43.1–.24), and all of them are distinct from those types seen in members of the subgenus *Pholadoris* and in Sophophoran species. The type shown in Figure 42.17 is restricted to members of the virilis group. Branches are heavy, nearly erect, and recurved only at their tips. There is a very marked and regular increase in length from basals to antibasals. In most species the median edge of the spiracle (from which the antibasals arise) is extended as a lip. This is most extreme in *ezoana* (figured). There are thirteen branches in the outer ring and a cluster of about four centrals which appear to originate at the bases of the antibasals. There are also some rudimentary central branches. Branches are brown with dark tips.

Although the number and length of branches varies, most of the remaining species from the virilis-repleta section have spiracles of the same general type (the robusta type, Figure 42.19). That of *aureata* (Figure 42.23, and see Wheeler, 1957) is an aberrant type seen only in this one species. The type shown in Figure 42.20 is restricted to members of the annulimana group, to *eremophila* (mulleri subgroup of repleta group) and to *melanura* (melanica group). A very similar type (Figure 42.21) is seen in several members of the fasciola subgroup of the repleta group. Among these last species there is an intergradation of types, with *fulvalineata* having spiracles of the robusta type and others being intermediate between the robusta type and the annulimana type. The remainder of the species from this section (Figures 42.24–.25; 43.1–.7) are all of the robusta type. In all of these the branches are heavy and increase regularly in length from basals to antibasals. There are always at least two centrals present and the basal gap is always distinct. Generally the color of the branches is brown with dark tips but in *castanea* the branches are almost black.

There are several distinct spiracle types from species in the quinaria section (Figures 43.8–.24). Those of species in the funebris group vary (Figures 43.8–.10). In one, *funebris*, the spiracle is of the robusta type. In *subfunebris* the basals are very small and two of the centrals have moved out so that they are almost on the ring. In *macrospina* the basals are absent and there are four long branches in their position. Judging from the situation in *subfunebris*, it would appear that the basals have been lost and the centrals have moved out to the ring, but this is only speculative. For convenience, these long branches in the basal position will be referred to as pseudobasals. The present terminology should be considered only as descriptive. Homologies of various branches can probably be determined eventually, but they must remain uncertain for the present. The type seen in *macrospina* is seen in the great majority of species from the quinaria section. This type can be defined as follows: basal gap very small or absent, pseudobasals present and longer than laterals, generally equalling antibasals in length, centrals always present. All species of the immigrans group have spiracles of this type, and it will be referred to as the immigrans type (Figure 43.11). Those of *spinofemora* have two pseudobasals, those of *hypocausta* (figured) and *immigrans* have four. Laterals are very short and the number of centrals varies from three to six. Spiracles from some species in the quinaria group resemble the annulimana type (compare 42.20 and 43.12). Although the number of branches varies, *innubila* (figured), *phalerata*, *falleni* and *transversa* are all of this type. Species J (Figure 43.13) and *occidentalis* differ from this type primarily in having a short and erect pair of pseudobasals. The type seen in *guttifera* and *tenebrosa* is the same as the latter type except that the branches are longer (Figure 43.14). The spiracles of *palustris* (Figure 43.15) and *subpalustris* are completely of the immigrans type. Several species from the tripunctata, testacea, macroptera and rubrifrons groups have spiracles resembling those seen in *tenebrosa* (a pair of short pseudobasals), and these can easily be confused with the robusta type if they are not checked closely. In these species the branches are longer and more slender than those of *tenebrosa*. Most species of the tripunctata group and all species of the guarani and calloptera groups (*schildi* not seen) have spiracles of the immigrans type. Three representatives are shown in face view (looking

directly toward the pseudobasals) in Figures 43.22–.24. The spiracle branches of *guaraja* are very long and delicate. They seldom stand erect, so this type appears to be distinct from the others. Relative length and position of branches, however, is as seen in the immigrans type.

The species of the cardini group (Figure 43.21) and species K (Figure 43.16) and L have unusual types of spiracles. In some of these species the number of branches is reduced (*neocardini* has the lowest number, *belladunni* the highest). There is a pair of very long and recurved pseudobasals, then a pair of up-curved small laterals. The remainder of the laterals are short and recurved, while the antibasals are long and tend to be recumbent rather than erect. Types having the higher branch numbers tend to resemble the immigrans type more closely, but most of them are distinct from it. Color of branches is variable from species to species, but most of the branches have black bases (and/or the edge of the spiracle itself is black) and the tips are pale. This same color pattern is seen in many species from other groups in the quinaria section.

Species from the subgenus *Hirtodrosophila* have very variable spiracles. Those of *duncani* are of the *Pholadoris* type with erect sub-basals (Figure 42.4). Those of *pictiventris* and *thoracis* (Figure 42.5) are of the immigrans type. Those of *histrioides* resemble the robusta type, except that the branches tend to be recumbent rather than erect. The spiracles of *busckii* resemble the immigrans type.

Spiracle types seen among species from other genera are shown in Figure 45. Those of *Chymomyza* species vary as shown in Figures 45.1–.3. That of *C. amoena* (Figure 45.1) is basically as in obscura group species, except that the rim of the spiracle is extended slightly as an antibasal lip. This extension is extreme in *C. procnemis*. Spiracles from *Scaptomyza* species (Figures 45.4–.5) more nearly resemble *Chymomyza* types than any other. The two species from the genus *Zaprionus* have two types of spiracles (Figures 45.6–.7). One is of the robusta type, the other of the immigrans type. The spiracles of *M. dimidiata* were difficult to draw. Basically there is an outer ring of eleven, increasing regularly from basals to antibasals, and an inner ring (all almost equal) of seven. Some of the centrals tend to crowd the basals and may give the appearance of pseudobasals.

In summary (Figure 44) types of anterior spiracles provide very useful characteristics, both for separating subgenera and for separating some groups within subgenera. When investigated in greater detail the arrangement of branches may also provide useful specific characteristics. The figures give only a limited indication of the distinctive features of each type. The types seen in species of the melanogaster group were particularly difficult to draw. However, once seen, melanogaster types cannot be mistaken for other types, at least among the species included in the present discussion. Among species from the subgenus *Drosophila*, spiracle types rather sharply distinguish species of the quinaria section from those of the virilis-repleta section.

*Other characteristics of the pupae*—Aside from the characteristics of the anterior spiracle, presently useful characteristics of the pupae are limited. Morphological characteristics of the posterior spiracles vary both within and between species and they follow no distinct pattern. Color of posterior spiracles is generally that of the puparium. In several species from the quinaria section the posterior

spiracles are dark, at least basally, and in some they are shining black. If no distinction is made between these two types, the distribution is as follows: immigrans, 2/3; guarani, 3/5; tripunctata, 9/13 (the fraction indicates the proportion of the group having dark posterior spiracles).

A feature of possible future use may be found in the characteristics of the pair of anal plates which lie lateral to the anal pore. These vary in shape and distinctness from species to species. The anal pore also varies in shape and in some species it appears double. These features have not been investigated in any detail. Color and position of posterior spiracles appears to be the same in both larvae and pupae, so characteristics of the larvae will not be considered separately.

*Egg Filaments*—Information regarding egg filaments is summarized phylogenetically in Figure 46. The phylogeny is the same as that used for spermathecae, etc. Types seen in *Scaptomyza* and *Chymomyza* species are also included in this figure, but they are not placed in the phylogeny. Egg filaments in species from the subgenus *Pholadoris* are thin, irregular in position, and variable in number. In the few eggs checked from these species counts ranged from five to nine. Wheeler (1949a) tabulates filament numbers from three species (*victoria*, *nitens*, *latifasciaeformis*) with a range from two to eleven. An egg with six filaments is shown in Figure 46 since this is near the modal number for the group as a whole. All Sophophoran species have eggs with two filaments. Those of *populi* are short and heavy, and they continue as narrow ridges to the posterior end of the egg. In the melanogaster group all takahashii and montium subgroup species (except *nikananu*) have egg filaments which are thick at the base, then irregularly tapering. The egg filaments of *nikananu* are of the more usual type for the subgenus and are seen also in species from the obscura, saltans and willistoni groups. In this type the filaments are heavy at the base, then very much flattened and expanded apically (see Figure 46).

Egg filament number among species from the subgenus *Drosophila* ranges from one (e.g., *bandeirantorum*, tripunctata group) to four. The great majority of species have four-filament eggs. Aside from variations in length of filament, four-filament eggs can be roughly divided into two classes. In the first, the posterior filaments are thick and flattened basally, tapering gradually to a fine point apically. The anterior filaments are thin and fine. This type is seen in two species of the immigrans group, species of the calloptera group (*schildi* not seen), in species of the annulimana group and in *castanea* and species F. In the second type all four filaments are thin, although they may expand very slightly at the base. This type is shown in Figure 46 for species of the virilis group, funebris group, etc. It is present in approximately 80 per cent of the species in the subgenus. Species from all of the groups not included in Figure 46 have egg filaments of this type. *D. aracea* (not figured), a species not assigned to any group, has four-filament eggs in which both the anterior and posterior filaments are heavy and flattened basally, tapering gradually to fine points.

Two-filament eggs are present in all species of the melanica group, bromeliae group and nannoptera group. They are present also in *hypocausta* of the immigrans group and have been reported (Frota-Pessoa, 1954) in some members of the tripuncata group. *D. aureata* (not figured) also has eggs with two filaments. Egg filaments among species in the melanica group are thick and taper only

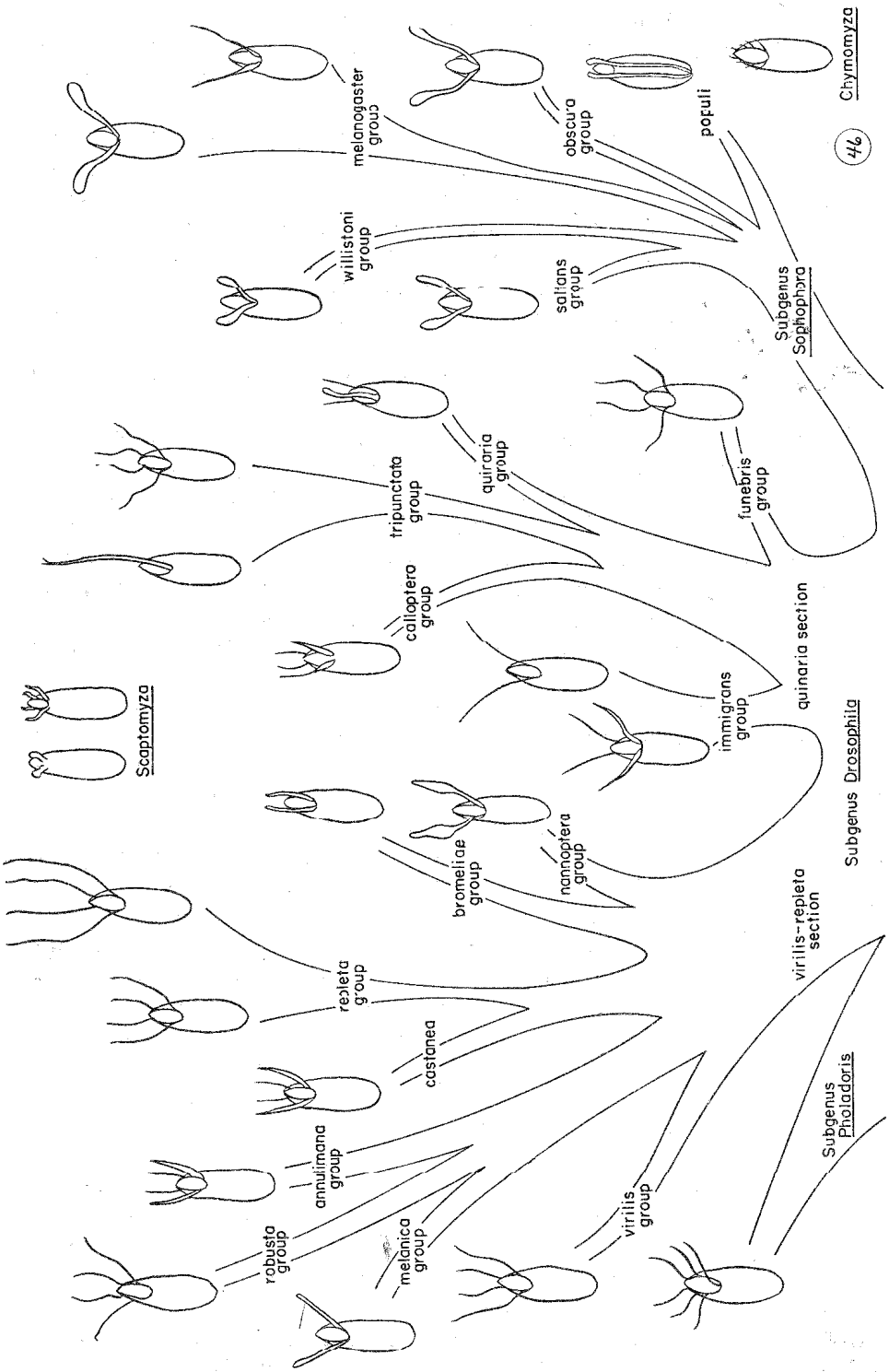


FIG. 46. Pictorial phylogeny of egg filaments. For further explanation see text.

slightly. Those of the bromeliae group are also thick and somewhat more distinctly tapering. In *nannoptera* they are flattened apically as in some *Sophophorans*, and in *hypocausta* they are very long, and thin throughout. In *aureata* the filaments are short, heavy and blunt.

Species of the quinaria group have three-filament eggs, the two anterior ones are thin, the single posterior one is short and heavy. Most species in the tripunctata group have the usual four-filament type, but some species have one (figured), two, or three egg filaments. Members of the subgenus *Hirtodrosophila* have four-filament eggs, with those of *thoracis* being rather peculiar. In this species the posterior filaments are short, blunt lobes, and the anterior ones are very short and extremely thin. In spite of their thinness they are quite rigid and perfectly straight. The eggs of the other three species are of the usual four-filament type, as are those of *busckii* (*Dorsilopha*). Eggs of *Phloridosa* species were not seen, but those of *floricola* are without filaments (Sturtevant, 1942).

Eggs from species in the genus *Chymomyza* are distinct. The general type is shown in the lower right corner of Figure 46. There are approximately eight short, in-curved filaments decreasing in size regularly from posterior to anterior. Types seen in *Scaptomyza* species are shown in Figure 46 also (upper center). That of *S. pallida* (left) has two short heavy posterior filaments. That of *S. adusta* (right) has four short, heavy and bent filaments. The eggs of *Zaprionus* species have four long filaments. They are all heavier than in the usual four-filament type. Eggs of *M. dimidiata* have four filaments. In all four the basal one-third is heavy, the remainder thin.

*Characteristics of the Abdominal Sternites of the Male*—Wheeler (1960) describes and discusses sternite modifications in male Drosophilidae. Number of sternites varies from six in more primitive forms to four in the more derived species. Reduction in number involves changes in, or eventual loss of, the first and sixth sternites. Detailed demonstration of sternite characteristics requires clearing of the ventral abdominal wall in phenol and examination at the higher magnifications of the compound microscope. Time did not permit a study of this sort, but it was possible to check those features which could be seen in the living fly using the dissecting microscope. At these magnifications well-chitinized and pigmented plates can be detected and, using the characteristics of the second sternite (see Wheeler, *op. cit.*) as a guide, the specific sternites represented could be determined. Results of this limited survey are of interest, and they are useful so long as the limitations of the observational methods are recognized.

Most species of the subgenus *Pholadoris* have visible remnants of the first and/or the sixth sternite. In species of the victoria group the first sternite is present as two plates (see Wheeler, *op. cit.*, for figure of *victoria*) and the sixth sternite is present as a plate comparable in size and appearance to the other abdominal sternites. In species of the coracina group no first sternite was visible. (In the following descriptions, "not visible" should *not* be interpreted as implying absence. The sternite in question may be present but not pigmented and of any of several forms, or it may actually be absent.) In *novopaca* the sixth is present much as in *victoria*. In *cancellata* and *lativittata* the sixth is present as a thin, chitinized bar. In *bryani* and *latifasciaeformis* the first is not seen. In

*bryani* the sixth is a narrow bar, concave posteriorly so that it has a shallow U-shape. A sixth was not seen in *latifasciaeformis*.

All Sophophoran species have the sixth sternite present as a well-chitinized, but generally not bristled plate. In most species this plate is somewhat polished in appearance. Shape of the plate varies. It is largest in *populi*, very narrow and almost interrupted in the midline in several species of the melanogaster group. In *populi* the first sternite is present as two bristled plates much as in *victoria*. In most of the remaining Sophophorans the first sternite is not seen. Two small dark plates in this region are seen in *neoelliptica*, *emarginata* and *milleri* (saltans group), although there is apparently individual and interstrain variation of the first sternite in some of these species (Malgalhaes, this Bulletin).

With two exceptions, first and sixth sternites are not seen in species from the subgenus *Drosophila*. In *montana* (virilis group) there may be a thin bar in the position of the first sternite, and there are faint indications of this in *littoralis* and *ezoana*. In the last two species there is considerable individual variation. If the presence of the first sternite *as a bar* is verified, this fact would be of considerable interest. The available evidence, while fragmentary, suggests that in most evolutionary lines in the genus the first step toward loss of the first sternite has involved its separation into two pieces. The presence of a complete, but narrow, bar in some species of the virilis group would suggest that loss of the first sternite may have evolved independently in this group. More detailed investigation of this characteristic may prove fruitful. In the remainder of the species belonging to the virilis-repleta section there was no visible evidence of either the first or the sixth sternites. In some species there were a few scattered bristles in the region of the sixth (in position suggesting that the sixth had split in the midline, then moved laterally to fuse with the seventh tergite) but observations were too limited to warrant further description.

The second exception involves the presence of the sixth sternite in *testacea*. In this case the sternite is rather polished and equals the fifth sternite in width. There is a conspicuous bristle at each lateral margin. Among species of the quinaria section no other structures were seen which could be interpreted unequivocally as a sixth sternite, although many species of the cardini group appear to have the anterior wall of the genital chamber weakly chitinized, suggesting the possible presence of a remnant of the sixth sternite in this region.

The first and sixth sternites are absent in *Phloridosa* species, in *buscki* (*Dorsilopha*), and in most species from the subgenus *Hirtodrosophila*. In *duncani* the sixth is present as a narrow bar.

The sternites in members of other genera are variable. The first and sixth were not visible in *R. obesa*. (In this species, *something* is present in the position of the sixth sternite, but its characteristics could not be determined by methods used here.) In *G. americana* the first is a very large plate, wider than the other sternites and concave at its anterior margin. The sixth is present, but turned on edge to lie along the anterior wall of the genital chamber. In *Zapriothrica dispar* the first is a broad plate, concave at its posterior margin, and the sixth is present as a narrow, rectangular and weakly bristled plate. In species from the genus *Chymomyza* both the first and the sixth sternites vary. In *C. aldrichi* the first and the sixth are both present as paired plates. In *C. amoena* the first is not visible

and the sixth is present as a large U-shaped plate (arms directed posteriorly), and the arms of the U are weakly bristled. In *C. procnemis* the first was not seen, the sixth is present as paired plates (see Wheeler, *op. cit.*, for figure). No first or sixth sternites were seen in *Scaptomyza, Zaprionus* or *Mycodrosophila* species.

## General Approach for the Analysis of Phylogenetic Relationships

It has often been stated that the characteristics of a population reflect its evolutionary history, and this postulate should be applicable to the characteristics of groups of species as well as to those of single populations. Most often, this statement is applied to the genetic structure of a population, but morphological characteristics are a reflection of the genotype, and if this truism is to have more than an abstract significance it should be possible to infer the history of different genotypes from the distribution of the morphological characteristics which they determine. The basic problem of phylogeny is that of detecting genetic continuity, and it is therefore necessary that phylogenetic analysis be made in terms of genotypes and their history. If the characteristics of populations actually do reflect the history of the populations, such an analysis should be possible. The following discussion is directed toward developing a method of phylogenetic analysis based on this assumption.

When phylogenetic analysis is to be approached in genetic terms it is first necessary that correlations be made between morphological characteristics and the genotypes which determine them. The difficulties inherent in making such correlations are well recognized, and some of the problems involved have been discussed by Dobzhansky (1959), Mayr (1959), Simpson (1961) and others. For the most part these discussions emphasize the fact that morphological identity and genetic identity need not be precisely related, and emphasis of this point is certainly justified. Intentionally or not, however, such discussions recognize only the negative aspects of the problem, and they appear to assert that inferences regarding the history of a genotype cannot be drawn from consideration of morphological characteristics. Emerson (1961) has pointed out a logical deficiency of such a position, and a completely negative approach does not appear to be justified.

Several considerations suggest that a certain amount of correlation between genotype and phenotype can reasonably be assumed. First among them is the fact that any operational, and to a large extent any theoretical, approach to phylogenetic analysis must be concerned primarily with the most probable course of events in an evolving system. It is certainly necessary to recognize that some things, e.g., mimetic mutations, can happen. It by no means follows that one must assume such events to happen routinely, or even with such frequency as to seriously affect the validity of conclusions regarding the phylogeny of a group as a whole. As stated by Emerson (1961), "the replicative capacity of the genetic system is even more apparent and precise than its capacity to change," and it is this stability which provides a reasonable basis from which to infer the most probable ways in which genotypes may come into being and change during the course of evolution.

The stability of the genetic system, and its limitations with respect to its

capacity to change rapidly (Haldane, 1957), suggest that a considerable portion of the genetic material in related species will have been derived substantially unchanged from their ancestral populations. Still, genetic differences below the level for morphological detection must be recognized as possible, and it is therefore necessary to make a distinction between gene homology and genotypic homology as these terms are to be used in making taxonomic comparisons. For gene homology the basic unit of reference is a specific allele at a specific locus. For genotypic homology the basic unit of reference is the portion of the gene pool which directly influences the expression of a given phenotype. When making comparisons between species, and when inferences are drawn regarding genotypic homologies underlying their morphological similarities, the genetic components compared *cannot be less* than the total controlling the expression of the characteristic throughout the range of the species and throughout their seasonal fluctuations. A given morphological feature of the individuals within a species will often, if not always, be under the control of a large number of genes, and the characteristics of any two individuals may not rest on identical genotypes. It would therefore be quite unrealistic to adopt a criterion for genetic homology which could not be applied uniformly to comparisons between individuals of one species. On this basis, for example, complete gene identity would not be a suitable criterion.

Simpson (1961, p. 78) has defined morphological homology as "resemblance due to inheritance from a common ancestry," and this definition applies equally well to genotypic homology. In the case of genotypic homology we deal with the inheritance from a common ancestry of the genetic elements which comprise the complex genotypes underlying the morphological expression of a given characteristic seen in several species. A large number of loci will be involved and complete identity of all alleles at all loci is not required, both because apparently identical phenotypes within a species may not rest on identical genotypes and because complete identity of structure between species may not be involved. In this sense then, the taxonomist deals with the *degrees* of genotypic homology rather than with the *absolutes* of gene homology, and the assumption that more nearly identical phenotypes reflect more nearly identical genotypes appears to be justified. Genotypic homology neither requires nor precludes complete gene identity. It does require that a large fraction of the genes controlling the expression of a given characteristic be derived from a common ancestral gene pool.

A special aspect of the problem of genotypic homology relates to the possibility that a developmental pattern, and therefore a particular phenotype, may be adaptive and so be perpetuated, but the genotype controlling this pattern may be sharply changed during evolution (Dobzhansky, 1959). Since the simplest and perhaps most effective way to retain a developmental system is to retain the genotype which originally produced it, this type of change may not be an inevitable consequence of evolution. Operationally, however, the reality of this possibility does not influence phylogenetic analyses. If a specific developmental pattern is to be *perpetuated* in two separately evolving lines, it must have been present in their common ancestral population. When morphological homologies are identified and genotypic homology inferred, the proper inference is not that the present genotypes are identical, but that the ancestral genotypes at the time

of the initial divergence were identical. Genetic continuity is determined by conditions at the time of separation of two populations, not by the terminal genotypes of these populations. Whether the terminal phenotypes result from retention of identical genotypes or from "gradually evolving functional analogy" (Emerson, 1961), the phylogenetic relationships suggested by these phenotypes will remain the same.

Finally, if gene complexes within the species gene pool are taken as the basic unit of reference for making taxonomically relevant identifications of genotypic homology, the possibility for the origin of identical or closely similar phenotypes through parallel mutation becomes rather small. For this to happen, substantially similar mutations would have to occur at a large number of loci and in such a genetic context that selection could mold them into an integrated gene complex which would be expressed as a phenotype so similar as to be confused with that produced by another system of independent evolutionary origin. If only one gene is involved this chain of events is conceivable. When gene complexes are considered, it becomes highly improbable. The possibility is certainly not great enough to invalidate the assumption that morphological identity indicates substantial genotypic homology, or to require us to discard concepts of genotypic homology as theoretical or operational tools. This should not be taken as implying that the possibility for independent origin of "homologous" phenotypes is something to be ignored or lightly dismissed. The problem still exists, but it is not a problem which should be of *first* concern when preparing a working model for phylogenetic analysis.

In passing it may be well to emphasize one feature of the chain of events just outlined which will be of significance later. This is the fact that, at the time of its mutational origin, whether this involves one mutational event or several, a necessary condition is that a new gene be adaptive. And this requires not only a congenial genetic background but also a congenial environment. Therefore, if a particular gene or genotype is to be established in a population, it must not only occur in a gene pool in which the appropriate genetic background is available (i.e., a background in which it may be expressed as a specific or mimetic phenotype), but this gene pool must also be present in an environment such that the new allele be adaptive. The establishment, which is *not* to say the fixation, of a gene in a population, i.e., its primary integration into the gene pool, is the critical evolutionary step which makes that gene or genotype available for future change. It is this integration, rather than the origin of the gene by mutation, which has such a low probability of occurrence in independently evolving gene pools. The same mutational events probably occur many times, but only those which occur in the "right" context are of phylogenetic significance. The probability that the "right" context has been produced independently several times in several phyletic lines is almost surely small.

*The possible fates of genotypes in evolving systems*—As stated previously, the basic problem of phylogeny is that of detecting genetic continuity. Before this problem can be approached it is necessary to determine the ways in which genotypes may traverse a succession of gene pools and the ways in which morphological characteristics may be distributed to descendent populations. For simplicity, the genotype for only a single trait will be followed. Also for simplicity,

it is convenient to treat the genetic system which determines the trait as involving only a pair of alleles at a single locus. Thus, the following discussion is a great over-simplification, phrased in the language of "bean-bag genetics" (Mayr, 1959), but it illustrates in general terms the possible sequences of genetic events which may have occurred during the speciation processes which established the various phyletic lines within the genus.

As a starting point a hypothetical population, genetically uniform (AA) with respect to the determiners for a given characteristic, may be assumed. In the course of time, through mutation and selection, this uniformity is lost and new genetic complexes arise (AA′) which have a direct effect on the trait in question. It is not strictly necessary that the phenotypic expression at this time (AA′) be specified, but it seems probable that it would be shifted somewhat from the earlier form and that this altered phenotype, whether defined in morphological or physiological terms, would be maintained in the population by one or several of the balance mechanisms currently postulated as being of importance in the perpetuation of genetic heterogeneity within a population. Mechanisms adequate for preserving heterogeneity have been proposed and discussed by many workers (e.g., Dobzhansky, 1951, 1955; Dempster, 1955). For the present discussion, mechanisms, so long as they exist, are not the important thing. What is important is that, during the course of evolution, the transition from a population showing one form of expression of a trait to another showing a derived form of the trait must involve temporally intermediate populations heterozygous for the genotypes determining the alternative forms of expression. If such a heterozygous population can come into being and be maintained for an evolutionarily significant period of time, there is a distinct problem concerning the fates of the possible genotypes (AA, AA′, A′A′) which will be produced within this gene pool. Theoretically, there are three possibilities. First, the old genotype may be completely replaced by the new (AA to AA′ to A′A′). Second, an equilibrium level may be attained and perpetuated so that both genes (genotypes) persist in the population (AA to AA′ to AA′, etc.). Third, the direction of selection may be reversed, and the old genotype will again predominate (AA to AA′ to AA). There is, in addition, the problem of the hypothetical, heterozygous population if, for one reason or another, it becomes subdivided and its subdivisions diverge genetically to such an extent that they would be recognized as distinct species. Again, the possible fates in these subpopulations are the three just listed, and we cannot say what would happen in any instance. There is no *a priori* basis for asserting that *only* one or another of these events occurs during evolution.

The first alternative (AA to AA′ to A′A′) is often the only one recognized by practicing taxonomists or phylogeneticists. A discussion by Hennig (1956) provides an example of an approach which recognizes only this alternative. Here he says, "Nothing is more obvious than the conclusion that two species which share the derived form of a characteristic have acquired this from a common stem species. *This would, accordingly, indicate that they are more closely related to each other than to species which do not show this form of the characteristic*" (writer's translation and italics). The general sequence of mutation and speciation events which would be required for the second statement (italicized) to hold true is shown in Figure 47 (left). Minor changes could be made in this sequence.

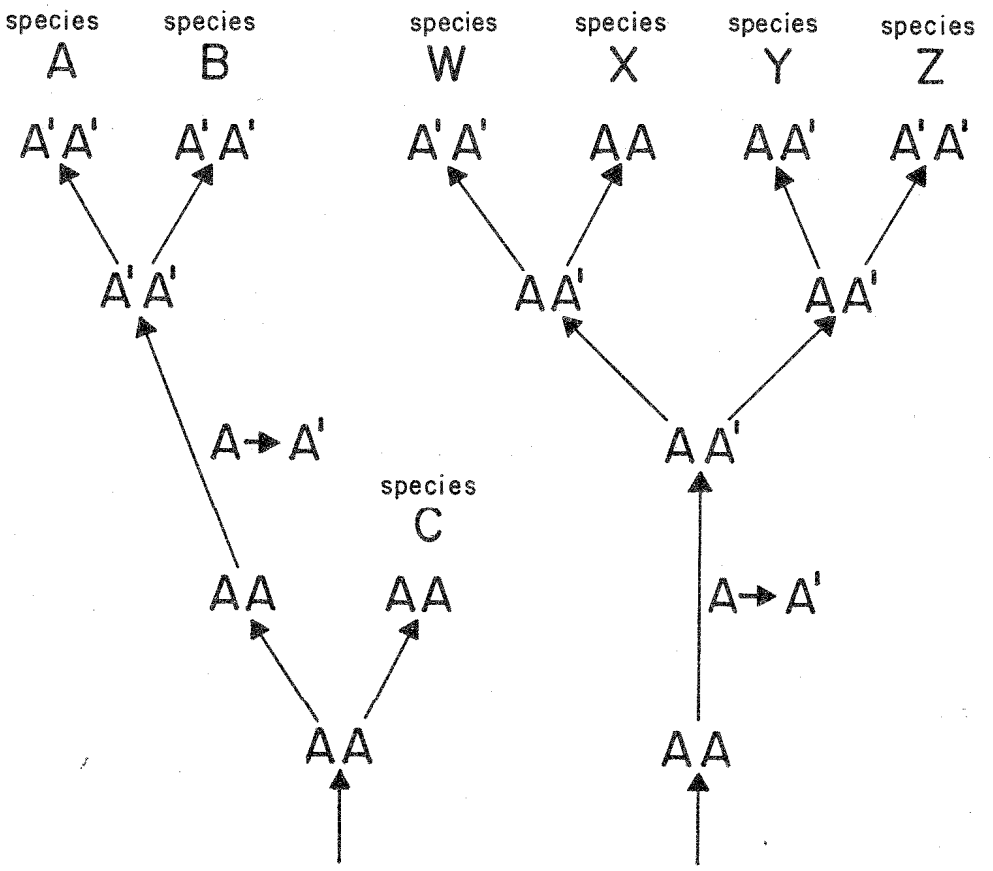


FIG. 47. Two alternative evolutionary sequences. The one on the left illustrates evolution according to the "classic" hypothesis. The one on the right illustrates a possible sequence based on the "balance" hypothesis. See text for further explanation.

For example, the mutations of A to A′ could have occurred during the first speciation process, but only in the subpopulation shown on the left. Or, if they occurred in both subpopulations, which seems more probable, those in the subpopulation at the right would have experienced strong negative selection while those in the subpopulation at the left would have been strongly selected for. Such slight shifts, however, would not alter the overall requirements or the general pattern. In this sequence species A and B share the derived form of the trait and are phylogenetically more closely related to each other than to species C, which shows the primitive form of the trait. Such a series of events has undoubtedly occurred during the formation of many groups of species. However, other sequences must be recognized as possible, and one such is shown in Figure 47 (right). In this sequence, the A′ allele is established, but not fixed, in the population prior to the first speciation event, and heterozygosity at this locus is maintained during and after speciation. Thus, the next speciation event in each new phyletic line involves a population heterozygous for the locus in question, and the alleles (genotypes) may segregate by selection in various ways to descendent populations. Among the possibilities shown, species W and Z share the derived form of the

trait. Species X shows the primitive form and species Y might show some intermediate form of expression. If genetic and speciation events followed some pattern such as this, at least in some instances, the assumption that two species showing a derived form of a characteristic are most closely related would not hold true. Species W and Z share the derived form of the trait, but species W is phylogenetically most closely related to species X, which shows the primitive form of the trait.

The two models, Figure 47 left and right, are related somewhat in the same manner as are the "classic" and "balance" hypotheses of population structure outlined by Dobzhansky (1955). That on the left follows the "classic" system, with its populations being primarily homozygous and its genetic diversity "either neutral, or transient or morbid" (Dobzhansky, 1955). In the example, the genetic diversity is transient and interposed between speciation events. The sequence on the right incorporates elements of the "balance" hypothesis, at least insofar as it emphasizes the role of heterozygous populations in evolution. To some extent, events shown in the sequence on the right may involve homoselection and hetero selection as defined by Carson (1959).

With reference to problems of phylogeny, if speciation may occur in this fashion (Figure 47, right; and see Carson, 1959), the inevitable result will be the perpetuation, for a considerable period of time, of a heterozygous population potentially capable of giving rise to descendent populations homozygous or heterozygous for alternative genotypes (species Y in Figure 47 could continue to evolve and speciate in this fashion, etc., etc.). Thus, within a phyletic line where evolution has occurred on some variation of this pattern, alternative forms of a trait may be distributed somewhat at random. *Possession by two species of a particular characteristic will indicate only that they are derived from some common heterozygous population*, which may be quite distant from either of them. They may belong to separate phyletic lines, each taking its origin from the heterozygous population in which the potentiality for segregation first appeared. A "sequence" of morphological types will not necessarily indicate an evolutionary sequence in the sense that species showing the primitive form appeared first, those intermediate appeared second, and those showing the derived form appeared last. Thus, before one can determine what significance is to be attributed to a morphological sequence of types, one must determine whether the evolution of the group has followed a pattern similar to that shown on the left in Figure 47 or similar to that shown on the right. These two patterns themselves only represent extremes, particularly if more complex genetic systems are considered and if several characteristics are considered simultaneously. A complete spectrum of alternatives lies in between, and the actual evolutionary pattern of a group should fit somewhere in the spectrum of possibilities.

Although the alternatives just noted appear to be possible, this alone does not make all of them probable. Events may be possible without their making any significant contribution to evolution. It is therefore necessary to seek evidence which might give some indication that evolution would be expected to follow, and at times has followed, a pattern similar to that outlined to the right in Figure 47. At a general level, the fact that heterozygosity contributes to the evolutionary potential of a population is widely recognized, and there is more than ample

evidence that heterozygosity exists in *Drosophila* populations in nature (e.g., Dobzhansky, 1951; da Cunha, *et al.*, 1959). When developing a phylogeny from living species, only those ancestral populations which gave rise to descendent species are detected. These are the populations which, at a given time level, had the greatest evolutionary potential, and, as a rough approximation, their evolutionary potential can be related directly to the number of species derived from them. It seems reasonable to assume that the detectible populations depended, at least in part, upon heterozygosity for their demonstrated capacity to evolve. Further, it seems reasonable to assume that those populations which continued to evolve most rapidly, and which produced the most descendent species, were able to do so because they *fell heir* to a part, or all, of the heterozygosity of their ancestral population and did not have to accumulate a complete new store of variability from which to mold adaptive genotypes. Conversely, a population which lost a large share of its genetic diversity, perhaps through homoselection, would have little, or at least less, capacity to proliferate rapidly. Before such a population could proceed to further subdivide and speciate, it would need to build up a new store of variability from which to produce a diversity of adaptive genotypes. Thus, in rapidly evolving populations heterozygosity may have been perpetuated, and, if so, it will have provided some of the genetic variability from which descendent adaptive genotypes have been fashioned. The assumption that all, or even most, speciation events entail a restriction or elimination of genetic variability, so that future evolution must depend on and reflect *only* variability injected into the gene pool by new mutation, seems unnecessarily rigid and not completely consistent with available information. Some speciation events almost surely do entail a substantial loss in heterozygosity, but it is not anticipated that populations passing through such evolutionary bottlenecks will have contributed as significantly to living populations as have those whose genetic variability has been less restricted. And there is no reason to assume that genetic determiners for characteristics of taxonomic value have been immune to retention or perpetuation in the heterozygous state, or that stem populations for families, genera, species groups, etc., have been conveniently homozygous.

While these speculations may have some merit of plausibility, it is still necessary to obtain more nearly objective evidence that such evolutionary sequences have occurred. For this we turn to an evaluation of the distribution of morphological characteristics among species for which a defined phylogeny is available. The most convincing and extensive evidence to this end comes from species in the repleta complex of the subgenus *Drosophila*. Extensive investigations, primarily by Wasserman (Wasserman, 1960, and This Bulletin), have provided a cytological phylogeny for these species (see Figure 25), and this can be correlated with the morphological characteristics already described. Such correlations have been made for the characteristics treated in this study, and they all show the same general pattern. Thus, details for only two of these, the characteristics of the spermathecae and of the ejaculatory bulb, will be summarized.

The complete data for spermathecae from species of the repleta complex are given in Figure 29. Figure 48 shows a portion of this data as it appears when combined with phylogenetic information from Figure 25. It should be emphasized that the cytological data show the various branches and single species to be de-

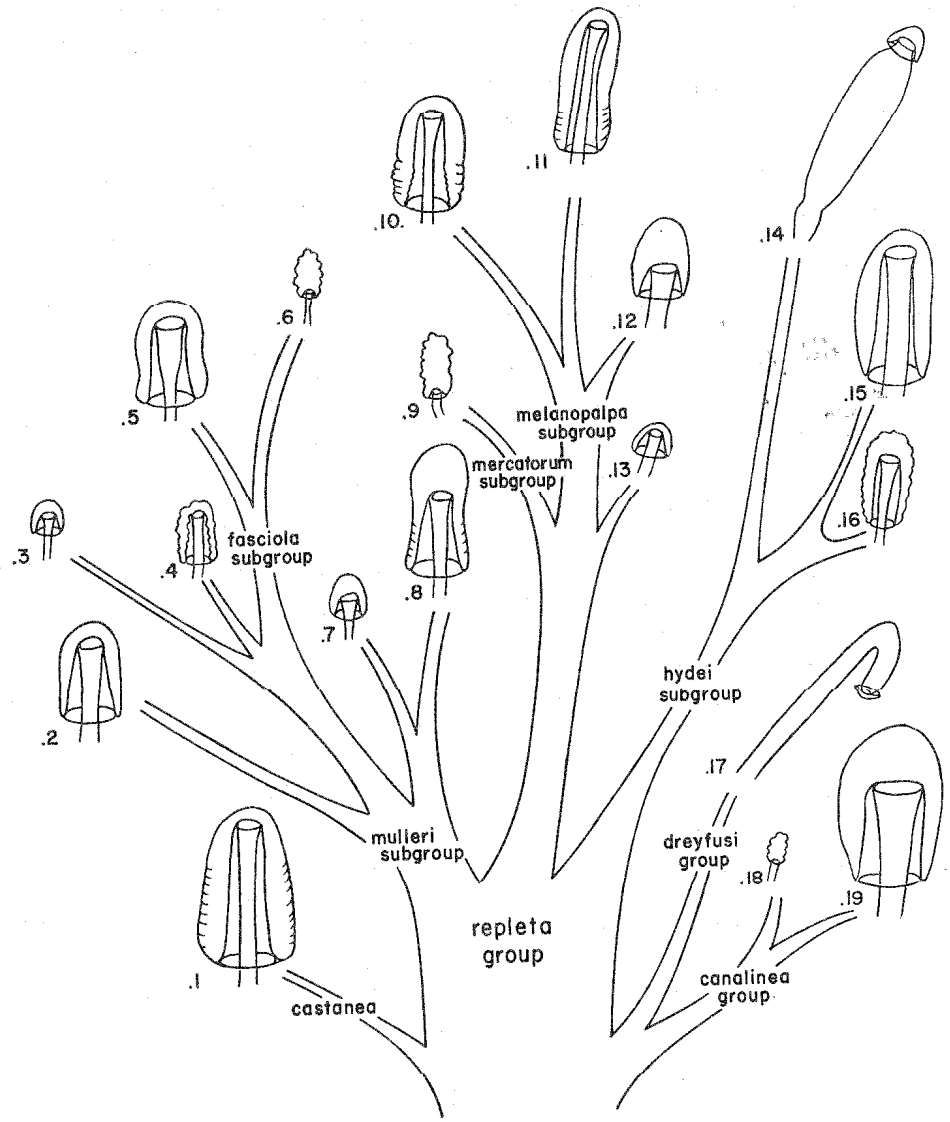


FIG. 48. Evolution of spermathecae in the repleta complex. Based on the cytological phylogeny given in Figure 25.

.1 castanea
.2 meridionalis
.3 mulleri
.4 pachuca
.5 fulvalineata
.6 moju
.7 buzzatii
.8 pegasa
.9 mercatorum
.10 limensis
.11 canapalpa
.12 fulvimacula
.13 peninsularis
.14 hydei
.15 nigrohydei
.16 bifurca
.17 briegeri
.18 paracanalinea
.19 canalinea

rived from the repleta standard gene arrangement. Cytological data give no information regarding the order or position of these branches relative to each other. The arrangement of branches in Figures 25 and 48 is that most compatible with morphological characteristics of the different species. In Figure 48 the

position of *castanea* and of species of the canalinea and dreyfusi groups has been shifted downward. This was done for convenience in making the figure and no particular significance should be attributed to it at the present time.

As has been noted previously, no primitive spermathecal type can be designated, although that shown in Figure 48.18 seems a reasonable choice. Since the major problem is that of the role of heterozygous populations during evolution, designation of primitive types is not critical. The behavior of primitive types can be followed more readily with characteristics of the ejaculatory bulb, and this aspect of the problem will be considered later.

Species of the repleta complex present several spermathecal types, and, as can be seen from inspection of Figures 27–30, the majority of these types are restricted to this section of the genus. The distribution of types among these species is, however, not completely regular. If only major branches are considered, the type shown in Figure 48.3 is seen in members of two branches (Figures 48.3 and .13) and in several species derived independently from the standard (e.g., Figure 48.7). The majority of species having this type, however, belong to the mulleri complex shown to the upper left in Figure 25. The type shown in Figure 48.6 is seen in the majority of species from the fasciola subgroup and in both species from the mercatorum subgroup. Again, each subgroup belongs to a different phyletic line. The type shown in Figure 48.14 is seen in three members of the hydei subgroup, and a very similar type is seen in species of the dreyfusi group (Figure 48.17). Other types (Figures 48.4 and .16; 48.2, .5 and .15; 48.18 and 29.27; etc.) show similar distributions, but their resemblances are not as sharp as those within the types first cited. It is obvious that a basic tenet of the classical approach to phylogeny, that species sharing a given trait are phylogenetically more closely related to each other than to species lacking the trait, does not hold when applied to relationships among species of the repleta complex.

Almost exactly the same type of distribution is seen with the characteristics of the ejaculatory bulb (Figure 49). We have good reason to assume that the simple ejaculatory bulbs (e.g., Figure 49.4) are the more primitive. Ejaculatory bulbs shown in Figure 49.4 and 49.8 are virtually identical, and they are presumably near the primitive, not only for the repleta complex but also for the genus as a whole. The type shown in Figure 49.5 is derived, and it is widely distributed among the various branches of this complex (Figures 49.2, .5–.7, .9–.10, .20). The type shown in Figure 49.3 is found in only two of the branches (Figures 49.3, .11, .13–.14), and that shown in Figure 49.12 has thus far been identified only from members of one branch (Figures 49.12, .15–.16). Here also then, we have a pattern similar to that shown to the right in Figure 47, and a comparable pattern is seen for the distribution of number of testis coils among these same species (see numbers in parentheses with the species names in Figure 25). There is, therefore, more than ample indication that independent phyletic lines have been initiated by populations heterozygous for genotypes determining several forms of expression of a given trait. Further, there is also evidence (e.g., Figures 49.3–.5 and 49.7–.8) that primitive genotypes may be "fixed" in species descended from populations presumably heterozygous for primitive and derived genotypes. Thus, we do see a type of pattern among these species which is sub-

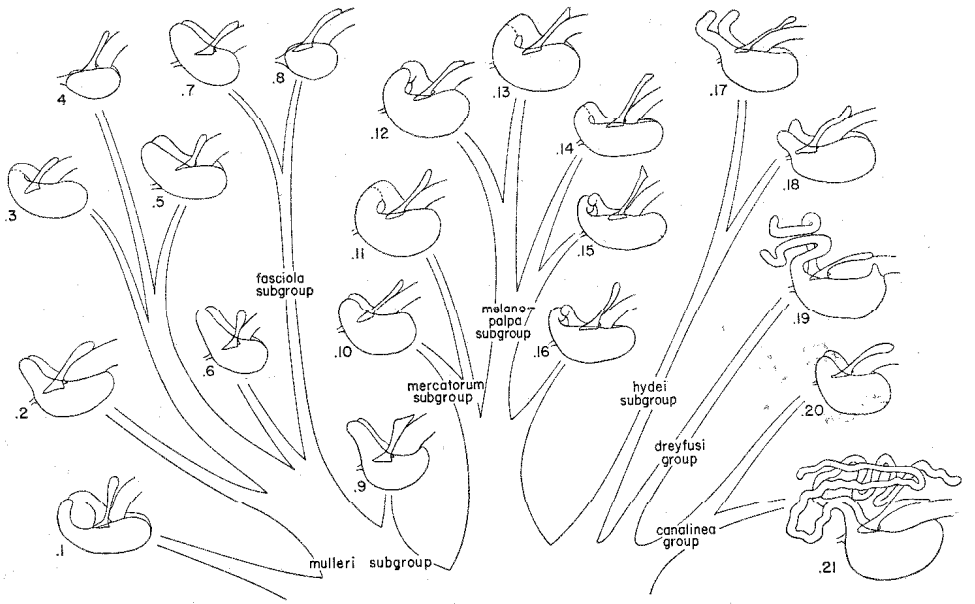


FIG. 49. Evolution of the ejaculatory bulbs of the repleta complex. Based on the cytological phylogeny given in Figure 25.

| | | |
|---|---|---|
| .1 castanea | .8 moju | .15 fulvimacula |
| .2 meridionalis | .9 pegasa | .16 peninsularis |
| .3 arizonensis | .10 mercatorum | .17 hydei |
| .4 mulleri | .11 paranaensis | .18 bifurca |
| .5 aldrichi | .12 limensis | .19 camargoi |
| .6 tira | .13 canapalpa | .20 paracanalinea |
| .7 mojuoides | .14 fulvimaculoides | .21 canalinea |

stantially that predicted if heterozygous populations play a major role in evolution and if genotypes may traverse gene pools in substantially the way outlined earlier. In short, if we take this data at face value, without elaborate rationalization and without attempts to explain it away, we see a pattern of evolution consistent with the best documented characteristics of evolving systems and of the behavior of the genetic material itself.

Before turning to comparable evidence from other sections of the genus, the implication of morphological evolution in the repleta complex can be evaluated in somewhat greater detail. It would appear that the population ancestral to these species was moderately heterozygous at the loci whose activity is detected morphologically in characteristics of the spermathecae, the ejaculatory bulbs and the testes. Its complete store of variability was not passed on equally to all subpopulations deriving from it, or, more correctly, as its subpopulations diverged, re-integration of their gene pools resulted in somewhat different gene complexes being maintained in each. Thus, while some morphological types are common to two or more lines, the total pattern of variability in any one line is slightly to markedly different from that seen in other lines. These different patterns may have arisen in two different ways. From a given array of genetic material, several to many adaptive combinations may be possible, and we may

infer that most of these different combinations will be reflected in slightly different phenotypes. Thus we see an array of types (Figures 48.2, .5, .8, etc.) which are substantially the same in general features but which differ from each other in detail. Such slight variations reflect either the different ways in which substantially similar genotypes may be integrated with each other and with other elements of the gene pool, or they reflect the contribution from new mutations which have occurred subsequent to the separation of the phyletic lines, or they reflect both of these factors. The contribution of mutation to the diversity of phenotypes can be inferred chiefly through noting the number of phenotypes *distinctive* to any one line. That shown in Figure 48.11 may be one such. This method of assessing the contribution of mutation is, of course, only an approximation, since the "distinctive" genotype may actually have been potential in the ancestral population but eliminated from all but one of the descendent populations. Or it may still be "potential" in genotypes of some of the existing species from several of the phyletic lines. We have no way of predicting the morphology of individuals of ancestral populations, and thus we cannot detect species retaining *in toto* the ancestral genotype. In spite of the limitations of the estimate, it seems probable that new mutations have contributed very little to the morphology of the species in this complex. By far the greater proportion of phenotypes are of such wide distribution that their genotypes must have been derived from genes already established in the ancestral population common to these species. Thus, distinctive phenotypes may owe their origin to new mutation or to an integration of a different array of genes from an ancestral population. The *major features* of types recurrent in several phyletic lines take their origin from the gene pool of ancestral populations, and hence they evidence genotypic homology.

In the light of this situation, the implications of genotypic homology must be reassessed. Genotypic homology does not require the direct derivation of a genotype from a population expressing that genotype. To return to the earlier, simple model, the genotype, A′A′, is homologous with other A′A′ genotypes regardless of whether it was derived from a population having the genotype A′A′, or from one having the genotype AA′. In the model shown in Figure 47, species W and Z have homologous genotypes, but neither of them was derived from a population expressing this genotype. Genotypic homology may be determined by common ancestry from an original population in which the genotype, A′A′, was potential, as well as from a population in which this genotype was fixed. Thus, a given genotype may be produced independently in separate phyletic lines, but the production of this genotype can only take place from a gene pool in which the necessary elements were already established. Genotypic homology requires *genetic* continuity, but it does *not* require *genotypic* continuity. Parenthetically, it may be noted that recognition of this fact removes so-called parallel evolution from the ranks of the inexplicable to those of the expected.

If we do not accept independent integration of genotypes in descendent populations as an adequate explanation of data such as is shown in Figures 48 and 49, we are left with a remarkable system of convergent evolution. The series of types seen on the central branch of Figure 49 may serve as one of many possible examples from the repleta complex. The type of ejaculatory bulb shown in Figure

49.11, .13 and .14 must have arisen independently at least three times on this branch alone and probably at least three times in species from the mulleri subgroup; once in the population from which the sibling species *arizonensis* and *mojavensis* arose, once to produce *anceps*, and probably once also to produce *serenensis* (see Figure 17). This type is not seen elsewhere in the genus, and its appearance within this closely related cluster of species strongly suggests that its occurrence here results from genetic continuity between these species. The distribution of this type is such, however, that its appearance cannot be attributed to genotypic continuity. In the figure, the type shown in 49.13 and .14 and the type shown in 49.12 and .15 cannot both owe their origin to genotypic continuity. One or the other must have originated independently, and from the standpoint of the present discussion it is immaterial which one did. In all probability, both owe their appearance to independent integration of their genotypes from a common heterozygous gene pool. The simplest alternate to this explanation would assume that the type shown in Figure 49.13 differs genetically from that seen in close relatives by a very low number of genes. Thus, the conversion of the type shown in Figures 49.12 and .15 to that shown in Figures 49.13 and .14 might involve a relatively simple genetic change and hence may have arisen independently by mutation. This interpretation would not be too difficult to accept if we had to consider only the sequence from Figure 49.12 to 49.15. At their time of divergence the ancestral gene pools would have been very similar. Specific mutations would probably occur with about equal frequency in each of these diverging gene pools and *might* be integrated independently in each. This possibility, however, becomes increasingly unlikely when we consider the distribution shown in Figure 49.3 to .5 and 49.10 and .11. The basic question here is whether origin by independent mutation or by independent integration of the genotype is more probable. If we assume common origin from a heterozygous gene pool, all that is required is a reintegration of the gene pool from elements *already tested* and probably at frequency levels such that the changes involved could be quite rapid. If we must start each time with mutation as the origin of the genotype, the appropriate genes must first arise, then they must be brought to a frequency level such that selection may act, then they must be integrated with the gene pool, and finally they must reach a level of integration such that they are uniformly expressed as characteristic of the species in question. While such a sequence is conceivable, it hardly seems to represent the most probable explanation. The time element alone, and the expense to the population (Haldane, 1957), would seem to preclude this. Only the last step is required if separation of the phyletic lines involved populations already heterozygous for the requisite genotypes. The origin of the genes and their primary integration into the gene pool need only have happened once. The expense of completely replacing one gene with another would be spread through a long period of evolutionary time and would not be borne by a single population during the formation of a single species. Once the primary integration into the gene pool is completed, the fates of potential genotypes are determined by adaptive situations to which the populations and their subdivisions are subjected. The number and distribution of types seen in the repleta complex suggests that, from the original heterozygous gene pool, only a limited number of adaptive combinations of genes controlling a given characteristic was possible.

These combinations have recurred, and one or another of these has been incorporated into the gene pool of each descendent species. Contributions from mutation subsequent to separation into independent gene pools cannot be excluded, but they seem to have played a minor role in determining the phenotypes expressed. Mutations which have been incorporated into the gene pool of a species *since* that gene pool became an independent evolutionary entity will, in most cases, have their major effects on the phenotypes to be produced in the future. Rapid evolutionary change (*phenotypic* change) is possible from such mutations, but evidence from the repleta complex suggests that it is not common.

Genes and genotypes controlling separate characteristics, e.g., those of the spermathecae and those of the ejaculatory bulbs, have been relatively independent of each other. The origin of the phyletic line leading to the mulleri complex species apparently involved a population in which the genotypes for characteristics of the ejaculatory bulbs (Figure 17) were relatively heterozygous while that for spermathecal type was substantially fixed. The line leading to the melanopalpa subgroup retained heterozygosity for characteristics of both the spermathecae and the ejaculatory bulbs, etc. (The term, fixed, as used here and elsewhere in this communication, must be interpreted rather loosely. At present there is sufficient uncertainty regarding the genetic structure of populations, e.g., Crow, 1961, to require a somewhat imprecise form of reference. The term, stabilized, might be a suitable alternative.) Thus, the general pattern of evolution during the transition from one morphological type to another becomes evident. It need not, and probably does not, involve a stepwise alteration of the phenotype. Rather, it involves the production of an array of types. When two or more systems are in transition in the same gene pool a great number of genotypic combinations are potential, and many of these are actually realized in descendent species. If evolution within the genus has been of this general type, it will be necessary to modify methods of phylogenetic analysis to accommodate for the irregular distributions of characteristics which result from such a system. Evidence that this has been the general pattern of evolution throughout the history of the genus comes from several sources and from all of the species groups available for study (except those consisting of only a single species). Only two examples will be treated in any detail.

First, we can see that evolution of this type is not peculiar to species of the repleta complex by analyzing the distribution of characteristics among species of the virilis group. A cytological phylogeny for these species is also available (Stone, *et al.*, 1960) and is shown, correlated with certain of the morphological characteristics, in Figure 50. The species of this group do not vary morphologically among themselves to the extent that species of the repleta complex do. Still, the same pattern of distribution is seen here. Two general types of paragonia are present in the group (e.g., that of *virilis* and that of *ezoana*). The population designated as Primitive I was probably heterozygous for genotypes determining these two types. The derivation of the population designated as Primitive II from this population may have involved homoselection toward one of these genotypes, but the population designated as Primitive III apparently retained heterozygosity for both genotypes. The population from which *ezoana* and *littoralis* were derived likewise retained heterogygosity, but that from which

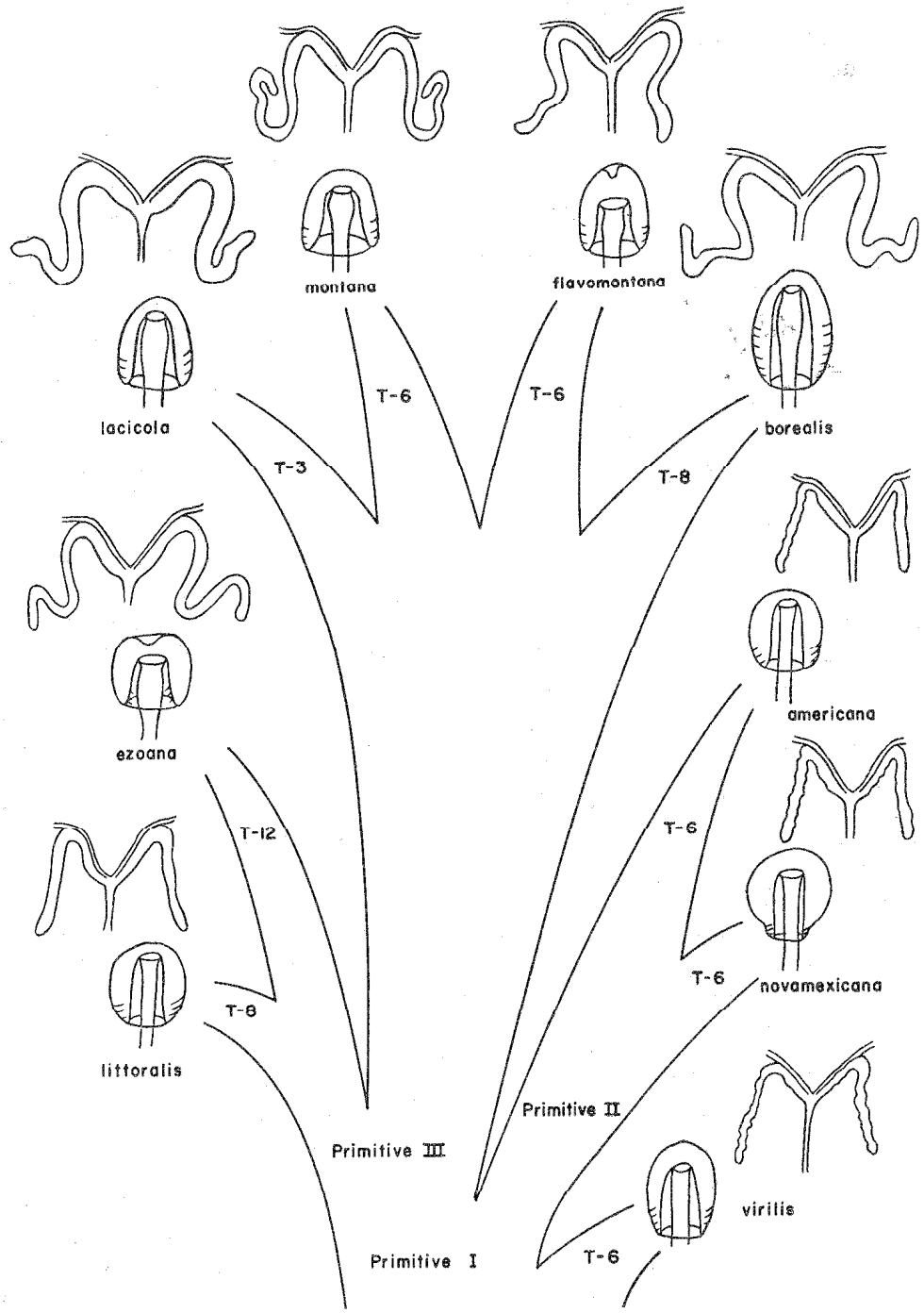


FIG. 50. Evolution of paragonia, spermathecae and testes of species in the virilis group. Correlated with cytological phylogeny of Stone, *et al.* (1960).

the other four species were derived became homozygous for the second genotype. A comparable pattern of distribution is seen for characteristics of the spermathecal duct and for number of testes coils (T-6, T-8, etc. in Figure 50). Other characteristics of these species, e.g., those of the ejaculatory bulb (Figure 16), those of the anterior pupal spiracle (Figure 42.17), etc., are distinctive to this group and almost completely constant within it. Apparently their genotypes originated and were fixed prior to the speciation events which produced the group as we now see it. These then, are the "good" taxonomic characteristics for the group. The others, those which are variable within the group and which do not define phylogenetic separations, are "bad" characteristics, and they fall in this category because the species showing them originated from populations in which genotype fixation had not yet occurred.

Extensive cytological phylogenies are not available for other groups of species, so further evidence for this type of evolution must be obtained in a different way. The evidence required can be deduced from consideration of the type of character distribution expected if evolution has followed the "classic" pattern (Figure 47, left). In such a case, genotype fixation is assumed to occur during each speciation process, those which established populations initiating phyletic lineages as well as those producing individual living species. Thus, a complete correlation of all characteristics is required. Any deviation from this pattern is assumed to result from mutation following speciation, and recurrence of types in separate phyletic lines should be rare. It should be possible to arrange morphological types in a stepwise sequence, and the sequences for one characteristic should correspond to those for any other—not necessarily in the sense that each morphological characteristic changed at the same rate and time as any other, but in the sense that primitive characteristics will generally be associated, derived characteristics will always be associated, and intermediate associations will show a recognizable sequence from the primitive association to the derived. Admittedly, these specifications are extreme, but they indicate the general rquirements of such a system. One example will serve to show the type of character distribution actually encountered in all species groups in the genus. Characteristics from eight of the thirteen species investigated from the quinaria group are shown in Figure 51. Space does not permit showing all thirteen in one figure, but these suffice to indicate the general pattern. The morphological sequence from left to right is arbitrary and follows the evolutionary change in the ejaculatory bulb (line 3 of Figure 51). Those types shown at the right in line 3 are distinctive, and their genotype presumably arose during the evolution of this group. If this sequence is held constant (changing the order within one of the bulb types will not help matters), then characteristics of the spermathecae, paragonia, vasa deferentia, anterior pupal spiracles and testes follow no regular pattern. There is a recurrence of general types, but it is impossible to arrange these species in any order (branching or linear) such that a regular sequence for all types is seen. The situation is further complicated by the fact that many of the types seen here are also seen in species outside of this group. The general type of anterior pupal spiracle seen in *tenebrosa* and *guttifera*, for example, occurs in species from the testacea group and in many species from the tripunctata, rubrifrons and macroptera groups. It must have arisen at least twice in the quinaria group, or

FIG. 51. The arrays of types produced by evolution from a heterozygous stem population. For further explanation see text.

else the type of ejaculatory bulb seen in *guttifera* arose twice. It must also have arisen many other times in other species of the quinaria section, but not elsewhere. Either the recurrent types arose by mutation, or these characteristics represent an array of types derived through evolution from a common heterozygous gene pool, and, on the whole, the latter process is more probable. Evidence that this type of evolution has occurred throughout the history of the genus will become more evident as the phylogeny is developed. To anticipate this, however, data included in the pictorial phylogenies (Figures 14, 20, 21, 24, 26, 31, 32, 40, 41, 44, 46) show that the stem populations from which major lines developed were themselves heterozygous for genotypes which later became fixed and characteristic of groups of species. Thus, analysis of phylogenetic relationships within the genus will require methods which recognize the taxonomic implication of heterozygous populations in evolution.

*Method for analyzing phylogenetic relationships*—Standard correlation methods (Sturtevant, 1942; Malogolowkin, 1953) are adequate for deriving static classification, but they are not completely suitable for analyzing the dynamic pattern of change which attends the evolutionary development of large groups of species. They establish with reasonable certainty what groups exist, but they do not tell how these groups came to show their particular combinations of characteristics. As indicated by Michener and Sokal (1957), the systematist must first determine relationships in a nonhistorical sense, and correlation methods are suitable to do this. The systematist may then *decide* on the most probable lines of descent. Some of the factors which must be considered when such decisions are made have already been discussed. The following method of analysis is one which takes into account the possibility that evolution may have proceeded from heterozygous populations. It is, however, quite suitable for analysis of phylogenetic relationships in groups whose evolution has followed the classic pattern, so it is not strictly necessary that the manner of evolution be known before the method is applied. The results of the analyses themselves will indicate the type of evolution involved.

Three major correlations may be made when a phylogenetic analysis is begun. The first of these is the total phenotypic comparison of the type described by Michener and Sokal (*op. cit.*). This is the correlation most useful to the systematist in determining a basis for classification, and its chief purpose is to subdivide large complexes of species into groups of closely related forms. Such correlations may provide some indication of phylogeny, but their chief usefulness is in breaking a group down into subunits which may be conveniently analyzed. The second type of correlation is one involving only primitive characteristics, and the third is one considering only derived characteristics. These last two correlations often need not be made in a formal sense. Inspection of the distributions of such characteristics among previously established groups will usually be sufficient. The purpose of these correlations is to establish major dichotomies and to indicate evolutionary trends in the group as a whole. Often, but not always, they will be a routine part of a taxonomist's evaluation of species relationships. Since these phases of analysis are generally standard practice, and since descriptions of general methods applicable to such study are readily available (Sturtevant, 1942; Michener and Sokal, 1957), they will not be considered here.

For the purpose of phylogenetic analysis, the genus *Drosophila* provides exceptional material. Classification within the genus has been established on a firm and objective basis by Sturtevant (1942), and this classification has been confirmed and extended by many workers since that time (Wheeler, 1949b; Patterson and Stone, 1952; Okada, 1956; etc.). Of primary importance for this analysis is the fact that the objective reality of the taxonomist's estimation of relationship has been substantiated by genetic and cytological investigation. Such studies have been made for many of the major species groups within the genus, and they confirm the geneological propinquity of species placed together in species groups (see Patterson and Stone, 1952, for summary to that time). It is therefore possible to restrict analysis of phylogeny to relationships between species groups rather than to relationships between individual species, and this greatly simplifies the problem. From genetic and cytological evidence, we may infer that species within a species group have originated from a common ancestral population and that this population was evolutionarily distinct from other such populations. The absolute validity of this inference is not required since the methods to be developed will indicate occasional erroneous classifications. Its validity is more than adequate for the present purpose.

Once groups of species have been established, further analysis proceeds by examination of the distributions of single characteristics. When living species provide the material investigated, phylogeny is determined by inference, and specifically by inference regarding the history of distinct genotypes. It is not to be expected that genotypes for several characteristics will have identical histories, and therefore they cannot be considered *en masse*. Herein lies one of the disadvantages of character correlations as indications of phylogeny. Of necessity, correlations must deal with many characteristics (genotypes) simultaneously, and hence they cannot give a detailed picture of the history of any one of them. They do give a picture of total genotypic change, but sequence of change must be determined subjectively if such methods are used.

As already indicated, the method for analysis of phylogenetic relationships within the genus must be one which does not involve the assumption that gene fixation has occurred at any one level. When and where character fixation first appeared may be inferred after the major outline of the phylogeny is determined, but not before. The method of analysis is eminently simple and may represent only a formalization of intuitive methods utilized by many systematists. Its steps are as follows:

1) Determination of general direction(s) of change for all characteristics. This need only be done tentatively since the major purpose of this step is to provide orientation for further analysis. This part of the present analysis was included with the earlier descriptions and need not be recapitulated here.

2) Analysis begins with choosing one characteristic, preferably one which allows subdivisions into the largest groups or which appears to separate distinct phyletic lineages. Characteristics of the paragonia, for example, are of this type and were used as the starting point for phylogenetic analysis of the genus. Characteristics of the spermathecae, on the other hand, are much too detailed to be used in initial stages. They could be used. In fact, *any* characteristic could be used, but the analysis is simplified if it is begun with the more general character-

istics. The distribution of the different forms of a trait among the species in each species group indicates the genotypes which were potential in the stem population for that group. From the inferred genotypes of these stem populations, one then infers the genotypes of the populations from which they were derived, etc. When two or more stem populations appear to be derived from populations having the same general genotypic composition, lines coalesce into single populations. Essentially, we are tracing geneological streams *back* through time to their point of confluence (Hennig, 1956). Examples will be given later to illustrate the steps and interpretations involved. A basic assumption to be made at all levels is that the population antecedent to the one in question was heterozygous for alternate genotypes, even though the population *from* which one is proceeding (backward) may appear to have been homozygous. The antecedent population may actually have been homozygous, but assumption of heterozygosity is *least limiting*. It is the most objective assumption which can be made at the start of the analysis. As analysis of other characteristics proceeds, accumulating evidence will often indicate general levels at which fixation of a given genotype has occurred.

3) Analysis of remaining characteristics is carried out in the same manner, each time working backward from the stem populations of species groups. Characteristics are analyzed one at a time, in sequence from the more general to the more specific. The results of each analysis are superimposed on the cumulative pattern produced from the previous ones. Addition of information from each new characteristic may suggest shifting the point of origin of the stem populations for various species groups, but the magnitude of these shifts will be limited within the pattern already adduced.

4) It will often be convenient to omit difficult groups from the first analysis. Once the major sequences of populations have been determined, genotypic characteristics of the stem populations of the difficult groups can be inferred. These can then be compared with the genotypes already established within the major pattern to determine their probable origins. As information from additional species becomes available it can be treated in the same fashion, as can information from species in other genera, etc.

5) The last step in the analysis is the determination of the histories of the genotypes of the individual characteristics. Once the major sequences of intermediate populations have been inferred from *all* characteristics, individual genotypes may be followed *from* the stem population to the terminal groups of the established phyletic lineages. This constitutes the final evaluation of the phylogeny.

Complete details of this analysis for the genus *Drosophila* are too voluminous to be outlined in the space available. A representative analysis, utilizing only a limited number of characteristics, will be given.

*Abbreviated analysis of relationships within the subgenus Drosophila*—The salient features of the method can be seen most easily when a limited number of species groups from the subgenus *Drosophila* is used. The first two steps in the analysis of this subgenus are shown in Figure 52. For brevity, three general types of paragonia may be identified, and the distribution of these types is shown in the inset at the upper left of the figure. The two major types are both robust forms, one having a single, high arch; the other having multiple folds. Species from the tripunctata, cardini, guarani, quinaria and calloptera groups all have

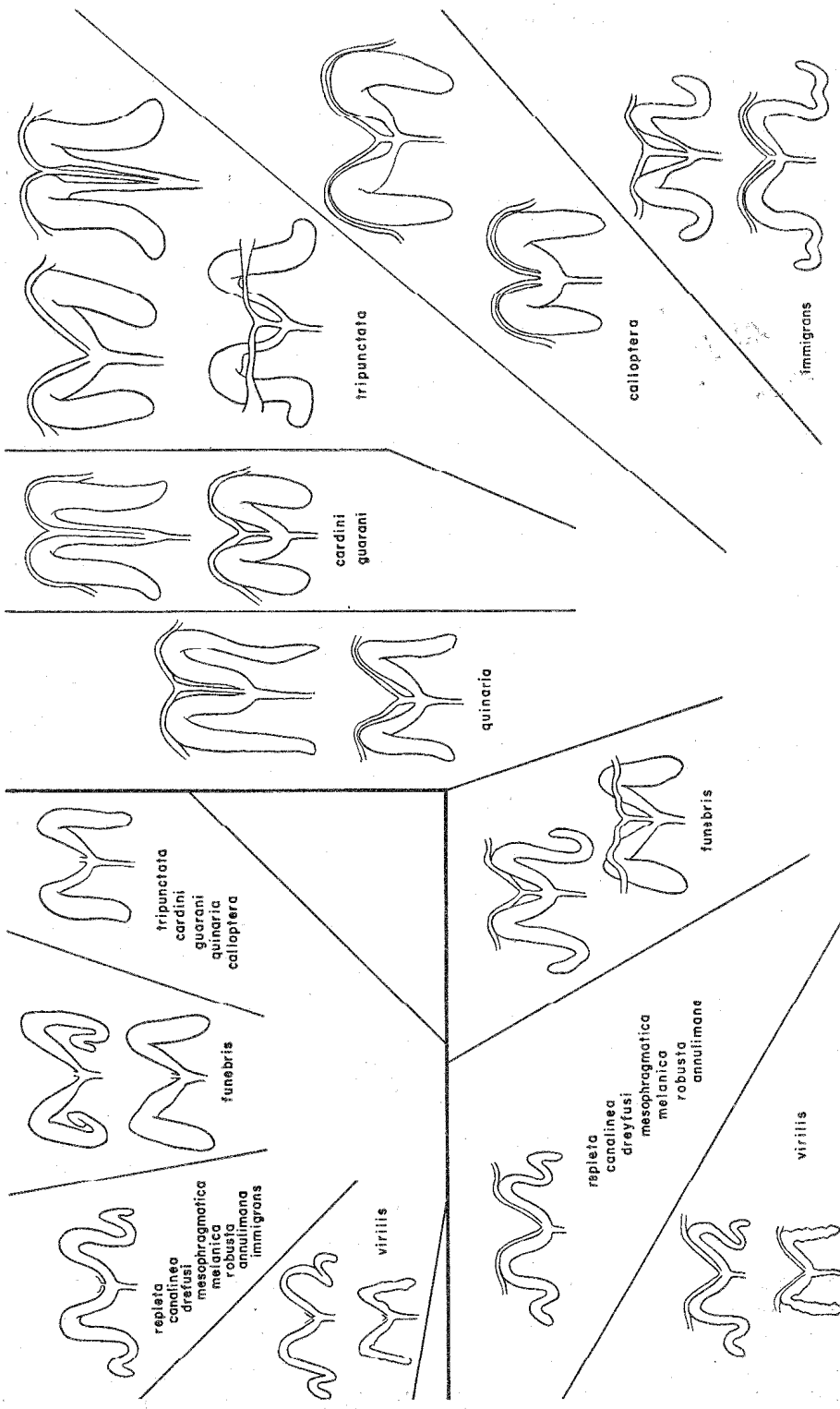


FIG. 52. Schematic representation of the first two steps in the phylogenetic analysis of the subgenus *Drosophila*. For further explanation see text.

paragonia of the first type (Figures 10–12), their stem populations are assumed to be derived from a common gene pool (not necessarily directly), and this stem population is assumed to have arisen from an earlier, heterozygous population. A similar situation exists for members of the repleta, canalinca, dreyfusi, mesophragmatica, melanica, robusta and annulimana groups, except that the paragonia here are of the second type (Figures 5–9). Characteristics of the stem population for the immigrans group (Figure 5) are somewhat less certain, but, on the whole, these paragonia are also of the second type and the immigrans group is therefore included with these forms. The stem population for the funebris group was heterozygous for *both* types (Figure 6). This stem population is assumed to have been derived from a population likewise heterozygous for these types, and this establishes the heterozygous population from which the other two major groups of species were derived.

Essentially then, three major heterozygous populations are "identified," each heterozygous for approximately the same genotypes, and these coalesce as shown in the inset of Figure 52. Members of the virilis group have two types of paragonia (Figure 4). One type, not quite so robust as in other species, has multiple folds and suggests origin from the same gene pool as the repleta group and its neighbors. There is, however, another type which is seen nowhere else in this subgenus, unless the extremely shrunken types seen in species of the hydei subgroup of the repleta group are of this pattern (Figure 9). However this may be, the position of the virilis group is uncertain. For the time being, it is derived directly from the population ancestral to the whole subgenus. Although this by no means exhausts the information available from the paragonia, this part of the analysis will be considered as substantially complete, and the tentative phylogeny arrived at is that shown in the inset of Figure 52. It should be obvious, but perhaps it should be mentioned, that the "populations" inferred are not populations of the same type as are seen in living species. These hypothetical populations had an extensive time dimension and most probably consisted of complexes of species. Since we are inferring broad, temporal sequences rather than detailed speciation events, it would not be legitimate to refer to these populations as species or any comparable equivalent thereof. They represent clusters of gene pools having the same general properties relative to the genotypes under consideration and having an indefinite, but perhaps considerable, time dimension.

The next step in this analysis utilizes characteristics of the vasa deferentia. Again, any of the remaining characteristics could have been used, and the final result would be the same. The characteristics of the vasa are examined, as before, to determine the distribution of genotypes in the stem populations of species groups. Characteristics of these stem populations and the ways in which they appear to coalesce will confirm the previous disposition of the groups or will indicate alternate groupings. They may also suggest subdivisions of the groups and indicate the sequence in which various groups were derived.

With one exception, characteristics of the vasa confirm the unity of the cluster of species groups (repleta, etc.) to the upper left in Figure 52. The exception is the immigrans group. From the distribution of types within this group, it may be inferred that the genotypes of its stem population were not comparable with those of other species groups in this section. Further, the genotypes of this stem

population appear to have included more primitive alternatives, and so the group is tentatively derived from the stem population for the subgenus (lower right, Figure 52). Characteristics of the vasa of virilis group species follow the same pattern as for species in the repleta cluster. Its position in the phylogeny is left unchanged (lower left, Figure 52). Characteristics of the vasa in species of the quinaria section are variable (Figure 52, right; Figures 10–12), showing different degrees and types of association with the paragonia and different degrees of basal fusion. Species of the tripunctata group show almost the complete array of types, lacking only that type seen in species of the calloptera group and in two out of three species in the immigrans group. Stem populations of the quinaria, cardini and guarani groups were each heterozygous for two of the major types. The distinctive characteristics seen in species of the calloptera group suggest its derivation from a gene pool having somewhat different characteristics than that from which the other four species groups arose. Since its stem population appears to have had some genotypes in common with that of the immigrans group, they are both shown as derived from the same ancestral population, i.e., the stem population for the subgenus. The remaining groups in this section (quinaria, cardini, guarani and tripunctata) had stem populations of approximately the same character and so these unite as shown in the figure.

In species of the funebris group, characteristics of the vasa are substantially as seen in species of the quinaria section. They are not sufficient to allow the stem population of the group to be *definitely* related to that of the quinaria section, however, and the group retains its original position. Thus, we now have six tentative branches from the ancestral population, four of which are individual species groups. The two remaining branches are made up of several species groups, with the individual groups in each of these branches being phylogenetically equivalent, i.e., no subbranches are indicated at the present time. Grouping of the cardini and guarani species groups was done to save space in Figure 52 and has no significance.

From this point onward, complete details of the analysis need not be given. Most of the more useful information has been included in the pictorial phylogenies seen earlier. The major objective now is to determine to what extent clusters of species may be separated to indicate sub-branches within major lineages. Only those characteristics which establish cleavages will be considered. Characteristics of the ejaculatory bulb are useful here (Figures 16–21). Those of species in the virilis group are quite distinctive and do not closely resemble any of the types seen elsewhere in the subgenus. This suggests a less close relationship with other groups, i.e., a longer period of independent evolution, so this group remains as derived independently from the ancestral population. Species of the melanica and robusta groups show an array of types, with most of the ejaculatory bulbs having four caecae. Both groups have one species showing the most extreme type, that figured for *lacertosa* (Figure 16.15) of the robusta group being also present in *melanura* (not figured) of the melanica group. These two groups are assumed to have a common ancestral population in which the genotype for this extreme condition was potential. Species of the annulimana group (see Figure 16) also have ejaculatory bulbs of types seen in both the melanica and robusta groups, so we can tentatively place these three groups on

a common sub-branch (see Figure 53 for final disposition of groups). This sub-branch is, itself, derived from the stem population for the major branch, which takes its origin from the ancestral population for the subgenus. By inference, the stem population for this branch is assumed to have been heterozygous for the genotypes common to the melanica, robusta, and annulimana groups. It probably was not heterozygous for those determining the extreme types seen in species from the melanica and robusta groups, but it may have been. The remainder of the species groups (repleta, canalinea, dreyfusi, mesophragmatica) would be derived as the other sub-branch of this major stem. Some species of the robusta group and species of the dreyfusi group have the posterior part of the ejaculatory bulb expanded laterally, and this suggests that species of the dreyfusi group may be more closely related to the robusta complex than are the other remaining species groups. Subgroups within the repleta group can be handled just as if they belonged to species groups and their stem populations identified accordingly. Species of the mulleri, fasciola, mercatorum and melanopalpa subgroups have over-lapping arrays of bulb types and so would be assumed to stem from a population distinct from that for species in the hydei subgroup. Species of the canalinea group have one type of bulb in common with repleta species, so are assumed to be derived from the same ancestral gene pool and hence are derived at the same level (see Figure 53), etc.

Characteristics of the spermathecae also provide information concerning the subdivisions of this branch. The array of types seen in the melanica group somewhat resembles that seen in species of the virilis group (Figure 28), but since the stem population for this branch is derived from the same stem population as was that of the virilis group, this does not alter the position of the melanica group within the branch. Genotypes for these characteristics are presumed to have been derived from the stem population for the subgenus. Retained genotypes might potentially have been present in all the stem populations of this branch, so their distribution cannot determine the position of the group in question. Spermathecae of species in the robusta and annulimana groups have many characteristics in common (Figure 28), and also some characteristics in common with types seen in members of the immigrans group (compare Figures 28.19 and 28.24). This, together with information from the melanica group, indicates that we must consider the possibility that these three groups (robusta, melanica, and annulimana) belong to an independent line derived directly from the ancestral population for the genus, rather than from the stem population in common with the repleta group and its relatives. Without going into details, however, information from remaining characteristics tends to confirm the disposition shown in Figure 53. Spermathecae from the repleta, canalinea, dreyfusi and mesophragmatica groups (Figure 29) form a distinctive *array* of types which suggests that they have a stem population in common and that this population was distinct from that of the robusta complex. Distribution of spermathecal types among subgroups of the repleta group tends to confirm the relationships indicated by the characteristics of the ejaculatory bulbs. They indicate also that the dreyfusi group originated from much the same population as did the hydei subgroup and that the canalinea group originated from much the same population as did the mulleri subgroup. Hence the final disposition shown in Figure 53.

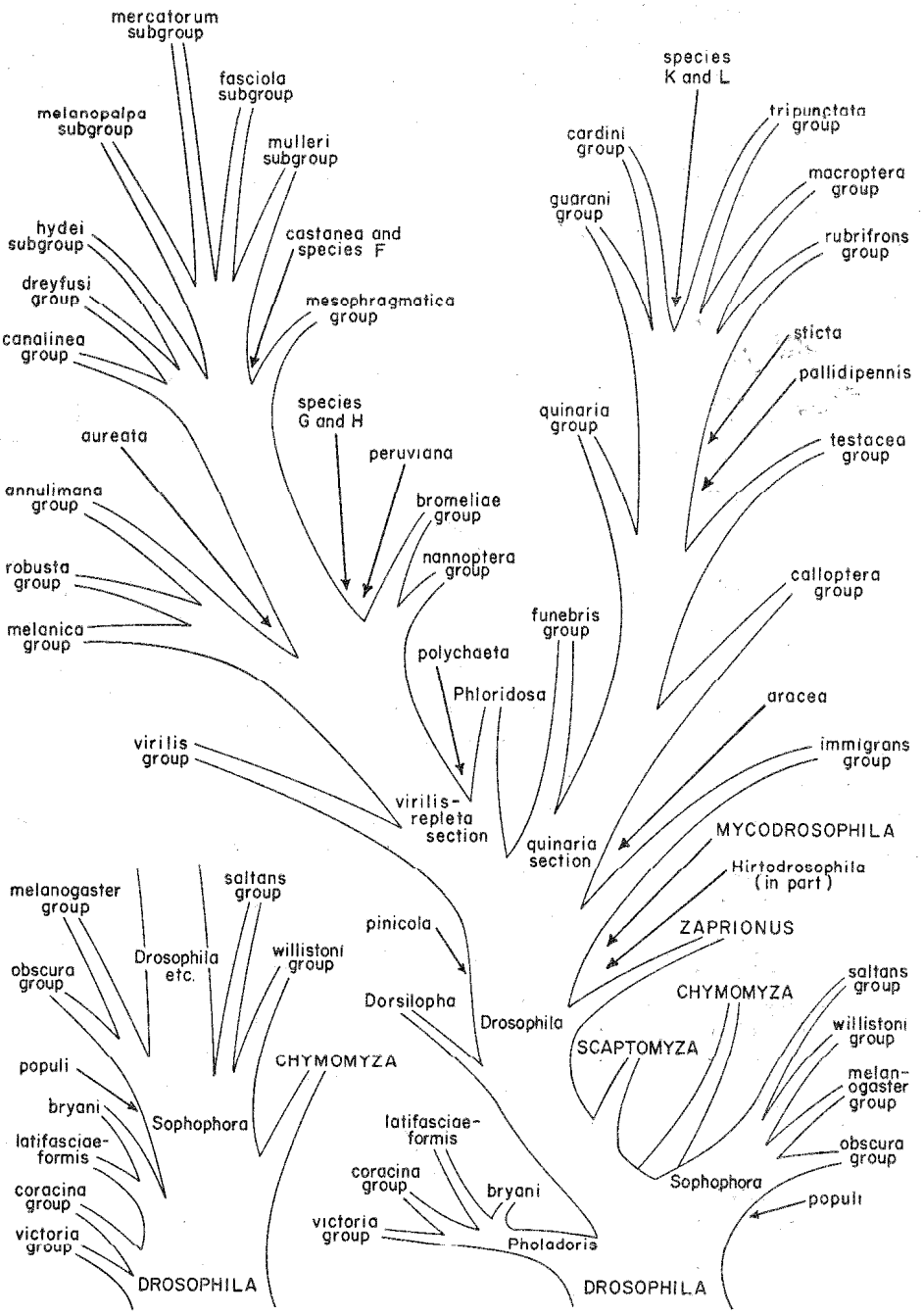


FIG. 53. The phylogeny of the genus *Drosophila* showing the relationships of other genera to the various phyletic lines within the genus. The inset (lower left) shows an alternate phylogeny which may be derived if subgeneric boundaries are ignored and the species groups are taken as the basic phylogenetic units. For further explanation see text.

While the analysis is by no means complete, this brief description has covered the major points of the methods involved. Similar methods are applied to species groups on the quinaria branch, and to groups from other genera and subgenera. This finally results in the delineation of a series of populations having intergrading or overlapping genotypic characteristics. Since the direction of evolution of each characteristic has been considered during the analysis, these populations are defined in a distinct time sequence. The genotypic change from one population to the next has been gradual, and some latitude exists as to the time of derivation of any particular group. However, change of all characteristics has not been synchronous, and the *total* genotypes at the different time levels are relatively unique and allow placing the various groups with some precision. The precision of such an analysis will increase as the number of characteristics considered increases, but it will always be limited by uncertainties regarding the length of time during which certain genotypes have been potential in the stem populations. In this manner, the extent to which stem populations have retained heterozygosity sets the final limit on the accuracy of *any* phylogeny, whether it be developed from living species, from fossil forms, or from chromosome structures.

*Phylogenetic relationships*—Figure 53 shows the phylogenetic relationships of genera, subgenera, species groups and species included in this study. Interpretation of this phylogeny must be made within the context of the methods utilized in obtaining it, and its major limitations and uncertainties will be pointed out in the following discussion. Points of origin of the various groups are essentially probability values based on consideration of the history and distribution of all characteristics and on an evaluation of the evolutionary characteristics of the alternate populations from which they may have been derived. The phylogeny is based completely on characteristics described earlier. It is, of course, not independent of other characteristics, since definition of species groups depends on total phenotypic comparisons among species. Figure 53 shows only the general sequence of origin of the different groups. Vertical separations and other dimensions were adjusted to avoid crowding in the figure, so they do not reflect an objective judgment of elapsed time.

In deriving the relationships shown at the base of the phylogeny, two alternative assumptions could be made. The relationships included in the total phylogeny result if it is *assumed* that both the subgenus *Pholadoris* and the subgenus *Sophophora* are monophyletic. If subgeneric boundaries are ignored and the species groups are taken as the phylogenetic units, the relationships shown in the insert (lower left, Figure 53) may be derived. The first alternative places both of these subgenera on side-branches. The second places both on the main sequence of evolution in the genus. Recalling the evolutionary models discussed earlier, there is no completely objective way to decide between these two alternatives. Consideration of other characteristics, for example, chromosome homologies or features of male genitalia, might provide information which would indicate the more probable relationship, but extension of the analysis to include such features is beyond the scope of the present discussion. The alternatives shown in the inset will not be included in the following discussions.

There can be little doubt that the stem population for the subgenus *Pholadoris*

originated at an earlier time than did those for the other subgenera. Almost all of the distinctive features of these species are primitive, and for most of those which are not, other evidence indicates either their independent origin within the group or their origin prior to the establishment of the stem population for the genus. The peculiar type of ejaculatory apodeme (Figure 15) seen in species of the victoria group falls in the first category. Characteristics of the Malpighian tubules (posterior tips apposed or fused rather than free) fall in the latter category. *Zapriothrica dispar*, which is surely more primitive than the complex associated with the genus *Drosophila*, has the posterior tips of the Malpighian tubules fused, indicating that genotypes for this fusion were potential in the stem population for the genus. The subgenus *Pholadoris* is the only group which includes species having an unreduced sixth sternite in the male, and most of these species have the primitive elliptical testes, the primitive vasa, etc. The only population which was potentially capable of producing these genotypic combinations was the stem for the genus.

The subgenus *Sophophora* was derived from a population which had undergone a certain amount of evolutionary modification and in which genotypes for several of the more derived types were potential. These genotypes may have been potential in earlier populations, but the evidence available at present suggests their origin during the time immediately following the separation of the *Pholadoris* stem population. As more extensive information from more primitive genera becomes available, it may be necessary to modify this assumption. This, incidentally, emphasizes the impracticality of attempting to determine the detailed phylogeny of a single genus without considering the characteristics of species in related genera. The major uncertainties of the present phylogeny stem almost directly from the fact that the available material from more primitive genera was not adequate to allow definition of the evolutionary events which preceded the origin of the stem population for the genus. Be that as it may, the available evidence allows tentative conclusions to be reached, and it would appear that genotypes for derived characteristics of the vasa, the testes, the ejaculatory bulb and apodeme, etc., either arose or reached greater significance as adaptive components of the gene pool during the period intervening between the time of origin of the stem population for the subgenus *Pholadoris* and the time of origin of the stem population for the subgenus *Sophophora*. The stem population for the genus *Chymomyza* arose from a population having the same general characteristics as that from which the Sophophoran stem originated. There is a distinct possibility that the genus *Chymomyza* was derived from the basic Sophophoran stem rather than from the major stem population for the genus. These two groups have in common characteristics of the ejaculatory bulb, the ejaculatory apodeme, the vasa, the ventral receptacle, the abdominal sternites, and the anterior pupal spiracle (see earlier descriptions). Since the genotypes for the distinctive features may have been present in the earlier population, Figure 53 shows *Chymomyza* to be derived at that level. This is the most objective assessment of its relationships. It *probably* was derived from the Sophophoran stem, but the evidence is not conclusive. The same general conditions also apply to the relationships of *populi*. This species was derived either from very near the base of the Sophophoran stem or directly from the major stem population of the genus.

Phylogenetic relationships within the subgenus *Sophophora* remain substantially as indicated earlier by Patterson and Stone (1952), except that no groups are visualized as derived from presently existing groups. The stem population for the subgenus became separated into three major subpopulations. Their sequence of origin is somewhat uncertain. The most obvious interpretation would be that the stem for the obscura group separated first. Then, following a period of evolution, the melanogaster stem separated from the saltans-willistoni stem. The alternative one would suggest that the stem population separated into two subpopulations, a saltans-willistoni stem and an obscura-melanogaster stem. These possibilities are equally likely, so the obscura, melanogaster and saltans-willistoni stems must be shown as derived at the same level. Evidence from the characteristics used here does not permit finer discrimination. As a convention in making Figure 53, groups were shown as derived at the same level when further details as to the precise sequence of origin could not be adduced from the data. Such a depiction is intended to be noncommittal. It does not indicate that the groups in question arose simultaneously, although they may have.

The evolutionary branch leading to the subgenus *Drosophila* was derived at approximately the same level as was the Sophophoran stem. Changes leading to the establishment of genotypes for still more derived conditions of the vasa, the ejaculatory bulbs, the ejaculatory apodemes, the ventral receptacles, the spermathecae and the anterior pupal spiracles were all in progress in this stem population. Since these changes were not synchronized, an overlapping pattern is produced which allows the origin of the various groups to be placed in an approximate time sequence. For example, the genus *Scaptomyza* arose at a time when genotypes for the primitive (Sophophoran) type of anterior pupal spiracle were still available in the gene pool. It arose at approximatedy the time when genotypes leading to an association between the vasa and paragonia were becoming available but before those for the coiled ventral receptacle became conspicuous. It arose at a time when the genotypes for the four-filament egg were becoming prominent, but before they had been stabilized to produce the typical four-filament egg of *Drosophila* species. It also arose from a population in which the genotypes for the spoon type of ejaculatory apodeme were potential. When all characteristics are considered, the genus *Scaptomyza* becomes one of the earliest branches to be derived from the stem population leading to the subgenus *Drosophila*. The subgenus *Dorsilopha* was also derived at the same general time, perhaps somewhat earlier, but probably somewhat later than was the genus *Scaptomyza*.

There is a considerable amount of character overlap between species of the genus *Scaptomyza* and those of the genus *Zaprionus*. Both have substantially the the same type of ejaculatory apodeme, the same type of ventral receptacle, and one species of *Zaprionus* has the same type of vasa as is seen in *Scaptomyza* species. Also, both *Scaptomyza* and *Zaprionus* species show the peculiar branched posterior caecae on the ejacultory bulb. All of these characteristics indicate that these two groups have originated from a common stem. Evidence from other characteristics indicates that these two genera originated in the sequence shown in Figure 53. Species of the genus *Zaprionus* have anterior pupal spiracles of the types seen in species from the subgenus *Drosophila*, one species has paragonia

and vasa of types seen in species from this same subgenus, egg filaments are basically of the *Drosophila* type, etc. The major alternative to the arrangement shown in Figure 53 would assume both *Scaptomyza* and *Zaprionus* to be derived from a single stem which was itself derived from the base of the branch leading to the subgenus *Drosophila.* This stem population would have been heterozygous for almost all of the genotypes seen in later species. During subsequent evolution of this stem population it would have become subdivided into the stem populations for the two genera. The origin of the *Zaprionus* stem would have involved homoselection toward almost *all* of the more derived genotypes and that of the *Scaptomyza* stem would have involved homoselection toward *all* of the primitive genotypes. This seems unlikely. In most of its characteristics, the genus *Zaprionus* shows a marked resemblance to species of the immigrans and funebris groups, and it is assumed to have arisen at a time either slightly antedating the origin of the immigrans group or even somewhat later. As far as the characteristics under consideration here are concerned, the genus *Zaprionus* is just a moderately distinctive species group in the subgenus *Drosophila.*

Most of the relationships within the subgenus *Drosophila* have been indicated during the earlier discussion. The immigrans group is derived almost directly from the stem population for the subgenus and the same is true for the virilis group. Within the virilis group the general combination of characteristics is that of the virilis-repleta section, and it is shown in Figure 53 as derived from the very base of this branch. Its most probable alternate position would be at an earlier level, derived directly from the stem population for the subgenus and independent of all other groups.

Evaluation of the relationships between the other groups from the virilis-repleta stem indicates three major clusters of species, the robusta complex, the repleta complex and the bromeliae complex. The latter cluster of species (*nannoptera, bromeliae,* etc.) is, for the present, a very ill-defined group. There is a relatively large number of undescribed or ungrouped species which are generally of the *peruviana* type, and its seems probable that as these species become more fully investigated and understood a third major phyletic branch, most closely related to the repleta complex, will be recognized. Even at the present time there would be some justification for more sharply separating the robusta complex from the other two, rather than deriving all three at the same level. As indicated previously, this method of depiction is intended to be noncommittal, and an objective evaluation of relationships does not allow finer subdivision at the present time. If, however, taxonomic studies of these species develop as anticipated, the virilis-repleta section will eventually be separated into two distinct phyletic lines. One will include the virilis group and the robusta complex. The other will include the repleta and bromeliae complexes.

Within the repleta complex itself, general relationships are as shown in the figure. Since this complex has already been discussed in some detail, and since a cytological phylogeny is available for many of these species, little more needs to be added. *D. aureata,* formerly placed in the repleta group (Wheeler, 1957), most probably arose much earlier. A conservative estimate of its position places it as shown in Figure 53. Alternate positions would range from this level downward about to the level indicated for the origin of *polychaeta.* The mulleri sub-

group of the repleta group is almost certainly not an evolutionary unit in the same sense that the hydei, fasciola, mercatorum and melanopalpa subgroups are. Many, if not most, of the species shown derived independently from the repleta standard gene sequence (see Figure 25) are probably of independent origin from the stem population for the repleta group. They might be shown as a scattering of "twigs" arising between the base of the hydei stem and the base of the mulleri stem as it is shown in Figure 53.

The position of *polychaeta* is somewhat equivocal. It shows some relationships with both sections of the subgenus, but the great majority of its characteristics are those of species in the virilis-repleta section. In characteristics of its ejacula tory bulb and apodeme it most nearly resembles *Phloridosa* species, and the subgenus *Phloridosa* itself is closest to the virilis-repleta section. It seems probable that both *polychaeta* and *Phloridosa* were derived from the early stem population for the virilis-repleta section.

The position of the funebris group remains doubtful. Since the majority of its characteristics are those of species in the quinaria section, it has been shown derived from the early stem population for this section. However, all of the genotypes for the characteristics seen in species of the funebris group were potential in the gene pools from which the stem populations for the two major sections took their origin. It is possible, for example, that the funebris group arose from an early population which was evolving *toward* the integration of virilis-repleta genotypes. It could simply have separated from this major population before the distinctive combinations of genotypes had been established. This is somewhat less probable than the alternative indicated in Figure 53.

Within the quinaria section itself, the calloptera group has a somewhat isolated position. It is unquestionably a member of this section, but its stem population appears to have separated from the main stem at an early time. The remainder of groups and species in this section form a rather closely knit cluster, with the quinaria group being the most distinct and derived earlier than the others. The terminal groups in this section appear to have been produced, as it were, by explosive subdivision of a large and complexly heterozygous stem population. Examination of all characteristics shows that the population at about the level of the quinaria group had retained a large amount of ancestral genetic variability, and it was acquiring and retaining a great deal more. The guarani, cardini and testacea groups are distinct and are each derived from stem populations which included a moderate "sample" of the genetic variability in the major stem populations. The remainder of these groups are much less well defined. It seems probable that species now placed in the tripunctata group have been derived from the *major* stem population *at a sequence of time levels*, and that the macroptera and rubrifrons groups are only moderately distinctive clusters of species derived in the same way. Thus, some of the species of the tripunctata group were probably derived at about the time of origin of the quinaria group, others somewhat later at about the time of origin of the guarani group, etc. This group or cluster of species probably provides the major exception to the earlier assumption that species in a group are derived from a single, *independent* ancestral population. Species of the mulleri subgroup of the repleta group are another exception, and it seems probable that the stem population for the subgenus *Drosophila* evolved

in much this same fashion. The semblance of order which is now seen at the base of the subgenus reflects the pruning effects of time, with most of the minor twigs removed and only the derivatives of the major populations remaining. Some twigs still exist, however. Both *carsoni* and *carbonaria* were probably derived from the stem population for the subgenus, and both have peculiar combinations of characteristics which can best be accounted for if they arose at an early time. *D. carsoni* most probably arose from a *very* early population of the virilis-repleta section, and *carbonaria* from an early population of the quinaria section. *D. tumiditarsus* likewise arose from an early stem population of the subgenus. It probably is a member of another major phyletic line of the subgenus, however, rather than an isolated "twig." From inspection of descriptions and figures by Okada (1956), it is apparent that several groups exist which probably represent an oriental equivalent of the primarily newworld, virilis-repleta stem. The branch to which *tumiditarsus* belongs is possibly the earliest branch from this stem, but it may have arisen independently from the stem population of the subgenus. There is probably also an oriental equivalent of the quinaria stem which originated at about the level indicated for *aracea* in Figure 53. Most of the oriental *Hirtodrosophila*, as well as several other oriental groups which Okada (1956) refers to the quinaria section, probably belong to this branch. *D. aracea* itself is most closely related to the immigrans group and is probably a member of still another independent lineage deriving from the early stem population for the subgenus.

Since only a single female was available for dissection, characteristics of *pinicola* (collected at Andreas Canyon, California) have not been discussed earlier. The characteristics of this female, however, are distinctive enough to allow it to be placed with reasonable certainty at about the level indicated in Figure 53. Thus far, this is the only species distinctly related to this stem which retains the first sternite as paired and pigmented plates. Its ventral receptacle is a mixture of the coiled and folded types, having about four large coils basally and a couple of flat loops distally. It is completely free and not appressed to the surface of the vagina. Except for the number of coils and folds, it is identical with that type figured for *histrioides* (Figure 35.4) and *M. dimidiata* (Figure 39.7). Its spermathecae are of the obscura or *Pholadoris* types, dark-pigmented and elliptical in outline with a slight apical indentation. The spermathecal duct is flexed just proximal to the spermatheca. The spermathecal envelope is thin and uniformly distributed. It is interesting to note that Sturtevant (1942) found the minimum number of differences between the species included in his study to be between *pinicola* and *Scaptomyza terminalis*, an observation which is completely consistent with the positions indicated in Figure 53. *D. pinicola* is one of the most primitive members of the subgenus *Drosophila*, but it can no longer be considered as near the primitive for the genus as a whole.

The position of the *Hirtodrosophila* must remain uncertain. As presently classified, they are almost surely a polyphyletic group. If subgeneric boundaries are ignored and the individual species placed as their characteristics would seem to indicate, *duncani* would be derived somewhat earlier than *Dorsilopha, pictiventris* at about the level of *Dorsilopha, thoracis* near *Mycodrosophila*, and *histrioides* at the level of the immigrans group. It would appear that the subgenus

*Hirtodrosophila* is badly in need of revision. Until it has been re-evaluated, these species cannot be placed in the phylogeny of the genus.

*Genotypic histories*—Once phylogenetic relationships have been indicated, final evaluation of the phylogeny is made through tracing the history of genotypic change for the individual characteristics. These analyses have the formal purpose of detecting inconsistencies and providing additional insight into the type of relationship which exists between the different groups. Since the genotypic histories for all characteristics follow substantially the same pattern, only one will be covered in any detail. Sufficient data have been provided in earlier sections so that the reader may develop the others if this seems desirable.

As described earlier, phylogenetic analysis proceeds backwards from the genotypes of the species in a group. From these genotypes, the genotypic composition of the stem population of the group is inferred, and so on until the stem population for the genus is reached. The summation of the analyses for all individual characteristics defines an evolutionary series of populations, the major genotypes potential within their gene pools, and the major changes which took place in these gene pools as subpopulations diverged from each other. Once this outline is complete and the major pathways of evolution established, the genotypic histories for individual characteristics may be traced, starting with the probable genotypes potential within the stem population for the genus. One of the more simple sequences of change, that involving the ejaculatory apodeme of Sophophoran species, has been diagrammed in Figure 54. If this figure is read from top to bottom, the phlyogenetic analysis based on the characteristics of the ejaculatory apodeme is followed. If it is read from bottom to top, the history of genotypic change is traced. The sequences leading to the terminal species of the subgenus *Drosophila* have not been included, although a much abbreviated summary for some distinctive types has been shown to the left-center in the figure. Since the method of phylogenetic analysis has already been described, this aspect of the figure will not be discussed. It should, perhaps, be emphasized, that the sequence of populations shown in Figure 54 is that determined by the *total* analysis and not just by the analysis of the ejaculatory apodeme.

In Figure 54, genotypes are shown figuratively by the phenotypes which they determine. At each level, an array of genetic variability existed, and an array of phenotypes is shown. It is not intended that these be considered the *only* genotypes potential in these populations. They are simply the genotypes which may be inferred to have been present then. When genotypes are shown as being perpetuated unchanged (solid arrows), it should be inferred that later genotypes were homologous in the sense defined earlier, but they were not necessarily *completely* identical with similar genotypes shown elsewhere. In most cases it seems probable that by far the major fraction of the genes comprising a given genotype were derived unchanged from ancestral gene pools. When new genotypes are indicated as originating (dashed arrows), most of these will have arisen by simple modification of a pre-existing genotype, not by a complete replacement of genes at all loci which influence the characteristic. A single possible exception to this will be noted later. Only the more general of the major genotypes are included in the figure. The complete array of variability, reflecting both the different ways in which these genotypes may be integrated with other elements of

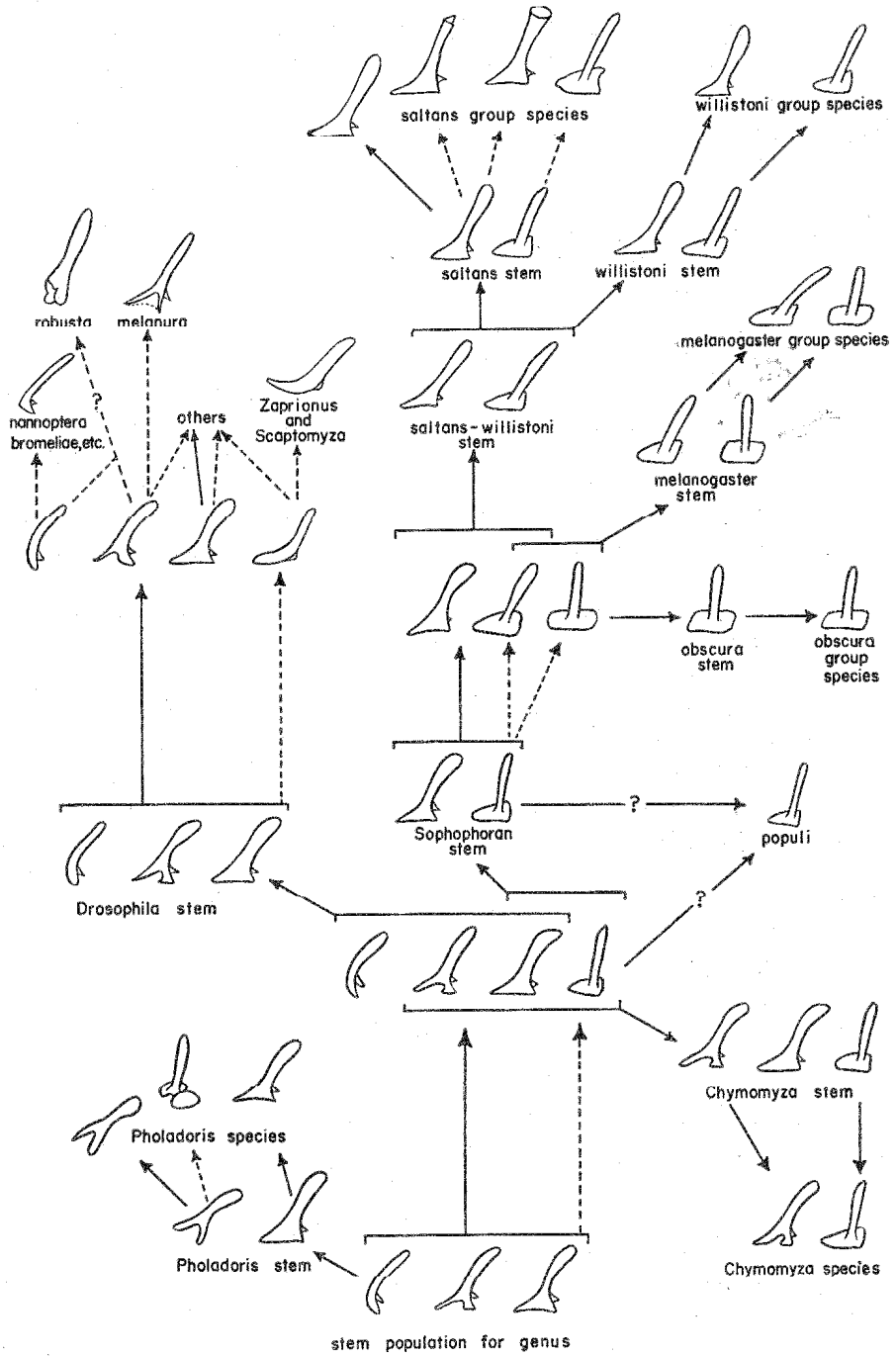


FIG. 54. The history of genotypic change for characteristics of the ejaculatory apodeme. For further explanation see text.

the gene pool and the addition of new genetic variability by mutation, can be determined by reference to Figure 15.

Available evidence suggests that the stem population for the genus was heter-

ozygous for the genotypes *potentially* capable of determining the array of types indicated at the bottom of Figure 54. Although it may not be obvious from the figure, the major difference between these types is a simple quantitative one involving the degree of chitinization of the ventral surface of the ejaculatory bulb. Weak chitinization produces the form shown at the left, and stronger chitinization produces that form shown at the right. For simplicity, the gene pool at this time may be visualized as including a complex of genes, with the "average" genotype producing a moderate amount of chitinization. Other genotypes are potential and probably recur at reasonable frequencies. Individuals whose genotypes incorporate a preponderance of "minus" modifiers will have ejaculatory apodemes approaching the type shown to the left, and those incorporating a preponderance of "plus" modifiers will have an apodeme approaching the type shown to the right. As this population, or its subpopulations, continues to evolve, selection may operate to swing the balance in either direction, or this section of the gene pool might be retained with only slight or no changes in frequency of the individual alleles involved. The subpopulations from which the subgenus *Pholadoris* was derived retained substantially the same type of heterozygosity as has been visualized for the stem population for the genus. Some species derived from the *Pholadoris* stem have established one or another of the genotypes, apparently very little modified, from the stem population. The difference between the Y-type of apodeme seen in some of these species, and the type shown to the left in the stem population for the genus, is primarily one of drawing perspective rather than of marked difference in structure. One of the types seen among the species of the victoria group is, however, a radical departure from previous types. This form has been indicated with a dashed arrow, which is somewhat unrealistic in the present instance. The victoria type of apodeme probably reflects a major reorganization of the genotype. Its antecedents are probably to be sought in a heterozygous gene pool which has since undergone reorganization with the addition of new genetic variability, rather than in some specific genotype which has been perpetuated and modified by the substitution or addition of a few genes.

During the continued evolution of the stem population for the genus ancestral heterozygosity was perpetuated, but the characteristics of the gene pool were being changed through the addition of new genetic variability. Genotypes for a new type of apodeme, with a flattened plate and the handle displaced slightly toward the center, became potential at about this time. Thus, the next major gene pool (second from the bottom in the center) has a more extensive array of potential genotypes. The stem populations for the subgenus *Drosophila*, the subgenus *Sophophora*, and the genus *Chymomyza* may all be traced back to this population, and each of these stem populations included a somewhat different "sample" (shown by brackets in the figure) of the genotypic variability potential at this time. The breadth of the sample itself is rather indefinite. Each stem population probably started with approximately the total array of variability but then passed through a phase during which there was restriction toward the particular genotypes actually detected as potential in each one. In few, if any, cases has such restriction been severe enough to completely erase the ancestral heterozygosity, at least in the major stem populations. It simply has limited the variability

somewhat, with further evolution of each stem being based on the ancestral genotypes which have been retained.

Sometime during the early evolution of the Sophophoran stem population, genotypes leading to the very heavy chitinization of the apodeme became prominent. Both the *Chymomyza* stem and *populi* appear to have arisen before such genotypes reached great significance. If they arose after this change was underway, subsequent evolution in each of these stems involved fixation of genotypes for the more weakly chitinized types. Also during the early evolution of the Sophophoran stem, genotypes capable of producing the obscura type of apodeme became potential, and it was approximately at this time that the saltans-willistoni, melanogaster, and obscura stem populations became distinct. Again, evolution of each stem population resulted in the retention of a somewhat different sample of ancestral variability. Evolution of the stem population of the obscura group apparently involved strong homoselection and virtual fixation of the genotypes producing the obscura type of apodeme. Parenthetically, almost all characteristics of the species in the obscura group show this same thing. Both internally and externally, these species are unusually uniform, suggesting that the stem population for the obscura group passed through a phase during which a severe restriction of its genetic variability occurred. Among the characteristics treated here, only those of the testes suggest a retention of ancestral heterozygosity. Evolution of the stem populations for many groups has involved homoselection toward one or another genotype, but only rarely has it involved anything approaching the almost total restriction indicated for the obscura stem. One other group in the whole genus, the virilis group, shows a similar pattern, and there restriction was by no means so extreme.

In the stem population for the melanogaster group genetic variability was restricted to the more derived genotypes. Species from this group show one or the other of the two types of apodemes indicated in the figure as potential in the stem population. In the saltans-willistoni stem population the more primitive of the available genotypes were retained, and this condition continued to exist until after this population had become subdivided into the stem populations for the saltans and the willistoni groups. During evolution of the willistoni stem these genotypes were retained substantially unchanged, and one or the other of them predominates in the gene pools of the individual species of the group. During the evolution of the saltans stem population, modifications were added, and a new and distinctive array of types is seen among these species.

The genotypic changes which occurred during the evolution of the stem population of the subgenus *Drosophila* will not be described in detail. Only those types which tend to justify inferences as to the basic genotypes are included in the figure. Thus, the existence of such types as are seen in species of the bromeliae and nannoptera groups suggests that genotypes producing weak chitinization were retained in the subpopulations leading to these groups. The basic spoon type of apodeme originated prior to the origin of the stem populations leading to *Scaptomyza* and to *Zaprionus*. It was subsequently much modified during the evolution of the quinaria stem, but it is seen relatively little changed in *polychaeta*, *tumiditarsus* and in species from the subgenus *Phloridosa*. The general pattern of change, while involving the origin of new and distinct types, is the

same for the two major subgenera. At each level some ancestral heterozygosity was retained. The "replacement" of one genotype by another was a long and gradual process, and the array of ancestral variability which was retained made very significant contributions to the characteristics of succeeding populations and to the phenotypes of species derived from them.

*General pattern of evolution in the genus Drosophila*—The earliest identifiable population contributing to the evolution of the genus *Drosophila* was heterozygous for genotypes influencing most, but probably not all, of the characteristics included in this study. These various genotypes (for the individual characteristics) were presumably integrated into a general population genotype which, by inference from current population studies (e.g., Dobzhansky, 1955; Lerner, 1954, 1958), was in a state of dynamic balance. Its continuing adaptation depended on the retention of heterozygosity, and hence genes potentially capable of being integrated in many other ways were maintained in the gene pool. These individual genes were retained as part of a balanced system, and selection operated to perpetuate this system. During the course of evolution, however, this population or some of its subdivisions encountered adaptive situations which resulted in the restriction of their genetic variability. In such cases heterozygosity at some loci was lost or sharply reduced, and these more nearly homozygous genotypes could be of many types. Thus, the original population was capable of meeting adaptive situations either by the retention of heterozygosity through changes in balanced gene complexes or through the generation of a multiplicity of adaptive types depending in part on homozygosity. Since well over 900 species of *Drosophila* alone (Wheeler, 1959) have been produced through evolutionary development from this population, it is hardly surprising to find that it had properties giving it the potential to undergo rapid adaptive change.

Most of the ancillary stem populations show evidence of a restriction of heterozygosity relative to the major population from which they originated. In retrospect, it is not possible to determine whether this restriction was an immediate consequence of the speciation process which produced the stem or whether it occurred at some later time. All of the major stem populations did, however, retain some degree of genetic variability. This tends to give the impression that speciation processes have rarely involved a sharp reduction in heterozygosity. Such an illusion, however, results from the fact that the data from which this inference is drawn come only from living species. At any time level one or more populations were probably adapted through retention of a heterozygous gene pool. In addition, several or many others were adapted, probably as more specialized forms, through homozygosity. Few of these specialized forms have survived to the present, either as isolated species or as sharply defined species groups. This should not mean that they were not produced, however. In the absence of fossil evidence we cannot know how many such types existed, but it seems probable that some were present at all levels and that the frequency of such forms among living species will be greatest among groups derived latest in time. Thus, the species groups in the subgenus *Sophophora* are compact, with most of the older intermediate forms or highly specialized types eliminated. Conversely, the subgenus *Drosophila* appears highly complex because it originated

much later in time, and many of the more specialized types remain as isolated or semi-isolated species or clusters of species.

Probably as a result of the pruning effects of time, evolutionary trends within the subgenus *Sophophora* are quite clear, and they can best be illustrated by considering the three major stems individually. The obscura stem, for example, underwent a period of extreme homoselection at some time during its early history. This period was probably followed by a slow increase in heterozygosity through the integration of new genes into the gene pool. As the capacity of the gene pool to generate a diversity of adaptive genotypes increased, the potentiality for the production of new species also increased. At present, this group appears to be in a proliferative stage following a period of stasis induced by an earlier loss of evolutionary potential through homoselection.

The population leading to the melanogaster group did not experience such an extreme reduction of heterozygosity, but there was a considerable restriction of genetic variability relative to the population from which it was derived. However, since it did retain some heterozygosity, it did not completely lose, and hence have to regain, its evolutionary impetus. Prior to the origin of the subgroups, sufficient time elapsed to allow stabilization of the genotypes which contribute to the distinctive phenotypes common to all members of the group (antero-postero orientation of the testes, etc.), and to make possible the integration of some novel genotypes which are common to species in several of the subgroups (the enlarged anterior ejaculatory duct, the peculiar anterior pupal spiracles, etc.). Thus, this group shows a history of slow change, with the gradual incorporation of new genotypes and the relatively uninterrupted proliferation of new adaptive types.

The population leading to the saltans and willistoni groups also retained some heterozygosity, and, like the stem population for the melanogaster group, passed through a stage during which new genotypes (e.g., for the saltans-willistoni type of ventral receptacle) became fixed. If much proliferation occurred during this time, the products have not survived or have not been included among the species used in this study. Evolutionary potential gradually increased, and the earliest subdivision resulted in the establishment of the stem populations for the saltans and willistoni groups. With time, each of these stem populations acquired a more extensive array of potential genotypes, since many of the most distinct features of species in the two groups appear to have originated after their stem populations became independent of each other.

Thus, after its origin from the major heterozygous population, the stem population for the subgenus *Sophophora* underwent a phase during which its genetic variability was somewhat restricted. During this period some new genotypes were incorporated into the gene pool (as is illustrated, for example, in Figure 54 for characteristics of the ejaculatory apodeme), and many others were substantially fixed (e.g., those for the free condition of the posterior tips of the Malpighian tubules). Still others were retained as potential in the more strongly heterozygous portion of the gene pool. Further evolution from this stem population resulted in the establishment of three new stem populations, and the genetic variability in *each* of these was restricted once again. From this point, continuing evolution in each stem population involved the integration of new genes and the gradual increase of heterozygosity to a level which has allowed renewed pro-

liferation and the generation of clusters of species rather sharply distinct from each other. The general pattern is one of adaptation through mild homoselection (probably) to a general adaptive niche, followed by specific adaptation of independent subpopulations to more restricted environmental niches by more extreme homoselection. Once established in these environmental niches, continuing evolution of each subpopulation involved the slow regeneration of heterozygosity, and this has made possible a new expansion or radiation from these types. At the present time most of the proliferation of Sophophoran species is probably based largely, but not exclusively, on new genetic variability which has been acquired since the stem populations became independent of each other. The pattern of evolution in the subgenus *Sophophora* more closely approximates the classic model of evolution discussed earlier (Figure 47, left) than does that of any other group in the genus. The resemblance, however, is superficial, since some ancestral heterozygosity has been retained at all levels. In this subgenus, the *appearance* of classic evolution results from the effects of time on the availability of living species for study. It is not actually a consequence of evolution proceeding after the classic pattern.

The evolution of the *Drosophila* branch of the genus has been in marked contrast with the Sophophoran pattern. Evolution here has been based on the perpetuation of the heterozygous stem population itself. Insofar as can be judged from the analysis of characteristics used in this study, the stem population leading to the subgenus *Drosophila* never experienced a sharp reduction in heterozygosity. Instead, one heterozygous genotype was gradually replaced by another, with, if anything, a slight increase in genetic variability. That is, new genotypes appear to have been incorporated somewhat more rapidly than older ones were lost. It is as if, through being highly heterozygous, the gene pool had a greater capacity for integrating and retaining new genes. This is probably in part a reflection of the environmental opportunity open to the population. A heterozygous population will be able to exploit a rich environment more fully, and such a population might also be able to increase its genotypic versatility until it effectively saturates all available sub-niches open to it. Thus, this stem population may have had many of the characteristics described by da Cunha, *et al.* (1959) for populations of *willistoni*, and for much the same reason.

From time to time, more restricted subpopulations have separated from the major stem. In most of these cases (e.g., *Scaptomyza*, *Zaprionus*, etc.) reduction in heterozygosity was only partial, as was the case for the stem population of the subgenus *Sophophora*. Further evolution in these side branches also appears to have followed the Sophophoran pattern in its general features. That is, there has been a period of adaptive restriction of the genotype during which the gene pool is reintegrated to produce a new and distinct population genotype. This period over-laps one during which new genes are integrated into the gene pool, and subsequent evolution depends both on retained ancestral genotypes and on new genotypes which are slowly being generated through new mutation and recombination.

At a relatively late time, the major stem population became subdivided almost equally (in terms of the gene content of the resulting gene pools), and both of the major subunits underwent some restriction of genetic variability. This di-

vergence was not abrupt, and many of the intermediate types still remain. The *major* subpopulations, however, constituted the stem populations for the virilis-repleta and the quinaria sections of the subgenus *Drosophila*. As they diverged from each other different heterozygous systems were stabilized in each, and different samples of ancestral variability were retained in each. The proliferation of new adaptive types apparently continued almost without interruption in each branch, and thus there is considerable over-lapping of character distributions for groups derived near the time when the initial divergence was occurring. Therefore, the phylogeny of this subgenus has a much more "brushy" appearance than does that of the subgenus *Sophophora*. This may be due, in part, to the fact that these groups originated relatively recently. However, it is probably also due to the fact that these stem populations were more highly heterozygous than were the early ones, and the adaptive restriction of ancestral variability in most of their subpopulations was less extreme. It probably also reflects the expansion of these subpopulations into a new, relatively rich and unexploited geographical area. Although such speculations must be extremely tentative, it seems probable that the original stem population developed in the old world. At a rather late time it was able to expand rather rapidly into the new world, and this first expansion may possibly have formed the virilis-repleta stem population. The major stem population continued to evolve in the old world and at a later time a new wave of expansion occurred which brought the stem population for the quinaria section to the new world. It is tempting to speculate that the "first" expansion occurred during an early interglacial period. A succeeding glacial episode isolated a new- and an old-world complex and these evolved independently of each other with the new-world complex being the virilis-repleta stem and the other the major stem population for the genus. In a later interglacial there was another major expansion of the stem population into the new world with, perhaps, a minor countermovement of some populations (from the robusta group, for example) back to the old world. There were almost surely other minor exchanges in both directions. When more of the Asian groups have been incorporated into the general phylogeny of the genus it will probably be possible to arrive at more reliable conclusions regarding the patterns of dispersion which have contributed to the present distribution of the species in the genus. Until then, very little can be said, but it does seem almost certain that the development of the early stem population for the subgenus *Drosophila* occurred in the old world. The new-world complexes of species are probably the result of the explosive radiation of new adaptive types into an unexploited geographical area rather than the result of evolution *in situ*.

In summary, during the evolution of the genus *Drosophila* the major evolutionary potential was retained primarily in one population which had continuity in time. Almost all major groups (*Pholadoris*, *Sophophora*, *Scaptomyza*, etc.) were derived at different time levels from this population. During or following their separation from the major stem, these populations passed through a phase of genotypic restriction, but the major population itself retained, and probably increased, its level of heterozygosity. Proliferation from most of the divergent branches was somewhat reduced, and none of them have produced as great a number of descendent species as has the heterozygous stem. It is probably no

coincidence that the stem population which retained the highest degree of heterozygosity also retained the greatest evolutionary potential. It is of interest to note that the stem population was also the population which retained the *primitive karyotype* for the genus (see Patterson and Stone, 1952, for published karyotypes). It is probable that the primitive karyotype (five rods and one dot chromosome) exhibits a higher degree of recombinational plasticity than do the more derived configurations involving fusions and consequent reduction in chromosome number. Recombinational plasticity has probably contributed markedly to the generation of adaptive genotypes and hence to the evolutionary potential of the groups which have retained the primitive karyotype. Simple retention of the ancestral karyotype has not, in itself, been sufficient to give evolutionary impetus, and heterozygosity alone (i.e., in a situation where recombination is sharply limited by inversions, etc.) would not be expected to induce rapid change. Thus, the distinctive properties of the stem population have probably resulted from a balance between heterozygosity and recombinational plasticity. Too little of either has been accompanied by a loss of evolutionary potential.

## Summary and Conclusions

During the earlier discussion and development of a method for phylogenetic analysis two major assumptions were made. The first of these was that genetic change during evolution is of necessity so slow that genes present in the gene pool of a population will be, for the most part, unchanged replicates of those from an ancestral population. Regardless of the population phenotype at any one time, a heterozygous gene pool will include within it many potential genotypes, and one or another of these may be expressed as a result of the reintegration of the gene pool which occurs when a population adapts to changing or changed environmental conditions. When the gene pool of a population changes with time, one array of potential genotypes only gradually replaces another through the integration of new genes into the gene pool and the loss of others. Between any two succeeding levels in time the arrays of potential genotypes will have more similarities than differences.

The second assumption which was made was that heterozygous populations may become subdivided, and their subdivisions may become evolutionarily independent, without completely losing their heterozygosity. Adaptive changes initiated by each new subpopulation may, and often must, depend on genotypes potential at the time of divergence. Thus, since the number of potentially adaptive genotypic combinations inherent in a given gene pool will be limited, it may be expected that certain phenotypes will recur among species derived from a common ancestral gene pool. Newly integrated genes may also make some contribution, but their effects on the characteristics of the population will be relatively much smaller than those of the ancestral genotypes.

For simplicity, two extreme patterns of evolution were visualized and contrasted. In the first, the classic pattern, evolutionary development proceeds through fixation of genotypes at each level, with subsequent change completely dependent on new genotypes which arise through mutation. In the second, the dynamic pattern, evolution proceeds from populations which have retained at

least some, and often much, heterozygosity. Thus, at any level, evolutionary change will depend both on ancestral variability and on mutation, with new mutations playing a much less significant role than they would under the classic system. Evolution following either of these patterns might be expected to occur at some times or in some groups, and the character distributions produced by each would be distinctive. Thus, the pattern of distribution of different characteristics among species for which an objective phylogeny is available will indicate the evolutionary system which operated to produce that distribution. This type of correlation was made, using the cytological phylogeny of the repleta complex, and the result of the analysis indicated that the combinations of characteristics seen among these species could only have been produced if the evolution of the complex had followed the dynamic pattern. Particular characteristics were distributed among these species in such a way that their origin could not plausibly be attributed to convergent evolution (the origin of identical phenotypes through new mutation). It was therefore necessary to develop a method of phylogenetic analysis which could indicate species relationships regardless of the pattern of evolution of the group.

One feature which both the classic and dynamic patterns of evolution have in common is that genotypes for derived characteristics must develop from a heterozygous gene pool. Regardless of the pattern of evolution, the transition from a primitive to a derived genotype must involve temporally intermediate heterozygous populations. The difference between the two types lies primarily in the duration of the heterozygous period and in the relationship of this period to the speciation events which initiate phyletic lines. For the classic system, the heterozygous period must be relatively short and interposed between speciation events. For the dynamic system, the heterozygous period may be very long, and it may span many speciation events. Hence, phylogenetic analyses may be made through identification of the ancestral, heterozygous populations which are common to two or more groups of species. Common possession by two species of a given characteristic indicates only that they are both derived from the same ancestral, heterozygous population, and by working backward from the species groups these heterozygous populations may be defined for each individual characteristic. Since the direction of evolution may be determined for most characteristics, and since most characteristics pass through sequences of change, a series of heterozygous populations is ultimately recognizable. The various species groups are shown to be related through origin from one or another of these populations.

When this method of analysis is applied to the genus as a whole, a phylogeny is developed which is broadly consistent with earlier phylogenies. All groups are found to be smoothly interconnected by heterozygous ancestral populations, and the general pattern is that predicted if evolution has followed the dynamic rather than the classic system. Within this pattern, no single instance of true convergent evolution is recognizable as such. The major potential for the evolution in the genus has come from one heterozygous population which had continuity in time. At various levels, ancillary stem populations have developed from this population. In most cases, the genetic variability in these stem populations has undergone some restriction. Almost all stem populations retained some heterozy-

gosity, however, and as a general rule the more heterozygous populations have left the greater number of descendant species.

During the evolution of the genus, adaptive change appears to have been of two types, that involved in the perpetuation of the major stem population and that involved during the establishment of ancillary populations. The two types of change are not mutually exclusive and should not be sharply differentiated. The major stem population changed through the slow replacement of one (presumably) balanced heterozygous system with another. Neither the loss of older genotypes nor the gain of new ones was precipitous, and many ancillary stem populations originated from this population as the slow replacement of one genotype with another proceeded. Thus, a considerable array of genotypes, both heterozygous and homozygous, were potential in this gene pool at all times. Since many genotypes remained potential over long periods, it may be inferred that the continuing reintegration of the population genotype depended more heavily on frequency changes of alleles already present than on substitution of new genes. Such substitution did occur, but it was neither extensive nor rapid.

The second type of change, that involved during the establishment of ancillary stem populations, entailed an adaptive restriction of genetic variability. It would appear that, during or following their separation from the major stem population, adaptive reintegration of these gene pools occurred under conditions which enforced higher degrees of homozygosity (or less heterozygosity). In these instances, fixation or loss of genes may have been quite rapid, but the genes involved were, for the most part, ancestral genes and not genes newly arisen by mutation. In most cases the restriction was not severe, with the individual genotypes for only certain characteristics becoming fixed. In some instances, however, it was pronounced, and ancestral variability was virtually erased. Overlapping and following the period of genotypic restriction, independent evolution of these ancillary populations proceeded much as did that in the major stem population. The older genotypes were gradually replaced by new ones, and other subpopulations were derived from them, again mostly through restriction of the genetic variability present in the population from which they originated.

The total pattern of evolution which can be derived from this phylogenetic analysis is, therefore, entirely consistent with the better documented elements of current evolution theory. This pattern of evolution depends almost entirely on the stability of the genetic material, on the capacity of the population gene pool to retain genetic variability, and on recombinational plasticity which allows distinct genotypes to recur and be reintegrated with different genotypes at succeeding levels in time.

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## Appendix

List of species and strains used, together with their collection numbers, localities and classification. Locally collected species, or strains not kept at the University of Texas Laboratories, are listed without collection numbers.

| Species | Collection number | Locality |
|---|---|---|
| | Family: Drosophilidae<br>Genus: *Drosophila* | |
| Subgenus: Pholadoris | | |
| **victoria group** | | |
| *victoria* Sturtevant | ..... | San Andreas Can., Calif. |
| *pattersoni* Pipkin | 2093.3 | Beirut, Lebanon |
| **coracina group** | | |
| *cancellata* Mather | 2372.1 | Queensland, Australia |
| *lativittata* Malloch | 2372.4 | Queensland, Australia |
| *novopaca* Mather | 2372.7 | Queensland, Australia |

| Species | Collection number | Locality |
|---|---|---|
| **latifasciaeformis group** | | |
| *latifasciaeformis* Duda | H75.4 | Heredia, Costa Rica |
| **bryani group** | | |
| *bryani* Malloch | 2535.2 | Ponape, E. Caroline Is. |
| Subgenus: HIRTODROSOPHILA | | |
| *duncani* Sturtevant | 2311.9 | Lake Wales, Florida |
| *pictiventris* Duda | 2264.40 | San Andres Tuxtla, Veracruz, Mexico |
| *thoracis* Williston | H400.40 | Palmar, Costa Rica |
| *histrioides* Okada and Kurokawa | 2531.4 | Irika, Japan |
| Subgenus: DORSILOPHA | | |
| *busckii* Coquillett | H360.127 | Boquete, Panama |
| Subgenus: SOPHOPHORA | | |
| *populi* Wheeler and Throckmorton | 3000.8 | Anchorage, Alaska |
| **obscura group** | | |
| obscura subgroup | | |
| *pseudoobscura* Frolova | 2258.1 | Zimapan, Mexico |
| *persimilis* Dobzhansky and Epling | 2529.6 | British Columbia, Canada |
| *miranda* Dobzhansky | 2529.5 | Big Basin, California |
| *ambigua* Pomini | 2529.7 | England |
| affinis subgroup | | |
| *affinis* Sturtevant | 2069.2 | Hastings, Nebraska |
| *algonquin* Sturtevant and Dobzhansky | 2528.5 | Iron River, Wisconsin |
| *narragansett* Sturtevant and Dobzhansky | 2528.9 | Nebraska National Forest |
| *tolteca* Patterson and Mainland | H316.1 | Medellin, Colombia |
| *athabasca* Sturtevant and Dobzhansky | 2528.2 | Cheboygen, Michigan |
| *azteca* Sturtevant and Dobzhansky | 2266.3 | Chilpancingo, Mexico |
| **melanogaster group** | | |
| melanogaster subgroup | | |
| *melanogaster* Meigen | Oregon R | |
| *simulans* Sturtevant | H48.3 | Zamorano, Honduras |
| *yakuba* Burla | 2371.6 | Ivory Coast, Africa |
| takahashii subgroup | | |
| *takahashii* Sturtevant | 2363.4 | Nepal |
| ananassae subgroup | | |
| *ananassae* Doleschall | H75.8 | Heredia, Costa Rica |
| *bipectinata* Duda | ..... | Nepal |
| montium subgroup | | |
| *rufa* Kikkawa and Peng | 1736.3 | Hangchow, China |
| *nikananu* Burla | 2371.5 | Ivory Coast, Africa |
| *serrata* Malloch | 2372.8 | Queensland, Australia |
| *auraria* Peng | 1736.1 | Hangchow, China |
| *seguyi* Smart | 2371.4 | Ivory Coast, Africa |
| *kikkawai* Burla | 2404.6 | Booyal, Queensland, Australia |
| **willistoni group** | | |
| *paulistorum* Dobzhansky and Pavan | 1957.2 | Belem, Brazil |
| *equinoxialis* Dobzhansky | 2533.3 | Teffe, Brazil |
| *tropicalis* Burla and da Cunha | 1975.1 | Belem, Brazil |
| *willistoni* Sturtevant | 2268.20 | Cuernavaca, Mexico |
| *pseudobocainensis* Wheeler and Magalhaes | H407.164 | Boquete, Panama |
| *sucinea* Patterson and Mainland | 2260.1 | Huauchinango, Mexico |
| *nebulosa* Sturtevant | 2373.9 | Tingo Maria, Peru |
| *changuinolae* Wheeler and Magalhaes | H403.11 | Changuinola, Panama |
| *capricorni* Dobzhansky and Pavan | H338.2 | Sao Paulo, Brazil |
| *fumipennis* Duda | H24.10 | San Salvador, El Salvador |

| Species | Collection number | Locality |
|---|---|---|
| **saltans group** | | |
| cordata subgroup | | |
| *neocordata* Magalhaes | 2536.7 | Boa Esperanca, Brazil |
| elliptica subgroup | | |
| *neoelliptica* Pavan and Magalhaes | 2536.5 | Sao Paulo, Brazil |
| *emarginata* Sturtevant | H158.2 | Turrialba, Costa Rica |
| sturtevanti subgroup | | |
| *sturtevanti* Duda | 2374.3 | Mato Grosso, Brazil |
| *milleri* Magalhaes | H129.17 | El Yunque, Puerto Rico |
| parasaltans subgroup | | |
| *subsaltans* Magalhaes | 2536.2 | Belem, Brazil |
| *parasaltans* Magalhaes | 2536.1 | Uaupes, Brazil |
| saltans subgroup | | |
| *lusaltans* Magalhaes | H411.20 | Petionville, Haiti |
| *nigrosaltans* Magalhaes | H360.92 | Boquete, Panama |
| *pseudosaltans* Magalhaes | 2536.10 | Sao Paulo, Brazil |
| *austrosaltans* Spassky | 2536.4 | Pirassununga, Brazil |
| *saltans* Sturtevant | H180.40 | San Jose, Costa Rica |
| *septentriosaltans* Magalhaes | H103.21 | Sevilla, Colombia |
| *prosaltans* Duda | H175.23 | San Isidro, Costa Rica |
| Subgenus: DROSOPHILA | | |
| virilis-repleta section | | |
| **virilis group** | | |
| *virilis* Sturtevant | 1801.1 | Texmelucan, Puebla, Mexico |
| *americana* Spencer | 1901.5a | Jackson, Michigan |
| *novamexicana* Patterson | 1720.7a | Cliff, New Mexico |
| *littoralis* Meigen | 2096.1 | Switzerland |
| *ezoana* Takada and Okada | 2531.1 | Raus, Japan |
| *montana* Stone, Griffen and Patterson | 1942.6J | Verdi, Nevada |
| *flavomontana* Patterson | 1951.1 | Hamilton, Colorado |
| *lacicola* Patterson | 1756.2b | Ely, Minnesota |
| *borealis* Patterson | 2077.5a | Itasca Park, Minnesota |
| **robusta group** | | |
| *lacertosa* Okada | 2531.2 | Usu, Japan |
| *colorata* Walker | 2551.1 | Petosky, Michigan |
| *sordidula* Kikkawa and Peng | 2531.3 | Sapporo, Japan |
| *robusta* Sturtevant | 2320.7 | Jamestown, South Carolina |
| **melanica group** | | |
| *micromelanica* Patterson | 2160.12 | Madera Canyon, Arizona |
| *melanica* Sturtevant | 1720.3 | Cliff, New Mexico |
| *paramelanica* Patterson | 2017.9 | Smokemont, North Carolina |
| *euronotus* Patterson and Ward | 2551.2 | Saint Louis, Missouri |
| *nigromelanica* Patterson and Wheeler | 2551.3 | Cold Spring Harbor, N.Y. |
| **annulimana group** | | |
| *gibberosa* Patterson and Mainland | 2530.1 | Mexico |
| species B | H42.38 | Volcan Boqueron, El Salvador |
| species C | H62.37 | La Palma, El Salvador |
| species D | H161.10 | Turrialba, Costa Rica |
| species E | H66.12 | San Salvador, El Salvador |
| **canalinea group** | | |
| *canalinea* Patterson and Mainland | H378.4 | Minatitlan, Veracruz, Mexico |
| *paracanalinea* Wheeler | H273.11 | Hardware Gap, Jamaica |

| Species | Collection number | Locality |
|---|---|---|
| **dreyfusi group** | | |
| *camargoi* Dobzhansky and Pavan | H231.10 | Georgetown, British Guiana |
| *briegeri* Pavan and Breuer | H322.5 | Palmira, Colombia |
| **mesophragmatica group** | | |
| *gaucha* Jaeger and Breuer | H347.1 | Rio Grande do Sul, Brazil |
| *pavani* Brncic | H347.6 | Santiago, Chile |
| **repleta group** | | |
| mulleri subgroup | | |
| *aldrichi* Patterson and Crow | 2552.1 | Austin, Texas |
| *anceps* Patterson and Mainland | 1808.4B | Oaxaca, Mexico |
| *arizonensis* Patterson and Wheeler | 2156.4 | Fairbanks, Arizona |
| *buzzatii* Patterson and Wheeler | 2093.10 | Ain Anub, Lebanon |
| *eremophila* Wasserman | H381.22A | Acatlan, Puebla, Mexico |
| *hamatofila* Patterson and Wheeler | 1981.1 | Fort Davis, Texas |
| *longicornis* Patterson and Wheeler | 2513.1 | Austin, Texas |
| *martensis* Wasserman and Wilson | H188.12 | Santa Marta, Colombia |
| *meridiana* Patterson and Wheeler | H305.5b | Madden Forest, Canal Zone |
| *meridionalis* Wasserman | 2507.21 | Angra dos Reis, Brazil |
| *mojavensis* Patterson and Crow | 2533.1 | Chocolate Mts., California |
| *mulleri* Sturtevant | 2533.2 | Austin, Texas |
| *nigricruria* Patterson and Mainland | H347.3 | Azapa, Chile |
| *pachuca* Wasserman | 2519.21 | Pachuca, Mexico |
| *pegasa* Wasserman | 2519.14 | Pachuca, Mexico |
| *peninsularis* Patterson and Wheeler | 2303.3 | Riverview, Florida |
| *promeridiana* Wasserman | H318.4 | Palmira, Colombia |
| *stalkeri* Wheeler | 2213.1 | Saint Petersburg, Florida |
| *tira* Wasserman | 2521D.2 | Mexico, D.F., Mexico |
| fasciola subgroup | | |
| *fulvalineata* Patterson and Wheeler | A2.4 | Patagonia, Arizona |
| *fasciola* Williston | H122.16 | St. Lucia, B.W.I. |
| *coroica* Wasserman | H346.43 | Coroico, Bolivia |
| *pictilis* Wasserman | H27.2 | Lago Pichichuela, El Salvador |
| *pictura* Wasserman | H109.28 | Port of Spain, Trinidad |
| *fascioloides* Dobzhansky and Pavan | H181.43 | Barro Colorado, Canal Zone |
| *moju* Pavan | H303.8 | Las Cruces Trail, Canal Zone |
| *mojuoides* Wasserman | H107.7 | Arima Valley, Trinidad |
| mercatorum subgroup | | |
| *mercatorum* Patterson and Wheeler | 2507.7 | Angra dos Reis, Brazil |
| *paranaensis* de Barros | H378.6 | Minatitlan, Veracruz, Mexico |
| melanopalpa subgroup | | |
| *fulvimacula* Patterson and Mainland | H302.28 | Las Cruces Trail, Canal Zone |
| *fulvimaculoides* Wasserman and Wilson | H163.31 | Turrialba, Costa Rica |
| *limensis* Pavan and Patterson | 1529.2a | Lima, Peru |
| *repleta* Wollaston | H118.1 | Barbados, B.W.I. |
| *canapalpa* Patterson and Mainland | 2251.8 | Gomez Farias, Tams., Mexico |
| *melanopalpa* Patterson and Wheeler | 2157.15 | Ramsey Canyon, Arizona |
| hydei subgroup | | |
| *nigrohydei* Patterson and Wheeler | 2510.1 | Antigua Road, Guatemala |
| *bifurca* Patterson and Wheeler | A2 × A8.10 | Patagonia × Klondike, Ariz. |
| *eohydei* Wasserman | H191.67 | Bucaramanga, Colombia |
| *neohydei* Wasserman | H207.26 | Carpentaro, Venezuela |
| *hydei* Sturtevant | 2360.3b | Cave Creek, Arizona |
| not placed in subgroup | | |
| *serenensis* Brncic | 2364.1 | Chile |

| Species | Collection number | Locality |
|---|---|---|
| ungrouped species near repleta group | | |
| *castanea* Patterson and Mainland | H360.24 | Boquete, Panama |
| species F | H407.140 | Boquete, Panama |
| *peruviana* Duda | H181.41 | Barro Colorado, Canal Zone |
| species G | 2507.20 | Angra dos Reis, Brazil |
| species H | H194.8 | Villavicencio, Colombia |
| *aureata* Wheeler | H180.42 | San Jose, Costa Rica |
| **bromeliae group** | | |
| species I | H435.21 | Leticia, Colombia |
| **nannoptera group** | | |
| *nannoptera* Wheeler | H381.6 | Acatlan, Puebla, Mexico |
| **polychaeta group** | | |
| *polychaeta* Patterson and Wheeler | H435.44 | Leticia, Colombia |
| quinaria section | | |
| **immigrans group** | | |
| *hypocausta* Osten-Sacken | 2502.5 | Ponape, E. Caroline Is. |
| *spinofemora* Patterson and Wheeler | 2372.16 | Queensland, Australia |
| *immigrans* Sturtevant | 2321.9 | Cross Anchor, South Carolina |
| **funebris group** | | |
| *macrospina* Stalker and Spencer | 1784.12 | Durango, Mexico |
| *subfunebris* Stalker and Spencer | 2181.3 | Willow Creek, California |
| *funebris* Fabricius | 2082.1 | Minneapolis, Minnesota |
| **quinaria group** | | |
| *innubila* Spencer | 2076.8B | Silver City, New Mexico |
| *quinaria* Loew | 1753.7 | Lake Shetek, Minnesota |
| *rellima* Wheeler | 2068.9 | Oakdale, Nebraska |
| *falleni* Wheeler | 1062.6 | Gt. Smoky Mt. N. P., Tenn. |
| *phalerata* Meigen | 1915.1 | Beirut, Lebanon |
| *occidentalis* Spencer | 2175.3 | Mt. San Jacinto, California |
| species J | 929.8 | Cave Creek, Arizona |
| *tenebrosa* Spencer | 2072.6 | Eagle Nest, New Mexico |
| *subquinaria* Spencer | 2516.1 | Aspen, B. C., Canada |
| *transversa* Fallen | 2549.1 | England |
| *subpalustris* Spencer | 1877.9 | Georgetown, South Carolina |
| *palustris* Spencer | 1757.13 | Bemidji, Minnesota |
| *guttifera* Walker | 2086.3 | Austin, Texas |
| **testacea group** | | |
| *testacea* von Roser | 3005.4 | Fairbanks, Alaska |
| *putrida* Sturtevant | 2539.1 | Austin, Texas |
| **calloptera group** | | |
| *ornatipennis* Williston | 2378.2 | St. Vicente, Cuba |
| *calloptera* Schiner | H192.11 | Rio Negro, Colombia |
| *schildi* Malloch | 2543.2 | Barro Colorado, Canal Zone |
| **guarani group** | | |
| guaramunu subgroup | | |
| *guaramunu* Dobzhansky and Pavan | 2271.1 | Brazil |
| *griseolineata* Duda | 2211.3 | Ponta Grossa, Brazil |
| *guaraja* King | H407.20 | Boquete, Panama |
| guarani subgroup | | |
| *guarani* Dobzhansky and Pavan | 2211.2 | Feliz, Brazil |
| *subbadia* Patterson and Mainland | 2262.24 | Huatusco, Veracruz, Mexico |

| Species | Collection number | Locality |
|---|---|---|
| **cardini group** | | |
| *dunni* Townsend and Wheeler | 2327.1 | Rio Piedras, Puerto Rico |
| *belladunni* Heed and Krishnamurthy | H356.3d | Hardware Gap, Jamaica |
| *nigrodunni* Heed and Wheeler | H247.1 | Barbados, B.W.I. |
| *polymorpha* Dobzhansky and Pavan | 2374.8 | Montes Claros, Brazil |
| *neomorpha* Heed and Wheeler | H51.10 | Lancetilla, Honduras |
| *neocardini* Streisinger | H339.1 | Angra dos Reis, Brazil |
| *parthenogenetica* Stalker | 1802.17 | Atlixco, Puebla, Mexico |
| *acutilabella* Stalker | 2378.3 | St. Vicente, Cuba |
| *procardinoides* Frydenberg | H346.8 | Corcico, Bolivia |
| *cardinoides* Dobzhansky and Pavan | H27.9 | Lago Pichichuela, El Salvador |
| *cardini* Sturtevant | 2263.6 | Tezuitlan, Puebla, Mexico |
| ungrouped species near cardini group | | |
| species K | H407.23 | Boquete, Panama |
| species L | H407.139 | Boquete, Panama |
| **rubrifrons group** | | |
| *parachrogaster* Patterson and Mainland | 1787.3 | Zacatecas, Mexico |
| *unimbes* Patterson and Mainland | H407.126 | Boquete, Panama |
| species M | 2510.6 | Guatemala |
| species N | H360.21 | Boquete, Panama |
| **macroptera group** | | |
| *submacroptera* Patterson and Mainland | 2522.9 | Antigua Road, Guatemala |
| *macroptera* Patterson and Wheeler | 2580.1b | Rocky Mt. Nat'l Park, Colo. |
| **pallidipennis group** | | |
| *pallidipennis* Dobzhansky and Pavan | H191.48 | Bucaramanga, Colombia |
| **tripunctata group** | | |
| *mediodiffusa* Heed and Wheeler | H352.4 | Ocho Rios, Jamaica |
| *albicans* Frota-Pessoa | H209.17 | Merida, Venezuela |
| *metzii* Sturtevant | H404.15 | Changuinola, Panama |
| *albirostris* Sturtevant | 2538.4 | Barro Colorado, Canal Zone |
| *unipunctata* Patterson and Mainland | H344.33 | Montero, Bolivia |
| *mediopunctata* Dobzhansky and Pavan | 2211.4 | Ponta Grossa, Brazil |
| *tripunctata* Loew | 2539.2 | Austin, Texas |
| *trapeza* Heed and Wheeler | H29.16 | San Salvador, El Salvador |
| *bandeirantorum* Dobzhansky and Pavan | H346.42 | Corcico, Bolivia |
| *paramediostriata* Townsend and Wheeler | H254.12 | Mayaguez, Puerto Rico |
| *mediostriata* Duda | H341.13 | Vila Atlantica, Brazil |
| *mediopictoides* Heed and Wheeler | H407.32 | Boquete, Panama |
| *crocina* Patterson and Mainland | H131.2 | Rio Piedras, Puerto Rico |
| ungrouped species near tripunctata group | | |
| *sticta* Wheeler | H51.15 | Lancetilla, Honduras |
| species not placed in section | | |
| *araccu* Heed and Wheeler | H46.28 | Santa Tecla, El Salvador |
| *carsoni* Wheeler | 2551.4 | Bridgewater, Vermont |
| *carbonaria* Patterson and Wheeler | 2540.2 | Austin, Texas |
| *tumiditarsus* Tan, Hsu and Sheng | 1736.6 | Hangchow, China |
| Subgenus: PILORIDOSA | | |
| species O | H444.2B | El Colegio, Colombia |
| species P | H444.2C | El Colegio, Colombia |
| species Q | H444.2D | El Colegio, Colombia |
| Genus: *Chymomyza* | | |
| *amoena* (Loew) | 2532.4 | Austin, Texas |
| *aldrichi* Sturtevant | 2199.9 | Morgan, Utah |
| *procnemis* (Williston) | 1782.7 | Durango, Mexico |

| Species | Collection number | Locality |
|---|---|---|
| | Genus: *Scaptomyza* | |
| *adusta* (Loew) | 2368.1 | Austin, Texas |
| *pallida* (Zetterstedt) | 2532.3 | Austin, Texas |
| *?hsui* Hackman | ..... | San Jacinto Mts., California |
| | Genus: *Zaprionus* | |
| *ghesquierei* Collart | 2371.3 | Africa |
| *vittiger* Coquillett | 1974.4 | Africa |
| | Genus: *Mycodrosophila* | |
| *dimidiata* (Loew) | 2540.1 | Austin, Texas |
| | Genus: *Zapriothrica* | |
| *dispar* (Schiner) | H444.2A | El Colegio, Colombia |
| | Genus: *Gitona* | |
| *americana* Patterson | ..... | Austin, Texas |
| *bivisualis* Patterson | ..... | Austin, Texas |
| | Genus: *Rhinoleucophenga* | |
| *obesa* (Loew) | ..... | Austin, Texas |
| | Family: DIASTATIDAE | |
| *Diastata vagans* Loew | ..... | Fairbanks, Alaska |
| | Family: SPHAEROCERATIDAE | |
| *Leptocera* sp. | ..... | Riverside, California |
| | Family: EPHYDRIDAE | |
| *Scatella stagnalis* (Fallén) | ..... | San Jacinto Mts., California |
| *Discocerina obscurella* (Fallén) | ..... | Riverside, California |
| | Family: PERISCELIDAE | |
| *Periscelis annulata* (Fallén) | ..... | Austin, Texas |
| | Family: SEPSIDAE | |
| *Sepsis violacea* Meigen | ..... | Austin, Texas |
| *Sepsidimorpha secunda* Melander and Spuler | ..... | Austin, Texas |
| | Family: CHLOROPIDAE | |
| *Oscinella carbonaria* (Loew) | ..... | Austin, Texas |
| *Oscinella cozendix* (Fitch) | ..... | Austin, Texas |
| *Thaumatomyia glabra* (Meigen) | ..... | Austin, Texas |
| | Family: ANTHOMYZIDAE | |
| *Mumetopia occipitalis* Melander | ..... | Austin, Texas |
| | Family: AGROMYZIDAE | |
| *Phytobia* sp. | ..... | Austin, Texas |
| | Family: AULACIGASTRIDAE | |
| *Aulacigaster leucopeza* (Meigen) | ..... | Austin, Texas |
| | Family: PIOPHILIDAE | |
| *Piophila casei* (L.) | ..... | Austin, Texas |
| *Prochyliza xanthostoma* Walker | ..... | Austin, Texas |