16. Genetics and Evolution of Hawaiian *Drosophila*

H. L. CARSON

Department of Genetics
University of Hawaii
Honolulu, Hawaii, U.S.A.

and

J. S. YOON   (1982).

Department of Biological Sciences
Bowling Green State University
Bowling Green, Ohio, U.S.A.

I. Introduction

The Hawaiian Islands have a rich but discordant insect fauna. Their remote ancestors apparently came from different directions across the Pacific Ocean. The endemic Drosophilidae of Hawaii consist of about 800 species belonging to one or possibly two major lineages (Hardy, 1965, 1974; Hardy and Kaneshiro, in Volume 3a; Carson and Kaneshiro, 1976). They are distributed throughout the high islands of the archipelago, displaying not only a highly characteristic single-island endemism but also extraordinary morphological diversity along with adaptations which show their intimate ecological relationship to the native flora. The original colonization of the archipelago may have involved ancient high islands now eroded nearly to sea level. This probably occurred in late Miocene or Pliocene and was then followed by exhuberant speciation southeastward along the volcanically developing archipelago.

Hawaii shows extraordinary isolation from the fringing continents and the other archipelagos of the Pacific basin. There is now and probably always has been more than 3500 km of open ocean between Hawaii and any significant source of terrestrial biota. The islands were and still are being formed from volcanic eruptions. These penetrate the Pacific tectonic plate, thrusting the lavas which form the islands above the sea. Of great significance for the evolutionist is the fact that the Hawaiian Islands display a linear sequence of ages. Thus, Kauai in the northwest (Fig. 1) is the oldest of the major high islands; potassium-argon measurements of its lava flows indicate an age of about 5·6 million years before the present. As one proceeds southeastward, each island and volcano is successively younger (McDougall, 1969). The youngest (and largest) island is called Hawaii (*sensu stricto*). It shows no lava flows older than 700,000 years; two of its five volcanos are great mountains rising to approximately 4200 m above sea level. A current widely-accepted theory holds that each successively younger island has been formed over a fixed "hot spot" in the earth's mantle as the Pacific plate has slowly moved northwestward (Dalrymple *et al.*, 1973).

The position of the Hawaiian Islands in the warm Pacific ocean just south of the Tropic of Cancer results in a tropical climate with abundant rainfall; this is especially heavy where the persistent northeast trade winds strike the cooler slopes of the mountains. These exuberant windward rainforests give way to xeric leeward areas and beaches. This creates rainfall gradients which, along with the range of altitudes and the variable ash and lava soils, produce a diverse series of abiotic

157° W. Longitude

N

KAUAI

OAHU

MOLOKAI

LANAI MAUI
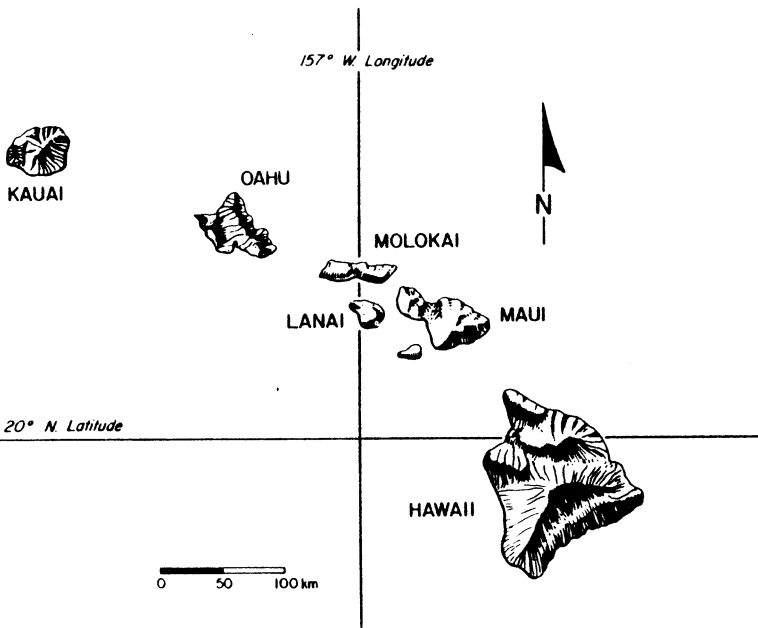
20° N. Latitude

HAWAII

0   50   100 km

FIG. 1. The main islands of the Hawaiian archipelago.

substrates for the development of ecosystems. These are often sharply discontinuous over short linear distances. This dissection of the environment is further affected by the way in which, on the slopes of an active volcano, the flows of lava from the rifts subdivide an older forest into isolated pockets of vegetation (kipukas). Such places are to be found most diagrammatically on the newest island, Hawaii ("The Big Island") where the flows are most recent (Fig. 2). Even on older volcanoes where rift flows have ceased, however, a mosaic of forest patches of different composition and age are left behind. Such a patchy environment is not easily homogenized and is conducive to odd distributions of species. In some cases specific isolations of the total population of a species are displayed.

On the older islands, the spottiness of biotic distributions due to the past history of volcanism and host distribution is often still discernible. Nevertheless, the older volcanic domes have been eroded and dissected into deep amphitheatre-shaped valleys in which the vegetation is more predictable and uniform than in the times of the early colonization phase. Many Hawaiian plants, which serve as hosts for *Drosophila*, are

FIG. 2. A kipuka at Mawae on Hawaii formed by an 1855 a'a lava flow. The large trees in the center are *Acacia koa* and reach heights of about 22 m. Photograph by H. E. Paterson.

themselves rare and local. This serves to explain the rarity of some of the *Drosophila* species which breed on them.

Although the endemic *Drosophila* fauna is rich in species, population sizes are generally small. This is due in part to the destruction of native forests by man and to the decline of these forests in the face of competition from exotic plant species. Nevertheless, it is clear that rare and local distribution phenomena are a characteristic feature of the island biotas quite apart from the influence of man.

From this diverse fauna, certain species and species groups have been selected for special attention. Our treatment in this paper emphasizes chromosomal characteristics. By far the most spectacular flies are the somewhat more than 100 species which are commonly called the "picture-wings" (Fig. 3). These are treated as a species group within the genus *Drosophila* (subgenus *Drosophila*). Fortunately, these species respond reasonably well to laboratory culture, have extremely favorable giant polytene chromosomes and are amenable to metaphase and electrophoretic examination. Behavioral studies and hybridization in the laboratory can also be widely employed as techniques for discerning relationships and evolutionary histories. Largely because of these features, much of the early work on the evolutionary biology of
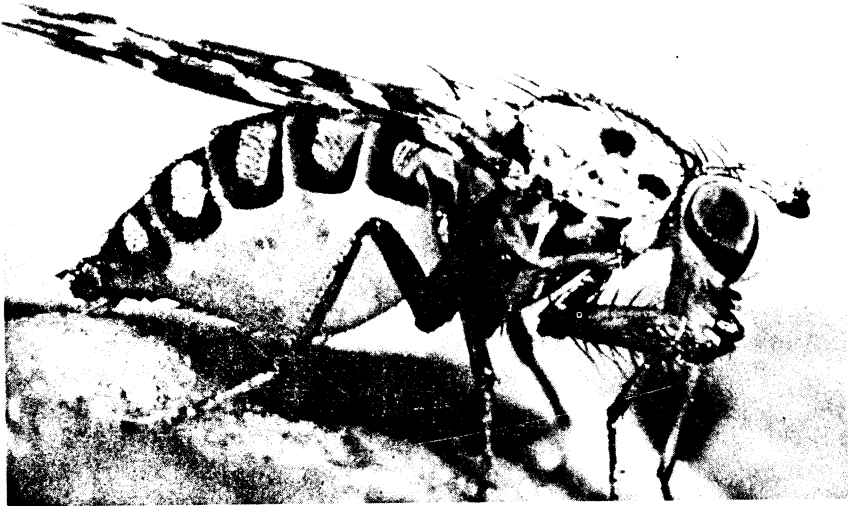
Fig. 3. A picture-winged species, *Drosophila grimshawi*. (male; ×22). Photograph by W. P. Mull.

Hawaiian *Drosophila* was concentrated on flies of this species group. Much information, however, is now available on several other groups of species which, like the "picture-wings", are also best relegated to the subgenus *Drosophila*, despite the fact that early taxonomic treatment resulted in some of them being placed in separate genera or subgenera (see Hardy, 1965).

Soon after experimental and laboratory studies of behavior were begun, however, it became apparent that many of the peculiar morphological features of these forms reflect sexual and agonistic behavior of males (Spieth, 1966). Indeed, many of these peculiar characters are wholly confined to the male sex and are unreliable as bases for erecting genera or subgenera. Accordingly, Kaneshiro (1976) has thoroughly revised generic and subgeneric concepts for the Hawaiian Drosophilidae. Effectively, he has reduced the endemic fauna to only two genera, *Drosophila* and *Scaptomyza*. Hardy (1977) has accepted most of these changes but, on the grounds of convenience, has suggested that one of the older generic names, *Antopocerus*, be retained as a subgenus in the genus *Drosophila*. In this paper, we have treated this group of fifteen

interesting species of flies as one of the many species groups within the subgenus *Drosophila* (e.g., "antopocerus species group"). Although extensively speciated, flies of the genus *Scaptomyza* have received relatively little attention either taxonomically, ecologically or genetically. This review, accordingly, emphasizes flies of the genus *Drosophila*.

In addition to its ancient endemic fauna, there are in Hawaii approximately 22 species which have been introduced inadvertently by man. Most of these introductions probably occurred after contact with European man (*c.* 1778). By and large, these species include most of the familiar cosmopolitan species found around the world. They will not be dealt with in this article.

II. Chromosomes

In spite of their morphological (Hardy, 1965), ecological (Heed, 1968) and ethological diversity (Spieth, 1966), the Hawaiian Drosophilidae constitute a closely related evolutionary group. Anatomical studies give further evidence for this (Throckmorton, 1966). Cytological studies of both polytene and metaphase karyotypes to some degree supplement the anatomical and behavior studies. In this section, the karyology of Hawaiian *Drosophila* is reviewed and discussed.

A. METHODS

Determination of metaphase and polytene karyotypes were made by slightly modified aceto-orcein methods. Heterochromatic bands were identified in polytene chromosomes by their amorphous appearance and by the fact that they stain darker than euchromatic bands. The latter comprise most of the polytene chromosome. The band (gene) sequences of *D. grimshawi* (Auwahi, Maui) for the picture-winged species group, and that of *D. hystricosa* (Kaupo Gap, East Maui) for the modified mouthparts species group were selected as the respective group "Standards" since these species have been used for the codification of polytene chromosome designations for Hawaiian *Drosophila* (Yoon and Carson, 1973). The banding patterns of other species wherever possible have been referred to these Standards. Inter- and intra-specific crosses were made using pairs or small mass matings in order to detect sexual isolation and to confirm inheritance of cytogenetic variations.

B. METAPHASE CHROMOSOMES

Karyotypes of 155 species of the genus *Drosophila* from Hawaii have been determined (Yoon *et al.*, 1972–1978; Clayton, 1976; Clayton and Wheeler, 1975). The results are summarized in Table I. Most species show 2n = 12: five acrocentrics and a microchromosome (5R1D) or six acrocentrics (6R) (Fig. 4). The latter condition appears to involve an alteration in the amount or position of heterochromatin in the genome. For example, *D. yooni* (*antopocerus* species group) shows six pairs of acrocentrics, indicating an alteration of the dot chromosome into a rod by heterochromatin addition.

TABLE I. Karyotypic differentiation in endemic members of the genus *Drosophila* in Hawaii.

subgenus	5R1D	6R	1V3R1D	Metaphase configurations distributed among species 2V1R1D and other	Total
Drosophila					
other species groups	117	10	8	6	141
antopocerus species group	6	4	—	—	10
Engiscaptomyza	—	—	4	—	4
					155

Reduction in chromosome number from the apparently primitive five rods and one dot (n = 6) to n = 5 or n = 4 has apparently occurred by the process of whole-arm fusion and centromere loss (Table I; Fig. 4; *D. crassifemur* belongs to the subgenus *Engiscaptomyza*). In Hawaiian flies of the genus *Drosophila*, about 12% (18 out of 155 species) have a reduced chromosome number while 46·7% of comparable non-Hawaiian species (114 out of 244 species) show such reductions (Clayton, 1976). Among the 155 Hawaiian *Drosophila* there are no reports of karyotypic change by pericentric inversion, while Patterson and Stone (1952) found a minimum of 17 such cases among 150 non-Hawaiian species (about 11%).

Hawaiian flies of the genus *Scaptomyza* have been studied less than *Drosophila*. Among about 18 species, all have at least one fusion, so that neither the 5R1D nor the 6R condition has been recorded.

FIG. 4. Metaphase plates of three species of Hawaiian *Drosophila*. Heterochromatic parts (darkly stained) of each chromosome are shown and the microchromosomes (M) are indicated. (a) *D. kambysellisi*, modified mouthparts species group (5R1D); (b) *D. yooni* (6R: a pair of enlarged acrocentric microchromosomes is shown); (c) *D. crassifemur* (IV3R1D).

C. POLYTENE CHROMOSOMES

Hawaiian *Drosophila* have six polytene elements, five long and one short, in their genome. The principal type of chromosomal variation among these Hawaiian species is the paracentric inversion, which is relatively easy to detect. Furthermore, most of these inversions have become fixed in the populations of the one or more species in which they are found. Therefore, phylogenetic relationships among many species have been established in detail.

Combining our data (Yoon *et al.*, 1972–1978; Carson and Kaneshiro, 1976) brings to 316 the total number of inversions thus far detected in the 155 species of Hawaiian *Drosophila* of which the polytene chromosomes have been studied. This means that, on average, two inversions per species have been fixed during their evolution. The two subgroups in the modified mouthparts groups are differentiated by eleven inversions.

Although the classical inversion mapping of polytene chromosomes continues to be employed for determining phylogenetic relationships among closely related species, this approach is not feasible for the study of more distantly related taxa. Yoon *et al.* (1972c) devised a phylogenetic measure utilizing the proportion of the polytene chromosome karyotype which could be homologized. Using this measure, chromosomal homologies were established among various morphologically quite dissimilar Hawaiian Drosophilidae.

Thus, homologous sections found in each of six chromosomes were compared among species within three species groups of Hawaiian *Drosophila*. The similarity varies considerably with the chromosome, suggesting that each chromosome has undergone different degrees of rearrangement during its evolution. In the case of the *antopocerus* and modified mouthparts groups (see Fig. 5) approximately 40% of the length is homologous in the second chromosome, while only 20% of the third, 17% of the fourth, and 15% each of the X and the fifth can be homologized. Thus, approximately 30% of the genome may be homologized. This is not greatly different from approximately 50% of the genome which may be homologized in the species group comparisons of other species groups in the subgenus *Drosophila*, e.g. between the modified mouthparts and the picture-winged species groups. Yoon and Richardson (1978b) have shown that the banding order of the polytene microchromosome, relative to the centromere, is reversed in the *antopocerus* species and *Engiscaptomyza* when the latter two are compared with members of the subgenus *Drosophila*, including the picture-winged species.
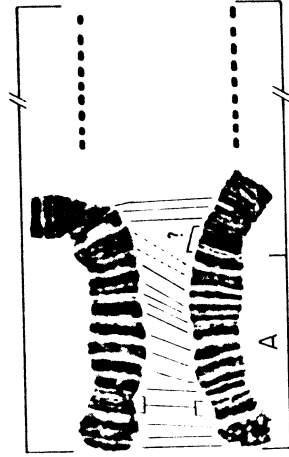
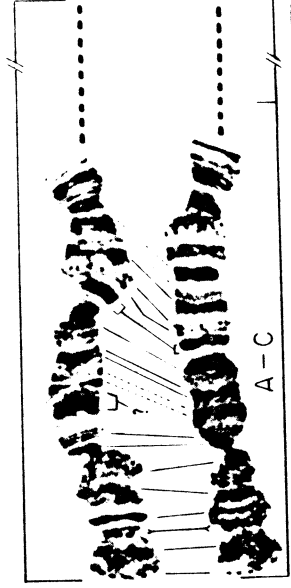Drosophila tanythrix

Drosophila mimica
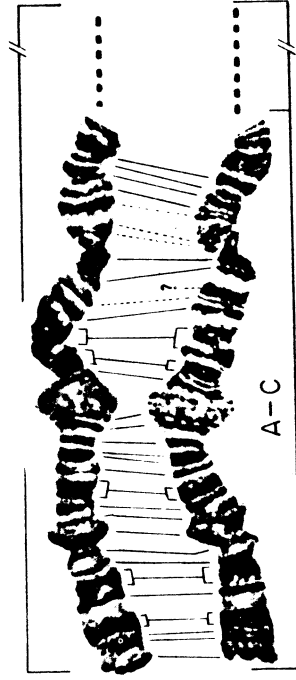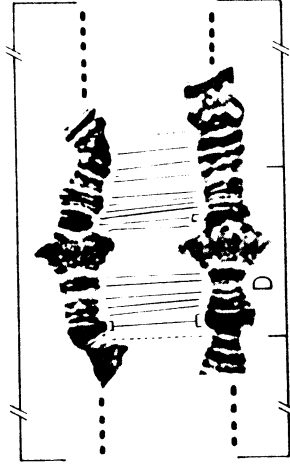
Inversions do not always differentiate species or species groups. Some inversions have remained as chromosomal polymorphisms in more than one species. For example, three species of the *hystricosa* subgroup (modified mouthparts) are polymorphic for some of the same inversions. These polymorphisms most likely arose in a common ancestor of these species (Yoon *et al.*, 1972a, b). Similar instances are known in the picture-winged group and will be described later.

The term "homosequential species" has been used to refer to those species which have the same sequential polytene chromosome karyotype (Carson *et al.*, 1967). Even though the polytene chromosome banding patterns are the same, however, the amount and distribution of heterochromatin sometimes differs, and, consequently, the metaphase karyotypes differ between such species. Yoon *et al.* (1972b) designated these cases as "anisohomosequential". Pairs of species with no detectable cytological differences are called "isohomosequential" species. Although true isohomosequential species may exist, the experience of one of us (JSY) has shown that, as the species are studied in greater detail heterochromatic differences usually become apparent.

D. Pseudochromocenter and Heterochromatin

In earlier studies of Hawaiian *Drosophila*, an additional chromocenter-like configuration was found in polytene nuclei of several species in addition to the true chromocenter (Fig. 6). Only specific sites of certain chromosomes join to give the characteristic configuration, which was designated as the "pseudochromocenter" (Yoon *et al.*, 1972a). These sites are apparently composed of intercalary heterochromatin and represent multiple points where non-homologous association occurs. Based on several lines of cytogenetic evidence, a model involving the pseudochromocenter in the production of chromosome rearrangements has been proposed (Yoon and Richardson, 1978a).

The wealth of cytological comparisons possible among the many relatively closely related species of Hawaiian *Drosophila* has been exploited in various ways. As can be seen from the above paragraphs and examination of Fig. 7, heterochromatin changes are striking between species of Hawaiian *Drosophila*. It has been suggested (White, 1973; Pathak *et al.*, 1973) that centromeric heterochromatin may play

---

Fig. 5. Chromosomal homology between *D. mimica* (modified mouthparts group) and *D. tanythrix* (*antopocerus* species group). Solid lines between chromosomal sections indicate homologous bands. The distal ends of the chromosomes are to the left. Arabic numbers refer to chromosome arms.
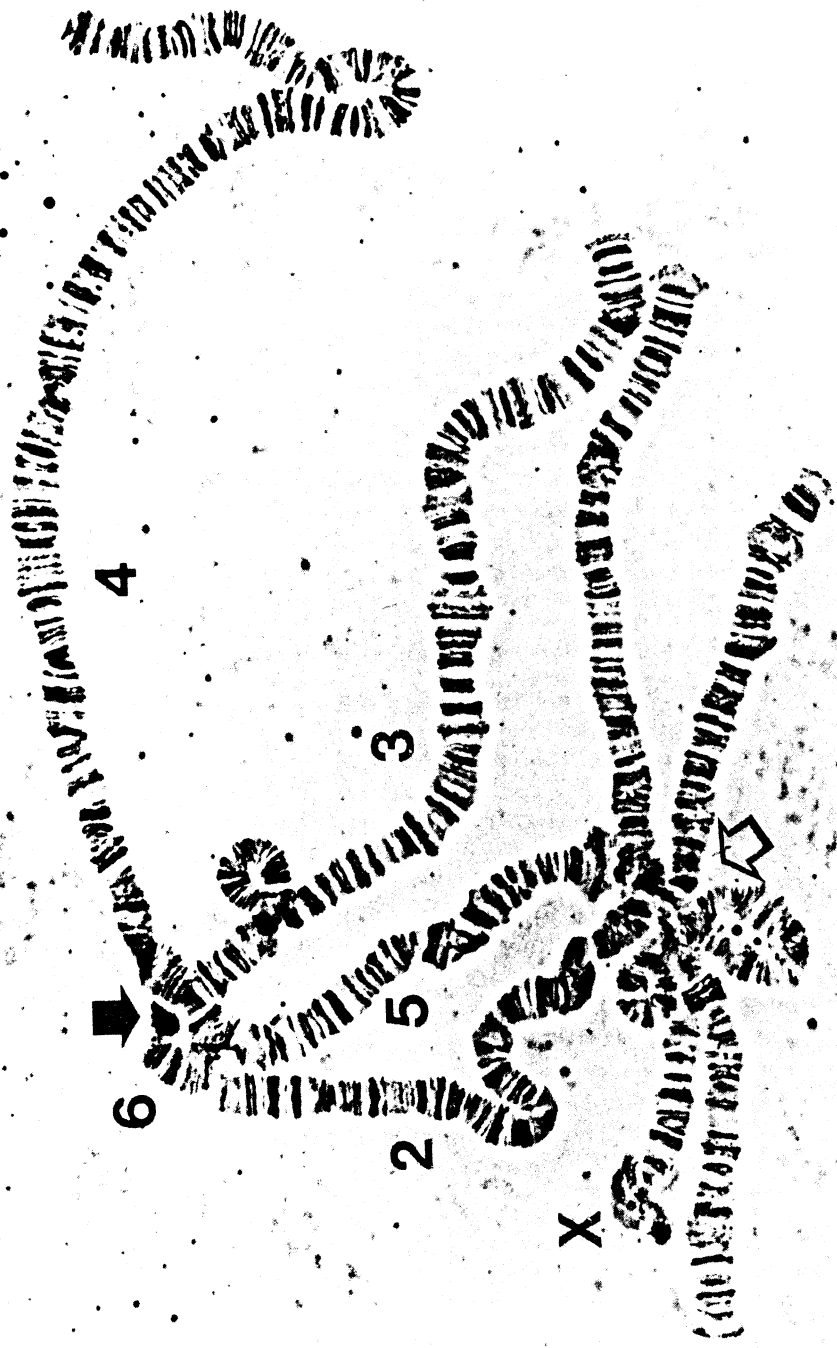
a significant role in the evolution of the karyotype of both plants and animals, being associated with changes in chromosome morphology through breakage and reunion.

Yoon *et al.* (1975) have discussed the phylogenetic changes of heterochromatic sites in polytene chromosomes of Hawaiian *Drosophila*, as well as total heterochromatin associated with individual metaphase chromosomes. It seems that the distribution of heterochromatin is also related to speciation. Several phylogenies in the Hawaiian *Drosophila* exhibit an accumulation of heterochromatin on the "dot" (micro-) chromosome, thereby converting it to a rod. These large accumulations of heterochromatin on microchromosomes have so far been observed only in species which appear to have been recently derived from, but which show no differences in gene sequence from, their nearest relatives (Yoon and Richardson, 1978c). Upon more detailed analysis of the metaphase and polytene chromosomes of 155 species (443 strains) of Hawaiian *Drosophila*, the following facts and interpretations have been made by these authors: (a) there are 14 species with large accumulations of heterochromatin on the microchromosome, converting the latter into a large acrocentric chromosome; (b) each of these 14 species is a member of a different set of species. Species within each set have identical polytene chromosome karyotypes; (c) each of these 14 sets of homosequential species is situated at what appears to be a terminal position of a phylogeny and, therefore, may represent the most recently derived species of that lineage. This provides a means of determining direction of evolution in several instances.

The facts also suggest that localized accumulations of heterochromatin are correlated with absence of chromosome rearrangements. In other words, concentrations of heterochromatin in localized regions may signal reduced potential for rearrangements to occur elsewhere in the genome. This suggests that until the intercalary heterochromatic sites form, the accumulation of inversions may be delayed, contributing to the accumulation of homosequential species at the phylogenetic termini. In addition to the effect on structural changes, the location and/or amount of heterochromatin may serve as a factor in differentiating newly-formed species (Yoon and Richardson, 1978c).

Fig. 6. Salivary gland chromosomes of *D. biseriata*. Both true chromocenter (solid arrow) and pseudochromocenter (open arrow) are shown. Chromosomes X, 2 and 5 are involved in the latter. Note that the centromere of the X chromosome was detached from the true chromocenter while being squashed for slide preparation.

A phylogenetic chart of Hawaiian *Drosophila* chromosomal inversions. Text labels and boxed inversion designations, read from top to bottom:

- murphyi Hawaii [3o/+]
- balioptera Maui, Molokai, Lanai
- orthofascia Maui, Lanai, Hawaii
- hirtipalpus Maui [Xq²]
- psilotarsalis Hawaii [Xp² 2j]
- heedi Hawaii [6R]
- recticilia Maui [5g/+]
- turbata Oahu [5g]
- ciliaticrus Hawaii
- affinidisjuncta W Maui
- gradata Oahu [3g]
- silvarentis Hawaii
- reynoldsiae Oahu [3o] — Xg — sobrina Oahu
- disjuncta Maui [4v/+]
- bostrycha Molokai
- lasiopoda Maui [6R]
- pullipes Hawaii
- [2b]
- gymnobasis Maui
- flexipes Oahu
- musaphilia Kauai
- engyochracea Hawaii [4l]
- orphnopeza Maui [3o/+]
- sodomae Molokai
- atrimentum Oahu
- [5R 1D]
- X 2 3 4 5 6 STANDARD
- villitibia Molokai
- formella Hawaii [4j³/k³]
- hawaiiensis Hawaii
- [Xa² 4u]
- grimshawi Kauai, Oahu, Lanai, Molokai, Maui
- sproati Hawaii [Xb²]
- obatai Oahu
- claytonae Hawaii [5r]
- villosipedis Kauai
- crucigera Oahu, Kauai [Xa]
- limitata Maui [6R]
- sejuncta Kauai
- ochracea Hawaii
- [5a]
- pauciciliа Oahu
- gymnophallus Oahu [Xh] — liophallus Maui
- paucipuncta Hawaii
- punalua Oahu
- spaniothrix Oahu
- odontophallus Maui
- psilophallus Oahu [6R]
- ocellata Kauai
- macrothrix Hawaii [3i] — tarphytrichia Oahu
- uniseriata Oahu [6R]
- hexachaetae Oahu [3q]
- digressa Hawaii
- virgulata Maui [5d]
- vesciseta Maui
- pilimana Oahu
- glabriapex Kauai
- aglaia Oahu
- conspicua Hawaii [Xmn 4m]
- discreta Maui [Xc 3ch/p]
- [4c]
- lineosetae Maui [Xz²a³ 2a/+ 3b/c]
- basisetae Hawaii [4u²]
- prolaticilia Hawaii [5b]
- [Xef 3z 4efg]
- divaricata Oahu
- distinguenda Oahu [Xc³d³ 2r]
- inedita Oahu
- [4b]
- [2a/+ 3b/+ 3c/+ 4c/+]
- fasciculisetae Maui [2a/+ 3b 4c/+]
- prostopalpis Maui [Xh³4R1V1D]
- montgomeryi Oahu [6R]
- assita Hawaii [Xk³l³]
- micromyia Kauai [Xp³q³r³s³]
- alsophila Hawaii [Xj³ 4h³]
- silvestris Hawaii [3m/+] heteroneura Hawaii
- hemipeza Oahu [Xc²]
- planitibia Maui
- [Xf]
- differens Molokai
- nigribasis Oahu
- neoperkinsi Molokai [2m/+ 4f³/+]
- cyrtoloma Maui [Xu² 5V1J]
- oahuensis Oahu
- melanocephala E Maui [4w²/+ 5RIV]
- [2m] [Xt]
- hanaulae Maui
- [3c² 4f³ 4w²/+]
- ingens W Maui [Xi³ 4w²]
- obscuripes Maui [Xu²/+]
- [Xt/+ Xu²/+ 2m/+ 4f³/+]
- neopicta Maui, Molokai [Xt/+ 2m/+ 4f³/+]
- substenoptera Oahu [Xopqs]
- picticornis Kauai [Xdl]
- [3e 4hij 5e]
- setosifrons Hawaii [Xj 3d]
- [Xik Xo/+]
- primaeva Kauai [Xg²/+ 2gh 3x/+ 4y 5j]
- ochrobasis Hawaii [2k]
- [Xm² 3l 4b²]
- setosimentum Hawaii [Xn² 4c²d²e²f²n²o²]
- touchardiae Oahu [Xm³]
- adiastola Lanai, Maui [4p]
- spectabilis Maui, Molokai [4r]
- cilifera Molokai
- [Xz 2e]
- [3j]
- peniculipedis Maui
- neogrimshawi Oahu [3t]
- clavisetae Maui [4p]
- hamifera Maui
- varipennis Molokai
- paenehamifera Maui
- truncipenna Maui [Xf³]
- [Xvw 3f 4q 4p/+ 5f]
- [Xy² 4p]
- [Xo 2c]
- [Xuxy 2d 3k 3j/+ 4o]
- ornata Kauai [Xr²s²t² 3jw 4v²]
- mimica Hawaii [+ 45 inversions, 6R]
- [5h]
- primaeva Kauai [Xg²/+ 2gh 3x/+ 4y 5j]
- [Xd²e²f²h²i²j²k² Xg²/+ 2f 3x/+ 4wxz 5i]
- attigua Kauai [Xg²x² 2i 3xy 4x²y²z² 5o]

immediate origin of certain species but also to permit inferences about the very earliest colonization of the archipelago.

B. PICTURE-WINGED SPECIES

1. The basic data

Of the *Drosophila* of Hawaii, it is the picture-winged group which has proved most amenable to the application of chromosomal methods to the tracing of species origins. The data from which phylogenetic schemes may be constructed consist of readings of polytene banding sequences of each species (Fig. 7). In this work, begun about 1963 (initial report in Carson *et al.*, 1967), photographic polytene chromosome maps were prepared for *D. grimshawi* Oldenberg (monomorphic stock G1, Auwahi, Maui) which was chosen as the general Standard for the group. Details of the system for naming inversions are given in the caption of Fig. 7. To date, 213 inversions have been discovered in 103 picture-winged species (Table II). Cytological localizations of most of these inversion breaks are given in a series of published maps (references in Carson and Kaneshiro, 1976). Thirteen newly-discovered inversions have not yet had their break-points published. The many species in Fig. 7 have been informally split into five chromosomal subgroups, each bearing the name of a species for which separate maps were prepared. The subgroups are: *planitibia* (lower left of figure), *adiastola* (lower right), *punalua* (upper left), *grimshawi* (upper center) and *primaeva* (bottom). The number of species in each subgroup is listed in Table III. Nevertheless, all inversion breakpoints can be referenced to the maps of *D. grimshawi*. As will be discussed later, the general Standard does not consist of sequences which are currently judged to be the most primitive. For each of the picture-winged species, the polytene analysis of the entire genome is complete. Near the bottom of Fig. 7, *D. mimica* is

TABLE II. Number of fixed and polymorphic inversions among 103 picture-winged species of *Drosophila*.

Chromosome	X	2	3	4	5	Total
Fixed	59	11	14	32	11	127
Polymorphic						
confined to single species	10	7	13	32	10	72
not confined to single species	3	2	5	3	1	14
TOTAL	*72*	*20*	*32*	*67*	*22*	*213*

III. Chromosome Tracing of Species Origins

A. BACKGROUND

The cytological favorability of the giant chromosomes of many species of Hawaiian *Drosophila* has made possible sensitive cytological comparisons of species. These comparisons refer primarily to the basic polytene gene sequences of each chromosome. These are inferred from the banding orders in the giant chromosomes which may be directly observed under the light microscope. Species can be grouped according to the similarity of their gene orders. Those which share certain inversions, for example, can be grouped together in a scheme which directly reflects phylogenetic relationships between the species.

This type of study was first made on a large scale by Wasserman (1963) using species of the *Drosophila repleta* group. For successful application to the more distant members of a phylad, it is necessary, through sequence mapping, to identify sections of polyene chromosome which are inverted relative to an arbitrarily chosen Standard sequence. In most cases, these inversion differences must be capable of recognition in the homozygous state.

Most relationship-diagrams based on inversion similarities cannot be readily converted into unidirectional phylogenetic schemes, for the following reasons. When confronted with two sequences, for example, 1 2 3 4 and 1 3 2 4, the facts so displayed carry no information as to which of the two was the original sequence and which derived. Accordingly, without information outside of the chromosome sequences themselves, phylogenetic relationships between the carriers of the relevant gene orders must be represented with double arrows: i.e. 1 2 3 4 ⇌ 1 3 2 4. In the Hawaiian situation, however, powerful independent geological and geographical information makes possible the construction of very reasonable unidirectional phylogenies.

Regarding the unique species of the newest island, Hawaii, one may ask from where and from what specific ancestors did these species arise? Naturally, one looks to the fauna of an adjacent, somewhat older island. Such a hypothesis as to origin is strengthened if specific arrangements of genes are found to be restricted in geographical distribution to the newest island and if this condition can be traced, stepwise, back to sequences on even older islands. As will be described, these conditions are fulfilled in varying degrees by a number of Hawaiian drosophilid phylads. As a result, inversions may be used not only to trace the

entered. It belongs to a closely related group of species (modified mouth-parts). Six picture-wing inversions may be recognized in this species (Xiko, 2c, 4b and 5h). Thus *D. mimica* may ultimately serve as a link between the picture-winged and the modified mouthparts groups.

Examination of the "box" marked "Standard" in Fig. 7 will show that a total of ten species are homosequential for the general Standard gene order in all chromosomes. These are (clockwise from *grimshawi*): *atrimentum, orphnopeza, sodomae, disjuncta, affinidisjuncta, bostrycha, pullipes, obatai* and *villosipedis*. Four of the species are monomorphic as far as has been determined (*atrimentum, pullipes, obatai* and *villosipedis*). These ten species are quite different from one another and are easily diagnosed

FIG. 7. Informational display of polytene chromosome sequence formulae and meta-phase conditions in 103 species of picture-winged *Drosophila* of Hawaii. The sequences of *D. grimshawi* serve as an arbitrary General Standard for the group. Thus, X 2 3 4 5 is displayed in a box in upper center of the figure. Metaphase configuration of the Standard is five rods and one dot (5R1D). Boxes near the species names record any metaphase which differs from this, e.g. 6R (six rods). The polytene element represent-ing the microchromosome (6) is not included. Each inversion is represented by a lower case letter (employed in the order of discovery) used as a suffix to the number of the chromosome. The large number of inversions have made it necessary to use the alphabet several times, i.e. the inversions of chromosome 4, discovered following 4z, are desig-nated $4a^2$, $4b^2$, etc. (These symbols are read "four-a-two, four-b-two".) When a symbol stands alone (e.g. 4b or 2b) this means that the sequence represented is present in the homozygous state. Some, but not all, known inversion polymorphisms are repre-sented. Thus "4v/ + " (upper center) means that both the inverted sequence 4v and the relevant matching Standard section 4v+ (or simply, +) are segregating in the populations of the species. Most of the polymorphisms which are confined wholly to a single species are omitted from the diagram. Often, however, an inversion found polymorphic in one species will be found in another in either fixed or polymorphic state. A number of species may share the same fixed inversion but differ in other respects. For example, *orphnopeza* and *murphyi* (upper left) are polymorphic 3o/ + whereas several other species in the same phylad are fixed for either 3o or Standard, as the case may be. In some cases, repetition in the display of such facts has been avoided by adding to the diagram the inference that the species concerned arose from a common ancestral population having a certain inversion composition. The inversion formulae of these inferred ancestral populations are given within boxes with rounded ends. The conditions as directly observed within extant species have been placed within rectangu-lar boxes. The Species Standard sequences for any species in the diagram can be deter-mined by starting at the species name and reading the formula of fixed inversions cumulatively by following the lines through all boxes back to the General Standard. Several examples may be used to illustrate this procedure. *D. liophallus* of Maui (upper left) has the formula Xh 3i 5d 4b 2, or, when arranged in order, Xh 2 3i 4b 5d. *D. lineosetae* of Maui (center right) may be read as Xz^2a^3 2 3b 4bc 5. *D. attigua* of Kauai (bottom of figure) differs from *grimshawi* Standard by 27 inversions: $Xikod^2$-$e^2f^2g^2h^2i^2j^2k^2x^2$ 2cfi 3xy 4bwxzx^{2}y^{2}z^2 5hio.

morphologically. Numerous other examples of homosequential clusters of species can be found elsewhere in the diagram. It should be noted that the definition of a homosequential species does not require that such species also be monomorphic for gene arrangement.

Inversions in the picture-winged species are enumerated in Table II. Inversions found in the polymorphic state are of two kinds. Those confined to a single species total 72. Such inversions are of little use in phylogenetic constructions and most of them are omitted from the displays in Fig. 7. Nevertheless, the break points, species and geographical origin of most of these have been published in previous papers (references in Carson and Kaneshiro, 1976). Of greater interest are those 14 inversions which are not only found polymorphic in at least one species but are also present, in either fixed or polymorphic state, in other species. If this situation exists for a polymorphism, this fact is represented in Fig. 7. Certain of these situations will be considered later.

Routinely, each wild-caught female is induced to lay eggs in the laboratory and salivary gland smears from the resulting larvae are used not only to record the basic species Standard but also to record polymorphisms. The overall frequency of chromosomal polymorphism in the picture-winged species can be estimated from these data (Table III). Judgement that a species is monomorphic must of necessity be somewhat tentative since many of the species are rare and their populations have not been extensively sampled. Nevertheless the category is surely a real one. Thus, in the *adiastola* subgroup only *setosimentum* (9 inversions) and *ochrobasis* (6 inversions) are polymorphic. Samples amounting to about 2000 wild chromosomes from the other twelve species have proven entirely monomorphic (Carson, 1971).

As can be seen in Table III, well over half of the polymorphic species carry only a single inversion in the polymorphic state. In addition to the two *adiastola* subgroup species mentioned above, *D. silvestris* and *D. neopicta* are highly polymorphic with 12 and 8 inversions respectively. *D. disjuncta* (*grimshawi* subgroup) has six and *D. primaeva* seven. Thus the highly polymorphic species (6 or more inversions) are rare in the total picture-winged fauna, i.e. 6/103, or about 6%.

2. *Inference of phylogenies within the Hawaiian Archipelago*

The Continental origin of the fauna in general and the picture-winged species in particular will be considered in the next section. Here we deal with the use of the different specific chromosomal sequences to infer events which are relatively recent, that is, within the last six

TABLE III. Chromosomal polymorphism in 103 picture-winged species of Hawaiian *Drosophila*.

Subgroup	No. of species	per cent poly-morphic	Species polymorphic for one or more inversions								
			1	2	3	4	6	7	8	9	12
grimshawi – I											
Standard 4 phylad (*grimshawi* section)	23	43·5	7	2	—	—	1	—	—	—	—
Standard 4;2b phylad (*hawaiiensis* section)	14	35·7	4	1	—	—	—	—	—	—	—
Inversion 4b phylad (*glabriapex* section)	25	24·0	4	—	1	1	—	—	—	—	—
planitibia II	17	35·3	4	—	—	—	—	—	1	—	1
adiastola – III	14	14·3	—	—	—	—	1	—	—	1	—
punalua – IV	8	12·5	—	1	—	—	—	—	—	—	—
primaeva V	2	100·0	—	1	—	—	—	1	—	—	—
TOTAL	*103*	*31·1*	*19*	*5*	*1*	*1*	*2*	*1*	*1*	*1*	*1*

million years. Those inter-island founder events which are evidently the most recent are the easiest to construct. Particularly clear are those that involve new arrivals on the island of Hawaii. As one proceeds back to an old island such as Kauai, tracing becomes more difficult. Although it is a large island with five volcanoes, Hawaii is the newest in the chain (less than one million years old). As has previously been noted, Maui, Molokai, Oahu and Kauai are successively older (1·3, 1·8, 3·3 and 5·6 m.y. respectively).

As discussed in Section IV of this paper, the pivotal form for the broad phylogeny is *Drosophila primaeva*, a species endemic to Kauai. Carson and Stalker (1969) assayed all of its polytene chromosome sequences and showed that it stands as intermediate between those found in the other picture-winged species of Hawaiian *Drosophila* and those species which characterize the *robusta* group. The latter consists of species that are distributed on the continents or continental margins of the Palearctic and Nearctic regions.

Using the above relationship as a starting point, it is possible to visualize an early major evolution of picture-winged species on Kauai, the oldest of the major high islands. This suggestion, originally made by Carson *et al.*, 1970, still forms the major working hypothesis for the evolution of these species within the archipelago. Nevertheless, northeast of Kauai the Hawaiian chain extends, as a sparse series of very low islands and rocks, more than 2000 km to Midway Island. Beyond and northward there are a series of older submerged seamounts. Presently, these islands have no known drosophilid fauna so no direct evidence exists that any modern Kauai species is derived from ancient fauna which may have existed there.

Among approximately 12 picture-winged species presently found on the modern island of Kauai, there is at least one member of each of the five major subgroups. Both members of the *primaeva* subgroup-V (*primaeva* and *attigua*) are endemic to the island. Using the chromosome sequences and the data in Fig. 7, it is possible to construct a phylogenetic diagram based on inversions fixed between the species (Fig. 8). It should be borne in mind, however, that each inverted section repre-
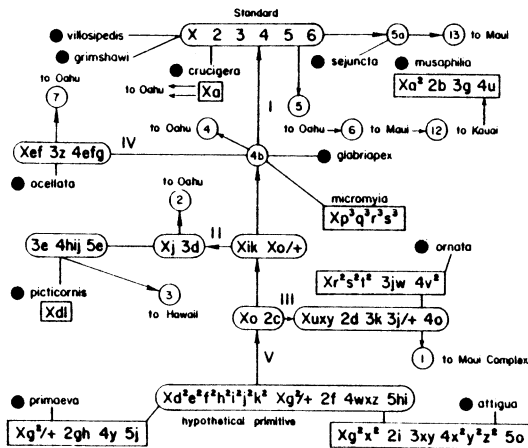
FIG. 8. Evolution of picture-winged Drosophilas on the island of Kauai. Chromosomal relationships between the two *D. primaeva* subgroup-V species and the other Kauai species of subgroups I, II, III, and IV are given. Closed circles: existing species with their inversion formulae; open circles and boxes with rounded ends: hypothetical ancestral populations. Arrows leading to encircled numbers indicate the origin of hypothetical founders which go to the other islands; their fate is shown in Figures 10–13. The direction of evolution is upward from the bottom of the figure, although the chromosomal sequence formula for each species may be read cumulatively from the Standard (box, upper center) as in Fig. 7.

sents a highly specific genetic label for the phylad of species to which it may be confined. The species closest to *primaeva* is *glabriapex* (21 inversions). This species is a member of the very large *grimshawi* subgroup of which about one half (25 out of 48) of the species share with *primaeva* the key gene arrangement 4b. In fact, *D. glabriapex* differs from *D. grimshawi* (also found on Kauai) by just this one inversion. Both "standard 4" and "4b" sections of the subgroup have produced many descendants on the newer islands and indeed a few on Kauai as well (see Fig. 7; selected phylogenies will be discussed in detail below).

Further reference to Fig. 8 will show that there are other Kauai species which are close to *glabriapex*. In fact, only 6 unique inversions away is *D. ocellata*, the only member of the *punalua* subgroup-IV on Kauai. *D. picticornis* is the only member of the important *planitibia* subgroup-II on Kauai. Although this species has 9 inversions unknown in other Kauai species, only two are unique to *D. picticornis* itself; the other 7 are found separately in two other divisions of the subgroup on other islands. Accordingly, the evolutionary series leading to *picticornis* has apparently been dispersed by two founder events from which lineages were established on the newer islands (see arrows leading to *planitibia* subgroup-II, Fig. 8).

Completing the roster of the Kauai species at the base of the proposed phylogeny is *D. ornata*, the only member of *adiastola* subgroup-III on the island. This species has seven inversions which it shares with others in the subgroup. Like *planitibia* subgroup-II, the later and major evolution of the *adiastola* subgroup-III occurred on the Maui complex of islands.

Differences between species based on the number of fixed inversions (as shown in Fig. 8) must be interpreted very cautiously. Inversions serve as important qualitative genetic labels for a phylad of species. Nevertheless, "distances" based on the number of fixed inversions between two species probably do not accurately reflect genetic or phylogenetic distance between such species. Not only are many obviously distant species homosequential but also many clear cases exist wherein multiple inversions have become fixed between very closely related species. For example, chromosome 4 of *setosimentum* differs from that of *ochrobasis* by six fixed inversions (see Fig. 7, center, right). As will be discussed later, electrophoretic distances appear to clock the time at which species diverged much more accurately than the inversions do.

3. Selected newer-island phylogenies

The biogeography of the rest of the islands may be best understood not only by recalling the serial origin of the islands and volcanoes from northwest to southeast but also by considering sea-level fluctuations. Figure 9 displays the outlines of the main Hawaiian Islands at the present stage of the level of the sea, juxtaposed to a second set of outlines at 305 m below the present sea level. The most striking feature of this comparison is the apparent reduction of the six major islands to four larger ones. The essential change is that the existing complex of three separate islands (Maui, Molokai and Lanai) become joined to form a single large island. This large ancient island is referred to as Maui Nui (greater Maui) by Macdonald and Abbott (1970). At several times in the past, especially during the Pleistocene, it is clear that the present central Maui complex of islands was joined into such a single large island. Interpretations of past history are accordingly somewhat simplified by these considerations.

Using Fig. 8 as a base, proposals will now be advanced to explain evolutionary patterns of the various picture-winged subgroups as they have occupied the newer islands. In these hypotheses, each group on a new island has been successfully linked to a Kauai relative. Figures 10 13 give the details of these colonizations. For consistency, no present-day species (closed circles) is shown as ancestral to any other species.
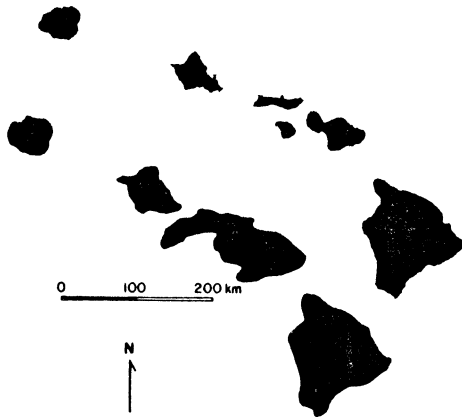
FIG. 9. The major Hawaiian islands. Upper: the islands at the present stage of the level of the sea. Molokai, Maui and Lanai form the "Maui complex". Below: the appearance of the islands at 305 m below present sea level. The large central island is the ancient land mass called "Maui Nui" (greater Maui).
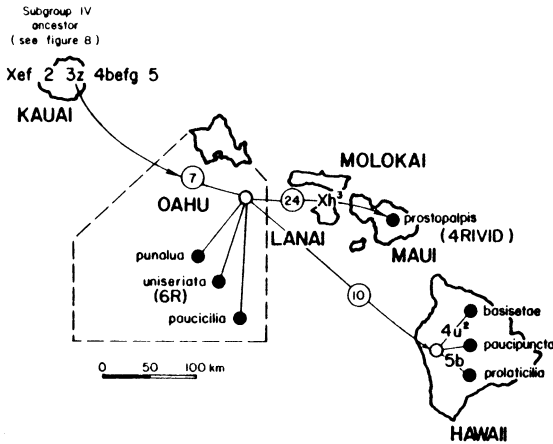
FIG. 10. Evolution of 7 species of *punalua* subgroup-IV on the southeastern Hawaiian Islands. All species are chromosomally very similar, if not homosequential, to *D. ocellata* of Kauai. Direction of evolution is assumed to be from older volcanoes to newer ones. Closed circles: existing species; open circles: hypothetical ancestral populations. Numbers within circles attached to arrows designate the particular founders proposed. All species have a metaphase of five rods and one dot except for *uniseriata* (6R = six rods) and *prostopalpis* (4R1V1D = four rods, one V and one dot). These newly acquired areas consist of heterochromatin.
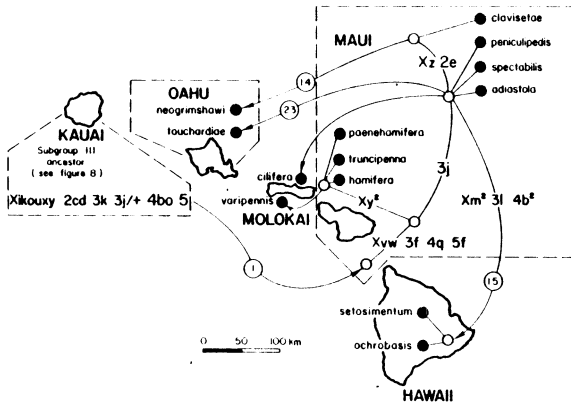
FIG. 11. Evolution of 13 species of *adiastola* subgroup-III on the southeastern Hawaiian Islands. Solid circles: existing species; open circles: hypothetical ancestral populations. Four hypothetical founder events (encircled numbers) and certain key fixed inversions are given. (For details, see Figs 7 and 8.)
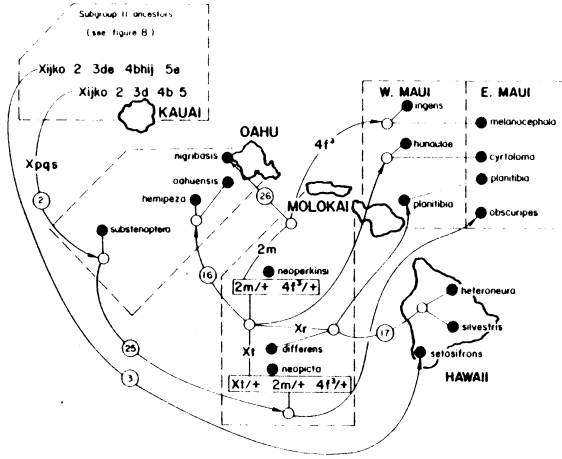
FIG. 12. Evolution of 16 species of *plantibia* subgroup-II on the southeastern Hawaiian Islands. Because of the currently existing key chromosomal polymorphisms in *D. neopicta* and *neoperkinsi* on Molokai, the major evolution in the subgroup appears to have occurred on that island. For explanation of symbols, see caption of Fig. 8.
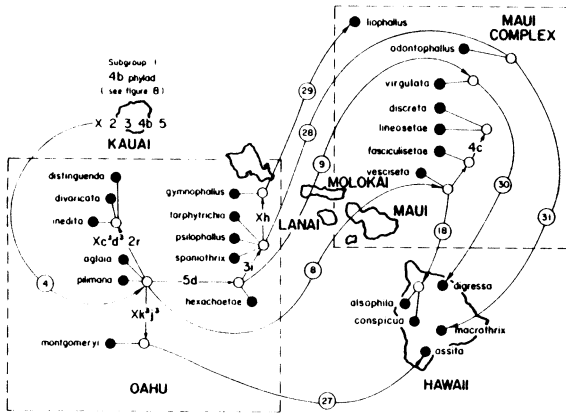

FIG. 13. Evolution of the 4b phylad of 23 species of the *grimshawi* subgroup-I on the southeastern Hawaiian Islands. For explanation of symbols, see caption of Fig. 8.

Each is assumed to be derived from a hypothetical ancestor, often with the same chromosomal constitution as the derived species. Inversions confined to single species, however, are generally not entered in these figures. The complete formulae of each species can best be determined from Fig. 7.

The *punalua* subgroup-IV (Fig. 10) represents a fairly simple case. None of the seven derived species shown deviates by more than one fixed inversion from the putative Kauai ancestor. This conservatism is underlined by the fact that four species, *ocellata, punalua, paucicilia* and *paucipuncta*, are homosequential in polytene chromosomes, lack intraspecific polymorphism and have indistinguishable metaphases.

The *adiastola* subgroup-III (Fig. 11) shows 13 species on the newer islands. All species have similar metaphase groups. Nine of the species are found on the Maui complex, and the distribution of fixed inversions among these suggests that the major evolution occurred on Maui, with subsequent spread of new species to Molokai and Hawaii. Morphological diversity among some of these species is very great. For example, the four "Xy^2" species are not only giant, chunky flies with short wings, but they show strong differences from one another in wing pattern and secondary sexual characters of males. The "Xz 2e" species (Fig. 11) show an extra crossvein in cell R_5 of the wing. The two Oahu members of this subgroup are very different from one another and are traceable to different sections of the subgroup on the Maui island complex.

The *planitibia* subgroup-II consists of a complex of 17 species (see Figs 7 and 12 and Table III). These flies are of special interest for several reasons. The subgroup contains some of the largest *Drosophila* species in the world; some specimens of *D. cyrtoloma*, for example, have a wingspread of 20 mm. All but two of the species in this chromosomal grouping show a striking extra crossvein in cell R_5 of the wing. This character led early taxonomists to erect a separate genus (*Idiomyia*) for these flies, but their anatomical and chromosomal similarity to species lacking such crossveins led, as in the case of the *adiastola* group Xz 2e species mentioned above, to the abandonment of this view. Longevity of most of the species is great and they show complex courtship and lek behavior.

The subgroup is named for *D. planitibia* of Maui, a large and cytologically very favorable species. Its gene sequences, relative to standard *grimshawi*, are Xijkopqrst 2 3d 4b 5. The fairly close affinity to the 4b section of the *grimshawi* subgroup is evidenced by the fact that chromosomes 2, 4b and 5 are homosequential with the members of that subgroup. The greatest divergence has been in the X chromosome, showing nine fixed inversions.

The key distinguishing sequences for the *planitibia* subgroup are 3d and Xijk. These four inversions are found in all members of the subgroup including *D. picticornis* of Kauai. This forms the basis for including the latter species and its close relative *D. setosifrons* in the subgroup despite their lack of the extra crossvein.

Like the *adiastola* subgroup, the *planitibia* flies had their greatest evolution on the central islands, in this case particularly on Molokai (see Founders 2 and 25, Fig. 12). Polymorphic conditions existing today within two modern species on Molokai are especially significant in this phylogeny (Carson, 1973). In particular, *D. neopicta* is polymorphic for three inversions, all of which have become fixed among a number of the more newly derived species. Inversion Xt, for example, is particularly instructive. This inversion is fixed in ten species of the *planitibia* subgroup (see Fig. 12) whereas the ancestral sequence (Xt⁺) is present in the other subgroup members as well as all other Hawaiian *Drosophila*. Arrangement Xt therefore, is clearly a derived arrangement but is still nevertheless rather ancient since it is fixed in quite a few species. Molokai *neopicta*, therefore, may be described as having preserved a chromosomal polymorphism in which both sequences are ancient. Reference to Fig. 12 will show the extensive evolution which has occurred in Xt species, including colonizations of Oahu, Maui and Hawaii.

D. neoperkinsi is a giant Molokai species which differs greatly in size, morphology and behavior from *D. neopicta*. Yet, like the latter, it retains two autosomal polymorphisms also found in *neopicta*. Like Xt, these inversions have become fixed in an additional array of newly-formed species, further indicating the Molokai populations to be of an ancestral nature.

As in all these diagrams, the number of inter-island founders has been assigned in a parsimonious manner. For example, Kaneshiro (1976) has argued on behavioral and morphological grounds that the "Xr" species on the island of Hawaii are derived from separate founder events although cytological and electrophoretic data suggest that the two Hawaii species are exceedingly close and may be easily derived from the single founder event postulated on Fig. 12.

The *grimshawi* subgroup-I includes 48 species. Figure 13 provides a phylogenetic interpretation for 23 putative descendants of a *glabriapex*-like ancestor on Kauai (the 4b phylad). The chromosomal sequences and metaphase of *D. micromyia* collected from Mt. Haupu, Kauai (488 m) on November 24, 1978 by S. L. Montgomery are included in Figs 7 and 8 and have not previously been published. Characteristic of this subgroup is an exhuberant speciation on Oahu and the Maui

complex. Clusters of homosequential species have been formed and are widely distributed in the islands, whereas the *inedita* complex (Fig. 13) is confined to Oahu. The 4b 5d phylad has produced many species which have been widely disseminated to Maui and Hawaii, mostly without further chromosome differentiation. As in other cases, these homosequential species are for the most part very disparate in morphology, behavior and ecology. Only one of the species in this subgroup has acquired extra metaphasic heterochromatin.

The 23 species most closely related to *D. grimshawi* (the "Standard-4" phylad of subgroup-I, Fig. 7) have been considered in several recent publications (Carson, 1980, 1981). Many new data have been recently obtained on these species. Accordingly, a summary of their chromosomal evolution will not be attempted here.

4. Estimation of chronology

One of the unique properties of the evolution of the Hawaiian *Drosophila* is the possibility of dating past events using the age of the islands as a clue. As previously mentioned, the oldest lava flow which has been measured in the present group of high islands is found on Kauai. These rocks have been determined to have a Pliocene age, that is, older than 5·6 m.y. It is possible, of course, that the base of the island may be somewhat older. Nevertheless, the time frame into which evolution of high-altitude terrestrial organisms such as *Drosophila* of the southeastern high islands must be fitted seems well circumscribed. Protein differences between a newly-evolved species on the newest island (Hawaii) and successively older chromosomally related species on the older islands suggests that protein evolution is rapid, indeed in the order of one Nei's D (Nei, 1972) in two million years. The exuberant evolution of flies on Molokai, Maui and Hawaii (64 species) in the last two million years (i.e., 32 species per million years) suggests that there was ample time for early evolution on Kauai prior to the emergence of the next island, Oahu. Accordingly, eletrophoretic and chromosomal data are in accord with the view that all the modern species now found on Kauai evolved on that island. It seems probable that their ancestors came from ancient islands to the north and west along the Hawaiian Ridge towards Midway Island. Although now eroded to very low islands or seamounts, the flat-topped nature of the seamounts suggests former high islands. That the *Drosophila* fauna shows affinities to Palearctic elements from the fringe of the Asian continent does not mean that colonization of Hawaii was accomplished only by long-range dispersal to the present island of Kauai.

C. Non-picture-winged Species

1. D. crassifemur *complex*

The species of this complex are of special interest since they are intermediate between *Drosophila* and *Scaptomyza*. In recognition of this unique status, Kaneshiro (1969) placed these species in a separate subgenus (*Engiscaptomyza*) on the basis of typical *Scaptomyza*-like internal anatomy, egg characteristics (Throckmorton, 1966) and mating behavior (Spieth, 1966) but left it in the genus *Drosophila* on the basis of external morphology (see also Takada, 1966).

Each of the four described "sibling" species of the *D. crassifemur* subgroup is endemic to one of the major island areas of the Hawaiian archipelago (Fig. 14; the data are from Yoon *et al.*, 1975). The band sequences in each chromosome from material of *D. amplilobus* collected · at Halemanu, Kauai (Q76Y62) was arbitrarily selected as the "Standard" for this complex and the banding patterns of corresponding chromosomes of the other species were referred to this set. The nomenclature of the chromosome arms (X, 2, 3, 4, 5, 6) indicates the observed homology to the arms of the picture-winged and modified-mouthparts species. The only variation in polytene sequences between species involves paracentric inversions. Seven different inversions, six structurally homozygous (U, V, P, S and W) and one heterozygous
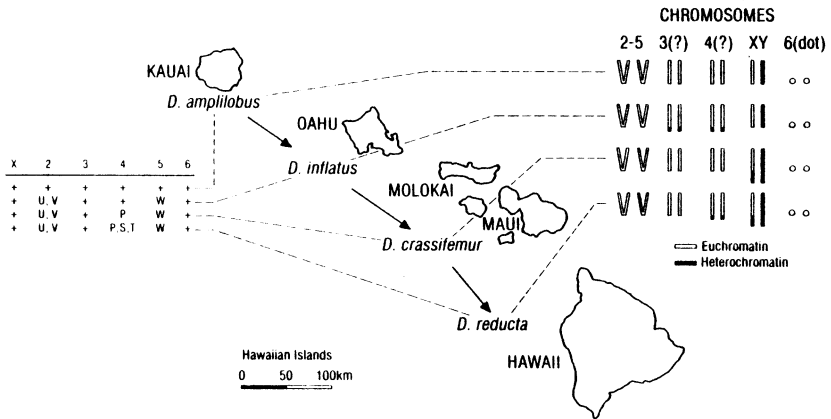

FIG. 14. Phylogenetic relationships of the *crassifemur* complex of species and the presumptive colonization steps among the Hawaiian Islands. Above: metaphase karyotypes; below: polytene formulae. + = Standard gene orders; letters = inversions acquired after each colonization (arrows).

(Q) have been found in the 23 strains of these four allopatric sibling species.

Chromosome element 2 of *D. inflatus*, *D. crassifemur* and *D. reducta*, which forms one arm of the metacentric chromosome, differs from the Standard by two fixed inversions, U and V. The other arm of the metacentric, element 5, is also identical in *D. inflatus*, *crassifemur* and *reducta*, all three differing from the Standard of *D. amplilobus* by a single inversion (W). Chromosome 4 of *inflatus* and *amplilobus* appears identical in band sequences. In contrast, chromosome 4 of *crassifemur* and *reducta* differs from the Standard by one homozygous inversion (P) and one heterozygous inversion (Q/+). Chromosome 4 in *reducta* has two additional small inversions (S and T), thereby differentiating *reducta* from *crassifemur*. Thus, all of the observed inversions are located in chromosomes 2, 4 and 5. Chromosomes X, 3 and 6 (dot) are structurally conservative, being free of inversions.

Chromosomes of Standard *D. amplilobus* are similar to those of *D. hystricosa* (the Standard of the modified-mouthparts group). Both have the same metaphase configuration (1V 3R 1D) and at least 30% of their total polytene chromosomes not only have readily identifiable homologous banding patterns, but also have remained in approximately the same relative position in the chromosome arms. In both species groups, the metacentric chromosome is composed of the same two polytene elements (2 and 5).

Although all strains of all species examined have the same basic metaphase karyotype (1V 3R 1D), heterochromatic differences were found. These are diagrammed in Fig. 14. *D. amplilobus* has the least amount of heterochromatin whereas *reducta* has the most. *D. inflatus* and *crassifemur* have different distributions of heterochromatin, making quantitative comparisons somewhat difficult. *D. inflatus* has small heterochromatic blocks on the rod autosomes (3 and 4). In *D. crassifemur*, the sex chromosomes have a large heterochromatic component. The general trend is for increasing amounts of heterochromatin from *D. amplilobus* on Kauai to *D. reducta* on Hawaii.

On the basis of the presence and distribution of paracentric inversions, the phylogenetic sequence is believed to be: *D. amplilobus* → *D. inflatus* → *D. crassifemur* → *D. reducta*. *D. amplilobus*, from the oldest island (Kauai), is tentatively identified as the primitive species of the complex, based on limited ecological and geological information.

2. D. hystricosa *complex*

This complex belongs to the subgenus *Drosophila* and is included in the modified-mouthparts group. All species have a karyotype of 3V 1R 1D (2n = 10). As in *Engiscaptomyza*, the metacentric is composed of polytene elements 2 and 5. Four species (*D. furvifacies, D. biseriata, D. hystricosa*, and *D. mitchelli*) within this complex were studied (Yoon, *et al.*, 1972a). The island origin and the inversion formulae for each species is shown in Fig. 15. *D. hystricosa* was chosen as Standard.
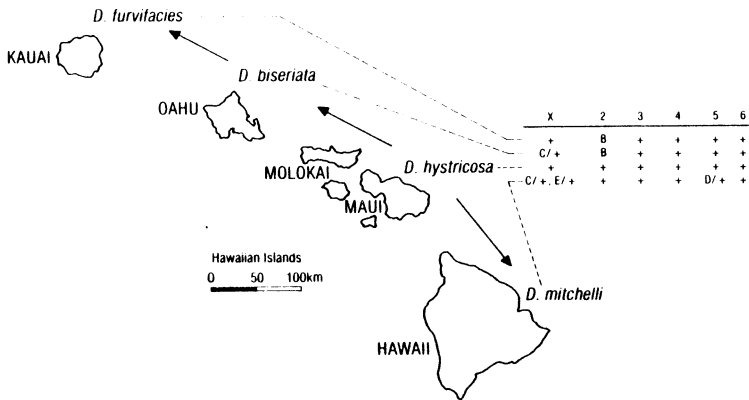
FIG. 15. Phylogenetic relationships of four species of the *hystricosa* subgroup, their polytene formulae and their proposed migration and speciation. + = Standard; letters = inversions acquired after each colonization (arrows).

As previously noted, *D. biseriata* displays a pseudochromocenter in the polytene chromosomes (see Fig. 6). The other species of the complex also have a tendency to form this arrangement, although in these species it is not expressed in all cells. Specific interstitial heterchromatic loci on the X, second and fifth chromosomes are involved in the ectopic joining. This heterochromatin of the pseudochromocenter, furthermore, is frequently associated with the true chromocenter. This feature suggests that some centromeric heterochromatin is related to that in the areas forming the pseudochromocenter. A very striking feature of this situation is that all inversion breakpoints in this complex of species coincide with the loci forming the pseudochromocenter (see Yoon and Richardson, 1978a).

The data suggest that *D. hystricosa* of the Maui complex occupies a central position in the phylogeny of these species. Descendants of

this supposedly ancestral stock are considered to have given rise to *mitchelli* (Hawaii). Other migrants appear to have been responsible for the formation of a species on Oahu (*biseriata*) and then Kauai (*furvifacies*).

3. *The* antopocerus *species group*

There are fifteen moderately large species in this group. They are characterized primarily by the males having highly modified antennae and forelegs. Their habitat is the rain-forest above 700 m elevation, where larvae mine fallen leaves of several plant species (Heed, 1968). They are found on all major high islands except Kauai.

Six of the species have been studied cytologically (Yoon and Richardson, 1976); their polytene relationships are given in Fig. 16. The Standard arms are homologous to those of the other species groups described in this article.
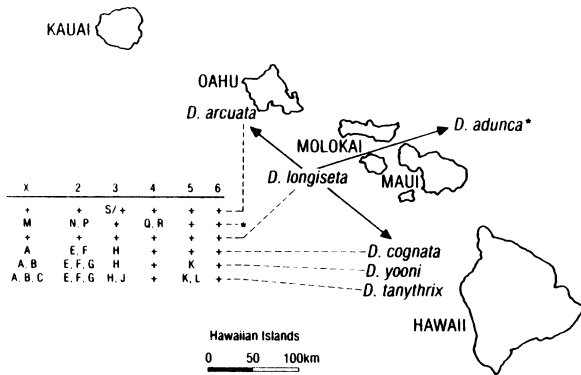

FIG. 16. Phylogenetic relationships of six species within the *antopocerus* species group. + = Standard; letters = inversions fixed in each species.

The X chromosome of *D. cognata* differs from the Standard *longiseta* by one inversion, designated as A. *D. yooni* has this and an additional overlapping inversion, B, and *tanythrix* has still another included inversion, C. The phylogenetic arrangement, based on the overlapping inversion, must be *longiseta* ↔ *cognata* ↔ *yooni* ↔ *tanythrix*. *D. arcuata* of Oahu is homosequential with *longiseta* of Molokai, thus these two are very closely related. The remaining species, *adunca* of Maui, could have been derived from or given rise to either *D. longiseta* or *arcuata* but cannot be considered as an intermediate between the two.

Two of the species, *adunca* and *yooni*, have a rod-shaped chromosome pair 6 at metaphase; this condition is also known in a third species, *entrichocnema* (Clayton, 1971).

As discussed under the picture-winged species, chromosomal phylogenies must be supplemented by other data (biogeographical or behavioral) if direction of evolution is to be inferred. Spieth (1968) considers *tanythrix* and *cognata* to be derived species both from their biogeographical position on Hawaii and their behavior. We follow these arguments and may add that *yooni* has the derived 6R metaphase. When the behavior of the very similar *arcuata* and *longiseta* is compared (Spieth, 1968), it appears that *longiseta* has the more primitive type. Accordingly *longiseta* may be considered to be the best representative of a primitive species in our phylogenetic scheme. These putative relationships are entered as single-headed arrows in Fig. 16. If this is true, *antopocerus*-like flies arose no more than 1·8 million years ago, since this is the age of Molokai, the oldest portion of Maui Nui (Macdonald and Abbott, 1970). It is of interest that quite different evidence has also placed Molokai at the center of evolution of the *planitibia* subgroup-II of the picture-winged species. Just as in *antopocerus*, the Oahu species appear to be back-migrants, derived from a burst of evolution on ancient Maui Nui (see Fig. 12).

IV. Continental Origin of the Fauna

The problem of the ultimate origin of Hawaiian *Drosophila* remains unsolved. However, there is some chromosomal and other evidence that they have originated from a continental source in either the Nearctic or Palearctic regions.

A. EVIDENCE FROM CHROMOSOMAL SEQUENCES

In regard to the relationships of Hawaiian and Continental species groups, Stalker (1972) reported that the *D. robusta* group is a more reasonable choice as an Hawaiian ancestral group than are other groups of the continental species (the *melanica*, *carbonaria*, *carsoni*, *polychaeta*, *virilis*, *funebris*, or *repleta* groups) which he has studied. He also confirmed the earlier conclusion that *D. primaeva* and *D. attigua* are intermediates with regard to the other Hawaiian picture-winged species and continental species, primarily based on the distribution of inversion "h" in chromosomal element 5 (see Stalker, 1972; Carson and Stalker, 1969).
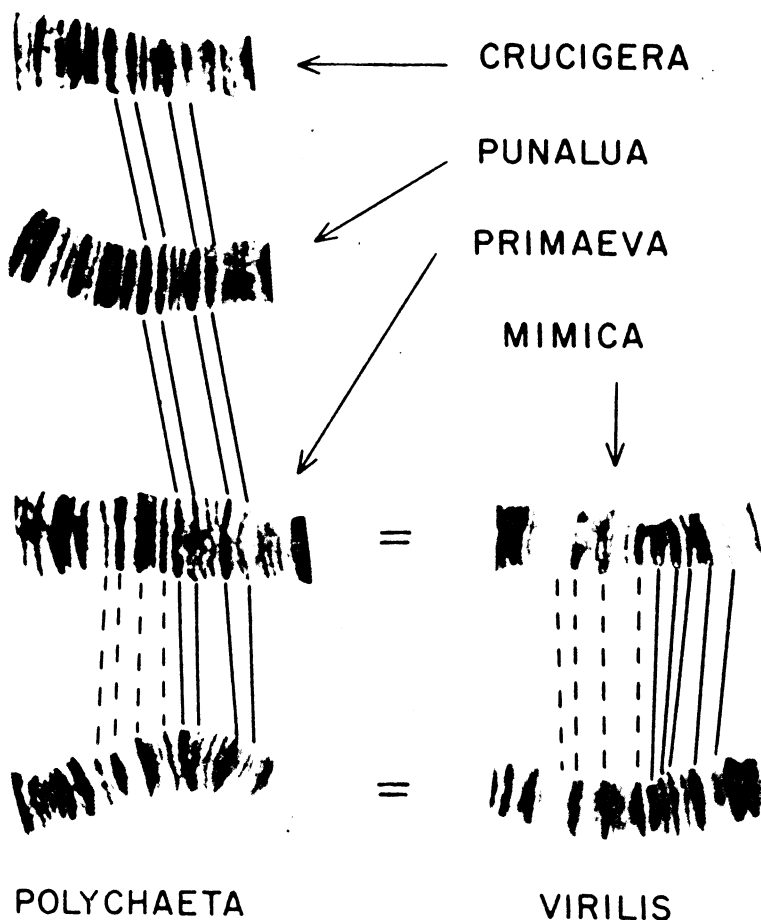
FIG. 17. Polytene chromosomal homology in a section of chromosomes between four Hawaiian species (above) and two continental species (below). A "run" of about 15 polytene bands can be homologized between the continental species and the Hawaiians *D. primaeva* and *D. mimica*. This run is reduced to about 7 bands by the occurrence of inversion 5h in all the picture-winged Hawaiian species from the newer islands (represented here by *crucigera* and *punalua*). After Stalker (1972).

As shown in Fig. 17, a long homologous section is found in *D. primaeva* and *D. attigua* and in the continental species, but only part of it can be seen in the remaining Hawaiian picture-winged species groups. The reduction of the homologous section is the result of occurrence of the 5h Hawaiian inversion. However, it should be noted that the absence of the 5h inversion in *D. primaeva* and *D. attigua* exists also

in *D. mimica* (a member of a different species group of Hawaiian *Drosophila*, the "modified mouthparts" group: see Fig. 7).

In this connection, it should be mentioned that the above Hawaiian species (*primaeva* and *attigua*) could be in an ancestral position to the picture-winged group phylogeny, but there is no assurance that they are in that position with respect to the entire Hawaiian phylogeny, including the modified-mouthparts species group.

B. OTHER LINES OF EVIDENCE

From extensive studies of internal morphology, it has been suggested that the Hawaiian drosophilids originated in East Asia (Throckmorton, 1966). Since then, investigations have tended to confirm the idea that they arose from among the clusters of species groups of *Hirtodrosophila* (Throckmorton, 1975; Okada, 1967, 1971). This cluster is distributed in Asia from the northern subtropics to the cool temperate zone. Some representatives of these lineages are also found in the New World (the *melanderi* and *pinicola* groups). These latter are specialized in one way or another, thus giving less probability that they were themselves ancestral to the Hawaiian drosophilids. However, because of the geographical distribution of the *D. pinicola* group and its ecological and behavioral similarities with Hawaiian *Drosophila*, it has been suggested that species of this group (*D. pinicola* and *D. flavopinicola*) are the descendants of an ancestral population that was closely related to the ancestor which gave rise to Hawaiian *Drosophila* fauna (Spieth and Heed, 1975). Chromosomal evidence on these theories, however, is not available.

The highest concentration of related types (of Old World *Hirtodrosophila*) is in the northern subtropics of East Asia, and it is most probable that the Hawaiian lineages derive from there by some more-or-less direct route. Furthermore, populations with the requisite genotypes were in existence in that region by Miocene times at the latest, so they were easily available to colonize the Hawaiian chain during the Pliocene times when the present high islands developed, or they could have colonized earlier islands of the central Pacific before the existing Hawaiian Islands emerged (Throckmorton, 1975).

It is not clear whether the immediate ancestor of the Hawaiian *Drosophila* was of an Asiatic or a New World form. However, from the fact that a relatively high percentage of chromosomal sections can be fully homologized among all species groups of Hawaiian *Drosophila* and since the homologous sections have remained in almost the same regions of each chromosome, it could be true that all Hawaiian *Drosophila* have

a common ancestor, whether only one or two separate migrations from one continent to the Hawaiian Islands occurred during their evolutionary history.

V. Population Studies of Selected Species

For reasons which will now be discussed, the most intensive work on the genetics of natural populations of the Hawaiian *Drosophila* has utilized species from the island of Hawaii itself (the "Big Island"). That this entire large island is geologically very new has become unequivocally established following the work of McDougall (1969) whose potassium-argon studies indicate that even the most eroded of the five volcanoes (Kohala, see Fig. 18) is less than one million years old. With the possible exception of the Ninole section at the south end of the island (between Kahuku and Waihaka, Fig. 18), all the rest of the land mass is clearly far younger than this; indeed, Mauna Loa and Kilauea volcanoes are active at the present time.

This extraordinary newness of the island has great attraction for those interested in the dynamic processes of evolution, since this recency implies that species endemic to the island must themselves also be new.
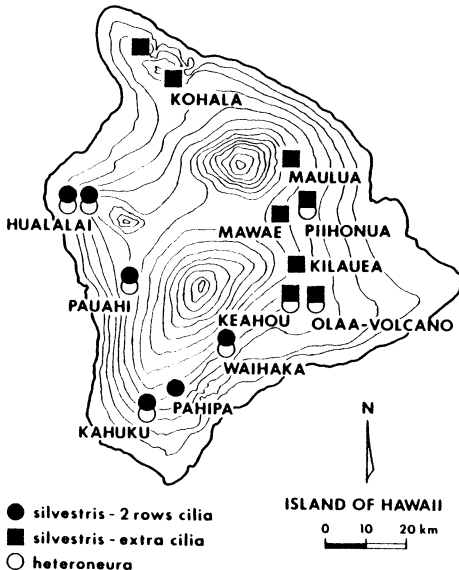

FIG. 18. Distribution of *D. silvestris* and *heteroneura* on the island of Hawaii. At localities where two symbols are in contact, the two species are sympatric.

This idea is further supported by a study of the systematics of the species of the Big Island. Of the 26 picture-winged species which have been described and chromosomally characterized, all but one have been judged on both morphological and genetic grounds to be unique species found only on this island. Presumably these species have evolved *in situ* since the island was formed.

Although moderately large (10,458 km²), about half of the island of Hawaii is inhospitable to vegetation and thus supports little or no drosophilid fauna. The non-forested half includes the high altitudes above 2300 m, the extremely xeric leeward lowlands and large areas of new lava flows and cinder cones which, even in mesic areas, are still in a pioneer stage of colonization. Patches of older forest surrounded by newer lava flows (kipukas) are frequent and even what appears superficially to be a rather uniform dense forest will show spotty biological discontinuities due to ancient kipuka formation.

These factors result in a basically patchy and discontinuous set of environments. Thus the island supports populations of plant species and associated insects like *Drosophila* that are frequently confined to exceedingly small areas. Both plant and insect display either extraordinary rarity or limited geographical distributions. The purpose of these remarks is to emphasize the fact that the figure of about 50% of the island area being habitable for *Drosophila* gives an erroneous impression. Most species are strictly limited by their host-plant distribution. Some of these hosts, such as *Sapindus saponaria* or *Charpentiera obovata* are exceedingly rare and local trees. The rarity of most of the *Drosophila* species thus finds a ready explanation.

The modern biologist must continually remember that the inroads of human activity such as logging, forest clearance for pasture and agricultural crops have been very great in Hawaii as elsewhere. Nevertheless, the patchiness described above is clearly a natural ecological outcome on these shield volcanoes. It is not, as one might think, the direct result of environmental degradation by man. Large areas in which the environment is reasonably intact still exist. In these, the patchiness may be clearly observed; furthermore, this phenomenon was repeatedly emphasized by such early perceptive botanists as Hillebrand (1888) and Rock (1913). Man certainly has accelerated the destruction of both species and habitats but is not the initial cause of their rarity.

A. *D. silvestris* AND *heteroneura*

In recent years a number of population studies have been devoted to these two very large and spectacular picture-winged species. By most

criteria, these species may be judged as having been produced by relatively recent evolutionary events. Attempts have been made to define which, if any, of their characteristics may be important in the study of the origin of species.

These species belong to the *planitibia* subgroup-II, which is well developed on the nearby Maui complex of islands. Among the species found there, two are homosequential with, and thus most closely related to, the Big Island forms. These are *differens* (Molokai) and *planitibia* (East and West Maui). All four species have identical metaphase chromosome configurations consisting of five rods and one dot, with no discernible variations of heterochromatin.

Although *heteroneura* and *silvestris* are confined to montane rainforests between about 1000 and 1600 m in altitude, the species are sympatric over a considerable part of their ranges (Fig. 18); *D. silvestris*, nevertheless, reaches higher altitudes (e.g., Pahipa, Kilauea, Mawae, Maulua). *D. heteroneura* appears to be wholly absent from the Kohala range.

To a considerable degree, the species are still further restricted in distribution, since their principal host plants for oviposition are certain species of the lobeliad genus *Clermontia*. The thick fleshy branches of these plants sometimes die and are prone to fermentation; this site serves as a substrate for oviposition not only for *silvestris* and *heteroneura* but several other *Drosophila* species as well. These plants occur in rare and local patches, especially on lava flows within the *Metrosideros* forests. The species are exceedingly close ecologically; in several instances, individuals of both species have been reared from a single piece of

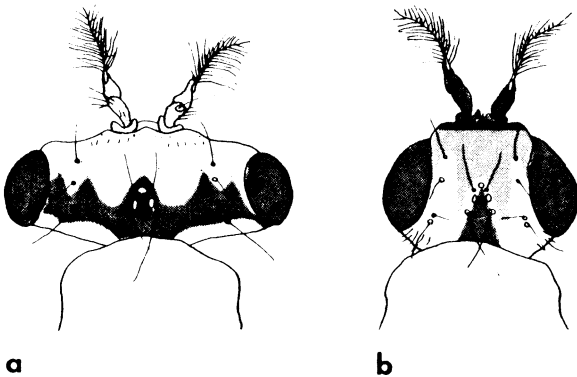**a**   **b**

FIG. 19. Differences in head shape and coloration between males of (a) *D. heteroneura* and (b) *D. silvestris*. After Val (1977).

fermenting branch only about 20 cm in length. Where sympatric, the adults of the two species are closely associated in their microdistribution. Leks of the two species are frequently close together and reciprocal encounters between both sexes of both species have been observed (Conant, 1978).

D. silvestris and *heteroneura* are similar biochemically, chromosomally and in internal anatomy; reciprocal F_1 and F_2 hybrids between the species are vigorous and fertile (for review, see Carson, 1978). Thus it may at first seem paradoxical that this quite fundamental similarity does not extend to external morphology. Indeed, the two species are easily distinguished in both sexes by wing markings, face and pleural color, thorax and abdomen patterns, sculpturing on the egg chorion and, most strikingly, by the width of the head. Some of these characters are expressed in both sexes, with the width of the head, however, being notably exaggerated in the male *heteroneura* (Fig. 19). Thus a number of these characters are of a secondary sexual nature.

Recently (Carson and Bryant, 1979) a secondary sexual difference has been found between populations of flies which have been included within what is normally described as *silvestris*. Thus, in populations from the south and west parts of the island the dorsal surface of the foreleg tibia in males bears two longitudinal rows of long recurved hairs (cilia). Each row has about 25 cilia; they are separated by a naked area. At a crucial point in courtship, the male places this leg surface against the dorsum of the female's abdomen and vibrates it. This character and the specific use of the fore tibia is very similar in the Maui and Molokai species as well as in *heteroneura*.

In contrast, *silvestris* males from the localities on the north and east sides of the island (solid squares, Fig. 18) show a foreleg tibia having many extra cilia on this surface (means of about 14 to 30). These occupy the area between the two major rows of cilia and are distributed in several irregular rows. Although the number of these cilia varies considerably, there is no overlap between males from the two regions. Accordingly, this morphological character may be used as a reliable means of diagnosis for the geographical origin of any single *silvestris* male. An assessment will be made of intraspecific variability in *D. silvestris* following further consideration of other similarities between *silvestris* and *heteroneura*.

As mentioned earlier, *silvestris*, *heteroneura*, *differens* and *planitibia* share a common set of Standard gene sequences in all chromosomes (i.e., are homosequential). Nevertheless, both Big Island species are polymorphic for inversions. A total of twelve have been found in *silvestris* and only one in *heteroneura*. The latter is an ubiquitous polymorphism

(gene arrangement 3m), which is identical to one of the *silvestris* polymorphisms.

Eight of the twelve chromosomal polymorphisms of *silvestris* are widely distributed in the species, including the 3m polymorphism. The geographical distributions of the common polymorphisms of this species have been summarized by Carson and Bryant (1979). Populations of the two different bristle types display almost identical altitudinal clines of gene arrangement frequency, although two inversions not involved in clines are confined to north and east populations.

Extensive electrophoretic surveys of both *heteroneura* and *silvestris* have been made (Sene and Carson, 1977; Craddock and Johnson, 1979). These species display high average heterozygosity and are highly polymorphic, yet show an extraordinary similarity. Both within and between the species, similarity coefficients are above 0·9 and, using standard starch-gel techniques, no diagnostic alleles at any locus have been discovered. It has been suggested that this very great similarity is due to the recency of the cladistic event which separated the two species (see Carson, 1976). As might be expected, the two *silvestris* bristle types are also indistinguishable electrophoretically.

Contrasting with the electrophoretic situation, the species are behaviorally highly distinct. In "male choice" experiments in which a single virgin male is placed in a mating chamber with one virgin female of each species, homogamic mating is almost the invariable rule. This result is obtained whether or not the specimens are drawn from sympatric or allopatric populations (Kaneshiro, 1976).

When rare interspecific matings do occur, however, the resulting F_1 flies from the reciprocal crosses are fertile in both sexes (Val, 1977). Generally the F_1 hybrids are phenotypically intermediate, although there is a tendency for the F_1s to resemble the female parent, indicating a sex-linked and/or maternal effect. Analysis of F_2 segregation shows a considerable spread of variability in most characters, suggesting that they rest on a polygenic basis. Val (1977) has estimated that the species may differ at between 15 and 19 loci which regulate these morphological differences.

At one locality (Kahuku), hybridization between these two species has been found in nature (Kaneshiro and Val, 1977). Phenotypically the hybrids give evidence that the cross consisted of *silvestris* ♀ X *heteroneura* ♂; it is this combination that succeeds most easily in the laboratory. In this particular population, hybrids constitute only a little over 1% and the evidence indicates that backcrossing has not been extensive. Accordingly, there is apparently no extensive breakdown of the integrity of the gene pool of either species in this area. Despite their

close sympatry over much of their ranges, the two species appear to have remained distinct; even the episodes at Kahuku do not seem to have had a major effect.

The suggestion has been advanced (Carson, 1978) that behavioral differences, especially those related to courtship, constitute the most important basis of the specific distinctness of the two species. The morphological differences between them appear to be related to the complex lek and courtship behaviors displayed by the species. Although the matter must be regarded as unsettled, the senior author of this article suggests that the morphological and behavioral differences between the species were established largely in allopatry with only minor reinforcement following the acquisition of sympatry.

The ultimate origin of *silvestris* and *heteroneura* forms a grand puzzle to which there is no obvious simple solution at the present time. Figure 12 represents a single founder arising from a *differens*-like ancestor on Molokai. After reaching Hawaii a further allopatric separation is suggested. This scheme reflects the bias of the senior author for parsimonious allopatric schemes. Alternate schemes are certainly possible (Craddock, 1974; Kaneshiro, 1976) but a full discussion of these arguments does not seem appropriate in this review.

B. GENETIC DIFFERENTIATION WITHIN *D. silvestris*

In recent years special attention has been given to genetic variation within this species. As mentioned earlier, *silvestris* shows a striking difference in the forelegs of male flies taken from the two sides of the island. Despite this non-overlapping difference, all populations show not only close electrophoretic similarity but also display parallel altitudinal clines of frequencies of four specific inversions. Thus, one is led to the view that the morphological bristle character difference is a more recent evolutionary event than the chromosomal or biochemical differentiation just mentioned. This follows despite the fact that heterozygosity for both allozymic and chromosomal variants is very high in all parts of the range of the species.

Tibial cilia of male *silvestris* from the south and west parts of the island closely resemble both *heteroneura* and the Maui and Molokai species of this complex of species. This suggests the conclusion that the north and east populations of the species are the most newly evolved, since only they show the newly-embellished leg character. Accordingly, particular attention is currently being focussed on these populations (solid squares, Fig. 18), with a view to attempting to determine which of them is the oldest and which is the newest.

A new approach has recently been made to the solution of this problem. Experiments with laboratory strains have revealed that females from ancestral populations are reluctant to accept the sexual advances of males from more newly-founded populations. Females from derived populations, however, do not show this tendency (Kaneshiro, 1976; Arita and Kaneshiro, 1979; Ohta, 1978; Ahearn, 1979). The resulting "asymmetrical isolation" may be used to place a series of populations in a precise phylogenetic order according to their time of founding (Kaneshiro, 1980).

C. *D. setosimentum* AND *ochrobasis*

Like *heteroneura* and *silvestris*, these species constitute a pair of closely related forms endemic to the Big Island. *D. setosimentum* is found in many of the same localities as *silvestris* (see Fig. 18) where it occupies very similar breeding sites. In a number of these localities, *setosimentum* is replaced at higher altitudes by the very similar but considerably rarer second species, *D. ochrobasis*.

These are the only two species of the *adiastola* subgroup endemic to the Big Island, whereas on Maui considerable diversification of these forms has occurred. As can be seen in Fig. 11, there are three fixed inversions common to the two Big Island species which are not found in the putative Maui ancestors. Additionally, there is a considerable number of inversions in *setosimentum* which are fixed relative to *ochrobasis*. The latter thus tends to remain closer to the Maui forms, especially in chromosome 4.

The chromosomal and electrophoretic characteristics of these species have been studied by Carson and Johnson (1975) and by Carson *et al.* (1975a).

Both species are characterized by extensive chromosomal polymorphism (Table III); in fact, these are the only polymorphic species among fourteen known in the *adiastola* subgroup. Both species show complex geographical and altitudinal distribution of these inversions. In particular, populations of *D. setosimentum* from the Kona coast (Hualalai and Pauahi, Fig. 18) display three fixed cytological differences relative to populations on the rest of the island. This includes a metaphase difference (Kona populations show "large dots", that is, microchromosomes which have a relatively large amount of added heterochromatin). These populations are also monomorphic for the *adiastola* Standard second chromosome. All other *setosimentum* populations show the fixed inversion 21 and all *ochrobasis* populations show fixed 2k. Accordingly, Kona *setosimentum* is one step closer to the putative Maui ancestor.

Over most of the island, *setosimentum* demonstrates a rather broad altitudinal range, permitting, as in *silvestris*, the identification of frequency differences of inversions with changes in altitude. Thus the populations in the Olaa-Volcano area at about 1200 m altitude are highly polymorphic for a number of inversions. These decline to virtual monomorphism at nearby areas at about 750 m altitude in the Puna District.

Although it is a rare high-altitude form, samples of *D. ochrobasis* have been obtained and studied chromosomally in several parts of its range. Chromosomal distinction from *setosimentum* is easily apparent due to several fixed differences between the two. In one locality (Kahuku Ranch), however, a series of F_1 hybrids and one backcross hybrid between the species have been recognized in the natural population (Carson et al., 1975b). Hybrids are few (about 2%) and there is no indication that the integrity of the gene pool of either species is breaking down in the area. These hybrids appear to be formed at rather high altitude in an area where *setosimentum* is relatively infrequent.

Study of various high altitude areas (for example, Mawae, Fig. 18) indicates that both species are exceedingly variable chromosomally. Each species appears to have, segregating within its populations, unusual homologues which show a series of constrictions distributed along the euchromatic arms. These have been called "complex chromosomes" by Carson and Johnson (1975) and appear to be lethal in the homozygous state. Complex chromosomes found in progenies of *setosimentum* females display a number of sections which appear to be derived from *ochrobasis*. Comparable chromosomes found in *ochrobasis* may be derived from *setosimentum*. Carson and Johnson (1975) interpreted the facts to indicate that this condition exists because of past hybridization between the species, possibly of the kind currently being observed at Kahuku.

Carson et al. (1975a), using allozymic and chromosomal data on these two species, have calculated a series of similarity coefficients, both within and between the species. The resulting indices show that, as in the case of *heteroneura* and *silvestris*, allozymes do not differentiate well between the species. Thus Nei's I (coefficient of identity, Nei, 1972) for allozymic comparison of the species is about 0·9. As in *heteroneura* and *silvestris*, this great similarity is ascribed to the relative recency of the cladistic event which separated the species. In this case, however, chromosomal methods are much more efficient in revealing specific genetic distinctions than are the biochemical ones.

D. *D. grimshawi*

An old problem in evolutionary biology is sometimes encountered in the Hawaiian *Drosophila*. This is how to deal taxonomically with very similar populations which are wholly allopatric, indeed often found on different islands. Might not morphological similarity be a façade, as it is in the case of sympatric sibling species, which masks the fact that two differently adapted integrated gene pools (i.e., species) exist?

The taxonomy of the picture-winged *Drosophila* of Hawaii, in most cases, does not present a serious problem, inasmuch as morphological secondary sexual and behavioral differences are usually strong between species. Supplementary data on chromosomal and biochemical characters as well as hybridization studies can aid in such judgements. The result has been that the great preponderance of the picture-winged *Drosophila*s (100/103, see Fig. 7) have been judged to be full species which are endemic to single islands (the Maui complex is considered a single island for this purpose). The totals are: Kauai, 10; Oahu, 27; Maui-complex, 38; Hawaii, 25.

The three species judged to be distributed on more than one major island, therefore, are of some interest. They are: *crucigera* (Kauai, Oahu), *grimshawi* (Kauai, Oahu, Maui-complex) and *orthofascia* (Maui-complex, Hawaii). Detailed electrophoretic, behavioral and hybridization data on populations within and between islands are available only for *D. grimshawi* Oldenberg (Ohta, 1977, 1978).

Flies morphologically very close to the original Oldenberg description are found on all six major islands, including Hawaii. In 1972, Hardy and Kaneshiro described the forms from the island of Hawaii as a separate species, *D. pullipes*. This was based on differences in leg and pleural color and on the fact that crosses to the standard Maui form yielded sterile F_1 males. For convenience, all populations will be here referred to as "*grimshawi*-like", without regard to systematic judgements.

The *grimshawi*-like flies divide into two strikingly different ecological types. Thus, Kauai, Oahu and Hawaii populations all display a high degree of specialization in that oviposition is confined to decaying bark of one genus of endemic trees, *Wikstroemia*. This tree shows considerable variability throughout the islands and has been divided into a number of species. Apparently, only certain of these are suitable for oviposition by *D. grimshawi* and these species are quite infrequent. Accordingly, *grimshawi*-like flies are very rare on Kauai, Oahu and Hawaii; specimens are never taken very far from fallen or damaged *Wikstroemia*.

On the other hand, *grimshawi*-like flies from the three Maui-complex

islands of Molokai, Lanai and Maui will oviposit on decaying plant material from 12 different plant families, most of which are indigenous to the Hawaiian islands. Thus, the Maui-complex forms are breeding-site "generalists" whereas the others are "specialists".

Ohta (1978) has presented data on the mating behavior of these various populations. As Kauai and Oahu are geologically older than the islands to the southeast, the proposal is made that the specialist *grimshawi* populations on these older islands may be considered closer to the ancestral forms than the generalists of Maui. Using choice experiments similar to those of Kaneshiro (1976), Ohta showed that the Kauai form in particular shows mating behavior in which the females are extremely reluctant to accept males from the newer islands. The reverse is not true. In fact, females from the Maui-complex and Hawaii readily accept males from the older islands. Thus, behavioral tests have a strong asymmetrical character. Accordingly, a phylogenetic scheme has been constructed to explain the origin of the various island populations based on these behavioral data (Fig. 20). Allozyme data also show that the most distant and possibly ancestral population is that from Kauai.
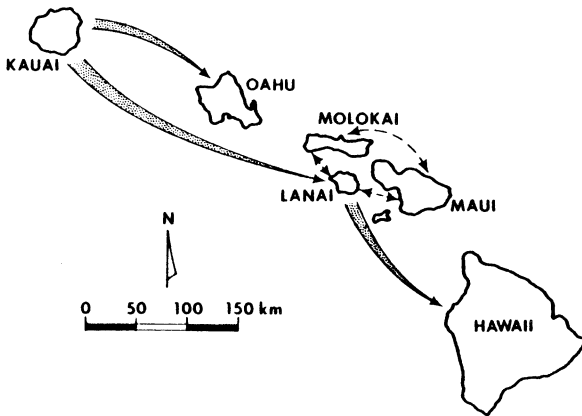

FIG. 20. Presumptive colonization scheme for populations conforming to *Drosophila grimshawi* and *D. pullipes* (Hawaii island only). After Ohta (1977).

Except when populations from Hawaii are used, fertile offspring are obtained in inter-island crosses. Despite their electrophoretic dissimi-larity, crosses between Kauai and Oahu specialists show a high degree of interfertility. In contrast, when a generalist and specialist are crossed (Oahu X Maui or Oahu X Molokai) the F_1 flies are highly fertile but a striking F_2 breakdown occurs even though the populations are

allozymically rather close. This suggests that when ecology is different, isolated populations may build up coadapted gene complexes which are prone to breakdown on crossing, especially when F_2 recombination is permitted (Ohta, 1979).

All these populations are chromosomally homosequential and, except for a single polymorphic inversion in Maui-complex populations, are monomorphic. Metaphase chromosomes do not differ. Differentiation in sexual behavior appears to occur through a sequential series of founder effects. The salient feature of this situation, however, appears to be the adaptational divergence associated with generalist or specialist ecology. This divergence appears to result in an F_2 fitness breakdown suggesting that distinctly different coadaptive complexes have built up in the isolated populations. Chromosomal, electrophoretic or behavioral elements do not appear to be very intimately associated with this type of differentiation. Such cases as this one and that of *heteroneura* and *silvestris* suggest that the key element in speciation is a reorganization of the genome through a process of "shifting balance" as Wright (1978) has termed it. Shifts in balance, however, whether adaptational or sexual, appear to be promoted by an attenuation of the gene pool, mediated in these Hawaiian cases largely by the founder effect.

References

AHEARN, J. N. (1979). Evolution of behavioral isolation in a laboratory stock of *Drosophila silvestris*. *Experientia* **36**, 63–64.

ARITA, L. H. and KANESHIRO, K. Y. (1979). Ethological isolation between two stocks of *Drosophila adiastola* Hardy. *Proc. Hawaii. ent. Soc.* **13**, 31–34.

CARSON, H. L. (1971). Speciation and the founder principle. University of Missouri. *Stadler Symposia* **3**, 51–70.

CARSON, H. L. (1973). Ancient chromosomal polymorphism in Hawaiian *Drosophila*. *Nature, Lond.* **241**, 200–202.

CARSON, H. L. (1978). Speciation and sexual selection in Hawaiian *Drosophila. In*: "Ecological Genetics: The Interface" (P. Brussard, ed.), pp. 93–107. Springer-Verlag, New York.

CARSON, H. L. (1980). Chromosomes and evolution in some relatives of *Drosophila grimshawi* from Hawaii. *Symp. Roy. Ent. Soc. Lond.* **10**, 195–205.

CARSON, H. L. (1981). Chromosomal tracing of evolution in a phylad of species related

to *Drosophila hawaiiensis*. *In*: "Evolution and Speciation" pp. 286–297. (W. R. Atchley and D. S. Woodruff, eds). Cambridge University Press, Cambridge.

CARSON, H. L. and BRYANT, P. J. (1979). Genetic variation in Hawaiian *Drosophila*. VI. Change in a secondary sexual character as evidence of incipient speciation in *Drosophila silvestris*. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1929–1932.

CARSON, H. L. and JOHNSON, W. E. (1975). Genetic variation in Hawaiian *Drosophila*. I. Chromosome and allozyme polymorphism in *D. setosimentum* and *D. ochrobasis* from the island of Hawaii. *Evolution* **29**, 11–23.

CARSON, H. L. and KANESHIRO, K. Y. (1976). *Drosophila* of Hawaii: Systematics and ecological genetics. *A. Rev. Ecol. Syst.* **7**, 311–346.

CARSON, H. L. and STALKER, H. D. (1969). Polytene chromosome relationships in Hawaiian species of *Drosophila*. IV. The *D. primaeva* subgroup. *Univ. Texas Publ.* 6918, 85–94.

CARSON, H. L., CLAYTON, F. E. and STALKER, H. D. (1967). Karyotypic stability and speciation in Hawaiian *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* **57**, 1280–1285.

CARSON, H. L., HARDY, D. E., SPIETH, H. T. and STONE, W. S. (1970). The Evolutionary Biology of the Hawaiian Drosophilidae. *In*: "Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky" (M. K. Hecht and W. C. Steere, eds.), pp. 437–543 Appleton-Century-Crofts, New York.

CARSON, H. L., JOHNSON, W. E., NAIR, P. S. and SENE, F. M. (1975a). Allozymic and chromosomal similarity in two *Drosophila* species. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4521–4525.

CARSON, H. L., NAIR, P. S. and SENE, F. M. (1975b). *Drosophila* Hybrids in Nature: Proof of Gene Exchange Between Sympatric Species. *Science* **189**, 806–807.

CLAYTON, F. E. (1971). Additional karyotypes of Hawaiian Drosophilidae. *Univ. Texas Publ.* **7103**, 171–181.

CLAYTON, F. E. (1976). Metaphase configurations in *Drosophila*. A comparison of endemic Hawaiian species and non-endemic species. *Arkans. Acad. Sci.* **30**, 32–35.

CLAYTON, F. E. and WHEELER, M. R. (1975). A catalog of *Drosophila* metaphase chromosome configurations. *In*: "Handbook of Genetics" (R. C. King, ed.) Vol. 3, pp. 471–512. Plenum Press, New York.

CONANT, P. (1978). Lek behavior and ecology of two sympatric homosequential Hawaiian *Drosophila*: *Drosophila heteroneura* and *Drosophila silvestris*. M. S. Thesis, Department of Entomology, University of Hawaii, 191 pp.

CRADDOCK, E. M. (1974). Degrees of reproductive isolation between closely related species of Hawaiian *Drosophila*. *In*: "Genetic Mechanisms of Speciation in Insects" (M. J. D. White, ed.), pp. 111–130. Australia and New Zealand Book Company, Sydney.

CRADDOCK, E. M. and JOHNSON, W. E. (1979). Genetic variation in Hawaiian *Drosophila*. V. Chromosomal and allozymic diversity in *Drosophila silvestris* and its homosequential species. *Evolution* **33**, 137–155.

DALRYMPLE, G. B., SILVER, E. A. and JACKSON, E. D. (1973). Origin of the Hawaiian Islands. *Amer. Sci.* **61**, 294–308.

HARDY, D. E. (1965). Insects of Hawaii. Vol. 12. Family Drosophilidae. Honolulu: University of Hawaii Press. 814 pp.

HARDY, D. E. (1974). Evolution in the Hawaiian Drosophilidae: Introduction and background information. *In*: "Genetic Mechanisms of Speciation in Insects" (M. J. D. White, ed.), pp. 71–80. Australia and New Zealand Book Company, Sydney.

HARDY, D. E. (1977). Review of the Hawaiian *Drosophila* (*Antopocerus*) Hardy. *Proc. ent. Soc. Wash.* **79**, 82–95.

HARDY, D. E. and KANESHIRO, K. Y. (1972). New picture-winged *Drosophila* from Hawaii. Part III. *Univ. Texas Publ.* **7213**, 155–161.

HARDY, D. E. and KANESHIRO, K. Y. (1981). Drosophilidae of Pacific Oceania. *In*: "The Genetics and Biology of *Drosophila*" (M. Ashburner, H. L. Carson and J. N. Thompson, eds.) Vol. 3a. Academic Press, London and New York.

HEED, W. B. (1968). Ecology of the Hawaiian Drosophilidae. *Univ. Texas Publ.* **6818**, 387–419.

HILLEBRAND, W. (1888). "Flora of the Hawaiian Islands". Hafner Publishing Company, New York. 673 pp. (Reprinted 1965).

KANESHIRO, K. Y. (1969). The *Drosophila crassifemur* group of species in a new subgenus. *Univ. Texas Publ.* **6918**, 79–83.

KANESHIRO, K. Y. (1976). Ethological isolation and phylogeny in the *planitibia* subgroup of Hawaiian *Drosophila*. *Evolution* **30**, 740–745.

KANESHIRO, K. Y. (1980). Sexual selection, speciation and the direction of evolution. *Evolution* **34**, 437–444.

KANESHIRO, K. Y. and VAL, F. C. (1977). Natural hybridization between a sympatric pair of Hawaiian *Drosophila*. *Amer. Nat.* **111**, 897–902.

MACDONALD, G. A. and ABBOTT, A. G. (1970). "Volcanoes in the Sea". 441 pp. University of Hawaii Press, Honolulu.

McDOUGALL, I. (1969). Potassium-argon ages from lavas of the Hawaiian Islands. *Bull. geol. Soc. Amer.* **80**, 2597–2600.

NEI, M. (1972). Genetic distance between populations. *Amer. Nat.* **106**, 283–292.

OHTA, A. T. (1977). Evolutionary relationships among six populations of Hawaiian *Drosophila* in the *grimshawi* complex of species: a multidisciplinary approach. 93 pp. Ph.D. thesis, University of Hawaii, Honolulu.

OHTA, A. T. (1978). Ethological isolation and phylogeny in the *grimshawi* species complex of Hawaiian *Drosophila*. *Evolution* **32**, 485–492.

OHTA, A. T. (1979). Coadaptive gene complexes in incipient species of Hawaiian *Drosophila*. *Amer. Nat.* **115**, 121–132.

OKADA, T. (1967). A revision of the subgenus *Hirtodrosophila* of the Old World with descriptions of some new species and subspecies (Diptera, Drosophilidae, *Drosophila*). *Mushi* **41**, 1–36.

OKADA, T. (1971). Systematic and biogeographical analysis of the *denticeps* group, with descriptions of two new species (Diptera, Drosophilidae). *Bull. biogeogr. Soc. Japan* **26**, 29–38.

PATHAK, D., HSU, T. C. and ARRIGHI, F. E. (1973). Chromosomes of *Peromyscus*. IV. The role of heterochromatin in karyotype evolution. *Cytogenet. Cell Genet.* **12**, 315–326.

PATTERSON, J. T. and STONE, W. S. (1952). "Evolution in the genus *Drosophila*". 610 pp. The Macmillan Company, New York.

ROCK, J. F. (1913). "The Indigenous Trees of the Hawaiian Islands". 548 pp. Charles E. Tuttle Company, Rutland, Vermount. (Reprinted 1974).

SENE, F. M. and Carson, H. L. (1977). Genetic variation in Hawaiian *Drosophila*. IV. Allozymic similarity between *D. silvestris* and *D. heteroneura* from the island of Hawaii. *Genetics* **86**, 187–198.

SPIETH, H. T. (1966). Courtship behavior of endemic Hawaiian *Drosophila*. *Univ. Texas Publ.* **6615**, 245–313.

SPIETH, H. T. (1968). Evolutionary implications of the mating behavior of the species

of *Antopocerus* (Drosophilidae) in Hawaii. *Univ. Texas Publ.* **6818**, 319–333.

SPIETH, H. T. and HEED, W. B. (1975). The *Drosophila pinicola* species group. *Pan-Pacif. Ent.* **41**, 287–295.

STALKER, H. D. (1972). Intergroup phylogenies in *Drosophila* as determined by comparisons of salivary banding patterns. *Genetics* **70**, 457–474.

TAKADA, H. (1966). Male genitalia of some Hawaiian Drosophilidae. *Univ. Texas Publ.* **6615**, 315–333.

THROCKMORTON, L. H. (1966). The relationships of the endemic Hawaiian Drosophilidae. *Univ. Texas Publ.* **6615**, 335–396.

THROCKMORTON, L. H. (1975). The phylogeny, ecology and geography of *Drosophila*. *In*: "Handbook of Genetics" (R. C. King, ed.), Vol. 3, pp. 421–469. Plenum Press, New York.

VAL, F. C. (1977). Genetic analysis of the morphological differences between two interfertile species of Hawaiian *Drosophila*. *Evolution* **31**, 611–629.

WASSERMAN, M. (1963). Cytology and phylogeny of *Drosophila*. *Amer. Nat.* **97**, 333–352.

WHITE, M. J. D. (1973). "Animal Cytology and Evolution, 3rd Ed." 961 pp. Cambridge University Press, New York.

WRIGHT, S. (1978). "Evolution and the Genetics of Populations. Vol. 4. Variability within and among Natural Populations", 580 pp. The University of Chicago Press.

YOON, J. S. and CARSON, H. L. (1973). Codification of polytene chromosome designations for Hawaiian Drosophilidae. *Genetics* **74**, s303–s304.

YOON, J. S. and RICHARDSON, R. H. (1976). Evolution of Hawaiian Drosophilidae II. Patterns and rates of chromosome evolution in an *Antopocerus* phylogeny. *Genetics* **83**, 827–843.

YOON, J. S. and RICHARDSON, R. H. (1978a). A mechanism of chromosomal rearrangements: The role of heterochromatin and ectopic joining. *Genetics* **88**, 302–316.

YOON, J. S. and RICHARDSON, R. H. (1978b). Evolution of Hawaiian Drosophilidae III. The microchromosome and heterochromatin of *Drosophila*. *Evolution* **33**, 475–484.

YOON, J. S. and RICHARDSON, R. H. (1978c). Rates and roles of chromosomal and molecular changes in speciation. *In*: "The Screwworm Problem" (R. H. Richardson, ed.), pp. 129–143. The University of Texas Press, Austin.

YOON, J. S., RESCH, K. and WHEELER, M. R. (1972a). Cytogenetic relationships in Hawaiian species of *Drosophila* I. The *D. hystricosa* subgroup of the "modified-mouthparts" species group. *Univ. Texas Publ.* **7213**, 179–199.

YOON, J. S., RESCH, K. and WHEELER, M. R. (1972b). Cytogenetic relationships in Hawaiian species of *Drosophila* II. The *D. mimica* subgroup of the "modified mouthparts" species group. *Univ. Texas Publ.* **7213**, 201–212.

YOON, J. S., RESCH, K. and WHEELER, M. R. (1972c). Intergeneric chromosomal homology in the family Drosophilidae. *Genetics* **71**, 477–480.

YOON, J. S., RESCH, K., WHEELER, M. R. and RICHARDSON, R. H. (1975). Evolution in Hawaiian Drosophilidae: Chromosomal phylogeny of the *Drosophila crassifemur* complex. *Evolution* **29**, 249–256.

JONG SIK YOON
DEPARTMENT OF BIOLOGICAL SCIENCES
BOWLING GREEN STATE UNIVERSITY
BOWLING GREEN, OHIO 43403