

# The Evolutionary Biology of the Hawaiian *Drosophilidae*<sup>1</sup>

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<sup>2</sup> Wilson S. Stone possessed a powerful intellect, fertile with ideas, and analytical in action. As one of the Principal Investigators of the Hawaiian *Drosophila* Project, he effectively utilized these abilities in the planning, encouragement, and development of the diverse research efforts involved in the project. As early as 1966, recognizing the appropriateness of such a review as is presented in the following pages, he began the preliminary organization for the writing of the manuscript. His untimely death in February 1968 prevented participation in the final achievement, but because of his incalculable contributions to the entire Hawaiian *Drosophila* Project he is by unanimous agreement of the other three authors listed as a contributor to this review.

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## Introduction

Grimshaw (1901, 1902) and Perkins (1910) first focused the attention of dipterists on the aberrant characteristics and abundance of the Hawaiian Drosophilidae. They described 47 species; additionally, Perkins' extensive descriptions (1913) of the biotic habitat in which the insects live alerted biologists to the unique but unknown ecology of the flies.

Following T. H. Morgan's introduction of *D. melanogaster* as a valuable organism for the study of genetics and evolution, investigators in these fields became interested in the Hawaiian drosophilid fauna. Richard Goldschmidt planned to study in Hawaii but was prevented from doing so by the outbreak of the first World War; subsequently one of his students was likewise deterred from his proposed study of the Hawaiian drosophilids by the second World War (Zimmerman, 1958). In the early 1930's Curt Stern also unsuccessfully hoped to conduct studies in Hawaii. In the meantime various taxonomists had devoted some attention to the flies (see Hardy, 1965, pp. 21-22).

At the University of Texas, J. T. Patterson began his extensive study of the biology and evolution of the Drosophilidae in 1932. Patterson's collaborator, W. S. Stone, quickly appreciated the significance of the unique Hawaiian fauna, especially after Zimmerman published his introductory volume to the *Insects of Hawaii* (Vol. I, 1948). In 1946 G. B. Mainland,

a student of Patterson's, joined the staff of the University of Hawaii with the avowed intent of studying the Hawaiian flies. He was successful in collecting numerous species but was frustrated in his attempts to rear the species under laboratory conditions.

In 1948 D. E. Hardy became a member of the University of Hawaii faculty and began his taxonomic studies of the Diptera of the islands. As he acquired more intimate knowledge of the rich Hawaiian *Drosophila* fauna, he made an intensive effort to interest evolutionists and geneticists in the unique problems presented by these insects. In 1961, Stone and Hardy met and developed plans for the present Hawaiian *Drosophila* Project, which was initiated in early 1963 under the auspices of both the University of Hawaii and the University of Texas. Involved in the research efforts have been a number of senior investigators from various institutions, assisted by energetic and highly competent senior technical personnel, by graduate students, and by responsible and able undergraduates.

At the time of the initiation of the project, general skepticism was expressed by competent authorities as to the probability of any successful resolution of the problems that had prevented earlier investigators from conducting successful laboratory investigations of the Hawaiian species. In such successes as have been achieved to date, serendipity has occasionally played an important role, but the major factors have been the close cooperation and integration of the efforts of all personnel involved in the project. Be that as it may, today probably more is known about the ecology, evolutionary relationships, and cytogenetics of the Hawaiian Drosophilidae than of any other comparable complex of drosophilid species. As related below a number of new, often unexpected, findings have added significant new parameters to our understanding of the taxonomy, biology, and evolution of the family Drosophilidae.

## Habitat

### Land Areas

The Hawaiian Archipelago consists today of a series of atolls, reefs, islets, satellite islands, and islands oriented along a 1600-mile (2500-km) northwest to southeast axis and located between 154°41' and 171°75' W. longitude, 18°54' to 28°15' N. latitude. Numerous authors (including Zimmerman, 1948; Fosberg, 1961; Stearns, 1966) have presented detailed information containing the geological history and existing nature of the Hawaiian Islands. The material presented here is minimal and has been selected for its pertinence to the analysis of the drosophilid fauna and its evolution.

The Archipelago can be characterized as the result of a series of volcanic





episodes that began tens of millions of years ago at the northwest end of the chain and is still continuing at the southeast termination. Cores from drill holes on Oahu and Midway (Stearns, 1966) substantiate that the western islands are much older than the eastern ones. Further, Stearns (1966) shows that each of the Hawaiian volcanoes experienced a more or less similar historical development.

As a result of erosion, both fluvial and marine, aided by subsidence, the older, westernmost islands such as Kure, Midway, Pearl and Hermes Reef, Lisianski, and Laysan (Fig. 1) have been reduced and planed off from original high islands into reefs. French Frigate Shoals today consist of small pinnacles of volcanic rock, while Necker, Nihoa, and Kaula have been reduced to rocky islets. These islets, pinnacles, and reefs are known as the leeward chain in comparison to the eight younger islands, which form the windward or main islands of the Archipelago (Table I).

The eight windward islands are scattered over a 400-mile stretch of the ocean, separated from each other by channels of varying widths and depths (Table II). Their total area amounts to 6435 sq. mi. (16,667 km<sup>2</sup>), which is approximately half the size of the Netherlands (12,978 sq. mi.) and slightly less than that of the Fiji Islands (7070), but more than Jamaica (4411 sq. mi.). Each island consists of one or more volcanic domes. Niihau is a low remnant of an original single dome. Kauai is mainly a large symmetrical dome with a smaller dome on its southeastern flank. The potas-

TABLE I  
Land Area, Maximum Elevation, and Median  
Rainfall of the Main Hawaiian Islands

Island	Area (sq. mi.)	Max. Alt. (ft.)	Median Annual Rainfall (inches) <sup>a</sup>
Hawaii	4,030	13,796	75
Maui	728	10,023	75
Oahu	604	4,046	58
Kauai	555	5,170	99
Molokai	260	4,970	42
Lanai	141	3,370	21
Niihau	72	1,281	—
Kahoolawe	45	1,477	—
	6,435		

<sup>a</sup>From Taliaferro (1959).

TABLE II  
Approximate Distances and Minimum Depths of  
Channels Between the Hawaiian Islands

Islands	Channel Name	Distance (miles)	Depths (ft.)
Niihau-Kauai	Kaulakahi	17.0	2568
Kauai-Oahu	Kauai	73.0	9630
Oahu-Molokai	Kaiwi	26.0	1722
Molokai-Maui	Pailolo	8.5	564
Molokai-Lanai	Kalohi	9.0	264
Lanai-Maui	Auau	9.0	138
Maui-Kahoolawe	Alalakeiki	6.0	276
Maui-Hawaii	Alenuihaha	30.0	6180

sium-argon (K-Ar) method of aging indicates that Kauai (which has a minimal age of 5.6 to 3.8 million years) is older than the more eastern islands and as a consequence has suffered much greater erosion than have the younger islands. Oahu is composed of the remains of two domes, i.e., the older Waianae Range (3.4 to 2.7 million years of age), and the younger Koolau range (2.5 to 2.2 million years). Molokai is also formed from two domes, the western lower one having an age of 1.8 million years, the eastern one 1.5 to 1.3 million years. Maui likewise is the result of the fusion of two domes, West Maui with an age of 1.3 to 1.15 million years and East Maui less than 1 million years old. Hawaii, the most eastern and largest island of the windward group, consists of five fused volcanic domes, each being less than one million years of age. The northwest Kohala dome is the oldest, followed by Mauna Kea, Mauna Loa, Hualalei, and Kilauea (Table III).

The pairs of volcanic domes which form each of the present islands of Oahu, Molokai, and Maui, arose independently and originally formed independent islands, separated by narrow channels that were eventually obliterated by subsequent vulcanism, primarily from the eastern member of each pair, whose lavas now overlie those of the western member at the point of junction between the two domes (Stearns, 1966). In each instance the eastern dome is also 200,000 to 300,000 years younger than the western member of the pair.

The history of Molokai, Lanai, West Maui, East Maui, and Kahoolawe has been especially complex, a fact of importance to the evolution of the *Drosophilidae* fauna, as is shown later. This complexity was caused by

three features, i.e., (1) the younger age of East Maui; (2) the low elevation of the land that joins East and West Maui; and (3) the narrow and shallow channels that exist between the four islands (see Table II). During periods of worldwide glaciation during the Pleistocene, the ocean levels were lowered due to removal of water to the extent that some or all of these islands were joined together at least twice. According to modern estimates, the duration of the Pleistocene was of such a magnitude that the entire geologic development of these islands is encompassed in this period. During interglacial periods when the ocean levels rose, not only were the present islands separated by oceanic channels, but even East and West Maui must have been separated by a shallow channel.

During periods of glaciation, accompanying the lowering of the ocean levels there was a general lowering of temperature, as a result of which the forests probably moved downward and then upward during the interglacial periods. In any case, it is clear that forests containing trees (such as *Cheirodendron*) and other plants of the same genera as are now present in the forests and upon which many of the Drosophilidae are dependent did move up and down the mountain slopes, as shown by Selling's studies (1948) of the fossil pollens.

Other channels, excepting those between the islands of the Maui-

TABLE III  
Estimated Ages of Hawaiian Island Areas<sup>a</sup>

Island	Million Years
Kauai	5.6-3.8
West Oahu (Waianae Mts.)	3.4-2.7
East Oahu (Koolau Mts.)	2.5-2.2
West Molokai	1.8
East Molokai	1.5-1.3
West Maui (Wailuku Mts.)	1.3-1.15
East Maui (Halaekala)	0.8
Hawaii (Kohala Mts.)	<1.0
Hawaii (Mauna Kea)	0.6
Hawaii (Mauna Loa)	<0.5
Hawaii (Puu Waawaa)	0.4

<sup>a</sup>From Stearns (1966). The data were derived by the K-Ar technique. Although the samples are relatively few in number, the results are of significance in showing the relative ages of the islands.

Molokai complex, are of such depths and widths (Table II) that the individual islands have been separated throughout their entire history by ocean waters. Thus, the migrations of *Drosophilidae* from one to another of these land masses must of necessity have been accomplished by air-borne adults or by individuals that were able to tolerate movement of floating debris. Considering the nature of the developmental forms, the latter possibility (i.e., floating debris) seems highly improbable as a mechanism for safely allowing immigrants to be carried across these oceanic stretches.

The ages given for the islands represent the time when the major domes were built up above sea level, and do not signify the cessation of lava eruptions. These are still occurring on the island of Hawaii; during the period from 1850 to 1950 Mauna Loa liberated approximately 4 billion ( $4 \times 10^9$ ) cubic yards ( $3 \times 10^9 \text{ m}^3$ ) of lava (Stearns, 1966); Kilauea since 1823 has extruded 5-billion cubic yards or 0.92 cubic miles of lava; Hualalei last erupted in 1800-1801.

Especially when the eruptions issue from the major vent or caldera on the top of the dome or from rifts high on the flank of the volcano, the lava flows downhill, often reaching the sea. In doing so it cuts a swath through the existing vegetation. Often the flow is such that numerous large areas of forest are surrounded and cut off, thus forming islands (or kipukas) within the river of fresh lava (Fig. 2). Isolating barriers are thereby created, which may at least partially if not completely stop gene flow between adjacent populations for considerable periods of time.



*Fig. 2. Kipuka on the saddle between Mauna Loa and Mauna Kea, Hawaii. The picture is taken from a distance of 1 km; the trees are about 12 m high; the area of kipuka is about 25000 m<sup>2</sup> (6 acres) and is basically square in shape. Photo by M. P. Kambysellis.*

All evidence indicates that the older islands experienced a similar history to that which has occurred within historical time on Hawaii. Furthermore, late in the life of a volcanic island, it may after a long period of erosion and submergence experience a rejuvenation of volcanicity with the occurrences of secondary eruptions mostly along or near the coasts. Diamond Head, Kaau Crater, Punch Bowl, Round Top, Sugar Loaf, and Tantulus on Oahu are products of this secondary vulcanism; the last three are only about 5,000 years old (Stearns, 1966). Thus the fauna and flora of the Hawaiian Islands have evolved with vulcanism as a constant companion, a companion which created barriers to gene flow and provided new sere areas of considerable size for reinvasion by the already established organisms from surrounding regions.

### *Rainfall*

The windward islands all lie in the path of the persistent northeast trade winds. The flowing air as it strikes the islands is forced upward, rain clouds form, and the resultant rain drenches the windward-facing slopes and crests. On the leeward slopes the precipitation falls off rapidly and progressively. Thus on Kauai the Waialeale Station at 5075 foot elevation over a period of 46 years recorded a median rainfall of 465.5 inches (11.8 m), while Waimea on the coast directly southwest and only 13.4 miles (21.4 km) from Waialeale received 21.6 inches (Table IV). Likewise, on Oahu the Pauoa Flats Station at 1800 feet elevation recorded during 31 years a median of 162.4 inches, while the Honolulu Substation only 4.25 miles away had a median of 25.5 inches during 51 years. Trade-wind-engendered precipitation also decreases at elevations above 7000 feet (2100 m). Only East Maui (Haleakala) and Mauna Kea and Mauna Loa on Hawaii exceed this elevation; the uplifted trade winds do not go over their tops but rather flow around their flanks. A north-south transect of stations on East Maui, extending from Punaluu on the windward side to Kahikiniu on the leeward side of Haleakala (Table IV) shows the combined effect of elevation and rain shadow occurring over a distance of less than 20 miles. Additionally, as Fosberg (1961) points out, on these high islands the phenomenon of invection intrudes, with the result that certain leeward sites which normally would be expected to be arid do receive afternoon rains.

Finally, it is to be observed that there are periods of varying length of time when the trade winds cease and are replaced by air movements from other directions, typically by southern (Kona) winds. As an example, such a cessation of trades happened between early December 1967 and the beginning of March 1968. Associated with this period were a number of winter frontal-type storms that drenched the islands and especially the

TABLE IV  
Rainfall Data Showing Effect of Elevation and Rain-shadow<sup>a</sup>

Station Number	Name	Elev. in Ft.	Number Years	Annual Rainfall Inches			Approx. Distance between Stations
				Max.	Median	Min.	
<b>Kauai</b>							
1047	Waialeale	5075	46	624.0	465.5	218.0	} 13.4 miles
947	Waimea	20	45	42.2	21.6	7.0	
<b>Oahu</b>							
784	Pauoa Flats	1800	31	239.2	162.4	98.2	} 2.50 miles
706	Pacific Heights	680	29	95.2	67.3	39.8	
704	Honolulu Subst.	12	53	45.0	25.5	10.3	
							1.75 miles
							4.25
<b>Maui</b>							
447	Punaluu	700	51	220.6	123.3	76.9	} 1.20 miles
449	Waikamoi Gulch	1200	51	392.1	199.2	96.7	
336	Waikamoi Dam	4250	47	427.6	225.7	48.7	} 4.65 miles
341.2	Waikamoi Runoff #2	7200	6	128.1	112.1	39.3	
338.3	Haleakala	9750	8	76.4	32.6	27.5	} 3.00 miles
254	Kahikiniu	1400	32	43.9	28.4	9.7	
							3.15 miles
							7.25 miles
							19.25
<b>Hawaii</b>							
39	Mauna Loa Slope Observatory	11,146	5	28.11	21.85	14.28	-

<sup>a</sup>Data from Taliaferro (1959), except for Station 39, Mauna Loa Slope Observatory. The data for this station are from years 1963-67 inclusive, as presented in the monthly publication *Climatological Data, Hawaii*, U.S. Dept. of Commerce.

"normally" leeward or southern and southwestern portions. Thus Waimea (Station 947), with a median annual rainfall of 21.6 inches, received 17.59 inches during these three months; and the Honolulu Substation (704) received 18.58, as against its median annual amount of 25.5. Likewise the gauges at high elevations registered increased amounts. During the two months of December and February, Haleakala summit received 21.85 inches. The trade winds returned in March but were frequently interrupted in April and replaced with winter storms; Haleakala summit then received an additional 19.04 inches, some of which fell in the form of 9 inches of snow accompanied by 29°F temperatures. These winter storms may result in extraordinary amounts of precipitation within a short period of time, e.g., the maximum recorded rainfall on the islands for a 24-hour period is 38.5 inches at Kilauea, Kauai in January 1956. In January 1969, Kokee, Kauai, recorded 34 inches in a 24-hour period (personal communication, Saul Price, Regional Climatologist).

The fluctuating interactions of frontal storms and northeast trade winds are a consistent phenomenon, and their interplay appears to have been a constant phenomenon during the Pleistocene. These interactions result in great variations in the annual rainfall at any given location on the islands, as can readily be seen by studying the data presented in Table IV. An important corollary of these fluctuations is that when a period of low rainfall occurs, the high porosity of the underlying rocks quickly results in the occurrence of drought conditions.

### The Forests

The endemic Hawaiian flora, derived from a few original stocks (Fosberg, 1961), evolved therefore under conditions of great diversity of physiography and climate, accentuated still further by the fluctuations caused by Pleistocene glaciation (Selling, 1948). The unique assemblage of endemic vegetation types that evolved under these conditions displays enormous diversity, occupying most available habitats and consisting of a number of forest types. It is within certain of these forest types that the great array of endemic Drosophilidae are found. Various authors have proposed classifications of the Hawaiian vegetation (Hillebrand, 1888; Rock, 1913; Hosaka, 1937; Robyns and Lamb, 1939; Ripperton and Hosaka, 1942; Selling, 1948; Krajina, 1963). Fosberg (1961) distinguishes 18 forest and scrub ecosystems. Four of these are formed by recently introduced plants and in all instances have replaced native forests. Of the remaining 14, 4 serve as the home for almost all of the drosophiloid<sup>1</sup> and scaptomyzoid species. These are (1) the cloud forest; (2) the dryland sclerophyll forest; (3) the *Metrosideros* forest; and (4) the mixed mesophytic forests.

**METROSIDEROS FOREST.** This forest is found in moderately moist to wet localities, from fairly low to middle elevations. Exhibiting considerable variation in composition, complexity, and structure at different sites, it may consist of almost pure strands of *Metrosideros* trees, especially on young lava flows and ash beds. In the older forests, species of many other trees and shrubs are to be found mixed with the *Metrosideros*. In areas of high precipitation, from 2000 to 5000 feet, these older forests become highly complex, forming a true montane rainforest (Fig. 3). In such situations other trees may share dominance with *Metrosideros* and a second tree layer is formed, while shrubs create a still lower layer. Ferns of various types, including the tree ferns of the genus *Cibotium*, occur in abundance, and climbers such as *Freycinetia* and an abundance of bryophytes and lichens grows on the trunks and limbs of the trees. Most importantly for the drosophiloid species, their prime host plants such as *Tetraplasandra*,

<sup>1</sup> I.e., *Drosophila* and closely related genera.



Fig. 3. *Metrosideros* forest, Kula pipeline, Waikamoi, Maui. Typical rainforest habitat of *Drosophilidae*. Photo by L. H. Throckmorton.

*Ilex*, *Myrsine* and especially *Cheirodendron* and the lobeliads *Clermontia* and *Cyanea* are common members of this complex assemblage.

CLOUD FOREST. Above and contiguous with the *Metrosideros* forest there often is found a cloud forest (Hosaka, 1937). This is a complex forest with an abundance of shrubs, ferns, mosses, and hepatics. In sheltered areas such as ravines, the vegetation reaches forest stature while on the exposed slopes and ridges it is dwarfed and tangled, reduced to scrub status or even boggy grass areas (Fosberg, 1961). In the sheltered areas drosophiloids are found along with their major host plants such as the lobeliads and *Cheirodendron*.



**MIXED MESOPHYTIC FOREST.** These are forests of very diverse composition, found at low to moderate elevations and also at higher elevations. They differ from the rain forests in receiving less precipitation, typically 50 to 100 inches, but do not suffer from an actual moisture deficit (Fosberg, 1961). *Cheirodendron* and the lobeliads are characteristically lacking from such forests, but other host plants of the drosophiloids such as *Sapindus*, *Osmanthus*, and *Myrsine* are common components. Furthermore, these forests have considerable numbers of fungi, sometimes large in size and abundant in number, which serve as food for the adults of numerous species and as the developmental sites for a number of species, including the entire fungus-feeding drosophiloid group. At higher elevations these forests reach their most exuberant development in the rich soils of the kipukas such as Kipuka Puaulu and Kipuka Ki on Hawaii. These kipukas with their relatively low temperatures have amazingly rich drosophiloid faunas. Few of the endemic drosophiloids are able to tolerate the temperatures that exist below 1000 feet elevation, even on the cooler windward exposures. Those species which do live at lower elevations seem typically to be most commonly found in the mixed mesophytic forest areas.

**DRYLAND SCLEROPHYLL FOREST.** Only a few remnants remain of this type of forest, which Rock (1913) indicated was species-wise the richest of all Hawaiian forests. Originally, large areas of the dry coastal slopes and higher rain shadows up to at least 5000 feet were covered with this scrub-type forest. The Auwahi forest on the south slopes of East Maui appears to be the sole remaining remnant on Maui. Amongst the 50 species of trees and shrubs that Rock (1913) found here are a number of host plants for drosophiloids, e.g., *Cheirodendron*, *Charpentiera*, *Tetraplasandra*, and others. Despite the declining fate that this forest, located at an elevation of 3500 to 4050 feet, is experiencing, it still is a rich drosophiloid collecting area. In its pristine state an enormously rich and varied drosophiloid fauna must have inhabited this ecosystem.

All of these four types of forest have been ravaged by the activities of man. The native forests originally covered the islands from seashore to timber line (Zimmerman, 1948). The arrival of the Polynesians introduced fire and caused a rapid retreat of the lowland and dryland forests, but the real destruction began with the arrival of Western man in 1778. Today the dry sclerophyll forest is almost extinct, and introduced plants and animals insure its eventual demise. The mixed mesophytic forest has been radically reduced and only in areas such as the National Parks is it partially protected. The *Metrosideros* and cloud forests have been the least disturbed, but even they have been seriously modified as man and his feral and domestic animals have slashed their way through the vegetation. The drosophiloids, like the honeycreepers, have suffered severely; probably many species and certainly countless populations of drosophiloids have been

destroyed during the past 300 years. Fortunately most of the extant species dwell in the *Metrosideros* and cloud forests at elevations of 2500 to 5000 feet on the windward or wetter areas of the islands. These forests are relatively much more resistant to the intrusion of man than is any other area of the islands, as any *Drosophila* collector well knows, and additionally they have relatively high recuperative powers.

### *Drosophilid Fauna*

The family Drosophilidae is most remarkably developed in the Hawaiian Islands and represents one of the most unusual faunas of any area of the entire world; no other drosophilids are so diversified morphologically and biologically. We have here a striking example of rapid, explosive evolution that is almost unparalleled in the animal kingdom, and this group is most ideally suited for evolution and genetic studies. That the islands have served as an area of spectacular adaptive radiation is exemplified in many different groups of native plants and animals, but the dipteran family Drosophilidae is the most unusual of any animal group that has been studied.

As now known, the Hawaiian drosophilid fauna is composed of almost 500 species. About 460 endemic species have been named to date and 17 introduced species are present; 96.4 percent of presently known species are endemic. Of the named endemic species, approximately 330 belong to *Drosophila* Fallén and closely related genera (drosophiloids), and 132 belong to *Scaptomyza* Hardy and related genera (scaptomyzoids). These represent approximately one third of the total number of *Drosophila* species known for the entire world, and twice the number of species of *Scaptomyza* known for the world, excluding Hawaii. In spite of the large number of known species, the taxonomy of this group is still in a preliminary state. It is now obvious that the total fauna consists of 650 to 700 species. It is probable that an additional 125 species of *Drosophila* and 75 to 100 *Scaptomyza* remain to be described.

Only the basic morphological descriptions have been given for roughly two thirds of the species in the fauna, and since it has become so obvious that it is impossible to determine relationships based on morphology alone, it has not been feasible to date to set up a detailed phylogenetic arrangement for the species. The team approach, which is now being used to study the evolution and genetics of Hawaiian Drosophilidae, gives a unique opportunity to gain a thorough understanding of one of the most complex and remarkable groups of animals known in the world. To my knowledge, this is the first time that a group of highly qualified specialists have pooled their efforts for determining paths of evolution and the factors affecting speciation rates in animal groups under certain insular conditions. Aside from the spectacular accomplishments resulting from the varied aspects of this study, the

combined results are providing a sound taxonomic basis for this family. Phylogenies are being worked out as a thorough understanding of various species groups is being gained, based upon internal and external morphology, genetics, hybridization, behavior, ecology, ovarian transplants, and biochemical studies of enzyme systems. Such detailed knowledge has rarely been obtained for such a group of animals and the combined efforts will result in a classical example of the proper approach to systematics. Carson's arrangement of the picture-winged flies based upon comparisons of the gene sequences of the giant salivary gland chromosomes (p. 520ff.) is the most significant development that has occurred regarding our understanding of systematics of Hawaiian *Drosophila*. The detailed studies of the male genitalia by Takada (1966) and Kaneshiro (1968) correlate closely with the arrangement of Carson in most details. Following the work of Okada (1953, 1954, 1955), Kaneshiro has demonstrated that the character of the male aedeagus is of considerable importance in determining relationships between species.

The Hawaiian drosophilids have not only developed fantastic numbers of species, but exhibit the greatest diversity of form and habits in any known area of the world. A great many structural peculiarities are found that apparently do not occur in other drosophilid faunas. Some of these structural modifications are illustrated in Figure 4. Most of them are found in the male sex only; this is especially true of the mouthpart and leg modifications. The behavior studies of Spieth (1966b, 1968b) have demonstrated that most of these structural peculiarities are directly associated with courtship and mating. Various parts of the body are modified; examples are provided in the following paragraphs.

Head: extremely broad, showing beginning of stalk-eyedness (Fig. 4.37); elongate (Fig. 4.47); lack of frontal bristles (Fig. 4.50); excess of hairs (Fig. 4.46); pectinate antennae (Fig. 4.43,49); pointed second antennal segment (Fig. 4.48); preapical arista (Fig. 4.30); modified mouthparts (Fig. 4.34-36, 39-42, 44); bristles on palpi (Fig. 4.31-33).

Legs: "spoon-tarsi," with front basitarsus of males short, flat and concave on upper surface and modified into a spoon-like structure (Fig. 4.1-2); "forked-tarsi," males having an appendix developed at the apex of the front basitarsus and only four tarsomeres present (Fig. 4.10-15). The male of *D. freycinetiae* Hardy, however, has an appendix at the base (rather than the apex) of the front basitarsus and has the normal complement of tarsomeres (Fig. 4.3); "bristle-tarsi" have the apex of the front basitarsus of the male with a comb-like arrangement of bristles or strong setae at the apex of the front basitarsus (Fig. 4.6-7); in the *adiastola* complex the front basitarsus of the male is flat and densely setose (Fig. 4.5,16); clubbed middle tibia of male (Fig. 4.8-9); hook on tibia of male (Fig. 4.17-18); long ciliation of male forelegs (Fig. 4.19-26); knobbed tibia (Fig. 4.4).

Wings: "picture wings," characterized by rather elaborate patterns of

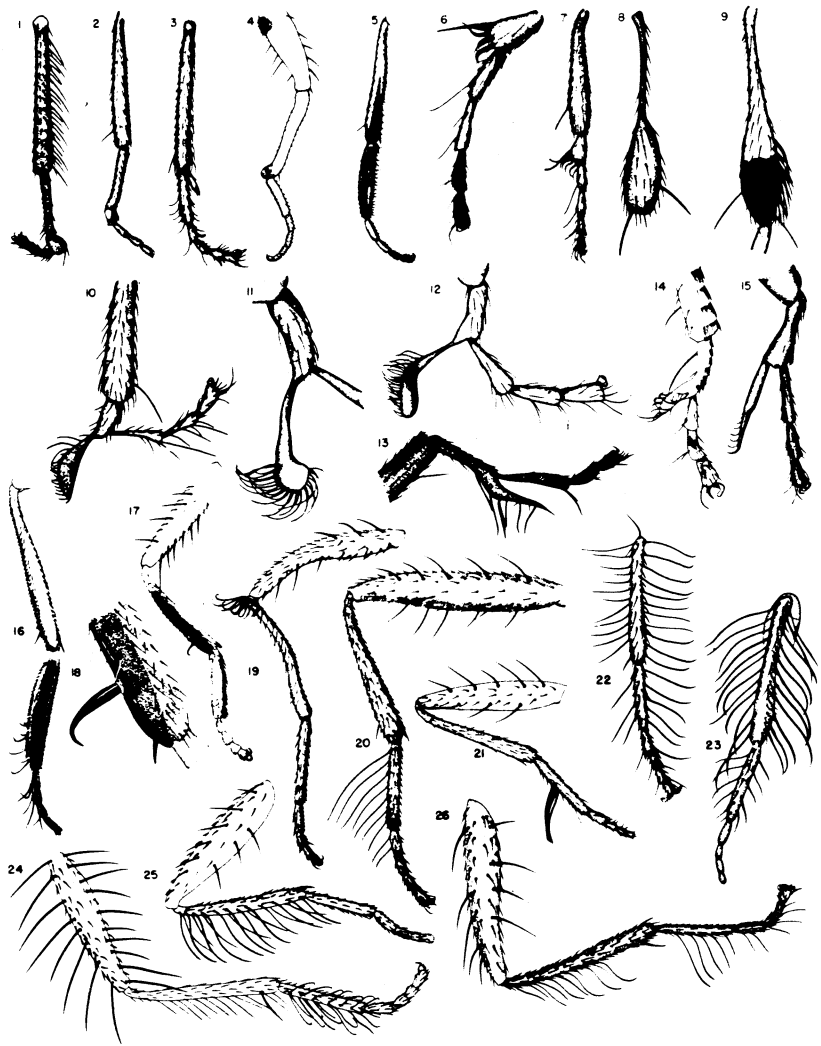


Fig. 4a. Legs of *Drosophila* spp.: 1, *dasycnemia* Hardy; 2, *percnosoma* Hardy; 3, *freyrcinetiae* Hardy; 4, *anomalipes* Grimshaw; 5, *adiastola* Hardy; 6, *expansa* Hardy; 7, *perissopoda* Hardy; 8, *clavitibia* Hardy; 9, *fuscoapex* Hardy; 10, *clavata* Hardy; 11, *capitata* Hardy; 12, *attenuata* Hardy; 13, *fundita* Hardy; 14, *spiethi* Hardy; 15, *enoplotarsus* Hardy; 16, *aethostoma* Hardy and Kaneshiro; 17-18, *hamifera* Hardy and Kaneshiro; 19, *flexipes* Hardy and Kaneshiro; 20, *gradata* Hardy and Kaneshiro; 21, *lineosetae* Hardy and Kaneshiro; 22, *silvarentis* Hardy and Kaneshiro; 23, *liophallus* Hardy and Kaneshiro; 24, *vescisetia* Hardy and Kaneshiro; 25, *hirtipalpus* Hardy and Kaneshiro; 26, *glabriapex* Hardy and Kaneshiro.

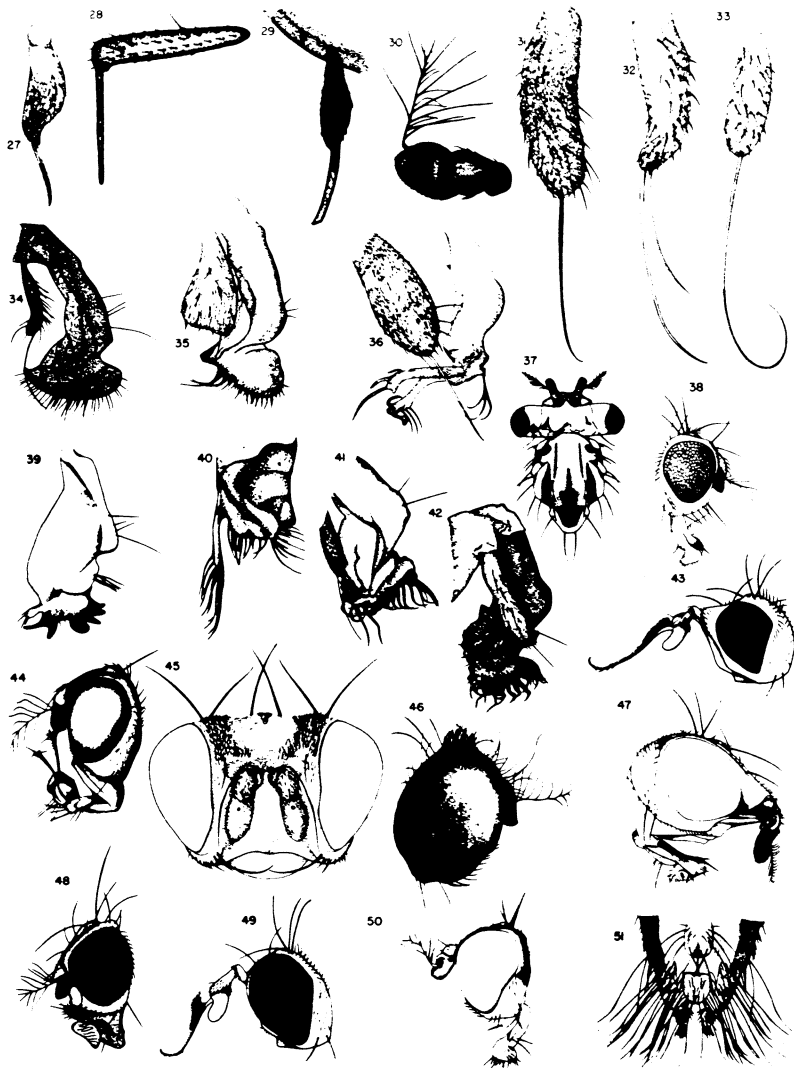


Fig. 4b. 27, Palpus of *Drosophila basisetæ* Hardy and Kaneshiro; 28, palpus, *Nudidrosophila gemmula* Hardy; 29, *N. lepidobregma* Hardy; 30, antenna, *Ateledrosophila preapicula* Hardy; 31, palpus, *D. hirtipalpus* Hardy and Kaneshiro; 32, palpus, *D. prostopalpis* Hardy and Kaneshiro; 33, palpus, *D. macrothrix* Hardy and Kaneshiro; 34, mouthparts, *D. cilifemorata* Hardy; 35, mouthparts and palpus, *D. acanthostoma* Hardy and Kaneshiro; 36, mouthparts and palpus, *D. aethostoma* Hardy and Kaneshiro; 37, head and thorax, *D. heteroneura* (Perkins); 38, *Celidosoma nigrocincta* Hardy; 39, mouthparts, *D. ceratostoma* Hardy; 40, mouthparts, *D. apoxyloma* Hardy; 41, mouthparts, *D. artigena* Hardy; 42, mouthparts, *D. scolostoma* Hardy; 43, head, *Antopocerus longiseta* Hardy; 44, head, *Drosophila adventitia* Hardy; 45, head, *D. setosifrons* Hardy and Kaneshiro; 46, head, *D. (Trichotobregma) petalopeza* Hardy; 47, head, *Drosophila planitibia* (Hardy); 48, head, *Grimshawomyia palata* Hardy; 49, head, *Antopocerus diamphidiopodus* Hardy; 50, head, *Nudidrosophila aenicta* Hardy; 51, apex of male abdomen, ventral, *D. clavisetæ* (From Hardy, *Insects of Hawaii*, 1965. Courtesy of University of Hawaii Press.)

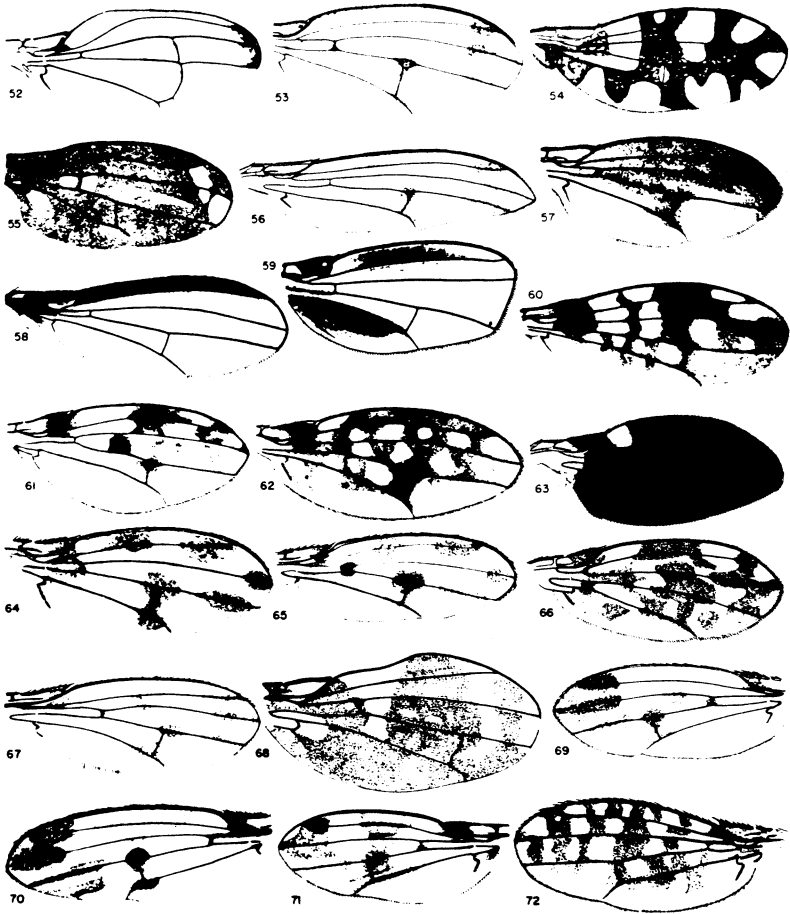


Fig. 4c. Wings: 52, *Drosophila melanocephala* (Hardy); 53, *D. deltaneuron* Bryan; 54, *D. fuscoamoeba* Bryan; 55, *D. varipennis* (Grimshaw); 56, *D. lanaiensis* Grimshaw; 57, *D. seclusa* Hardy; 58, *D. eurypeza* Hardy; 59, *D. truncipenna* Hardy; 60, *D. adiastrala* Hardy; 61, *D. clavisetae* (Hardy); 62, *D. spectabilis* Hardy; 63, *Celidosoma nigrocincta* Hardy; 64, *D. discreta* Hardy and Kaneshiro; 65, *D. ochracea* Grimshaw; 66, *D. crucigera* Grimshaw; 67, *D. flexipes* Hardy and Kaneshiro; 68, *D. hamifera* Hardy and Kaneshiro; 69, *D. liophallus* Hardy and Kaneshiro; 70, *D. virgulata* Hardy and Kaneshiro; 71, *D. silvarentis* Hardy and Kaneshiro; 72, *D. ochrobasis* Hardy and Kaneshiro.

markings (Fig. 4.52-72); brown marks may be over one or both crossveins (Fig. 4.64,70); variously arranged maculations (Fig. 4.54-55,68); the wing may be predominantly brown with hyaline spots (Fig. 4.60,62,66); nearly all brown (Fig. 4.63); extra crossvein in cell R-5 (Fig. 4.52,61); arcuate costal margin (Fig. 4.68); long and pointed (Fig. 4.56); *m* crossvein split (Fig. 4.53).

Abdomen: with clavate setae on venter of male (Fig. 4.51).

The *Drosophila* may be divided into rather rough groupings of species according to morphological characteristics. In some cases, these groupings are artificial and are not subgeneric units or even species groups. As indicated in the descriptive portion above, however, some of the peculiarities are useful as group characters. Some of the more obvious species groups that can be recognized are listed in the following paragraphs.

The "picture-winged" species include most of the flies with conspicuously marked wings, although such maculations have apparently arisen independently in more than one species group. *Idiomyia* was erected as a new genus by Grimshaw (1901) based upon the presence of an extra crossvein in cell R5 (Fig. 4.52). This would appear to be a most excellent generic character, very distinctive and characteristic of only this group of Hawaiian species. As pointed out by Hardy (1965), however, the "idiomyia" are nothing more than very large *Drosophila* with a freak wing-venation. Spur-vein abnormalities are sometimes found in field-collected and laboratory strains of Hawaiian *Drosophila*, suggesting that there may be genetic variability for this character in nature. Carson et al. (1967) expressed the opinion that the genus should be abandoned because of close chromosomal similarity to other picture-winged species of the subgenus *Drosophila*. The wing-vein character is obviously not even of species-group importance. Nevertheless, this subgroup is interesting because among them are the giants of the family; some species have a wing-spread of as much as 18 to 20 mm. *D. cyrtoloma* (Fig. 5) is probably the largest species of *Drosophila* known in the world. The *eurypeza* (Fig. 4.58) and *semifuscata* (Fig. 4.57) complexes include a number of species with characteristic wing markings. Approximately half of the Hawaiian *Drosophila* have brown or fuscous markings of some sort on their wings.

Several species groups can be recognized among those flies having modified mouthparts. In addition to those which have strong bristles or processes developed from the labella or on the palpa, there is a distinct group which has a heavily sclerotized rim around the apex of each labellum (Fig. 4.34). These latter form a complex ("white-tipped scutellum") of at least 50 species, which are further characterized by being predominantly black, usually polished, having the scutellum black with a small yellow to white spot at the apex. Some of these (*polita*-complex) have the wings entirely hyaline; others (*haleakalae*-complex) have the wings clouded at the apex and over the *m* crossvein. The species of this group are all apparently associated with fleshy fungi.

As described above, a number of groups of species may be characterized by having modified leg parts in males. In the work so far, they have been conveniently referred to as the "bristle-tarsi," "spoon-tarsi," "forked-tarsi," and "clubbed-tibiae." Each are groups including numerous species.

The preliminary classification of the Hawaiian fauna (Hardy, 1965)

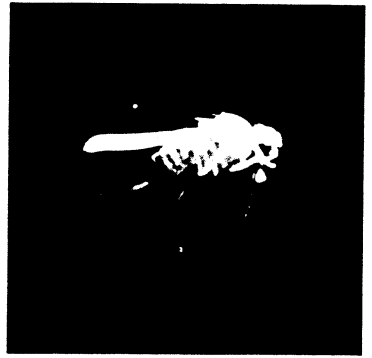


Fig. 5. (A), *Drosophila cyrtoloma* from Waikamoi, Maui compared with (B), *Drosophila melanogaster*. Both figures x 10. Photographs by H. D. Stalker.

divided the family into thirteen genera and ten subgenera. Two of these, *Gitonides* Knab, represented by one species, and *Pseudiasata* Coquillett, represented by three species, were purposely introduced for biological control of mealy bugs. Only *G. perspicax* Knab became established. Two were accidentally introduced. *Chymomyza* Czerny, represented by one species, and *Dettopsomyia* Lamb, represented by two species. The remaining nine "genera" are endemic, with thirteen species of *Drosophila* and one of *Scaptomyza* having been accidentally introduced from other areas.

The following introduced species have been recorded: *Gitonides perspicax*



Knab; *Chymomyza procnemis* (Williston); *Dettopsomyia formosa* Lamb; *Dettopsomyia nigrovittata* Malloch; *Drosophila (Drosophila) bizonata* Kikkawa and Peng; *D. (Drosophila) busckii* Coquillet; *D. (Drosophila) carinata* Grimshaw; *D. (D.) funebris* (Fabricius) (known from only one specimen collected at Kula Pipe Line, Maui, 4500 to 5000 feet, March 1932—this region has been collected very thoroughly, and *funebris* is obviously not established in the Islands); *D. (D.) hydei* Sturtevant; *D. (D.) immigrans* Sturtevant; *D. (D.) nasuta* Lamb; *D. (D.) polychaeta* Patterson and Wheeler; *D. (D.) repleta* Wollaston; *D. (Sophophora) ananassae* Dole-schall; *D. (S.) kikkawai* Burla; *D. (S.) melanogaster* Meigen; *D. (S.) simulans* Sturtevant; and *Scaptomyza (Parascaptomyza) pallida* (Zetterstedt).

It is quite remarkable that even more species of this family have not reached Hawaii and become established here. We receive immigrant insects at an alarming rate; over the last 20 years or so an average of 16 additional species of insects from various parts of the world have become established each year.

Subsequent studies have demonstrated that the earlier generic classification was not entirely accurate. The amazing array and diversity of the morphological characters displayed by the Hawaiian species and the obviously high degree of convergence and/or parallelism that has occurred require that concepts distinctly different from those employed for other world areas be used in dealing with this fauna. Characters that would normally be considered of generic importance in other parts of the world are often found to be only species-group characters, and in some cases are of no importance in grouping the species and represent cases of convergence or parallelism. Hence, it becomes quite impossible to determine phylogenies based on morphological characters alone.

The synonymy of *Idiomyia* with *Drosophila*, being based on a character not even of species-group importance, was noted above.

*Nudidrosophila* Hardy (1965) is of very questionable status. It would appear to be strikingly distinct from any known genus in the family by the males lacking the major head bristles; the reclinates, the proclimates, and the ocellar bristles are completely lacking; the front is entirely bare except for microscopic pubescence, or recumbent scale-like setae (Fig. 4.50). The ocellar triangle has a series of laterally directed, recumbent, very fine and inconspicuous hairs, and a thick, rather flattened spine-like development is present at the apex of each palpus (Fig. 4.28, 29). All of the above are male characters, and the females have not been associated with some of the species. As discussed by Hardy (1966), those females of *Nudidrosophila* which have been associated with the males exhibit typical *Drosophila* characteristics; and in the case of *N. aenicta* Hardy the females show remarkable resemblance to *Drosophila hirtitibia* Hardy. Based upon the females, these species would appear to be very closely related. In most

charactersitics these species appear to be identical, and they probably occupy the same or similar habitats. It is necessary that this complex be studied in detail before definite decisions can be made, but I believe it is evident that *Nudidrosophila* should not be retained as a genus. It may be necessary to synonymize it with *Drosophila*, even though on the basis of the males the striking differences would seem to be of generic importance: the male characters depart radically from those of *Drosophila*, according to present concepts. Five species have been described as *Nudidrosophila*.

*Antopocerus* Hardy consists of nine known endemic species, characterized by modifications in the antennae of males: first segment large, extending well beyond the margin of the front, concave on ventral surface, subequal in length to the third antennal segment, and the antennae strongly porrect with arista densely short-haired dorsally and bare ventrally (Fig. 4.43,49) except near the apex in some species. This genus is an offshoot of *Drosophila*.

*Ateledrosophila* Hardy, with two included species, is distinguished from *Drosophila* by having the arista preapical in position (Fig. 4.30), by having no discernible anterior reclinate or ocellar bristles, and no preapical dorsal bristles on the front tibiae. The group is poorly known. We have no information on the biology, behavior, or genetics. It is obviously close to *Drosophila*.

*Celidosoma* Hardy has one known species, which approximates *Chaetodrosophila* Duda in Wheeler's key to the genera of Drosophilidae of the Pacific islands (1952), but differs by lacking a comb of short spines on the front femur; by having a very weakly carinate face rather than a prominent face with a well developed carina; by having two strong sternopleural bristles rather than one strong bristle and two hairs present on upper portion of each sternopleuron; and by having the second antennal segment rather strongly produced apically extending over the basal portion of the third segment, as well as by other details. It is distinguished from other Hawaiian drosophilids by having four or five pairs of dorsocentral bristles and only four rows of acrostichal setae; by having the anterior reclinate and the proclinate bristles situated near the anterior margin of the front; and by the pointed second antennal segment. In the latter regard, it is like *Grimshawomyia* Hardy (Fig. 4.48). These two genera somewhat resemble one another in other respects, but the resemblance is superficial and they differ strikingly as follows: *Celidosoma* has only four rather than six distinct rows of acrostichal setae; four or five pairs of dorsocentral bristles rather than two, with two pairs presutural in position; two strong humeral bristles; face flat medianly on lower half and slightly carinate above; head very differently shaped; anterior reclinate and proclinate bristles situated near the anterior margin of the front instead of the usual position; each tibia with two black bands; wings predominantly dark brown, hyaline only on the basal portion. This would appear to be a direct offshoot of *Scaptomyza*.

Further studies are necessary to determine its exact status. We have no knowledge of its biology.

*Grimshawomyia* Hardy is represented by two species. This genus is distinguished from *Drosophila* by having the second antennal segment sharply pointed at the apex, extending over the base of the third; sides of the vertex swollen, and vertical and upper ocellar bristles situated on the gibbose portion (Fig. 4.48); costal fringe elongate, extending nearly to apex of vein  $R_{4+5}$ ; the wing and genital characters are distinctive. We have no information on habits, biology, or genetics of this genus.

*Drosophila* Fallén presently consists of 343 described species, of which 13 are introduced. It is probable that when all of the species of the Hawaiian *Drosophila* are known, the total will be near 500. In the earlier classification, the genus was divided into four subgenera. The great bulk of our species have the external morphological characters of the subgenus *D. (Drosophila)*, as presently defined. Throckmorton (1966) stated "the characteristics of the egg filaments, ventral receptacles, and vasa strongly indicate a position on the branch leading to the subgenus *Drosophila*. All other characteristics are consistent with this interpretation." More recently, Throckmorton (in litt.) has concluded that on the basis of internal anatomy, the Hawaiian *Drosophila* appear to be more closely related phylogenetically to the subgenus *D. (Hirtodrosophila)*. It is probable that when more complete information has been obtained, it will be logical to divide the genus *Drosophila* into several subgenera and many species groups. It is obvious, however, that this cannot be based upon morphological characters alone, and must entail all possible information concerning the ecology, genetics, behavior, biochemistry, and so forth.

Subgenus "*Hyponomyia*" Grimshaw was described as a genus by Grimshaw (1901) and was reduced to a subgenus by Hardy (1965). Grimshaw based this "genus" upon the dense clumps of bristles on the lower angles of the face, which is characteristic of the type, *D. varipennis* Grimshaw. This character has been found to have little or no value in grouping Hawaiian species, but *varipennis* and related species do have rather remarkable characters that set them apart. The males are characterized by the presence of a large preapical dorsal hook on the front tibia (Fig. 4.17, 18), by having the mouthparts highly modified and the costal margin of the wing strongly arched, and the male aedeagus flat and strongly clavate, spade-like. As discussed by Hardy and Kaneshiro (1968), in light of the extraordinary range of divergence of the morphological characters found in the Hawaiian species, we feel it best to treat *Hyponomyia* as nothing more than another unusual species group of *Drosophila*. This opinion is shared by Carson, who has found chromosomal similarities with *D. adiaestola*. We have thus synonymized *Hyponomyia* with *Drosophila*. Three known species are in the *varipennis* group.

Subgenus "*Trichotobregma*" Hardy (1965), with only one known species,

*D. petalopeza*, is probably a synonym of typical *Drosophila* and nothing more than another unusual manifestation of characters. It is obviously a bristle-tarsus *Drosophila* with unusual head hairs and modified middle tarsi. This species differs strikingly from others of *Drosophila* by lacking proclinate frontal bristles and by having numerous long bristle-like hairs on the lower sides of the front, completely obscuring the reclinate bristles in the male (Fig. 4.46). In the female, two lower reclinate bristles are present as well as a series of four to six short bristle-like hairs on the orbits between the anterior and posterior reclinates. The male is also characterized by having very tiny ocellar bristles, about equal in length to the fine hairs on the ocellar triangle.

Subgenus *D. (Sophophora)* Sturtevant contains four introduced, cosmopolitan species.

Subgenus *D. (Engiscaptomyza)* Kaneshiro (1969) contains six species of the *crassifemur* and *nasalis* complexes. This is a group that Throckmorton (1966) on the basis of internal morphology placed in his *Scaptomyza*-like grouping; he refers to these as the "scaptoids" (genera or groups related to *Scaptomyza*; it should be spelled "scaptomyzoid"). Also based on mating behavior, Spieth (1966b) placed these as *Scaptomyza*-like species. I would normally prefer not to use the terms drosophiloid and scaptomyzoid, even though the words are correctly formed, with the suffix -oid or -oides (meaning "like, resembling") because there is chance for confusion since the first could represent the adjectival form of the superfamily Drosophiloidea. In dipteran taxonomy terms like "drosophiloid," "muscoid," etc., are properly used only in the superfamily sense. However, in dealing with the Hawaiian fauna it is convenient to use such terminology and as long as our meaning is understood there should be no chance for confusion. On the basis of external characters, Mr. Kaneshiro has described *Engiscaptomyza* as a new subgenus under *Drosophila*. Moreover, the work of Takada (1966) emphasized the drosophiloid nature of the genitalia of these flies. It is not practical to differentiate genera strictly upon internal characters even though it is apparent from the excellent works of Throckmorton that the characteristics of the internal reproductive systems of both sexes are more reliable for determining phylogenies than are external characters.

In the present classification the scaptomyzoids are divided into two genera, *Scaptomyza* Hardy and *Titanochaeta* Knab. *Titanochaeta*, represented by 11 species, is very close to *Scaptomyza* although their biology is very distinctive; these species are apparently all predators upon the eggs of endemic spiders. Members of this genus are characterized by having the head equal to or narrower than the thorax, rather than distinctly broader; by having the front slanted, distinctly oblique rather than almost straight; compound eyes densely pilose, except in *S. vittiger* Hardy, rather than bare or nearly so; lower margin of head short, less than one-half as long as front

(except in *vittiger*) rather than having the lower margin of the head approximately equal in length to front; and arista lacking ventral rays except in *S. contestata* Hardy, which has one ventral ray. The male genitalia are rather similar to those of some *Scaptomyza* (*Trogloscaptomyza*) but the female ovipositor is slender, sharp-pointed, rather needle-like.

The scaptomyzoids contain 132 presently known species, 11 *Titanochaeta* and 121 *Scaptomyza*, sensu lato; 86 in the subgenus *Trogloscaptomyza* Frey, and 35 arranged in six other subgenera. These include *S.* (*Alloscaptomyza*) Hackman, an endemic group containing eight known species; *S.* (*Bunostoma*) Malloch, described from the Marquesas Islands, but best developed in the Hawaiian Islands, where eight endemic species are known at present.

*S.* (*Exalloscaptomyza*) Hardy was erected to contain one species, *mauiensis* (Grimshaw), which is characterized by having very short rays on the arista and short anterior dorsocentral and sternopleural bristles. The genitalia of both sexes are very distinctive. When the preliminary study was made *mauiensis* was thought to occur on all of the main islands. After detailed studies (Hardy, 1966), what was originally taken as *mauiensis* has been divided into six species. The islands of Kauai, Oahu, Maui, and Molokai each have one distinct species of *Exalloscaptomyza*, and the island of Hawaii has two species. All members of this subgenus breed in the flowers of morning-glory in the semi-wet areas of the islands, usually at elevations of 1,000 to 3,000 ft.

*S.* (*Parascaptomyza*) Duda is introduced and contains a cosmopolitan species, *S. pallida* (Zetterstedt). *S.* (*Rosenwaldia*) Malloch was described from the Marquesas Islands and contains six Hawaiian species. *S.* (*Tantalia*) Malloch is an endemic subgenus containing six known species.

*S.* (*Trogloscaptomyza*) Frey is an enigma. Eighty-six species are now known from Hawaii, 87 for the entire world. The type of the subgenus, *S. brevilamellata* (Frey), was described from the island of Tristan da Cunha, in the middle of the Atlantic Ocean. The group is unknown elsewhere in the world, except for the Hawaiian islands. Dr. Walter Hackman of the University of Helsinki, has done extensive studies on the genus *Scaptomyza* (1955, 1959). He has compared species of Hawaiian *Trogloscaptomyza* with Frey's type and has decided that they are congeneric. Most *Scaptomyza* over the world are characterized by having two to four rows of acrostichal setae. Many of the species of *Trogloscaptomyza* in Hawaii have six well-developed rows of acrostichals.

Throckmorton (1966) placed *Drosophila parva* Grimshaw in the scaptomyzoids. Likewise, Spieth (1966b), using mating behavior, places *parva* as a *Scaptomyza*-like species, as does Clayton (1968) on the basis of metaphase chromosomes. I have since checked *parva* again in more detail and find that it definitely is a *Scaptomyza*. It also is apparent that this is a species-complex, and from the preliminary study it appears that each

island has a distinct species of this complex. It will be necessary to assign this complex to a new subgenus under *Scaptomyza*. The genitalia are definitely *Scaptomyza*-like, as is the body form, coloration, and shape of legs. The arista also is not too atypical for some *Scaptomyza*, having three dorsal and one ventral ray in addition to the large apical fork.

The following is a summary of the present taxonomic arrangement of the genera and subgenera of the Hawaiian Drosophilidae:

#### Subfamily Amiotinae

*Gitonides* Knab, 1 introduced species

*Pseudiasata* Coquillett, 3 introduced species (not established)

#### Subfamily Drosophilinae

*Drosophila*-like genera (drosophiloids)

Genus *Antopocerus* Hardy, 9 endemic species

*Ateledrosophila* Hardy, 2 endemic species

*Celidosoma* Hardy, 1 endemic species

*Chymomyza* Czerny, 1 introduced species

*Dettopsomyia* Lamb, 2 introduced species

*Drosophila* Fallén

Subgenus *Drosophila* Fallén, 305 endemic species,  
10 introduced species

Subgenus *Sophophora* Fallén, 3 introduced species

Subgenus *Engyscaptomyza* Kaneshiro, 6 endemic species

324 total

*Grimshawomyia* Hardy, 2 endemic species

*Nudidrosophila* Hardy, 5 endemic species

*Scaptomyza*-like genera (scaptomyzoids)

Genus *Scaptomyza* Hardy

Subgenus *Alloscaptomyza* Hackman, 8 endemic species

*Bunostoma* Malloch, 8 endemic species

*Exalloscaptomyza* Hardy, 6 endemic species

*Parascaptomyza* Duda, 1 introduced species

*Rosenwaldia* Malloch, 6 endemic species

*Tantalia* Malloch, 6 endemic species

*Trogloscaptomyza* Frey, 86 endemic species

121 total

Genus *Titanochaeta* Knab, 11 endemic species

Total 479 presently known  
species in Hawaii

One of the most remarkable features of the Hawaiian fauna is the definite intergradation that is obvious between the two major genera *Drosophila* and *Scaptomyza*. As known from other world areas, these are distinct, well-defined genera, clearly differentiated by *Scaptomyza* having only two to four rows of acrostichal setae on the mesonotum; lacking ventral rays on the arista; having more elaborately developed male genitalia; frequent occurrence of weakly sclerotized, nondentate female ovipositor; very short egg filaments; primitive courtship and mating behavior; and by the characteristics of the internal reproductive system of both sexes (Throckmorton, 1966). *Scaptomyza* also differs from *Drosophila* by being dark-colored, grey pollinose, small in size and distinctive in body form. The biologies are characteristic; for the most part, *Scaptomyza* from other parts of the world are primarily leaf-miners in fresh leaves.

In Hawaii, numerous cases occur where *Drosophila* and *Scaptomyza* intergrade to a point where they cannot be separated by the normally accepted external morphological characters and may be differentiated only on the basis of mating behavior, internal morphology, and egg structure. At least half of the species of *Scaptomyza* (*Trogloscaptomyza*) have six distinct rows of acrostichal setae. Furthermore, Heed (1968) stated "the question arises whether the scaptoid line of descent overlaps the drosophilid line in ecological habits or has taken a different route. With the information available, it appears that it has done both. The *Tantalia*, *Exalloscaptomyza* and *Titanochaeta* have their own niches, while the *Trogloscaptomyza* overlaps somewhat the habitat of the more aggressive drosophilids."

The scaptomyzoids have comparatively elaborate male genitalia. As has been pointed out by Spieth (1966b), *Scaptomyza* exhibits a very primitive mating behavior, and it is apparent that the elaborate genitalia act as barriers for mating between different species. In *Drosophila* and closely related genera, on the other hand, the genitalia are for the most part very similar, especially in closely related species; and the isolating mechanism preventing cross-mating is the elaborate courtship and mating behavior, as has been thoroughly discussed by Spieth (1952, 1966b).

The borderline Hawaiian forms between *Drosophila* and *Scaptomyza* are as follows:

A white-tipped-scutellum species group, consisting of a complex of species presently treated under *Drosophila*, is characterized by being slender-bodied with a low body profile, having a small white to yellow spot at apex of scutellum and each labellum of the male having a heavily sclerotized dark brown to black rim along the margin (except in the species *D. fungiperda* Hardy). The larvae are apparently all fungivores and breed in poly-porous and other fleshy fungi. These forms all possess the typically accepted characteristics of *Drosophila*, i.e., several ventral rays on the arista, and numerous dorsal rays, numerous rows of acrostichal setae on

the mesonotum and relatively simple genitalia. Throckmorton (1966) has placed the white-tipped species with *Drosophila*, although he has stated that they show several *Scaptomyza*-like characteristics. The egg of these species have very short filaments and are somewhat *Scaptomyza*-like. Throckmorton has indicated that the paragonia are fully *Drosophila*-like. Also the ventral receptacle is *Drosophila*-like as is the female ovipositor. Spieth (1966b) said "the members of this group are quite distinctive from all other Hawaiian species not only with respect to their anatomy but also with respect to their mating behavior. The male courtship is essentially *Scaptomyza*-like." It is evident that this group should be erected to the rank of a new subgenus under *Drosophila*.

The "*Drosophila*" *parva* Grimshaw complex of species, which in the preliminary classification (Hardy, 1965) was placed under genus *Drosophila* because of the presence of a ventral ray on the arista and numerous rows of acrostichal setae, has now been found to be a species complex, as has been stated above; it has probably speciated by islands. The group has not been studied in detail, but it obviously will be necessary to include it as a new subgenus under *Scaptomyza*.

The "*Drosophila*" *crassifemur-nasalis* complexes of species, on the basis of Throckmorton's studies, have been placed as scaptomyzoid; although he did state that the pigmentation of the vasa, the ejaculatory apodeme and the anterior spiracle of the pupa are *Drosophila*-like. Spieth (1966b) says that their mating behavior is typically scaptomyzoid; furthermore, the metaphase chromosomes resemble those of scaptomyzoids. As has been stated elsewhere, a more detailed study has demonstrated that these represent two distinct complexes of species, which form a new subgenus. On the basis of their external morphological characters, Kaneshiro (1969) has described this as *Drosophila* (*Engiscaptomyza*). It is obviously borderline between *Drosophila* and *Scaptomyza*. The subgenus contains four species in the *crassifemur* complex and two species in the *nasalis* complex.

It is probable that the species *D. reducta* and *D. taractica* Hardy should be placed under *Scaptomyza*. Although presently under *Drosophila*, the male genitalia are definitely *Scaptomyza*-like. These species have not been studied further since the original descriptions.

The *Drosophila anomalipes* Grimshaw complex of species, consisting of two species, *anomalipes* and *quasianomalipes*, which occur in the same habitat in the Kokee area of Kauai, obviously represent a primitive group. Throckmorton (1966) points out that the spermathecae are rather *Scaptomyza*-like. Spieth (below) discusses the mating behavior.

The *Drosophila primaeva* Hardy and Kaneshiro complex, consisting of two species (*attigua* and *primaeva*), which occur in the same habitat as *anomalipes* and *quasianomalipes* on the island of Kauai, is similar in many respects to the above complex but differs in a number of important respects



pointed out by Hardy and Kaneshiro (1968). The actual breeding habitat of these species is not known. The males are characterized by having a sclerotized rim on the labellum; all other known Hawaiian *Drosophila* that possess this character are thought to be fungus breeders. From preliminary cytological studies made by Stalker (1968) it appears that *primaeva* is exceptional in that one arrangement in chromosome 5 closely resembles the sequence found in certain mainland species.

The numerous examples of intergradation between *Drosophila* and *Scaptomyza*, coupled with the fact that *Scaptomyza* has attained its greatest development in the Hawaiian Islands (presently the known number of species is two times greater in the Hawaiian Islands than for the rest of the world), lead to interesting conclusions. The circumstantial evidence presented by these data would obviously indicate that the genus *Scaptomyza* originated in the Hawaiian Islands and that the entire Hawaiian fauna could have originated from one ancestral species. Throckmorton (1966) in his summary made the following conclusions with regard to the major groupings of Hawaiian species: "phylogenetically, both of these groups are very closely related to each other and they are derived from near the base of the major branch leading to the subgenus *Drosophila* of the genus *Drosophila*. Evidence is presented indicating that the genus *Scaptomyza* originated in Hawaii and arguments for and against this interpretation are discussed. If *Scaptomyza* originated in Hawaii, then the available evidence favors the introduction of only a single individual (basically a *Drosophila*) as the progenitor of the more than 400 endemic species of Drosophilids. At most, two introductions, presumably of a single individual each, are required if the Scaptoids are thought to have originated from an introduction separate from that of the Drosophiloids. Existing evidence indicates that the Drosophiloid introduction was from east Asia, perhaps from Japan, but this problem is still under investigation."

This theory has rather startling implications, since these islands are comparatively young (one to six millions of years for the present main islands) and it seems rather inconceivable that *Scaptomyza* could have spread over much of the entire world from an isolated group of small islands in the middle of the Pacific Ocean. Much remains to be learned concerning this question and also the distribution of *Scaptomyza* over the world. Throckmorton (1966, pp. 385-386) stated "Regardless of the geographical point of origin of the Scaptoids, present evidence requires that they share a close common ancestor with the Hawaiian Drosophiloids. If the origin of the Scaptoids occurred outside of Hawaii, then two successful trans-Pacific colonizations are needed. Each one of these was, in itself, an improbable event. That a successful introduction be made twice from the same family of Diptera is even less probable, and that the two successful introductions from the same family should involve species so closely related (but presumably al-

ready generically distinct) as to produce the existing patterns of variation in Hawaii is less probable still. And if we do not postulate extremely close relationships between the two original colonizers, we must then explain the broad overlaps between the *Drosophiloids* and *Scaptoids* as due to convergent evolution. This requires that we explain why the *Scaptoids* should have diverged in the direction of *Drosophila* (while they were competing with them among the vacant niches in Hawaii), and why, for some character states at least, they have happened to diverge specifically in the direction of the *Hawaiian Drosophiloids*. And why do the *Hawaiian Drosophiloids*, of all the *Drosophiloids* in the world, include a group of species ("white-tip scutellum" forms) that share characters with the *Scaptoids*, their nearest (geographical) relatives? . . . For the present, then, Hawaii must be considered to be the only place in the world where the otherwise sharp distinctions between *Scaptomyza* and *Drosophila* tend to disappear."

The *Hawaiian* fauna exhibits a high degree of endemism by islands. From the preliminary studies, it is not possible to make accurate assessments of the species that are restricted to single islands, since it has become obvious that a high percentage of those which have previously been recorded from two or more islands actually represent species groups. In almost all of those cases which have been investigated thoroughly, a distinct species has been found on each island.

For the purpose of analyzing island endemism it is necessary to treat Maui, Molokai, and Lanai as one biological island, since these three islands have been fused into a single land mass at least two times, during the Pleistocene, due to the periodic retreat of the sea level. Besides, these islands are presently separated just by narrow channels, as discussed by Spieth (1968b; see also below). Their faunas show much closer relationship, and more species-overlapping occurs than between other islands, due to the comparatively recent isolation and their close proximity.

From the records published in the 1965 monograph with some corrections that have subsequently been made, it would seem that approximately 85 percent of the endemic *drosophiloids* have speciated by islands, treating Maui, Molokai, and Lanai as a biological island. The *scaptomyzoids* have not been considered in this analysis, since they are still rather poorly known. It is highly probable that the actual figure for endemism by islands should be 95 to 98 percent.

The best understood of our species to date are the large *Drosophila* that have extensive maculations on their wings (picture-winged; Fig. 4.52-72). These have received special attention (Carson and Stalker, 1968a, b, c; and Hardy and Kaneshiro, 1968) due largely to the fact that they are so ideal for refined genetic and laboratory studies. For the first time we have enough detailed information concerning a large segment of native species to provide a basis for a sound systematic arrangement of the species. The

results achieved with this group mark a major milestone in our understanding of the Hawaiian drosophilids. It is however, apparent that the picture-winged species constitute a small segment of the total *Drosophila* fauna, perhaps 20 percent (Carson and Stalker, 1968).

A fairly accurate analysis of the degree of speciation by islands can be made using the picture-winged species. The following have speciated by single islands. All the members of the *pilimana-hawaiiensis* complexes (51 species, Hardy and Kaneshiro, 1968, 1969); all of the *adiastola* complex (7 species, Hardy and Kaneshiro, 1968, 1969); all of the *semifuscata* complex (4 species, Hardy and Kaneshiro, 1968); and all of the "idiomyia" complexes of species (15 species, Hardy, 1969); *varipennis* complex (3 species, Hardy and Kaneshiro, 1968 and 1969); *haleakalae* complex (17 species of fungus feeders); and miscellaneous *Drosophila* that have extensive wing markings such as *fuscamoeba* Bryan and related species; *peniculipedis* Hardy, recorded from Maui and Oahu (the latter is very probably an error); and *aglaia* Hardy, recorded from Oahu and Hawaii (the latter record based upon one headless female and is no doubt an error). Considering all of the species that have rather extensive maculations in the wing, approximately 126 species to date (including 17 species of the *haleakalae* complex), only two, *crucigera* Grimshaw and *grimshawi* Oldenberg, are found on more than one main island. From an intensive study of the picture-winged species, based upon morphology it would appear that approximately 99 percent of the species are restricted to single islands.

The greatest known concentration of species is on the island of Maui as pointed out by the works of Carson and Stalker (1968a, b, c), many species groups have apparently originated on Maui, have undergone extensive speciation there and have spread to other islands, especially Oahu and Hawaii. Also from Carson's studies, using the picture-winged complexes, it is apparent that in some cases migrations have occurred from Kauai directly to Maui (bypassing Oahu) and that at least a number of species groups have developed there and radiated out to other islands. The greatest number of known species from any area in the Islands is in the Waikamoi section on the slopes of Haleakala on Maui (Fig. 3). This is rain forest, ca. 4000 feet elevation, with rainfall of 150 to 200 inches per year, and where all of the important host plants are available. Sixty-three described species of *Drosophila* and related genera have been recorded to date from this area.

The oldest islands of the Hawaiian chain (the Leeward Islands) are now worn down to the point where they no longer support a native fauna of Drosophilidae except possibly for a few species of *Scaptomyza*. The native species are now confined to the main islands: Kauai, Oahu, Maui, Molokai, Lanai, and Hawaii. Niihau and Kahoolawe are not at present accessible for field study but apparently support no native fauna. These are low islands,

1281 and 1477 feet, respectively; both are in the rain shadow and have been completely changed by man. The ancestors of the tremendous present day fauna that has developed were doubtlessly from Kauai, and one would expect that since this is the oldest of the main islands, it would support the greatest number of species of native plants and animals; but at least with the Drosophilidae this is not the case. By comparison with Maui, Hawaii, and Oahu, Kauai has a rather small fauna. To date, 44 species of *Drosophila* are known from this island. Five of these have also been recorded from other islands, and apparently 39 species are restricted to the island of Kauai. It is interesting to note, and also highly significant in the study of the evolution of this group in Hawaii, that of the drosophiloids only the genus *Drosophila* occurs on Kauai; *Antopocerus* Hardy, *Celidosoma* Hardy, and *Nudidrosophila* Hardy are unknown from this island. Also the picture-winged species of the genus *Drosophila* are poorly represented, and the "idiomya" and *haleukalae* complexes are unknown. It is evident that several important species complexes have probably developed on the island of Maui and have radiated out from there to the other main islands (see section, below, p. 469 ff.). Maui is very rich in species by comparison to Kauai, with 106 recorded species of *Drosophila* and 7 species belonging to genera close to *Drosophila* (*Antopocerus*, *Grimshawomyia*, and *Nudidrosophila*) for a total of 113 drosophiloids; 5 of these species have been recorded on other islands besides Molokai and Lanai. Molokai has 55 recorded species of drosophiloids. Of these, 26 are presently known only from the island of Molokai. Twenty-two are also found on Maui, and 13 are recorded as occurring on Hawaii, Oahu, or Kauai; these need further study and many may be erroneous records. Lanai has a very impoverished fauna, the island having been completely altered by man, and most of its endemic fauna lost. Only 13 species have been recorded from Lanai, 10 of which are also found on Maui, and three may possibly be endemic to Lanai. Two of these three, *D. lanaiensis* Grimshaw and *polita* Grimshaw, are known only from female specimens collected in 1893. The other, *D. kraussi* Hardy, was described from one male specimen from Lanai, and a series of specimens from Mt. Kaala, Oahu, were also placed here. This needs further study and may represent two species. One hundred and sixty species are found on the three islands, with 142 apparently restricted to Maui (including Molokai and Lanai). This compares with 98 species from the island of Hawaii, 91 *Drosophila* and 7 species in genera related to *Drosophila*. All but 4 of these are apparently restricted to the island of Hawaii. Oahu has 84 species, 5 belonging to genera related to *Drosophila*. All but 8 species are apparently restricted to Oahu. It should be noted that the total count of species in the above analysis is greater than the presently described number, due to the inclusion of a number of recognized species complexes that have not yet been treated taxonomically.

One factor that has had and is still having a serious effect upon the native fauna is the presence of predaceous ants in the islands. Forty-two species of introduced ants have been reported. These are widespread over the main islands and have no doubt taken a toll of native insects. Surely our leaf-breeding drosophilids would be affected where ant populations are present. It is felt that this is one of the major reasons why the island of Kauai has such a comparatively small present-day fauna of *Drosophila*. Ants are widespread over this island up to an elevation of 4000 feet, at Kokee. Fortunately they have not invaded back into the native rainforest or the Alakai Swamp area, or the elevations above 4000 feet, but collecting is very poor over this island below this elevation, probably because of the effect of the ants. This is also very dramatically demonstrated on Mt. Tantalus, Oahu, where collecting is now extremely poor and where before the turn of the century when Perkins did the collecting for the Fauna Hawaiiensis, the fauna must have been comparatively rich. Several of the species (other families) that were collected on Tantalus by Perkins are now apparently extinct.

### *Biology and Behavior*

The Hawaiian species of the family Drosophilidae, as shown by Hardy (see above), form two interlocking groups: the drosophiloids and the scaptomyzoids, each consisting of a number of closely related genera, subgenera, and species groups. Ecological and behavioral data pertaining to the scaptomyzoids are presently more fragmentary and incomplete than are those available for the drosophiloids. The present section therefore emphasizes the drosophiloids, with references to the scaptomyzoids in those instances for which adequate information is available to illustrate differences or similarities between the two groups.

### *Life Cycle*

The eggs display interspecific and intergroup variety of considerable extent, especially involving size, sculpturing, and number of chorionic egg filaments (Throckmorton, 1966). Scaptomyzoid eggs have zero to four short, heavy chorionic filaments. Drosophiloid eggs have two basic patterns of filaments. The eggs of the white-tipped scutellum group have either two or four short, slender filaments while the remaining species all have four. Kambysellis and Heed (1969, in ms.) studied the eggs and their development in the ovaries of 60 species. Represented in their samples were both scaptomyzoid species (*Exalloscapteromyza*, 4; *Engiscapteromyza*, 6), and drosophiloids (*Antopocerus*, 5; *Drosophila*, 45). The number of ovarioles varied from 2 per fly in some species to as many as 100 in other species.

The distribution, however, was not normal and tended to cluster in three groups. Group I consists of 10 species with 2 to 5 ovarioles per fly, the majority having four. Group II encompasses 15 species with the ovariole number varying from 8 to 20, but with most species having 10 to 15. Group III had ovarioles numbering between 20 to 100, with the most common number being 40 to 50. A number of other morphological and functional features appear to be correlated with the distribution of the ovarioles for the members of each group, i.e., the number of mature eggs found in the ovaries of each individual, the ratio of length of egg filaments to length of egg, and the ovipositional behavior of the females.

The Group I flies, regardless of the number (2 to 5) of ovarioles per individual, have only one ovariole in each ovary functional at any given time and, further, these function in an alternating manner so that only one fully developed egg can be found in a mature female. Thus these flies have the potential of laying only one egg at a time and not more than one egg per day. The individual eggs are large in comparison to the size of the fly, and lack chorionic filaments. In the laboratory the eggs are deposited individually on the sides of the vial, on the paper surface or the culture medium. They are not pressed into the food surface. The natural ovipositional sites of the four species of scaptomyzoids of the subgenus *Exalloscapteromyza* are known to be the flowers of the morning-glory, in which they deposit eggs on the anthers or stamens. The natural ovipositional sites of the *Engiscapteromyza* and *Grimshawomyia* species are presently unknown. *Engiscapteromyza crassifemur* and *nasalis*, however, can be reared under laboratory conditions and do lay a single egg per day. The eggs of Group I species can be held in the vagina of the female until a full-grown first instar larva has developed, so that the flies behave as oviparous species. It is to be noted, however, that not all of the Hawaiian scaptomyzoids conform to the pattern displayed by the species that Kambysellis and Heed studied. For example, species of the subgenus *Alloscapteromyza* produce very small eggs (Throckmorton, 1966) but the details of their ovipositional behavior are not fully known.

Most species of the Group II flies have 10 to 15 ovarioles per fly but show considerable intraspecific variability. Kambysellis and Heed found that the mean number of ovarioles per fly is positively correlated with the size of the individual. Robertson et al. (1968) suggest that the differences in size of the individuals of a species probably are due to variation in larval food supply. The alternating function of the ovarioles, which is characteristic of Group I, also pertain to Group II but is modified to the extent that more than one ovariole in each of the two ovaries is functional. Kambysellis and Heed found no ovariole contained more than one mature egg at the time of dissection. Without exception, the Group II flies oviposit only in decaying leaves of various endemic plants (Heed, 1968) and, further-

more, they release the eggs singly. The eggs have four chorionic filaments, which typically do not exceed the length of the egg itself. The females possess elongate ovipositors, which enable them to insert the eggs into a decaying leaf, but the chorionic filaments remain outside the epidermis on the surface of the leaf. The eggs are deposited soon after maturation, and thus embryonic development does not precede oviposition.

The Group III species display much more interspecific variability than do those of Groups I and II. The majority of the species have 40 to 50 ovarioles per fly. The alternating function of the ovarioles does not occur, and some species may have mature eggs in three fourths of the ovarioles, others as many mature eggs as ovarioles, and still others more mature eggs than ovarioles. The eggs themselves show much interspecific variation. All species studied, with a single exception (*Drosophila conspicua*), have four chorionic filaments but the filament/egg-length ratio varies from 0.71 in *Drosophila truncipenna* to 3.93 in *D. sejuncta*. This ratio is correlated with the number of mature eggs present in the ovaries, i.e., the smaller the ratio the fewer the number of mature eggs present. The females of all these species insert the eggs into the substrate in which the larvae develop, but those having a filament/egg-length ratio of less than 1.5 deposit their eggs individually, while those with a higher ratio often will at a single insertion of the ovipositor deposit a cluster of eggs. Those species which oviposit their eggs in clusters appear to utilize specific substrates that are nutritionally rich, such as the decaying bark of *Cheirodendron* and *Clermontia*. Such substrates can support a considerable number of larvae. Those species of Group III which deposit their eggs singly typically utilize a variety of different types of substrates, e.g., *Drosophila mimica* uses fungi, leaves of several plants, and fruits of the soapberry *Sapindus*.

Kambysellis and Heed have thus been able to show that the various species of Hawaiian flies have evolved adaptive reproductive mechanisms that allow for a maximum utilization of the various types of nutritional sources that are available, at the same time minimizing the competition for food.

As indicated above, the females of the various species with certain exceptions oviposit on or in rotting (fermenting) vegetable material substrates. Heed (1968) lists nine major ovipositional substrates (see Table V). Characteristically the leaves used for ovipositional sites are those which have abscised from trees or shrubs and have fallen to the forest floor in such a situation that they not only retain their moisture but also have absorbed additional moisture from the rainfall and the wet substrate. Leaves that become entangled in the ferns or other lower story plants, or are exposed to sunlight, desiccate to a point that they are unattractive as ovipositional sites. The females almost invariably insert the eggs into the original lower surface of the leaves; the long egg-filaments of the drosophiloids extend on the outside so that the investigator can ascertain exactly how many eggs

TABLE V  
Substrate Distribution of Reared Endemic Hawaiian *Drosophilidae*<sup>a</sup>

	Leaves	Stems	Flow- ers	Fruits	Fungi	Slime Flux	Frass	Ferns	Para- site	No. of Species
<b>Drosophiloids</b>										
Picture-wings	4	8	4	4	2	3	2			13
Modified-mouthparts	10	13	3	7	4					23
Ciliated-tarsi	10	3		4	1					17
Unclassified	6	1		1						8
<i>Nudidrosophila</i>		2								2
<i>Antopocerus</i>	11									11
Bristle-tarsi	16									16
Fork-tarsi	11									11
Spoon-tarsi	14									14
White-tipped scutellum					9					9
Number	82	27	7	16	16	3	3	2	0	
Frequency	.51	.17	.05	.10	.10	.02	.02	.01	—	
<b>Scaptomyzoids</b>										
<i>Trogloscaptomyza</i>	6	2	9	8			1			19
<i>Parascaptomyza</i>	1		1		1	1				1
<i>Bunostoma</i>					1					1
<i>Tantalia</i>	3									3
<i>Exalloscaptomyza</i>			4							4
<i>Titanochaeta</i>									6	6
Number	10	2	14	8	2	1	1	0	6	
Frequency	.23	.05	.32	.18	.05	.02	.02		.13	
Total Number	92	29	21	24	18	4	4	2	6	
Frequency	.46	.15	.10	.12	.09	.02	.02	.01	.03	

<sup>a</sup>Modified from Heed (1968).

have been oviposited in each leaf. The broad-leaved evergreen trees such as *Cheirodendron*, *Clermontia*, and *Ilex* shed leaves throughout the year, thus making properly fermented ovipositional sites continuously available. The broken, rotting ends of small limbs and large twigs, especially of *Clermontia*, which has a thick soft bark, are regularly used for oviposition and feeding by the adults. When a *Clermontia* shrub or tree dies, then for a long period of time it will attract an amazing number of species. To a lesser extent bark of other host trees or shrubs, especially *Cheirodendron*, will be likewise used by the flies.

Fungi (Spieth, 1966b), slime fluxes, which are relatively rare, and frass occur at various levels in the forest and are also sought out by various



species of the flies. In general, however, the majority of the species oviposit in materials that are lying on the forest floor, i.e., fallen leaves, fallen fruits and flowers of *Clermontia* and other lobeliads, broken limbs, and the fungi on fallen trunks or limbs.

Some species and some species groups of both scaptomyzoids and drosophiloids are substrate specific (Heed, 1968). Thus, species of *Tantalia*, the spoon-tarsi, the fork-tarsi, the bristle-tarsi groups, and *Antopocerus* utilize only leaves; *Nudidrosophila* is restricted to stems; *Exalloscaptomyza* prefers the flowers of the morning-glory; the exceptional genus *Titanochaeta* uses the egg masses of spiders, while the white-tipped scutellum group oviposit only in fungi. In comparison, the picture-wings, modified-mouthparts, and ciliated-tarsi groups, and *Trogloscaptomyza* are more catholic in their ovipositional behavior. Significantly these latter groups are, by species, the largest in Hawaii.

The accumulated data (Heed, 1968) show that, excluding fungi and the spider egg-masses, 32 endemic and 6 introduced genera of plants belonging to 30 families serve as ovipositional sites for the endemic Drosophilidae. Of these the genera *Cheirodendron* (Araliaceae) and *Clermontia* (Lobeliaceae) serve as ovipositional and larval food sites for more than half of all the species that have been reared from all sources. The fermenting leaves, stems, and roots of *Cheirodendron* and the leaves, stems, roots, and fruits of *Clermontia* all serve as ovipositional sites. From *Cheirodendron* 51 species have been reared, and from *Clermontia* 38. Of these 89 species only three (*D. disticha*, *Trogloscaptomyza hackmani*, and *Tantalia gilvivilia*) came from plants of both genera. Interestingly, one introduced drosophiloid species, *D. immigrans*, also uses the fermenting leaves and fruits of *Clermontia* as a breeding site. The next most frequently used plant is the monotypic genus *Ilex*, from which 14 species of drosophilids have been bred to date, the majority of which do not use either *Cheirodendron* or *Clermontia*. Thus, less than 10 species of the three plant genera account for approximately two thirds of all the species that have been associated with their ovipositional-larval-developmental substrates. An understandable effect of this concentration of species depending on a limited variety of food types is that many species can be reared from an individual plant or even portions of an individual plant. Heed (1968) has reared 59 individuals representing nine different species from the leaves of a single, physically isolated *Cheirodendron* tree in Kipuka Puauu (see Table VI). Interestingly, also, the ancestral stocks that gave rise to *Cheirodendron* and *Ilex* originated in Asia, but apparently *Clermontia* and the other lobeliads came from the Americas.

The spectacular nature of the Hawaiian drosophiloids led investigators during the first half of this century to attempt to culture the flies in the laboratory. Consistently, experienced scholars found this impossible to ac-

TABLE VI  
 Specimens Reared from Leaves of a Single Isolated Tree of  
*Cheirodendron gaudichaudii* (D.C.) Sem., Kipuka Puauulu, Hawaii<sup>a</sup>

	13.IV.66	3.VI.66	XI.66	10.VII.67
<i>A. cognatus</i>				1
<i>A. tanythrix</i>			7	
<i>D. cneocopleura</i>				1
<i>D. disticha</i>	1	1		1
<i>D. neutralis</i>		3		
<i>D. sordidapex</i>		3		
<i>D. spiethi</i>	9	21		7
<i>D. trichaetosa</i>	2		1	
<i>S. hackmani</i>		1		
Total of 59:	12	29	8	10

<sup>a</sup>From Heed (1968).

comply. Neither the adult flies nor the developmental stages would tolerate in the laboratory those methods that were successful for rearing species from other parts of the world. New techniques and new culture methods were clearly necessary. After a number of unsuccessful attempts, Wheeler and Clayton (1965) devised a food medium that, when supplemented in each food vial with a strip of absorbent tissue, moistened on one end with a dilute sterile solution of Karo (maize-sugar syrup) and yeast hydrolysate extract and on the opposite end with distilled water, is adequate for the rearing of a number of species. Large vials are usually used but some of the larger species will oviposit more readily if maintained in small cages (Clayton, 1969). Moreover, adults of most species (both field-captured and laboratory-reared) can be maintained in good health in "sugar" vials (Spieth, 1966a) for prolonged periods of time.

Those species which can currently be reared in the laboratory belong with few exceptions to the modified-mouthparts and picture-wing groups of the drosophiloids and to the scaptomyzoid subgenera *Bunostoma* and *Trogloscaptomyza*. Excepting *Bunostoma*, for which Heed (1968) reports rearing only one species (see Table V), the other three groups are the most polyphagous of all the Hawaiian flies. The essentially monophagous groups such as *Antopocerus*, bristle-tarsi, fork-tarsi, and spoon-tarsi are currently unculturable even though their natural foods are known. Furthermore, many species of the picture-wing and modified-mouthparts species can

either not be cultured or at best can be maintained for only one or two generations.

Ever since Heed in the summer of 1963 discovered that fermenting *Cheirodendron* leaves serve as a prime developmental site for many species, attempts have been made by analyzing these leaves chemically and physically to gain leads and insights into the preparation of new media for the "leaf feeders." Robertson et al. (1968) attacked the problem by analyzing the *Cheirodendron* leaf and using *Drosophila disticha* as a test organism. *D. disticha* is a leaf-breeding spoon-tarsi species that occurs in abundant, easily collectable numbers in the Waikamoi area of Maui. Heed (1968) has reared from natural substrates many individuals, the vast majority of which came from *Cheirodendron*, and an occasional individual from *Tetraplasandra*, *Pittosporum*, and *Myrsine* leaves.

Robertson and his associates dissected and analyzed the contents of the digestive systems of field-captured adults and larvae. The crops of the adults carried both yeasts and bacteria. The yeast contents of the males were quantitatively lower than were those of the female. Also a spectrum of bacteria occurred in the males different from that found in the females.

The larval gut content varied from the adults in that all larvae carried at least one of several recognizably different bacteria, while yeasts were present in only 3 of 40 individuals dissected, and probably 2 of these 3 were contaminants. Thus unexpected differences were found, not only between the sexes of the adults but more importantly between the adults and the larvae.

Robertson and coworkers fractionated the *Cheirodendron* leaf into three parts: Fraction I contained the combined organic solvents, Fraction II the water soluble constituents, while Fraction III was presumed to be principally cellulose. By adding Fraction I to agar they were able to induce the females to lay eggs, whereas with plain agar the females refused to oviposit. Then with axenic techniques and Medium C (Sang, 1956) they attempted to rear the larvae. On Medium C alone the larvae died in first instar. By adding appropriate amounts of supplements of various sorts to Medium C, including the *Cheirodendron* fractions and the yeasts found in the crops of the adults, they found that in some instances the larvae were able to develop into "well-grown" instar II but never to maturity (see Table VII).

Robertson et al. (1968) concluded that they apparently were "dealing with rather extensive metabolic differences between *disticha* and the species of *Drosophila* whose nutrition had been so far examined" and further that "the nutrition of a species like *disticha* may have more in common with that of soil-living nematodes than with the yeast-feeding *Drosophilidae*."

Subsequent to the investigations of Robertson and coworkers, attempts have been made to devise new types of food medium. Kircher et al. (1968) prepared a medium that gave some but not complete success for the rearing

**TABLE VII**  
**Effects of Supplementing Medium C with Various Concentrates, Compounds, Leaf Fractions, and Yeasts<sup>a</sup>**

Supplement	Growth <sup>b</sup>
1. None	0
2. Yeast extract	0
3. Malt	0
4. Malt and Neopeptone	+
5. Malt and phospholipid concentrate	0
6. Malt and brewer's yeast/mushroom extract	+/++
7. Neopeptone	+
8. Phospholipid concentrate	+
9. Neopeptone and phospholipid concentrate	+/++
10. Fraction I	0/+
11. Fraction II	0
12. Fraction III	0/+
13. Fractions I and II	+
14. Fractions I and III	0/+
15. Fractions II and III	+
16. Fractions I and II and III	+
17. Fraction II and <i>Torulopsis</i>	+/++
18. Fraction II and <i>Candida</i>	+/++
19. Fractions I and II and <i>Torulopsis</i>	+
20. Fractions I and II and <i>Candida</i>	+
21. Fraction leaf homogenate	0

<sup>a</sup>Modified from Robertson et al. (1968).

<sup>b</sup>Symbols: 0, No growth beyond instar I; +, Survival to small-sized instar II larvae; ++, Survival to large-sized instar II larvae.

of the leaf-breeding flies. Recently Kircher (unpublished manuscript) has analyzed both green and decomposing leaves of *Cheirondendron* for sterols, triterpenes, and fatty acids. He ascertained that the fatty-acid portion consisted mainly of palmitic, linoleic, and linolenic acids, while the nonsaponifiable fractions contained the sterols  $\beta$ -sistosterol and stigmasterol plus the triterpene cycloartenal. This study complements that of Robertson et al. (1968), who showed that the leaves contain the usual amino acids and sugars plus bacteria during the process of decay. Kircher found the leaves to contain adequate amounts of sterols for the needs of the developing larvae during all stages of leaf decay. The fatty acids are the usual ones that are found in plants. Thus the evidence to date indicates that the *Cheirodendron* leaves neither possess some unique biochemical constituent nor lack categories of materials that set them apart from other plants. The most feasible interpretation as to why these leaves are such favorite substrates for the developing larvae appears to be the combination of a slow

rate of decay, the retention of moisture, and the lack of yeasts and moulds in the interior of the leaves. Kircher believes "the leaf breeders in Hawaii may have evolved to utilize the slow release of nutrients caused by bacteria decay of *Cheirodendron* leaves." An important corollary is the lack of moulds inside the leaves, which causes consistent troubles in the laboratory when Wheeler-Clayton medium is utilized.

The important problem of developing methods for the rearing of additional species under laboratory conditions invites the attention of insect nutritionists as well as *Drosophila* investigators, for until it is solved many aspects of the over-all investigation of the biology and evolution of the Hawaiian drosophiloids simply cannot be solved.

The natural food substrates of the adults are less well known than are those of the larval stages. A number of investigators have observed adults of various species engaging in feeding behavior in the field. The substrates involved included fermenting leaves and bark of *Cheirodendron*; the leaves, flowers, fruits, and bark of lobeliads, especially species of *Clermontia*; the leaves of *Pisonia*; various fungi, especially the bracket fungus *Polyporus*; and the exuding sap from the recently cut stumps of large tree ferns (*Cibotium*). A number of different species have been observed feeding upon each of the substrates listed, and all except the stumps of tree ferns are known to serve as ovipositional sites for one or more species of the flies. There is a general rule that applies to the great bulk of the world's drosophiloids, namely, that the adults of a given species will always feed upon the substrate into which the females oviposit, but additionally they will also feed upon other substrates that are not used for oviposition. Excepting the parasitic species of the scaptomyzoid genus *Titanochaeta*, the Hawaiian flies do appear to conform to this generalization in their feeding, ovipositing behavior.

With rare exceptions when the larvae of the drosophiloids attain maturity and are ready to pupate, they leave their food site regardless of its location, and burrow into the soil; pupae have been repeatedly recovered from the soil. This unusual habit was first reported by Wheeler and Clayton (1965). Many of the larvae exhibit the habit of skipping, and the mature larvae of fungus-feeders have been observed skipping off the fungus in which they developed and falling several feet to the surface of the soil, into which they immediately burrowed (Spieth, 1966b). In those species which are culturable in the laboratory, it is necessary to remove the cotton plugs of the food vials when the larvae are mature and to place the vials in a jar or similar container partially filled with moist sterile sand. The full grown larvae leave the food, crawl out of the vial, and crawl or skip onto the sand, into which they burrow and then pupate. When the adult emerges it forces its way out of the soil (or sand, in the case of laboratory flies) as a general individual. Many scaptomyzoids follow this same pattern of behavior, but at least some do not for they pupate upon the material where they spend the

larval developmental period. For example, the individuals of the scaptomyzoid subgenus *Tantiala* (Heed, 1968) spend their larval life scavenging the surfaces of leaves and simultaneously cover themselves with tiny bits of dirt and debris strategically placed along their bodies. They then retain this material on the pupal case when they pupate on the leaf. *Trogloscaptomyza cyrtandrae* lays its eggs on the leaves of the native *Cyrtandra* sp., and the larvae apparently feed on the exudations from the leaf hairs found on the under surface of the leaves. Pupation also occurs on the plant, with the pupal case tightly adhering to the leaf surface (Hardy, 1965). It is also of note that Swezey (1954) reported one true drosophiloid, *Drosophila sadleria*, as a borer in the parenchyma of the rachis of *Sadleria* fronds. Pupation of this species also takes place in the fronds.

The length of the life cycle is inordinately long when compared to that of other drosophiloids. The newly emerged adults typically reach sexual maturity 10 to 12 days after emergence; the females reach their peak of

TABLE VIII  
Developmental Cycle Time Span<sup>a</sup>

Group and Species	Age of ♀ at 1st Oviposi- tion (days)	Days to Hatching	Development from Eggs to Adults (days)
Picture-wings			
<i>D. adiastola</i>	16	5	32
<i>D. conspicua</i>	15	5	29
<i>D. crucigera</i>	14	5	28
<i>D. discreta</i>	18	4	36
<i>D. engyochracea</i>	25	5	48
<i>D. fasciculasetae</i>	16	4	32
<i>D. grimshawi</i>	16	5	32
<i>D. hawaiiensis</i>	14	5	30
<i>D. hemipeza</i>	15	5	30
<i>D. paucipuncta</i>	15	5	32
<i>D. picticornis</i>	18	5	35
<i>D. pilimana</i>	18	5	41
Modified-mouthparts			
<i>D. asketostoma</i>	9	4	21
<i>D. dissita</i>	10	4	20
<i>D. eurypeza</i>	12	3	24
<i>D. fuscamoeba</i>	12	4	24
<i>D. mimica</i>	10	3	21
<i>D. quadrisetae</i>	10	4	20

<sup>a</sup>Data supplied by Kathleen Resch, Genetics Foundation, University of Texas.

receptivity 14 to 18 days after emergence. Miss Kathleen Resch (personal communication) reports that at the University of Texas larvae and pupae are maintained at 20° to 24°C, and adults and eggs at 14° to 17°C. Under these conditions (Table VIII) eggs do not hatch until 3 to 5 days after oviposition; the period of time from oviposition to adult emergence varies from 20 to 48 days. In comparison, *D. melanogaster* eggs (25°C) hatch in 20 to 22 hours, and *D. funebris* eggs in 30 to 32 hours after oviposition. *D. melanogaster* at 25°C can complete a life cycle in 9 to 10 days. The adult Hawaiian drosophiloids also have a long life span as shown by the fact that field-captured adults have been maintained under laboratory conditions for prolonged periods, e.g., Carson (personal communication) captured an adult female of *D. grimshawi* on July 21, 1964. The specimen oviposited that same day, continued to lay mostly fertile eggs until March 22, 1965, and died on April 20, 1965. Since the fly was mature and inseminated, it must have been more than two weeks old when captured; it must have had a life span in excess of nine months.

The adults, as would be expected, do not randomly distribute themselves throughout the forest habitat but, rather, select and tend to accumulate in areas, often relatively small, that meet their specific ecological requirements. The major factors that control the distribution of the individuals have been delimited grossly and are as follows: wind intensity, humidity, temperature, light intensity, adult food sources, and acceptable ovipositional sites. Each species, of course, appears to have its own specific ecological requirements, but these appear to be so similar for many species that the adults of large numbers of species tend to accumulate in small areas or "pockets" of the forest. The adults of most species avoid even moderate wind currents and light intensities, humidities below 90 percent, and temperatures above 70°F (21.1°C).

For the Hawaiian flies, and especially for the drosophiloids, the temperature requirements appear to be quite exacting. Thus, in rearing the species in the laboratory, it is mandatory that the temperature be kept below 70°F, preferably about 65° to 68°F. Heed (1968) in rearing larvae kept his field-collected cultures (substrates) at 68° to 70° or temporarily at 50° to 55°F. One of the richest collecting sites known on the islands is located at an elevation of 4000 feet along the Kula pipeline at Waikamoi, Maui (Fig. 3). During the period from 13 July to 2 August 1965, a continuous record of the temperatures taken approximately 5 feet above the ground showed the temperature to vary between an average minimum of 56.7°F and an average maximum of 66.8°F. The highest daily maximum was 69°F and the lowest minimum 53°F. During this same period the relative humidity was 95 percent or higher for 77.5 percent of the time, and less than 90 percent for only 2 percent of the time. The surface of the forest floor in such situations hovers around 60°F during all times of the day. These are typical

summer temperatures, and, of course, are considerably lower during the winter season, although the flies are active the year round.

The combination of physical environmental requirements listed above is not too commonly found even within the rainforests, especially when added to these factors must be the presence of suitable feeding and ovipositional sites. The preferred sites, therefore, are usually small ravines or areas that are protected from the prevailing trade winds by a ridge or similar physical obstruction. Additionally, the area must have a high and relatively dense forest canopy, usually provided by *Metrosideros* or *Metrosideros* and *Koa* or other trees of comparable size and habit, an understory of shrubs and/or tree ferns (*Cibotium*), and a dense lower level of "bracken" ferns. If in addition ovipositional substrates are present, especially *Cheirodendron* and/or *Clermontia*, areas of less than one acre (4000m<sup>2</sup>) will serve as a focal point for the adults of numerous species of drosophiloids and scaptomyzoids.

Within such an area, the greatest density both of individual adults and of number of species will be found in the "bracken" fern or lowest level of vegetation. The flies prefer to sit on the under surfaces of leaves, fern pinnae, and horizontal branches of shrubs, and the like. Almost invariably they avoid sitting in a situation where light, even the low-intensity light of such forests, falls directly upon them from above. During periods of heavy overcast, when the relative humidity approaches 100 percent and especially if a mist-type of rain is falling and the light intensity is further reduced, then the flies often move upward into the vegetation and can be found on the under surfaces of leaves, limbs, etc., 6 to 10 feet above the ground.

When cloudless, sunny, warm periods occur and the humidity drops rapidly, the flies disappear, presumably seeking out small, poorly lit areas where the humidity is high and the light intensity is low. Likewise, torrential rains (but not moderate rains) will drive them from those places where the investigator can normally expect to see adults. During moderate rainfall individuals of species that normally are hidden in the dense low fern cover (e.g., *Drosophila disticha*) will move upward in the vegetation and, if the light intensity is low due to a heavy cloud cover, may sit on the upper exposed surfaces of leaves. The fact that a droplet of rain may and often does fall upon the insects does not appear to bother them in any way. A curious behavior with relation to water is displayed by species of the *Idiomya* group, which under laboratory conditions actually bathe themselves by immersing their bodies in shallow water. The bathing motions are ritualized and involve depressing first the sides and then the venter of the body into the water followed by a period of careful grooming.

A careful search of any given collecting site will disclose that there are a number of preferred microsites such as a particular horizontal limb, tree fern, shrub, perhaps the leaves of one limb of a shrub that is so located



that light, temperature, humidity, and spatial arrangements create a micro-area that is hyperattractive to the adults. Repeated visitations to such micro-sites over a period of months or even years will always result in the finding of adults of one to several species occupying that site.

Although the adults do accumulate in numbers in small areas where food, ovipositional sites, and the physical environment are optimal, they also migrate through other parts of the forest. Thus, if collecting is done for adults by sweeping and baiting in windswept areas such as ridges and other exposed places, few if any adults can be captured. Often in such places there may be numerous specimens of *Cheirodendron* and *Clermontia* trees, and the fermenting leaves on the forest floor will invariably have been used as ovipositional sites containing eggs and or larvae in various stages of development. Apparently mature females must regularly move through such areas ovipositing in the available substrates. Also, in the early morning just after dawn following a windy, rainy night, adults have been collected in open areas sitting on automobiles and buildings located at considerable distances from the nearest forest in which they normally dwell.

Finally it should be noted that those species which dwell in the dry forests, such as are found at Auwahi, Maui, are exposed to greater wind velocities and light intensities than are those which dwell in the rain forests and the mixed mesophytic forests. Experienced collectors, when they first see this forest, find it difficult to believe that a rich fauna of drosophiloids dwells there. This forest, however, is at an elevation of 3000 to 4000 feet, and the temperatures are therefore within the range that the Hawaiian drosophiloids demand and the food substrates are present.

Temperature, as noted above, seems to be a limiting factor in the distribution of the Hawaiian Drosophilidae. Consequently most of the species both of drosophiloids and scaptomyzoids are found about 1000-foot (300-m) elevations and reach their maximum at 3000 to 4000 feet. A few individuals of species such as *D. crucigera* (600 feet) and *D. flexipes* (800 feet) have been taken below 1000 feet but both species also range up to 4000 feet. The introduced scaptomyzoid *S. pallida* ranges from sea level to above 8000 feet, and a number of scaptomyzoids are found from 5000 to 9000 feet in areas that are unsuitable for almost all drosophiloids. *D. asketostoma*, which is almost invariably collected from the endemic silver-sword *Argyroxiphium sandwicense* in the crater of Haleakala, Maui, at 8000 to 9000 feet, seems to be living at the highest elevation of any Hawaiian drosophiloid.

Significantly, species of *Clermontia* and *Cheirodendron* that serve as larval substrates and adult food sources for many species are rarely found below 1000 feet and also are most abundant in the 3000- to 4000-foot level. Related genera, e.g., *Reynoldsia* (Araliaceae) that do grow at lower and warmer elevations do not serve as a substrate for the larvae, although

*Tetraplasandra* and *Pterotropia* belonging to the same family and growing above 1000 feet, often along with *Cheirodendron*, are used by a number of drosophiloid species. The intimate relationship that exists between the Hawaiian Drosophilidae and the *Clermontia* and *Cheirodendron* plants seems to be one of long standing.

### **Courtship Behavior**

The Hawaiian drosophiloids and scaptomyzoids exhibit contrasting courtship behavior. A sexually active scaptomyzoid male, like those of most other cyclorrhaphous species, orients upon an individual to which he has become alerted, be it male or female, then maneuvers to a position behind or at the side of the other individual and with a running lunge attempts to mount. Having mounted, the males of some species vibrate one or both wings, many do not; but the male of every species elongates and curls the tip of the abdomen down and under the other individual, seeking to make genitalic contact and intromission by repeatedly drawing the genitalia across that of the individual that he has mounted. A male or nonreceptive female reacts vigorously by kicking, by twisting the tip of the abdomen away from the seeking movements of the courting male, by shaking the entire body, and by violent wing vibrations. After a variable period of time, the courting male is either dislodged or dismounts of his own accord. Receptive females typically accept the male immediately after he has mounted. Males are persistent suitors and move rapidly about seeking out other individuals. Both males and nonreceptive females are wary of any other nearby moving individuals, typically orienting themselves so that they either face such individuals or escape from the immediate vicinity (Spieth, 1966b; 1968a). Observational data on mating behavior of the Hawaiian scaptomyzoids is relatively limited, and as yet unpublished. All species observed to date, both in laboratory and field studies, conform to the basic "assault" pattern described above. There is, however, no valid reason for assuming that such a simple-appearing courtship involves fewer stimuli than are involved in the quite different pattern displayed by the drosophiloids (Spieth, 1968a).

The Hawaiian drosophiloids, with certain exceptions noted later, do indeed display a courtship pattern that differs sharply from the scaptomyzoid type. After orienting, the male approaches and assumes a display posture close to a female typically at the rear with his head under her wing tips. A small number of species posture at the front of the female and still fewer at her side. In each instance, the position is species-specific.

Having assumed the display posture, the male then engages in a number of complex, ritualized, species-specific movements utilizing various parts of his body. Nonreceptive females respond to the male's overtures by flee-

ing, kicking with their hind legs, depressing the tip of their abdomen toward the substrate or simply ignoring his action. If the female is nonreceptive but does not flee, the males of some species after unsuccessfully displaying for a period of time will leave the head-under-wing position and circle to the front of the female and, facing her, will engage in further complex display, exhibiting movements that typically are quite different from those performed at the rear. After a period of frontal display, the male then returns to the rear position. Males typically court nonreceptive females for prolonged periods of time, sometimes for hours. A receptive female usually accepts the male's overtures rather quickly, indicating acceptance by spreading her wings horizontally, extruding the ovipositor and depressing her body toward the substrate. (See Spieth, 1966b, 1966c, and 1968b for details of specific courtships.)

The Hawaiian drosophiloids' courtship behavior thus conforms to the general pattern displayed by drosophiloids from other parts of the world. There are, however, consistent quantitative and qualitative differences that distinguish the Hawaiian species from those found elsewhere. A major difference is the exact site selected for courtship. The usual worldwide pattern involves the diurnal morning and evening assembling upon the feeding sites by adults of all ages. The females devote most of their time to feeding, but the males after feeding for a short time then move actively about, investigating other individuals of approximately their own size and facies. Thus, the males will orientate and initiate at least the beginning of courtship with any male or female of their own species or of other similar-appearing species. If a female of his own species has been approached, the male will then proceed to court in typical fashion. At any given feeding period, most of the females will be either too young to accept the male's overtures or else have been fecundated at a previous time.

Of the numerous courtships attempted by the males, only a very small percentage results in copulation. Further, males of most non-Hawaiian species after engaging in courtship display do try to mount nonreceptive females, e.g., *D. melanogaster* and *D. immigrans*. As a total result there is a great amount of activity at a feeding site, due especially to the males' persistent courting and the negative response by many females.

In comparison the Hawaiian species do not engage in courtship *on* the feeding site. All individuals, both males and females, are quiet, move about slowly, and show no avoidance or antagonistic reactions to each other. The males feed for only a short time and then with very rare exceptions (e.g., *D. anomalipes*) leave the food with a quick darting flight into the surrounding vegetation. The flies show little diurnality. During periods of low light intensity (e.g., on cloudy days) and high humidity, a feeding site may be continuously occupied from early morning to late afternoon.

During periods of sunlight and low humidity, the same site may be deserted except early in the morning and late in the afternoon. (See also Grossfield, 1968.)

Almost all of the Hawaiian males display extraordinary sexual dimorphism (see Figs. 4a, b; the great majority of these features are found in males only). The male structures involved in these dimorphic modifications include the antennae (all *Antopocerus* spp.); the mouthparts (all the modified-mouthparts group and most of the fungus-feeders plus several picture-wing species); the reduction and/or modification of the setae on the dorsum of the head (e.g., *D. petalopeza*); the wings (many species); the prothoracic legs (picture-wings, bristle-tarsi, ciliated-tarsi, spoon-tarsi, and split-tarsi groups); metathoracic legs (various species), and the eyes (*D. heteroneura*) or rarely the abdomen (*D. clavisetae*). A given species may exhibit only one structural modification; this is the most common situation but a number of species display two or more dimorphic characters—for instance, *D. petalopeza* has a modified setal pattern on the dorsum of the head, a bristle-tarsi type of forelegs and modified tarsal segments 2, 3, 4, and 5 on the middle legs. In all cases observed to date, whenever a male of a species possesses a dimorphic character, the modified body structure is utilized in the courtship. A small percentage of the drosophiloid species from other parts of the world possess dimorphic structures—such as the sex combs of *D. melanogaster* and *D. pseudoobscura* and their relatives, but these represent rare exceptions rather than the rule, while just the opposite pertains for the Hawaiian species.

The most typical display movements during courtship of non-Hawaiian drosophiloid males involves extending *one* wing from the resting position, then vibrating it in relatively small amplitude, the employment of the mouth parts to lick the area of the female's genitalia, and the forward extension of the forelegs followed by vibration or striking movement of the tarsi against the female's abdomen. The Hawaiian males also typically use these same structures, but those species which engage in wing movements use *both* wings, with rare exceptions, and the vibration movements may be of two or three different types that occur sequentially. Likewise, foreleg movements are complex and often consist of a compound movement of first lifting and folding the leg, followed later by extension and vibration against the female. Mouthpart movements are also often complex, a series of different movements following in repeated sequence during a courtship display. Many species of Hawaiian males also, simultaneously with other display movements, pulsate a droplet of fluid from the anal papilla, while others extrude the posterior end of the rectum as a small tubular structure. Many species simultaneously curl the abdomen excessively upward, sideways, or under, often to the extent that the tip of the curled abdomen is brought *over* or *beside* the male's head or thorax.

In sum, the Hawaiian male drosophiloid engages in complex movements usually of large magnitude and simultaneously involving various parts of his body (see Spieth, 1966b, 1968b).

The typical female acceptance response is also unique in that the female extrudes her ovipositor and depresses her entire body downward against the substrate. Occasionally a male may be unable to achieve intromission with an "accepting" female. The female then may remain in a trance-like state for several minutes after the unsuccessful male has dismounted.

During copulation non-Hawaiian drosophiloid females will readily move about carrying the male, fending with her legs against other flies, and avoiding other males that may attempt to interrupt the copulation. In comparison, a copulating pair of Hawaiian flies are with rare exceptions immobile, and other individuals may actually strike against or even clamber over the pair without eliciting any responses from the mating couple. Near the end of copulation a number of males, e.g., *D. crucigera*, completely release their grasp of the female, falling backwards in a trance-like state, in which they remain for a number of seconds after the termination of copulation. When compared to displays by non-Hawaiian species, those of the Hawaiian flies can be best described as the derivative and hypertrophied modification of the basic drosophiloid pattern overlaid with uniquely novel elements.

Courtship, as noted above, does not occur at the feeding sites but rather in some other part of the habitat. Field investigations have shown that males, presumably sexually mature, tend to assemble in considerable numbers at specific localized sites in the vegetation such as the trunk of a single tree, one or more horizontal tree limbs, the leaves of one branch of a shrub, the stems of the fronds of a particular tree fern, or even the individual pinnae of a single fern-frond. These sites are not randomly distributed in the areas where the flies live, and each species has special preferences, apparently determined by factors such as light, humidity, temperature, and spatial elements. Almost invariably they are in close proximity to the food sites. Such sites retain their attractiveness over prolonged periods of time; the males of some species occupy their particular sites for 24 hours of the day. Each male defends a limited area, e.g., a single leaf, a fern pinna, a section of a fern-frond stem, or a tree limb or small area of a tree trunk. Simultaneously the male also advertises his presence, either visually by body movements, especially specific wing movements, or by pheromones (Spieth, 1968a). Some species (e.g., *D. crucigera*, *grimshawi*, *villosipedis*) repeatedly curl the abdomen downward and drag the tip against the substrate, and in doing so deposit a thin film of liquid as they move. Many assume a ritualized stance and pulsate a droplet of liquid from the anal papilla. The males, but none of the females of many species, and all of those which engage in abdomen dragging, pos-

sess a unique anal structure, which Throckmorton (1966) has named the intra-anal organ. There is considerable interspecific variation but typically this organ consists of a pair of lobes covered with a dense pile and lying between the anal plates. Associated with the organ is a special slender sclerotized rod, which lies in the median line between the two lobes. These lobes are extruded when the male is engaged in abdomen-dragging. Following such a bout of activity the intra-anal organ is carefully cleaned by means of the hind tarsi before it is retracted into its resting position between the anal sclerites.

When another individual enters the small territorial area of an advertising male, it is immediately approached and investigated. If it is another male or an individual of another species, aggressive action against the intruder is initiated. This usually involves physical contact between the individuals; most species employ complex ritualized postures and movements (Spieth, 1966b). As a result of the encounter, one of the participants invariably is displaced from the area and apparently the vigor and size of the individual is the sole determinant of success. In a number of species the mere assumption of an aggressive posture or movement prior to physical contact is sufficient to cause one of the individuals to flee.

If a female, of either the same species as the male or of another species of approximately the same size as the male, intrudes on the male's defended area, then initiation of courtship occurs. If the female is nonreceptive or belongs to another species, then the male quickly ceases courtship and turns to aggressive behavior, which invariably causes the female to flee.

These behaviors of territoriality, aggression, and advertising on the part of the males have been observed for numerous species of various species groups both in the laboratory and in the field. It appears that the Hawaiian drosophiloids have evolved a true lek behavior, which has developed in conjunction with the spatial separation of feeding and courtship.

From field and laboratory studies on more than 60 species of drosophiloids, it is clear that each of the currently accepted species groups displays a basic, unique courtship pattern that is common to the species within the group. Each, however, exhibits a species-specific courtship display, and thus we have what can be described as variations upon a basic theme existing within each species group (Spieth, 1966b, 1968b). The basic theme involves not only the shared manner of utilizing such specialized dimorphic male structures as the antennae of the *Antopocerus* group, the specialized labellar structures of the modified-mouthparts group, and the modified tarsi of the bristle-tarsi, spoon-tarsi and fork-tarsi groups, but also the movements of wings, forelegs, abdomen, and the site of posturing, i.e., in front or at the rear of the female. The spectrum of variation upon the basic theme appears to vary between groups. Thus the amount of *inter-species* variation between members of groups such as the bristle-, fork-,

and spoon-tarsi is least, and that of the picture-wings and modified-mouthparts groups is greatest. These variations in amount of *interspecific* courtship display between groups are, as Heed (1968) noted, paralleled by variations in larval feeding-behavior.

A considerable number of species are as yet still unclassified as to species group. Furthermore, the relationships between some of the species groups that have been tentatively delimited are obscure, e.g., the bristle-, spoon-, and fork-tarsi. Inability to rear the species of these groups under laboratory conditions is the major block to the elucidation of these unknowns. The relationships of other groups such as the picture-wings and modified-mouthparts can be estimated on the basis of various lines of evidence, e.g., anatomy, salivary chromosomes, larval feeding habits, and courtship. For instance, the courtship studies clearly indicate that the *adiastola* subgroup of the picture-wings constitutes a connecting link between the two groups. *Drosophila ornata* from Kauai actually has modified mouthparts whose components appear homologous to the same structures of the true modified-mouthparts species. Additionally, both this species and *D. adiantola*, which lacks the mouthpart modification, display a courtship pattern that is similar in certain respects to that of the modified-mouthparts basic theme and considerably different from that of the other picture-wings.

Amongst the species that are currently unclassified as to species grouping are several, e.g., *D. (Engiscaptomyza) amphibobus*, *crassifemur*, *nasalis*, and the *Drosophila parva* species complex, whose courtship behaviors are clearly anomalous with respect to the other Hawaiian drosophiloids. Each of these species displays typical scaptomyzoid courtship behavior even though the external anatomy indicates that they are drosophiloids. Throckmorton (1966) in his detailed study of the reproductive anatomy of the Hawaiian *Drosophilidae* examined *crassifemur*, *nasalis*, and *parva* and found that each of the "species departs from the more usual *Scaptomyza* pattern some way," e.g., *parva* has drosophiloid paragonia, *nasalis* has drosophiloid coiling of the vasa and pattern of pigmentation along the vasa, while *crassifemur* has a typical drosophiloid ejaculatory apodeme.

Two other unclassified species, *Drosophila anomalipes* and *D. quasi-anomalipes*, also show a queer mixture of drosophiloid and scaptomyzoid characteristics plus certain non-Hawaiian features. Both of these species are restricted to the old island of Kauai, where they occur sympatrically in the drier portions of the *Metrosideros* forests in the area of Kokee. Their ovipositional sites are unknown and they can not as yet be reared under laboratory conditions. The courtship behavior of these flies has been observed both in the laboratory and in the field, and are basically similar, the differences being restricted to wing displays by the males and repelling responses by the females.

The basic courtship pattern is as follows: The male orients on another

individual, approaches and with a distinct movement taps with one of his forelegs, then postures in front of the female, engages in wing display, employing both wings, circles to the rear, assumes a head-under-wing posture, crouches slightly and curls the tip of his abdomen under, and lunges onto the female in a typical scaptomyzoid fashion. He then seeks to achieve intromission, not in the scaptomyzoid fashion of drawing his genitalia across those of the female but rather in the drosophiloid manner of pressing the genitalia against those of the female. Furthermore, he dismounts quickly when he fails to achieve intromission. The nonreceptive female extrudes her ovipositor either upward (*quasianomalipes*) or downward (*anomalipes*). When perchance the male is not directly positioned behind the female as he crouches preparatory to his lunge, she will curl the tip of her abdomen toward the face of the male. Despite many hours of observation in the laboratory and in the field, involving scores of courtships, no copulation has been seen. Clearly, however, the courtship behavior is a mixture of scaptomyzoid and drosophiloid elements. Highly significant also is the clear and distinct tapping movement of the males, the extrusion of the ovipositor, and the curling motions of the tip of the abdomen of the nonreceptive females. All of these are *non-Hawaiian* in nature and are not found in any of the Hawaiian species studied to date but are characteristic of drosophiloids from other parts of the world.

Many of the Hawaiian species are not attracted to fermenting baits such as are so commonly used to collect the various species in other parts of the world, but the majority of the picture-wings will come to banana bait especially if it has been inoculated with yeast derived from the lobeliad *Clermontia*. The standard method of baiting is to smear a modest amount of food on the smooth trunk of a tree, on the under surface of a limb or on other similar surfaces, selecting a site that has environmental conditions that the flies will accept.

Both *D. anomalipes* and *quasianomalipes* are readily attracted to bait and therefore can be observed feeding in the field. The males, after feeding for a short time, do not fly away as do the other Hawaiian males but move away just past the edge of the bait area. Usually they position themselves a few centimeters away, facing toward and watching the bait area. Whenever another individual of either *anomalipes* or *quasianomalipes* arrives on the bait, the male immediately approaches, taps, and investigates the newcomer. If it is a female of his own species, he will posture in front, circle to the rear, and assume the slightly crouched position behind her. As long as she is feeding or standing on the food mass, he will go no further with his courtship; that is, he will not lunge onto the female. The female for her part then typically extrudes her ovipositor as she feeds and the male will stand posturing behind her for prolonged periods. If and when she completes her feeding, she may walk off of the bait covered area onto



the surrounding substrate. The male then follows and, as soon as the pair is clear of the bait, the male will lunge onto the female.

Thus males of this species, unlike all other Hawaiian males, do not fly away from the food area into the surrounding vegetation to establish a separate mating site that they defend; rather, they merely move off the food, await the arrival of the females and do not defend the space about them. Also they will engage in courting while on the food, a typically non-Hawaiian behavior, but will not complete the courtship unless the female is spatially a short distance away from the food proper, a "primitive" expression of the typical Hawaiian behavior.

Further, the semicosmopolitan species *D. immigrans* has been introduced into Hawaii and has established itself in the same area as *anomalipes* and *quasianomalipes*. Specimens of *immigrans* also come in numbers to the baits. The males of the three species often compete with each other in investigation of "newcomers" as they arrive on the bait, and *anomalipes* has been observed courting large *immigrans* females. Picture-wings such as *D. villosipedis* and *picticornis* simultaneously come to the bait and none of the males of *immigrans*, *anomalipes*, or *quasianomalipes* pays any attention to these picture-wings, but the picture-wings are often disturbed and flee from the bait as a result of the commotion caused by the males of *anomalipes*, *quasianomalipes*, and *immigrans* vigorously competing for the privilege of investigating other females.

Throckmorton (1966) studied *anomalipes* (but not *quasianomalipes*), and found that the dorsal spermatheca is scaptomyzoid in construction and that the ventral receptacles are coiled, a non-Hawaiian drosophiloid type, while external anatomy and the remainder of the reproductive system and of the egg filaments are typically Hawaiian. Thus both structure and behavior exhibit a mixture that could well indicate that these two species arose from a primitive stock that still possessed many of the derivative characters of both the Hawaiian scaptomyzoids and drosophiloids as well as elements of the ancestral non-Hawaiian stock that colonized Hawaii.

It is interesting that the fungus-feeders, which form a distinct evolutionary branch of the drosophiloids and apparently evolved from the base of the ancestral Hawaiian stock, display a primitive lek behavior. The males of these species, after feeding, fly into the nearby vegetation only a meter or so from the feeding-ovipositional fungus and take up station on the dorsal surface of leaves. Here they orient themselves facing toward and watching the feeding site, vigorously defending their small territories. Periodically the males fly to another leaf on which they see another fly sitting. Usually they encounter another male; but females, as they leave the feeding site, also land on these leaves, and eventually a male encounters a female, whom he immediately courts.

This behavioral pattern is thus in a number of respects only quantitatively

different from that displayed by *anomalipes*, and lacks the specialized features of apparent pheromone production seen in the picture-wings and modified-mouthparts species. Throckmorton (1966) has shown that this fungus-feeding group is most closely related to ciliated-tarsi species such as *D. imparisetae*. These ciliated-tarsi species breed in rotting bark of *Clermontia* and *Cheirodendron*. Behavioral data indicate that they could well represent the stock from which many of the other more specialized species groups have radiated.

### *Evolutionary Implications of Courtship Behavior*

The major evolutionary development in the courtship of the Hawaiian drosophiloids, but not of the scaptomyzoids, was the innovation of a true lek behavior involving the spatial separation of courtship from feeding and oviposition. This intensified sexual selection, since the males under lek conditions must attract the receptive females to the lek or mating site instead of moving about on the feeding-mating-ovipositional site opportuning all available females, both receptive and nonreceptive. In other organisms the result of intense sexual selection appears typically to result in increased sexual dimorphism, and this has clearly happened to an extraordinary degree in the males of the Hawaiian drosophiloids but not in the scaptomyzoids, which do not exhibit lek behavior. Sibley (1957) observes in his discussion of bird sexual dimorphism that "the genetic basis for such characters may, and probably does, involve but a few genes and these may control only relatively superficial characters" of male structure and display movements. Bits of evidence indicate that the Hawaiian drosophiloids conform to this generalization:

(1) Many species have females that can not be separated even though the respective males differ sharply in both their structural characteristics and their courtships.

(2) The males of a number of species differing distinctly in both structure and courtship when placed with foreign females under no-choice conditions will often achieve insemination. In a considerable percentage of instances, viable  $F_1$  hybrid adults are produced, and a number of these are fertile (Yang and Wheeler, 1969; see next section).

(3) Carson (see below, p. 492, ff.) has discovered several groups of homosequential species, i.e., groups of species in which banding-sequences of each polytene chromosome are identical. Each of these groups forms the nucleus for a species cluster involving other species that differ from the homosequential members by only one or two inversions. In those species studied to date the homosequential species are distinguishable by the male's courtship pattern and sexual dimorphic characters.

Although the factors that created the selection pressure leading to the

evolution of lek behavior cannot now be specifically determined, certain inferences can be drawn. The drosophiloids congregate and feed in exactly those microhabitats and on those plants which are attractive to many of the endemic Hawaiian birds, especially the insectivorous elepaio (*Chasiempis sandwichensis*) and the creepers (*Paroreomyza* spp.). Furthermore, many other honeycreepers catch insects and other arthropods. Both the honeycreepers and the flies are attracted to the lobeliads, especially species of the genus *Clermontia* (Spieth, 1966c). Warner (1967) placed six iiwis (*Vestaria coccinea*) in cages of approximately 60 cubic feet (1.7 m<sup>3</sup>) and supplied the birds with a standard nectarivorous bird diet plus 50 live specimens each of *Bunostoma* sp., *Drosophila mimica*, and *D. grimshawi*. The birds avidly pursued the flies, captured and then displayed a ritualized method of eating the insects. The scaptomyzoid *Bunostoma* is small, *D. mimica* medium-sized, and *grimshawi* large. The first two were readily eaten but when a *grimshawi* specimen was captured, it buzzed violently upon being grasped by the bird's bill and was then quickly released, thus appearing to be immune to iiwi predation. Before the decimation that occurred during the 19th century, the native bird population was quite large. Today the numbers of individuals are drastically reduced and a number of species are now extinct. Individuals of some of the extinct species were larger in size than the iiwi, but many were not.

All of the known areas that are prime collecting sites for drosophiloids also have an abundant bird fauna; e.g., Kipuka Puauulu is commonly known also as Bird Park. Assuming the presence of food and ovipositional sites, then the quality of any previously uncollected area can usually be roughly gauged by the number of birds present.

The flies are also preyed upon by species of the dipteran genus *Lispocephala*. Various species of lisocephalids are abundant in the area where the drosophilids are found, and the adults lay their eggs on the same fermenting substrate as do the drosophilids; the *Lispocephala* larvae are voracious predators on the drosophilid larvae. The *Lispocephala* adults also capture and eat many of the drosophilid species, but like the birds, are unable, at least in experiments with caged individuals, successfully to attack the larger species such as the picture-wings.

Not only courtship behavior but also all other aspects of the drosophiloid's behavior indicate high adaptation to the avoidance of predators, and in the impoverished oceanic-type native Hawaiian fauna only the birds and the lisocephalids are plausible candidates for the role of major predators. Furthermore, it seems possible that the large size displayed by many Hawaiian drosophiloid species may be an evolutionary response to the selective pressure of bird and lisocephalid predation.

Finally, it is of interest that a number of the sexually dimorphic structural features of the anatomy of the males must have evolved subsequent to

the behavioral characteristics associated with these structures. Thus, the male of the *Antopocerus* species (Spieth, 1968b) during courtship lunges forward and upward over the abdomen of the female and forcibly spreads her wings apart about  $45^\circ$  and upward  $15^\circ$  to  $20^\circ$ . In doing so, the male's antennae are thrust against and slide across the under surface of the female's wing-vane. The male antennae are adapted for this function: (1) the first antennal segment is enlarged and elongated; (2) the arista is elongated, enlarged, and whiplike; and (3) the arisal setae are extremely short but numerous and restricted to one surface (i.e., lacking on the side that slides across the female wing-vane). Clearly the normal drosophiloid antennae would be poorly suited for such vigorous physical contacts, and significantly the *Antopocerus* females do possess essentially "normal" antennae. Thus, *Antopocerus* displays not only an example of a unique sexually dimorphic character but also one that must have evolved as a result of selection pressure created by a behavioral feature that must have evolved at an earlier date than did the physical dimorphism. Various other dimorphic characters must have also followed a similar evolutionary sequence, e.g., the dorsal cranial setal pattern of *D. petalopeza*, the armored mouthparts of the modified-mouthparts group, the modified labellar lobes of *D. crucigera*, which are uniquely adapted for clamping onto the anal papillae of the female, the sclerotized oral rim of the fungus-feeders, and the specialized tarsal armaments of the bristle-, split- and spoon-tarsi groups (Spieth, 1966b). In all instances these are sexual dimorphic structures that are utilized by the males for courtship functions.

When compared to drosophiloids from other parts of the world, the courtship behavior of the Hawaiian species is clearly hypertrophied in character, and the individual elements are obviously derivative. Spieth (1952) noted that species of the subgenus *Sophophora* utilized distance stimuli, whereas those of the subgenus *Drosophila* are characterized by using contact stimuli involving prolonged licking and leg-caressing movements on the part of the males. The basic pattern of Hawaiian species when analyzed indicates clear relationship to that displayed by the primitive species groups of the subgenus *Drosophila*, rather than to the more specialized pattern of the subgenus *Sophophora*.

### **Chromosomal and Genetic Characteristics**

#### **Metaphase Karyotypes**

Data on endemic Hawaiian species of Drosophilidae will be found in Clayton (1966, 1968, 1969) and Carson, Clayton, and Stalker (1967). Approximately 17 species of the family apparently have been introduced into the islands by man, either by the Polynesians or by later peoples. These

TABLE IX  
Metaphase Configurations in Four Genera of Hawaiian *Drosophilidae*

Metaphase	<i>Drosophila</i> species	<i>Antopocerus</i> species	<i>Scaptomyza</i> species <sup>a</sup>	<i>Titanochaeta</i> species	Total species
5R 1D	80	1	—	—	81
6R	7	3	—	—	10
1V,3R,1D	2	—	12	1	15
1J,4R,1D	1	—	—	—	1
2V,1R,1D	2	—	5	1	8
2V,2R,1D	—	—	1	—	1
5V 1J	1	—	—	—	1
Total	93	4	18	2	117

<sup>a</sup>Includes five *Drosophila* species with strong affinities to *Scaptomyza* (see text).

are omitted from this discussion. Squash preparations have been made either from the the brains of larvae reared from eggs produced by females isolated from nature or from the testes of adult males captured in nature (see Clayton, 1960). In some instances, adult-male and brain material from laboratory stocks was used.

Table IX gives data taken from Clayton (1968) on the karyotypes observed in 117 species of 4 genera. (These summaries do not include the information given in Clayton, 1969.) The genus *Antopocerus* is closely related to *Drosophila* (subgenus *Drosophila*), and *Titanochaeta* has been characterized as scaptomyzoid by Hardy (1966), Takada (1966), and Throckmorton (1966). Indeed, *Titanochaeta* metaphases are consistent with two karyotypes found among 17 *Scaptomyza* species from Hawaii (Table IX). One of these karyotypes is illustrated in Figure 6,d. Furthermore, these configurations are the same as two types described from non-endemic species of this genus. Thus, *Scaptomyza adusta*, like the endemic species *argentifrons*, has one V, three rods, and one dot; and *pallida* has two V's, one rod, and one dot. Although in all these cases the V's appear to represent "fusions" of originally separate rod-shaped elements, there is no assurance that the same elements are involved in each case, so that the resemblance of some karyotypes may be a superficial one. The enumeration of *Scaptomyza* species from Hawaii made in Table IX includes five (including *D. nasalis*: 2V, 2R, 1D) that have been assigned to the subgenus *Drosophila* (*Engiscaptomyza*) Kaneshiro (1969). Recognition of the intermediate position of these flies is certainly necessary.

Karyotypes of 93 species of the subgenus *Drosophila* (*Drosophila*) are

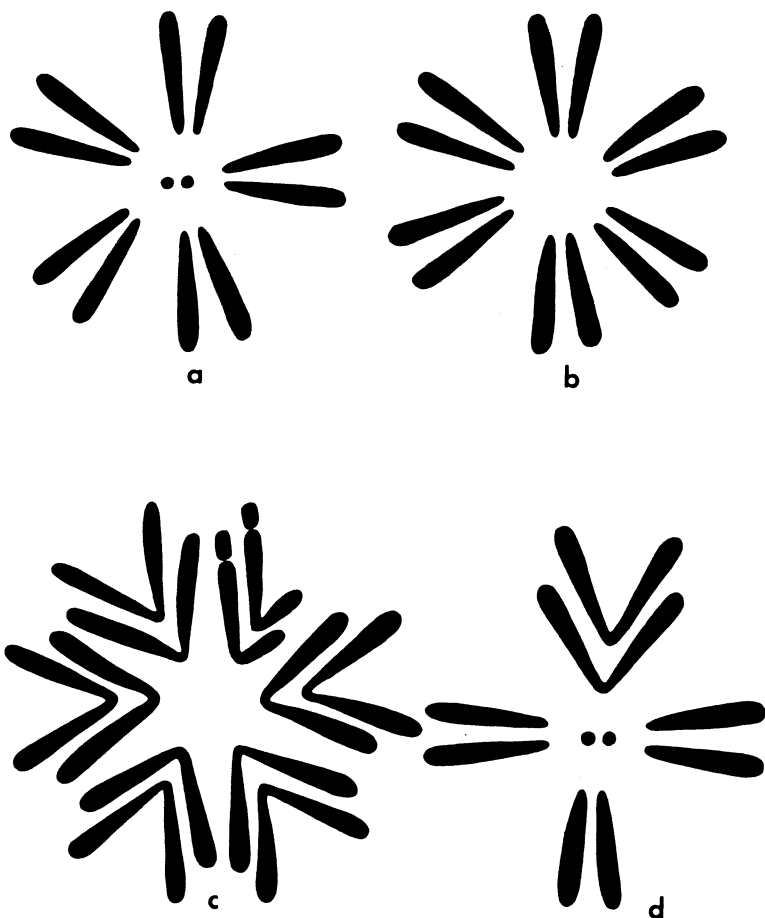


Fig. 6. Metaphase karyotypes among Hawaiian *Drosophilidae* (diagrammatic).  
 a, *D. grimshawi*; b, *D. mimica*; c, *D. cyrtoloma*; d, *S. argentifrons*.

entered on Table IX. The great majority of these (80) have a metaphase karyotype of five rods and one dot (Fig. 6). The other karyotypes having  $n=6$  (6R; 1J, 4R, 1D; 5V, 1J), furthermore, are clearly due merely to the addition of extra heterochromatin to one or more chromosome arms. In the case of 6 rods, the heterochromatin appears to have been added to the dot chromosome. Where J-shaped and V-shaped elements are found without change in chromosome number, the evidence is strong that this is likewise due to the addition of heterochromatin (Clayton, 1968). The most extreme case of this is the condition found in the giant species, *D. cyrtoloma* from Waikamoi, Maui (see Fig. 6). Although most of the other species so far described in its subgroup have five rods and one dot, this

species has acquired a full-length heterochromatic arm on each of five of the six chromosome-pairs. The remaining chromosome has become J-shaped also by acquisition of heterochromatin. This condition is not accompanied by any alteration of the usual salivary gland complement of five long and one dot-like polytene elements. The same is true of those forms which have six pairs of rods at metaphase, as well as a number of conditions, considered as basically "5R 1D" by Clayton, but which manifest, in some cases, an unusually long pair of rods or somewhat enlarged microchromosomes. No pericentric inversions have as yet been recognized in any Hawaiian species.

Accordingly, of the 93 species of the subgenus *D.* (*Drosophila*) indicated in Table IX, only four have had a reduction in chromosome number. This has apparently occurred by the process of whole-arm fusion and centromere loss. Three of these species belong to the modified-mouthparts group and one belongs to the bristle-tarsi group. These species are fairly close, however, and phylogenetically this may represent a single fusion, although this point remains uncertain. It is clear, however, that among the large group of picture-winged species (see Fig. 8) and the genus *Antopocerus*, there have been no fundamental changes from  $n=6$ .

The above is significant in view of the conclusion reached by Patterson and Stone (1952) that the five-rods, one-dot karyotype is a primitive one. Based on the study of 215 species of the genus *Drosophila* from areas other than Hawaii, these authors conclude that alterations of this primitive karyotype are the result of either: (1) fusion of whole arms with loss of centromere; (2) pericentric inversion; or (3) added heterochromatin. Clayton (1968) has compared the data on Hawaiian members of the subgenus *D.* (*Drosophila*) with the data on 150 non-Hawaiian species of the same subgenus taken from Patterson and Stone (1952) (Table X).

The data in this table underscore a most remarkable feature of the Hawaiian fauna, namely, its stability of chromosome number. Whereas there have been a minimum of 32 fusions in 150 continental species, only 4 of 93 Hawaiian species have had the karyotype so altered. On the other hand, changes via heterochromatin appear not to show very much difference from the continental situation (9/93 or 9.7 percent among insular compared with 19/150, or 12.8 percent among continental species). The count of nine Hawaiian species that have added heterochromatin, moreover, does not include those species which have a long pair of rods or which manifest rod-shaped microchromosomes. These variations also appear to be due to added heterochromatin, and inclusion of them would bring the island data even closer to that of the mainland with respect to this character.

In summary, then, it may be pointed out that endemic species of the Hawaiian *Drosophilidae* fall into two groups chromosomally: these groups

**TABLE X**  
**Comparison of Chromosome Numbers and Shapes from Hawaiian and Non-Hawaiian Members of the Subgenus *Drosophila* (*Drosophila*)<sup>a</sup>**

Haploid chromosome number	Non-Hawaiian species		Hawaiian species	
	No.	Percent	No.	%
seven	1	0.7	0	0
six	81	54.0	89	95.
five	36	24.0	2	2
four	29	19.3	2	2.
three	3	2.0	0	0
Number of species	150		93	
<b>Chromosome shapes</b>				
5R 1D	53	35.3	80	86
6R	7	4.7	7	7
3R 1V 1D	11	7.3	2	2
1R 2V 1D	15	10.0	2	2
other	64	42.7	2	2
Number of species	150		93	

<sup>a</sup>After Clayton (1968).

correspond with the genus *Scaptomyza* and the subgenus *Drosophila* (*Drosophila*). Flies of the genus *Titanochaeta* appear to relate to *Scaptomyza*, and members of *Antopocerus* to the subgenus *Drosophila*.

#### **Polytene-Chromosome Sequences**

Interspecific comparisons of giant salivary gland chromosomes have been published for 53 large endemic Hawaiian species belonging to the subgenus *Drosophila* (Carson, Clayton, and Stalker, 1967; Carson and Stalker, 1968a, b, c). This work has been aided by the fact that many of these species have exceptionally large and favorable salivary gland chromosomes (Fig. 7). This circumstance, together with use of a technique that permits table-level matching of unknown sequences under the microscope with photographic maps of known sequences, has permitted the description of all five major chromosome elements of all 53 species in terms of a single arbitrarily chosen set of Standard sequences.

In making comparisons between the species, it is apparent that virtually



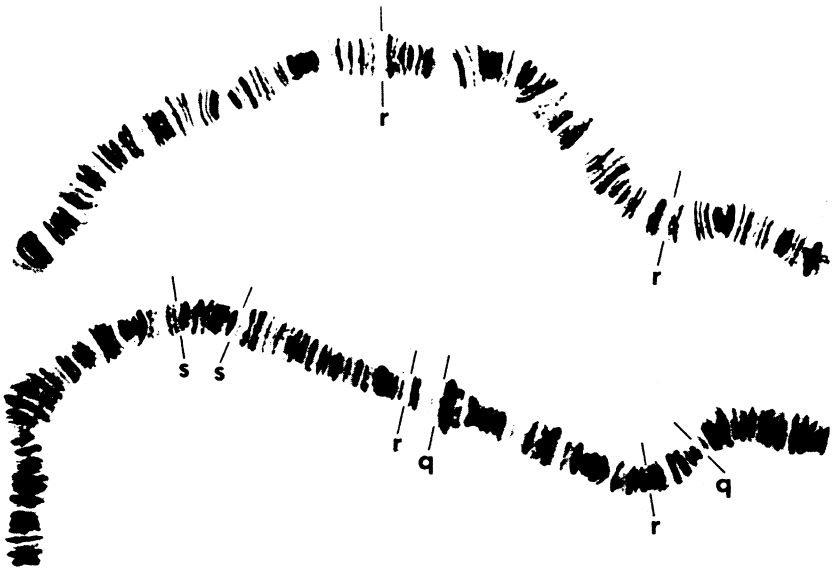
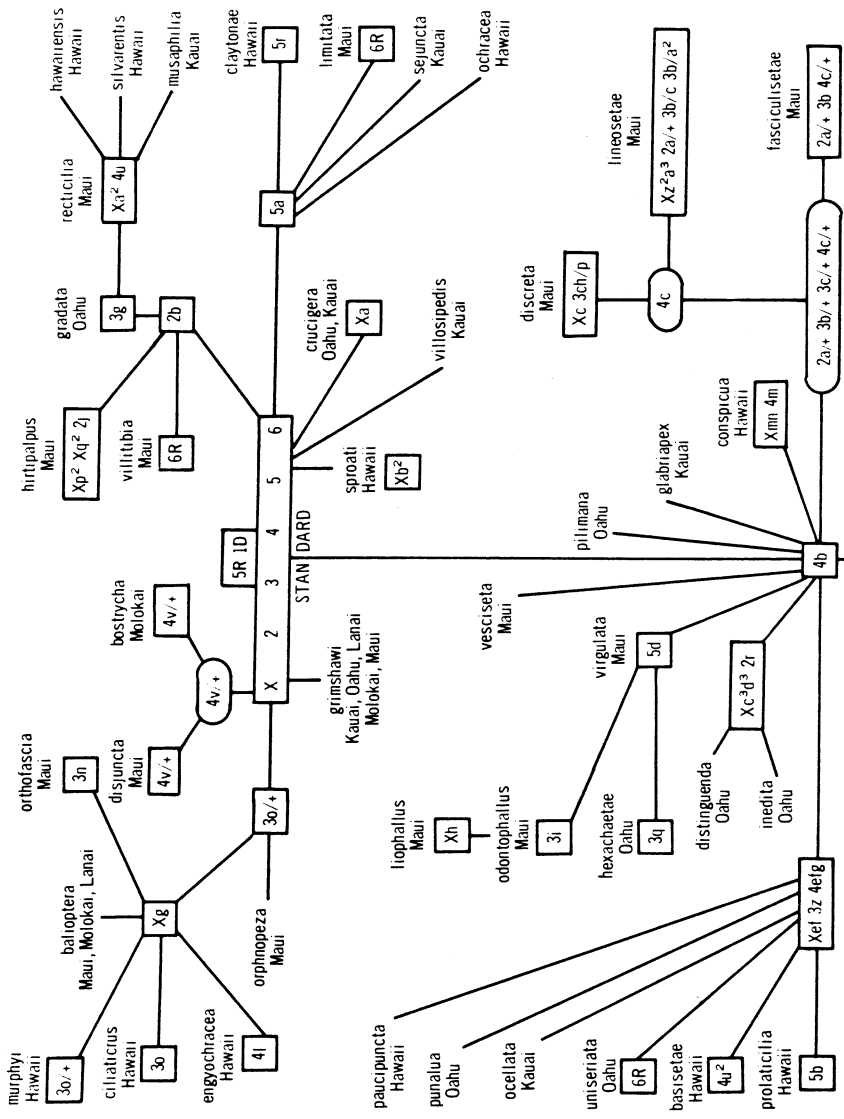


Fig. 7. Photographic chromosome maps of chromosomes 2 (upper) and 5 (lower) of *Drosophila crucigera*. The ordering of the bands shown is identical to the Standard *D. grimshawi*. The break-points of four previously unpublished inversions are entered; these are: 2r of *D. inedita*, 5q of *D. lineosetae*, 5s of *D. setosifrons*, and 5r of *D. claytonae*. The latter is based on the 5a arrangement (see Carson and Stalker, 1968a, Fig. 2).

all variation in sequences between species can be explained by paracentric inversions. Each inversion discovered has been designated by a lower-case letter, and the break-points determined. These break-points have been mapped and their exact positions entered on photographic chromosome maps. Despite the fact that the sequences of all species can be completely described in terms of the Standard *Drosophila grimshawi*, it has been found convenient to prepare five sets of chromosome maps made from each of the five species for which the subgroups are named (see Table XII, and Carson and Stalker, 1968a, b, c and 1969).

Figure 8 summarizes in a single master diagram the polytene and metaphase chromosome relationships for a total of 69 species. No species has





been included in this diagram unless the sequential order of each of its five major chromosome arms can be precisely and completely described in terms of the arbitrary Standard. The figure updates the information given on similar diagrams published in Carson, Clayton, and Stalker (1967) and Carson and Stalker (1968a, b, c). Sixteen species have been added and one removed. New names are provided for some species and a few corrections have been made. Details will be found in Table XI. In order that this review may include as much recent data as possible and not be overburdened with chromosome maps, documentation of the break-points of most of the new inversions will be deferred until later publications. This also refers to those chromosomal polymorphisms which are confined to a single species; the letter designations for these, if not entered on Figure 8, will be found in Table XI.

Most of the 69 species belong to what Hardy and Kaneshiro (1968) have called "picture-winged" species. These species are mostly large and among them are probably the largest *Drosophila* species in the world. Carson and Stalker (1968a, b, c) have divided these species into four subgroups on the basis of the similarity of polytene gene orders of their members. Each subgroup is named for the one species from which full chromosome maps have been made. In the present summation, a fifth subgroup has been recognized, centering on *D. primaeva*; this will be the subject of a forthcoming paper (Carson and Stalker, 1969). The five subgroups are listed in Table XII, which also shows the minimum number of inversions fixed in each subgroup relative to the others. From these facts it may be seen that the *D. grimshawi*, *planitibia*, and *punalua* subgroups are quite close to one another. Among these closely knit subgroups, the greatest "distance" is 14 fixed inversions. This occurs between the *punalua* and *planitibia* subgroups. The *primaeva* subgroup is not only the most removed from the Standard but is quite far from the other subgroups as well.

Figure 8 may be used to read the basic chromosome formula (both metaphase and polytene) of each species in terms of the Standard *D. grimshawi* chromosome sequences (X 2 3 4 5 6: center, upper portion of the figure). For example, the formula of *D. liophallus* may be read additively by following the lines leading from the Standard box, e.g.: Xh 2 3i 4b 5d; 5 rods 1 dot. Existing species are given within rectangular boxes, whereas formulas for ancestral populations inferred from the data are given within boxes with rounded ends. It is noteworthy as one studies Figure 8, that for every species listed each major chromosome has been completely characterized, from one end to the other. Each and every band of the Standard has been located and accounted for in each of the 69 species. This has been made possible only by the very great favorability of these species for chromosome study (see Fig. 7). Except for a 3-band deletion (Xd of *D. picticornis*), all aberrations given are inversions. The microchromosome (6), however, has not been systematically studied.

TABLE XI

Strains and Species of Hawaiian *Drosophila* for  
Which New or Corrected Data are Presented in Fig. 8.

Species	Locality and strain numbers	Number of wild chromosomes observed		Remarks
		auto-somes	X chromo-somes	
<i>D. attigua</i> <sup>a</sup>	Kahili, Kauai (2500') L41C12	4	3	Described by Hardy and Kaneshiro (1969, in press); intraspecific polymorphisms: 4c <sup>3</sup> /+; 4b <sup>3</sup> +
<i>D. claytonae</i> <sup>a</sup>	Upper Olaa For. Res. Hawaii L89L1	4	3	Described by Hardy and Kaneshiro (1969, in press); intraspecific polymorphism: 4e <sup>3</sup> /+
<i>D. cyrtoloma</i>	1. Kipahulu Valley, Maui L13G9-11 (mass) (Carson and Stalker 1968 b: as <i>D. neoperkinsi</i> ) 2. Waikamoi, Maui L18P4; L47C3; L86Q3	6	5	Described by Hardy (1969 in press); metaphase 5V 1J, see Clayton 1968 as " <i>perkinsi</i> ?"
<i>D. crucigera</i>	—	—	—	See Tab. 15
<i>D. distinguenda</i> <sup>a</sup>	Makaleha Valley, Oahu (1800') L92G1-4	12	10	Described by Hardy (1965)
<i>D. grimshawi</i>	—	—	—	See Tab. 16
<i>D. hanaulae</i> <sup>a</sup>	Hanaula, W. Maui (4000') L91Q10	4	3	Described by Hardy (1969 in press)
<i>D. inedita</i> <sup>a</sup>	Makaleha Valley, Oahu L88G1	4	3	Described by Hardy (1965)
<i>D. lineosetae</i> <sup>a</sup>	Hanaula, W. Maui L61C1-5 (mass); G1-3 (mass); L91B2	8	6	Described by Hardy and Kaneshiro (1968; intraspecific polymorphism: 5q/+)
<i>D. melanocephala</i> <sup>b</sup>	Waikamoi, Maui M12L2	4	3	Described by Hardy (1966; 5R 1D metaphase reported by Clayton 1968 may be a different species)
<i>D. murphyi</i> <sup>a</sup>	Pololu Stream, N. Kohala Hawaii (3300') L79G1, 2 Upper Olaa For. Res. Haw. L82B1, 2; G1; L89G5, 7, 10, 14, 18, 19, 21, 23, 25; C19, 24, 26, 27, 33	68	53	Described by Hardy and Kaneshiro (1969 in press); intraspecific polymorphism: 3ob <sup>2</sup> /+

Species	Locality and strain numbers	Number of wild chromosomes observed		Remarks
		auto-somes	X chromo-somes	
<i>D. musaphilia</i> <sup>a</sup>	Alexander Reservoir, Kauai (1700') L42G1	4	3	Described by Hardy (1965)
<i>D. neoperkinsi</i> <sup>a</sup>	Nawaihiluli Stream, Molokai (2500') L98G6; L98B4-12 (mass); L98G7,9,13 (mass)	8	7	See Hardy and Kaneshiro (1968) and redescription in Hardy (1969 in press)
<i>D. nigribasis</i>	Mt. Kaala, Oahu L87G3,4; L87G7-11 (mass); L87B5-9 (mass)	16	12	<i>New name</i> , in Hardy (1969 in press); metaphase 5R 1D, see Clayton (1968) as: " <i>brunneipennis</i> "
<i>D. ocellata</i> <sup>a</sup>	Mt. Kualapa, Kauai L45B1	4	3	Described by Hardy and Kaneshiro (1969 in press); 5 strains reported in Carson and Stalker (1968c) as "new species 'B'"
<i>D. ornata</i> <sup>a</sup>	Pouli Stream, Kauai Mt. Kahili, Kauai			Described by Hardy and Kaneshiro (1969 in press); 3 strains reported in Carson and Stalker 1968c as "new species 'A'"
<i>D. paenehamifera</i> <sup>a</sup>	Hanaula, W. Maui L61C13,L61B11 (mass)	4	3	Described by Hardy and Kaneshiro (1969 in press)
<i>D. peniculipedis</i> <sup>a</sup>	Hanaula, W. Maui L61G9-13,35	24	18	Aberration "4s/+" (Carson, Clayton and Stalker, 1967; Carson and Stalker, 1968c) appears to be a "kink" formed by constrictions in the homozygous state; it is not a heterozygous inversion
<i>D. perkinsi</i>	Waikamoi, Maui J24L10. See Carson, Clayton and Stalker (1967) Table 1, p. 1283 and Carson and Stalker 1968b as <i>D. neoperkinsi</i> , Waikamoi, Maui.			This record may be in error and is being withdrawn pending reconfirmation
<i>D. primaeva</i>	Mohihi, Kauai (3500')	2	2	Described by Hardy and Kaneshiro (1968); intra-specific polymorphisms: Xg <sup>2</sup> /+; Xg <sup>2</sup> 1 <sup>2</sup> /+ 2q/+; 4a <sup>2</sup> /+; metaphase 5R 1D (Clayton, 1968)
	G20.3B. Kokee, Kauai	4	3	
	J81C1. Pouli Stream, Kauai L37G2,3,7-9; B6	24	18	
	Mt. Kahili, Kauai L41C11,20,21; G8,11-14, 16,19,21,25,26; P4-6.	60	42	

Species	Locality and strain numbers	Number of wild chromosomes observed		Remarks
		auto-somes	X chromosomes	
<i>D. punalua</i>	All strains: see Carson, Clayton and Stalker (1967); Carson and Stalker (1968c).			Inversion previously considered identical with 3f of the <i>D. adiaastola</i> subgroup is now considered to be different and is designated 3z
<i>D. sejuncta</i> <sup>a</sup>	Mt. Kualapa, Kauai L45B3,6,10,11	16	12	Described by Hardy and Kaneshiro (1968)
<i>D. setosifrons</i> <sup>a</sup>	Upper Olaa For. Res., Hawaii L89C11-13; G1,2; L100P16	20	15	Described by Hardy and Kaneshiro (1968)
<i>D. silvestris</i>	Hawaii	—	—	<i>New combination</i> : Hardy and Kaneshiro (1968); this name replaces the combination <i>D. nigri-facies</i> used by Carson et al. (1967) and Carson and Stalker (1968b)
<i>D. truncipenna</i> <sup>a</sup>	Waikamoi, Maui L91C1-3 (mass)	2	2	Described by Hardy 1965; 6R metaphase reported by Clayton (1968) may be from a different species

<sup>a</sup>Metaphase reported as 5R 1D in Clayton (1969)

<sup>b</sup>Metaphase reported as 5R 1V in Clayton (1969)

**TABLE XII**  
**Cytological Subgroups of the Species of Hawaiian**  
*Drosophila* Diagrammed in Figure 8.

Number	Subgroup Name	Number		Minimum number of inversions fixed relative to subgroup number				
		Num-ber of species in sub-group	of species having at least one intraspecific polymorphism	I	II	IV	III	V
I	<i>D. grimshawi</i>	36	15	X				
II	<i>D. planitibia</i>	14	5	9	X			
IV	<i>D. punalua</i>	6	1	7	14	X		
III	<i>D. adiaastola</i>	11	2	17	17	21	X	
V	<i>D. primaeva</i>	2	2	22	23	27	28	X
	Total	69	25					

### *Intraspecific Chromosomal Polymorphism*

Immediately after its origin, a new inversion is of course polymorphic within the species in which it has arisen. This condition may persist or the new arrangement may become fixed in one or more descendent populations. The number of species showing at least one intraspecific polymorphism is given in Table XII. There are only 25 such species, that is, 36 percent of the total. Although polymorphism seems to be less in the *punalua* and *adiastola* subgroups, all subgroups show at least one polymorphic species. Certain species, which are known so far from only a few strains, may of course be erroneously judged to be monomorphic. On the other hand, the reality, in fact the preponderance, of monomorphic species can hardly be doubted (especially in view of the large number of natural samples that have been examined) for certain species, without giving any indication of the presence of chromosomal polymorphism (Carson and Stalker, 1968a, b, c). Documentation of intraspecific polymorphism can be gleaned from these papers as well as Table XI of the present review.

### *Fixed versus Polymorphic Inversions*

Further information on the distribution of inversion fixation and polymorphism will be found in Table XIII. Among a total of 178 inversions, 115 are wholly fixed. As several inversions are heterozygous in one species but fixed in one or more others, the number of fixations may be taken as somewhat greater than 115. In any event, the mean number of inversion fixations per species is slightly less than 2.

The circumstance that some inversions are polymorphic in some species and fixed in others has important phylogenetic implications. For example, *D. neopicta* of Maui is polymorphic for two inversions (Xt and 2m), which have subsequently become fixed in other lines of descent stemming from a common ancestral population (see Fig. 8). In such an instance, the polymorphic population retains a state resembling more closely the ancestral population than the other species do. As will be pointed out below (p. 508), the retention of such heterozygosity within existing species permits precise inference of ancient colonization routes for species-founder populations. Such cases, however, where an existing species actually retains the heterozygosity (boxes with square ends), should be distinguished from those wherein an ancestral population is merely inferred to have been heterozygous for a given inversion (boxes with rounded ends, Fig. 8).

Referring again to Table XIII, it may be seen that the chromosomes X and 4 have the greatest number of fixed inversions; this appears to be true in all subgroups. Conversely, chromosomes 2 and 5 (illustrated in Fig. 7) appear to be less often involved in rearrangements. In general,



TABLE XIII

Number of Fixed and Polymorphic Inversions Among Hawaiian Species of *Drosophila*. Inversions Common to More Than One Subgroup Have Been Entered Only Once. The Base Arrangements are Standard *D. grimshawi*

Chromosome	Subgroup Number												Total
	I. <i>grimshawi</i>		II. <i>planitibia</i>		III. <i>adiastola</i>		IV. <i>punalua</i>		V. <i>primaeva</i>		I-V		
	fixed	poly- morphic	fixed	poly- morphic	fixed	poly- morphic	fixed	poly- morphic	fixed	poly- morphic	fixed	poly- morphic	Total
X	15	0	12	4	12	3	2	0	8	2	49	9	58
2	3	1	0	3	5	1	0	0	4	1	12	6	18
3	4	10	2	2	6	1	1	0	2	0	15	13	28
4	4	12	4	4	8	9	4	0	7	3	27	28	55
5	3	4	2	0	2	1	1	2	4	0	12	7	19
Total	29	27	20	13	33	15	8	2	25	6	115	63	178

both fixed and polymorphic inversions seem to follow the same pattern, except that the number of X-chromosome polymorphisms is proportionally far less than that of the other chromosomes.

#### *Parallel Chromosomal Polymorphisms in Different Species*

Carson (1968, 1969) has made a study of intraspecific chromosomal polymorphisms in the central region of chromosome 4. This region is polymorphic in a number of closely related species of the *D. grimshawi* subgroup. Although in each instance the break-points of the inversions are closely similar, giving rise to strikingly parallel inversion configurations, these break-points are not identical. Thus, 4a of *D. grimshawi*, 4k and 4v of *D. disjuncta*, and 4c of *D. fasciculisetae* are closely similar. It was suggested that this region of the chromosome produces sectional heterozygotes yielding high fitness and that natural selection has selectively preserved similar, but not identical, inversions within different species in each case.

#### *Chromosomal Polymorphisms That Are Identical in Two Different Species*

Carson (1959) has pointed out the great rarity, among mainland faunas, of the condition where two descendent species have retained a polymorphism present in an ancestral population. This condition was ascribed to the

TABLE XIV  
Pairs of Closely Related Species that  
Have a Chromosomal Polymorphism in Common

Case no.	Species	Chromosomal subgroup	Chromosomal polymorphism in common
1	<i>D. bostrycha</i> <i>D. disjuncta</i>	<i>D. grimshawi</i> (I)	4v/+
2	<i>D. murphyi</i> <i>D. orphnopeza</i>	<i>D. grimshawi</i> (I)	3o/+
3	<i>D. fasciculisetae</i> <i>D. lineosetae</i>	<i>D. grimshawi</i> (I)	2a/+
4	<i>D. heteroneura</i> <i>D. silvestris</i>	<i>D. planitibia</i> (II)	3m/+
5	<i>D. neoperkinsi</i> <i>D. neopicta</i>	<i>D. planitibia</i> (II)	2m/+

tendency for species to arise from exceedingly small populations, which would be likely to be chromosomally monomorphic.

A significant number of exceptions to this rule have now been uncovered among Hawaiian species (Table XIV). In each of the five cases listed in the table the break-points of the inversion concerned have been determined as identical. In each case, there is strong evidence that the populations concerned are indeed species and are not merely subspecies. In each case, the two species appear to have inherited the polymorphism from a common ancestor.

TABLE XV

**Frequency (in %) of X- and Third Chromosome Gene Arrangements  
in Natural Populations of *Drosophila crucigera* from Kauai and Oahu**

Locality	No. of X chromosomes examined	Gene arrangement		No. of 3rd chromosomes examined	Gene arrangement	
		Xa	Xab		3	3a
<b>Kauai</b>						
1. Kokee	11	100.0	0.0	14	71.5	28.5
2. Halemanu	250	100.0	0.0	332	71.1	28.9
3. Kumwela Ridge	10	100.0	0.0	14	64.3	35.7
4. Alexander Reservoir	3	100.0	0.0	4	100.0	0.0
5. Kilohana Crater	3	100.0	0.0	4	100.0	0.0
6. Iliiliula River	7	71.4	28.6	10	90.0	10.0
7. Wailua River	9	33.3	66.7	12	100.0	0.0
8. Mt. Kualapa	27	81.5	18.5	36	100.0	0.0
<b>Waianae Range, Oahu</b>						
9. Peacock Flat	36	100.0	0.0	48	72.8	27.2
10. Makaleha Valley	33	100.0	0.0	44	59.1	40.9
11. Mt. Kaala	3	100.0	0.0	4	75.0	25.0
12. Palikea	9	100.0	0.0	12	66.7	33.3
13. Mauna Kapu	80	100.0	0.0	106	46.2	53.8
<b>Koolau Range, Oahu</b>						
14. Pupukea	15	0.0	100.0	23	95.7	4.3
15. Puu Kapu	38	0.0	100.0	49	98.0	2.0
16. Waimano Stream	3	0.0	100.0	4	100.0	0.0
17. Lulumahu Falls	42	12.0	88.0	62	88.7	11.3
18. Mt. Tantalus	20	0.0	100.0	26	88.5	11.5
19. Aihualama Stream	8	0.0	100.0	10	100.0	0.0
20. Manoa Falls	6	0.0	100.0	8	100.0	0.0
21. Pukele Stream	6	0.0	100.0	8	100.0	0.0
22. Kaau Crater	15	0.0	100.0	20	100.0	0.0
23. Wiliwilinui Ridge	9	0.0	100.0	12	100.0	0.0
24. E. Wailupe Gulch	20	0.0	100.0	28	100.0	0.0
25. Kului Gulch	9	0.0	100.0	12	100.0	0.0
26. Pia Valley	6	0.0	100.0	8	100.0	0.0
27. Kupaua Valley	27	3.7	96.3	36	91.6	8.4

*Quantitative Studies of Chromosomal Polymorphism in Selected Species*

From the inception of the Hawaiian project, data on chromosomal polymorphism have been obtained in such a way that reliable estimates of the frequencies of various gene arrangements within species could eventually be made. Because of the great diversity of species that has been uncovered, and because most species are rather rare, quantitative data have been accumulating very slowly. For several species, however, suggestive preliminary data will be discussed here.

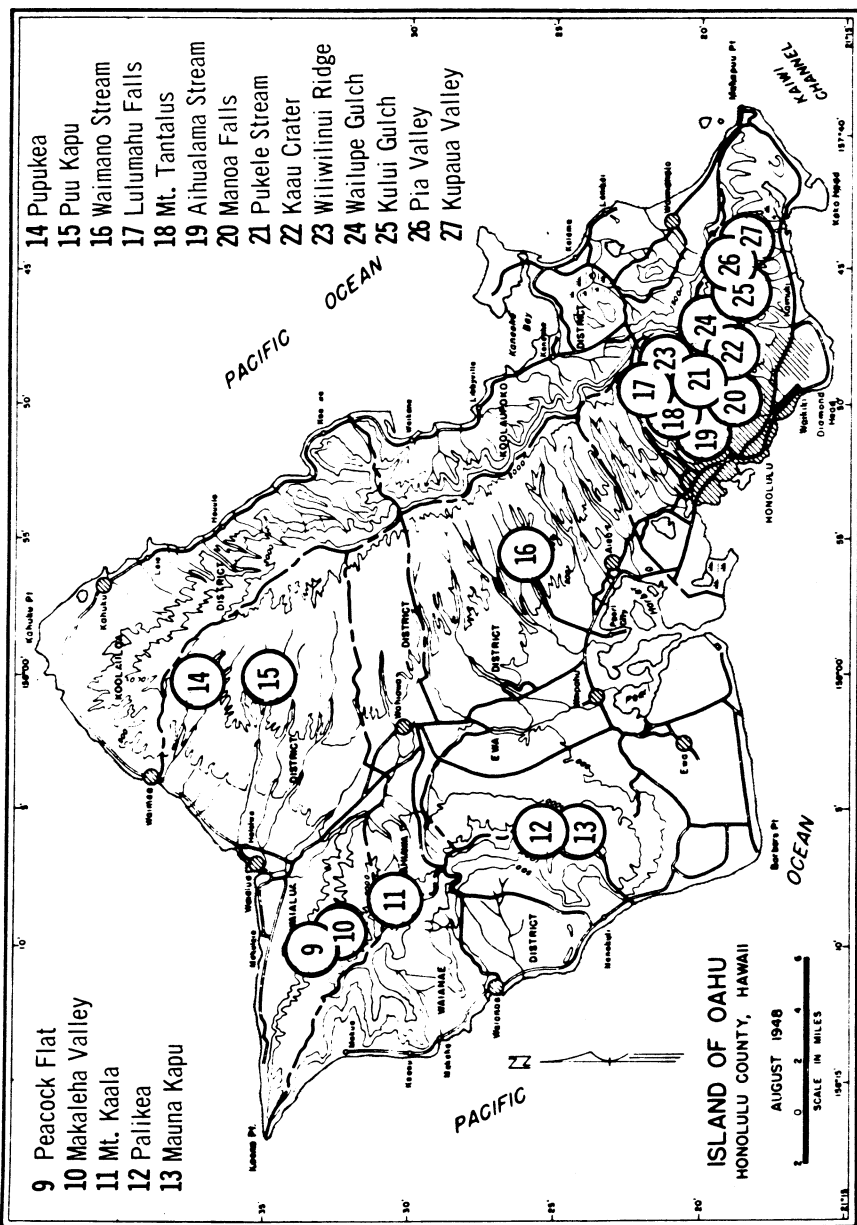
*DROSOPHILA CRUCIGERA*. This species occurs on both Kauai and Oahu. Carson (1966) has presented preliminary data on populations from the Koolau Range of Oahu and the Kokee region of Kauai. In the present publication, these data have been supplemented by studies of new collections from both islands. The methods used are the same as those described in Carson (1966).

Table XV presents all data, both new and old, for a total of 27 localities, 8 from Kauai, 5 from the Waianae Range, Oahu, and 14 from the Koolau Range, Oahu. Each locality is numbered and its location is given on Figures 9 and 10.

As will be documented below (p. 511), of the two X-chromosome arrangements, Xa is more ancestral, being only one inversion step away from the Standard X. Xb overlaps Xa and is inseparable from it, giving the derived arrangement Xab. Populations from the Kokee-Halemanu area of Kauai and the Waianae Range of Oahu are characterized by very high frequencies of Xa; in fact in all but three localities on the windward side of Kauai, which are polymorphic Xa/Xab, Xa is fixed. In contrast, populations from all but two localities in the Koolau Range, Oahu, show fixation for Xab, and where polymorphic populations occur (e.g., Lulumahu Falls) the frequency of Xab is very high. With regard to the polymorphism in chromosome 3, there is again a close resemblance between the Kokee-Halemanu area of Kauai and the Waianae Range of Oahu. Similarly, windward Kauai (low frequency of 3a) resembles the Koolau Range populations. Evidence will be presented below pointing to the origin of *crucigera* from a *grimshawi*-like ancestor on Kauai. The oldest population of *crucigera* is considered to be that from the Kokee-Halemanu area. It is suggested that the Waianae, Oahu population is descended from a simple intraspecific colonizer, which reached there directly from Kauai, with the modern Waianae populations being little changed from the ancestral.

Apparently, the Xb inversion arose in populations of windward Kauai, wherein the frequency of the 3a inversion was also reduced somewhat. It is proposed that the Koolau populations are descended from a second and separate simple colonization of Oahu from Kauai by this species. Sub-





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Fig. 10. Localities on Oahu from which *Drosophila crucigera* has been sampled cytologically. Localities 9-13 are in the Waianae Range, and 14-27 in the Koolau Range.

sequently the Xab arrangement and the Standard 3 arrangement have moved close to fixation in modern Koolau populations. Further discoveries may indeed invalidate these tentative conclusions although the writer feels that various alternate schemes lead to more difficult assumptions than the two-colonization hypothesis presented here.

The conclusion that *D. crucigera* populations of Kauai and Oahu indeed belong to the same biological species requires defense because of the exceedingly high degree of single-island endemism that is manifested in this group (see Table XVIII). In order to test the conclusion of Hardy (1965) that there is no morphological basis on which separate species could be constructed, the following genetic tests were made. Reciprocal crosses were made in the laboratory between an Oahu stock derived from a single female caught on Mt. Tantalus in the Koolau Range [C53.11] and a similar stock collected at Halemanu, Kauai [W(H)31C.2]. Each cross was made in duplicate and 10 virgin females and 10 males were used in each instance. Control crosses of the parental strains were carried out simultaneously. All cultures gave an abundant  $F_1$  without delay, and the sex ratios were normal. All  $F_1$  cultures, furthermore, produced vigorous and fertile  $F_2$  generations, with the interisland hybrids producing equally well if not better than the control strains. Male interisland hybrids (either Kauai  $\times$  Oahu or Oahu  $\times$  Kauai) were fully fertile in backcrosses to either Kauai or Oahu females. Crosses between wild males captured in the Waianae Range and C53.11 virgin females have been routinely made as a part of the study of chromosomal polymorphism. These crosses succeed easily and give normal and vigorous  $F_1$ 's. Accordingly, all crossing data support the conclusion that all these flies belong to the same biological species. Somewhat comparable data will be adduced below for *D. grimshawi* but, at the time of writing, these are the only two species of picture-wings that occur on more than one major island.

**DROSOPHILA GRIMSHAWI.** This is one of the easiest of all species to collect on Maui, Molokai, and Lanai. Almost all specimens from these islands are of large size and readily oviposit in the laboratory, giving vigorous  $F_1$ 's and laboratory stocks. At an early time in the investigations the chromosomal arrangements found in stock G-1 from Auwahi, Maui (see Carson, Clayton, and Stalker, 1967) was chosen as the arbitrary Standard with reference to which all the other species on Figure 8 have been described chromosomally.

Since the intensity of collections on Kauai and Oahu have increased in the last few years (1966-1968), a small number of specimens closely resembling *D. grimshawi* have been collected on both islands but they can certainly be rated as extremely rare, with not more than 10 wild females having been caught so far on each island. The specimens differ from those of Maui-Molokai-Lanai in being smaller as well as having a slightly different

TABLE XVI

Frequency (in %) of 4th-Chromosome Gene Arrangements in *Drosophila grimshawi*

Locality and collection no.	No. of 4th chromosomes examined	Standard 4	Gene arrangement 4a
<i>Kauai</i>			
1. Pouli Stream, Hanalei District (1500 feet) L37B4 ♂ × C134.7D virgin ♀, Keanae, Maui	2	100.0	0.0
2. Kokee, M9J1; M11J2	8	100.0	0.0
<i>Oahu</i>			
3. Kaau Crater, Koolau Range (1200 feet) L23G5 ♂ × PK-9 virgin ♀, Puu Kolekole, Molokai	2	100.0	0.0
<i>Molokai</i>			
4-6	50	74.0	26.0
<i>Lanai</i>			
7-10	20	80.0	20.0
<i>West Maui</i>			
11-12	38	21.1	78.9
<i>East Maui</i>			
13	74	100.0	0.0
14-15	28	89.3	10.7
16-19	40	0.0	100.0

position of one of the wing-spots. Despite the fact that *grimshawi* from the southern islands breeds exceedingly well, great difficulty has been experienced in breeding Oahu and Kauai flies. To date, only two females (M9J1 and M11J2, Kokee, Kauai; Table XVI) have produced progeny in the laboratory.

In two instances, wild-caught males have been successfully crossed to virgin laboratory females from stocks derived from the southern islands (Table XVI). In each case a vigorous  $F_1$  was obtained. The sex ratios of these progenies were normal and both wild males proved to be homozygous for the Standard 4th chromosome found in the Auwahi, Maui strain. What is even more significant in the present context, however, is the fact that the interisland  $F_1$ 's in both cases proved to be fertile.  $F_1$ 's from the Oahu male (L23G5) produced an abundant  $F_2$ ; the one produced by the Kauai male appeared to be less so.

More data concerning the Kauai and Oahu races of *D. grimshawi* are being actively sought. It may tentatively be concluded, however, that these



indeed are conspecific with *grimshawi* from the southern islands. The questions of which of these populations is ancestral and which derived will be discussed below, where it will be suggested that the Kauai and Oahu populations are primitive relicts.

Table XVI also gives data on 4th chromosome polymorphism in populations of *grimshawi* from Molokai, Maui, and Lanai. These data are in summary form; details will be presented in Carson and Sato (1970). The facts are also shown geographically in Figure 11. The Standard chromosome 4 runs 75 percent to 80 percent in most populations on Lanai and Molokai. West Maui, however, seems to be reversed, with a low frequency of Standard 4. On East Maui (Haleakala Volcano), populations from the leeward side (Auwahi) appear to have the Standard 4 in fixed condition. As one proceeds to the windward side, only the Waikamoi area (locality no. 15) shows polymorphism, and the 4a arrangement appears to be fixed in Kolea, Keanae, Hana, and Kipahulu. Thus it appears that chromosomal races can indeed be sharply manifested even over the small distances involved. Further documentation on this subject is being sought both for this species and for *D. bostrycha* and *D. disjuncta*, which are very closely related species occurring with *grimshawi* on both Maui and Molokai. *D. disjuncta*,

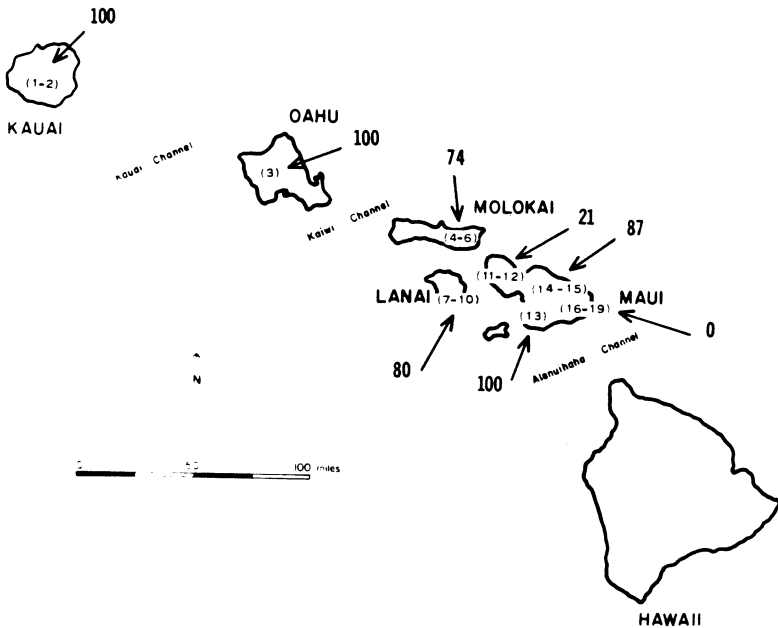


Fig. 11. Frequencies (in %) of Standard chromosome 4 of *Drosophila grimshawi* relative to its alternative, the derived gene arrangement 4a. Locality numbers (see Table XVI) are in parentheses.

in particular, appears to show striking chromosomal differences in different populations on the island of Maui. These will be the subject of further study in a forthcoming paper (Carson and Sato, 1970).

**OTHER SPECIES.** Carson and Stalker (1968a, b, c.) have recorded intraspecific polymorphism in a number of species. At least four species show considerably more polymorphism than either *D. crucigera* or *grimshawi*, but population samples are so far relatively small. These species are *D. setosimentum* and *silvestris* from the island of Hawaii, and *fasciculisetae* from Maui. Geographical data on the polymorphisms present in these species are being collected and will be presented elsewhere.

### **Homosequential Species**

Carson, Clayton, and Stalker (1967) introduced this term to refer to two or more species that have identical polytene chromosome-banding sequences in all chromosomes. If intraspecific chromosomal polymorphism is disregarded, there are 10 groups of species among the Hawaiian fauna that can be referred to as homosequential (Table XVII). A total of 31 species fall into this category; this amounts to more than one third of the species given in Figure 8. Among the extensively studied *Drosophila* species from mainland groups, several cases are known in the *D. mulleri* and *mercatorum* subgroups (Wasserman, 1963). In these cases, however, the species concerned are essentially sibling species, whereas this term cannot be properly applied to any of the cases listed in Table XVII. Not only is each species easily distinguished from all others morphologically, but in some cases the members of a homosequential group bear little superficial resemblance and would, in some cases, hardly be grouped together by ordinary taxonomic characters. The most striking cases are *D. vesciseta* and *pilimana*, *D. punalua* and *paucipuncta*, and *D. villosipedis* and *grimshawi*. Ward et al. (1968) have described what appears to be a somewhat comparable case among mainland species.

Homosequential species are significant because their very existence stresses the importance of point mutations as a source of variability for speciation and evolution. It stresses the fact that considerable evolutionary divergence can repeatedly occur in the absence of sectional (chromosomal) mutations.

Study of Figure 8 with special attention to the distribution of homosequential species shows another interesting feature. Homosequential species, or ones that are essentially so, occur in clusters; in most cases, a relatively small number of intermediate states is found. This suggests that, at intervals, a highly stable and successful karyotype is somehow achieved through normal evolutionary processes. This karyotype may then remain essentially in a fixed condition while multiple speciation based on

TABLE XVII

Groups of Homosequential Species Among Hawaiian *Drosophila*

Homosequential group no.	Species	Island	Chromosomal subgroup		Chromosome formula				
1.	<i>D. bostrycha</i>	Molokai	I	X	2	3	4	5	
	<i>D. disjuncta</i>	Maui							
	<i>D. grimshawi</i>	Kauai, Oahu, Maui, Molo- kai, Lanai							
	<i>D. orphnopeza</i>	Maui							
	<i>D. villosipedis</i>	Kauai							
2.	<i>D. glabriapex</i>	Kauai	I	X	2	3	4b	5	
	<i>D. pilimana</i>	Oahu							
	<i>D. vespiseta</i>	Maui							
3.	<i>D. limitata</i>	Maui	I	X	2	3	4	5a	
	<i>D. sejuncta</i>	Kauai							
	<i>D. ochracea</i>	Hawaii							
4.	<i>D. balioptera</i>	Maui, Molo- kai, Lanai	I	Xg	2	3	4	5	
	<i>D. murphyi</i>	Hawaii							
5.	<i>D. hawaiiensis</i>	Hawaii	I	Xa <sup>2</sup>	2b	3g	4u	5	
	<i>D. musaphilia</i>	Kauai							
	<i>D. recticilia</i>	Maui							
	<i>D. silvarentis</i>	Hawaii							
6.	<i>D. heteroneura</i>	Hawaii	II	Xijkopqrst	2	3d	4b	5	
	<i>D. silvestris</i>	Hawaii							
	<i>D. planitibia</i>	Maui							
7.	<i>D. neopicta</i>	Maui	II	Xijkopqs	2	3d	4b	5	
	<i>D. obscuripes</i>	Maui							
8.	<i>D. hanaulae</i>	Maui	II	Xijkopqst	2	3d	4b	5	
	<i>D. oahuensis</i>	Oahu							
9.	<i>D. adiastrala</i>	Maui, Lanai	III	Xikouvwxxy	2cd	3fjk	4bopq	5f	
	<i>D. cilifera</i>	Molokai							
	<i>D. peniculipe-dis</i>	Maui							
10.	<i>D. ocellata</i>	Kauai	IV	Xef	2	3z	4befg	5	
	<i>D. paucipuncta</i>	Hawaii							
	<i>D. punalua</i>	Oahu							
	<i>D. uniseriata</i>	Oahu							

point mutations may occur on this karyotypic base. The most striking cases of this tendency are the *D. punalua* subgroup and the cluster of species that are close to *D. grimshawi*, *planitibia*, and *adiastola*.

### *Sibling Species*

This term has been applied to two or more species that resemble one another morphologically so closely that they have been frequently overlooked by the usual taxonomic procedures. Genetic tests may disclose that within what has been considered a single taxonomic entity two or more gene pools coexist without admixture. If such is the case, the separate populations concerned may be referred to as sibling species. This term is frequently retained for such entities despite the fact that in a number of instances a minor difference in genitalia, for example, may be discovered, which permits ready species recognition.

From the inception of the Hawaiian project all workers doing breeding work were sensitive to the possibility of the presence of sibling species. For over three years no evidence of such cryptic species was found: in fact the existence of morphologically divergent species that were nonetheless homosequential seemed to be the antithesis of the concept of sibling species.

Accordingly it was of great interest when, in May of 1968, a classical case of sibling species was discovered. *Drosophila primaeva* is a characteristic and widely distributed fly in the wet forests of Kauai, and since the species has a series of interesting chromosomal polymorphisms, some quantitative data have been gathered (Carson and Stalker, 1969) from various populations. In screening a large number of  $F_1$ 's from Kahili, Kauai, a sibship was found, which showed numerous sequential differences from *D. primaeva*. Inspection revealed a difference of 13 fixed inversions from the latter species, which was sympatric with it. Rearing of  $F_1$ 's from this culture (L41C12) was accomplished. Morphologically, these flies are virtually indistinguishable from *D. primaeva* although a small difference in male genitalia was found (Hardy and Kaneshiro, 1969). This species has been described as *Drosophila attigua*. Its difference in chromosomal makeup from *primaeva* is diagrammatically represented in Figure 8 and listed in Table XI, where it may be seen that several unique polymorphisms were also discovered in it.

### *Interspecific Hybridization*

By and large, the species of *Drosophila* of the Hawaiian fauna are sharply distinguished from one another morphologically and behaviorally. This very discreteness of the species constitutes evidence against the widespread occurrence of interspecific hybridization in nature. In general, the cytological data regarding the picture-winged species also supports the conclusion

that natural interspecific hybridization is either absent or extremely rare. Many very similar allopatric species differ by one or more fixed inversions. Hybridization between these species could be quickly and easily recognized cytologically, but no such case has been found.

On the other hand, the existence of that small number of cases where two (or in one case, three) homosequential species are sympatric requires comment. In these cases, interspecific hybridization could not be detected cytologically. On the other hand almost every one of the species concerned displays unique morphological characters, which in themselves could serve to label  $F_1$  individuals as hybrids if such did indeed occur. Despite careful investigation of all these possibilities, no certain case of natural interspecific hybridization has been detected.

Under laboratory conditions, however, many interspecific hybridizations have been made. The most notable study is that of Yang and Wheeler (1969). These authors used 28 picture-winged species and attempted 278 interspecific crosses. In 50 instances,  $F_1$  hybrid larvae, pupae, or imagos were produced. This number includes 11 cases in which hybrids were produced from both reciprocal crosses.  $F_1$  imagos were obtained in crosses involving 20 species (Fig. 12). In this figure, the species are grouped according to their chromosomal similarities. Most of the successful crosses occur within the chromosomal subgroups; the rather large number of hybridizations within the *grimshawi* subgroup is not unexpected in view of the great chromosomal similarity of these species (Fig. 8). Although eight inseminations have occurred involving members of different cytological subgroups, imagos were produced in only one instance, that of *picticornis* females crossed to *crucigera* males. The three other inseminations of *D. picticornis* females by members of the other two subgroups may be related to the postulated relict nature of this species (see below).

In most cases,  $F_1$  female hybrids are fertile in backcrosses to one or both of the parental males. Of even greater interest, however, is the fact that in six instances  $F_1$  hybrid males were at least partially fertile in backcrosses or even in  $F_2$ 's (small darkened squares, Fig. 12). In each case, the strains involved are from different islands and morphological differences between the species are moderately great, and there appears to be no reason to insist that the data indicate the conspecificity of the entities involved. This is especially true because in only one case are the males fertile from both reciprocal crosses.

In the case of *crucigera* and *grimshawi*, Yang and Wheeler's data indicate fertility of  $F_1$  males from both reciprocal crosses. These data are in disagreement with the findings of Clayton (unpublished; cited by Carson, 1966), who made the same crosses and found the  $F_1$  males to be sterile. Such differences might be due to genetic differences between the strains used.

The *hawaiiensis-gradata* and *hemipeza-silvestris* cases are most interest-

	♂	♀	<i>bostrycha</i>	<i>disjuncta</i>	<i>grinshawi</i>	<i>villosipedis</i>	<i>balioptera</i>	<i>engyochracea</i>	<i>orthofascia</i>	<i>crucigera</i>	<i>gradata</i>	<i>hirtipalpus</i>	<i>hawaiiensis</i>	<i>silvarentis</i>	<i>pillimana</i>	<i>discreta</i>	<i>pauipuncta</i>	<i>uniseriata</i>	<i>planitibia</i>	<i>silvestris</i>	<i>hemipeza</i>	<i>picticornis</i>	
I. <i>D. grinshawi</i> subgroup																							
<i>bostrycha</i>		I	I																				
<i>disjuncta</i>		I	I																				
<i>grinshawi</i>																							
<i>villosipedis</i>																							
<i>balioptera</i>																							
<i>engyochracea</i>																							
<i>orthofascia</i>																							
<i>crucigera</i>																							
<i>gradata</i>																							
<i>hirtipalpus</i>																							
<i>hawaiiensis</i>																							
<i>silvarentis</i>																							
<i>pillimana</i>																							
<i>discreta</i>																							
II. <i>D. punalia</i> subgroup																							
<i>pauipuncta</i>																							
<i>uniseriata</i>																							
III. <i>D. planitibia</i> subgroup																							
<i>planitibia</i>																							
<i>silvestris</i>																							
<i>hemipeza</i>																							
<i>picticornis</i>																							

Fig. 12. Interspecific crosses of picture-winged species from which at least one  $F_1$  hybrid imago was obtained (after Yang and Wheeler, 1969). L=larva; P=pupa; I=imago; N=no insemination; S=presence of sperm in female reproductive organs; open squares=not tested;  $\square$ = $F_1$  males fertile; larger dark squares=chromosomal subgroups.

ing. In each case, one species is from Hawaii and the other from Oahu, and in both cases considerable fixed inversion and morphological differences separate the species. These cases suggest the conclusion that sexual isolation is an essentially fortuitous accompaniment of speciation. Such a conclusion is especially compelling in view of the evidence that speciation frequently has followed a single founder event (see below, p. 523). This means that complete reproductive isolation may not always accompany speciation.

The fact that the above cases involve species that are very different morphologically and chromosomally hardly tempts one to discard the previously held species criteria and declare the populations involved to be conspecific. In this connection, however, two other cases studied by Yang and Wheeler are particularly interesting since the species in both cases are morphologically and cytologically very close. *D. disjuncta* and *D. bostrycha* are allopatric species with the former confined to Maui and the latter to Molokai. They are not only homosequential but share a common polymorphism ( $4v/+$ ). Thus it is impossible to distinguish the species chromosomally. Morphologically, the only difference that can easily be used is the presence of several long dorsal hairs on the middle of the tibia of *bostrycha* (Hardy, 1965). Despite these great similarities,  $F_1$  males from both reciprocal crosses are sterile, confirming the conclusion that these two are indeed bona-fide species.

Crosses between the homosequential species *hawaiiensis* and *silvarentis* of Hawaii also resulted in sterile  $F_1$  male hybrids, again reinforcing the judgment of the taxonomists (Hardy and Kaneshiro, 1968).

In summary, Yang and Wheeler's study of hybridization shows that the rather free crossing between species of picture-wings parallels the other data in revealing biological closeness beneath a façade of striking morphological difference. Ethological isolation is not as pronounced as might be expected from the extensive development of secondary sexual characters. Failure to mate interspecifically seems to be at least in part a fortuitous species characteristic and in part a by-product of the strong tendency for lek behavior that these species show.

The potential for natural interbreeding and gene exchange between Hawaiian species of *Drosophila* is certainly great, especially through backcrossing of  $F_1$  hybrid females to males of parent species. Recognition of such cases should not be difficult since most species are well-marked morphologically and cytologically. The fact that no such case has as yet been recognized is noteworthy, but the search continues.

Kambysellis (1969) has studied the phylogenetic relationships of both picture-winged and other Hawaiian *Drosophila* by the method of interspecific ovarian transplantation. In contrast to what the same author found for mainland species groups of *Drosophila* (Kambysellis, 1968a, b),

interspecific incompatibility among Hawaiian species is far less pronounced. This has led to the conclusion that *Drosophila* species endemic to Hawaii manifest a marked uniformity of internal environment in contrast to the high diversity observed for mainland species.

The method used by Kambyzellis also leads to the possibility of the production of  $F_1$  interspecific hybrids. This possibility is enhanced by this technique because when a donor egg laid by a host female is fertilized by a host sperm, ethological isolation is effectively bypassed. *D. grimshawi* proved to be the best host; *hawaiiensis* and *hemipeza* were also used. Among 18 picture-winged species studied, the index of oogenesis for donor ovaries developing in *grimshawi* hosts is 100 except for members of the *adiastola* subgroup, where it is somewhat less. Two modified-mouthparts species, *D. mimica* and *eurypeza*, show indices of oogenesis of 100 with the picture-winged hosts. Of all species from which transplanations were made, *nigra* and *crassifemur* have the lowest indices of oogenesis (67 and 65, respectively). This is not wholly unexpected since *D. crassifemur* has *Scaptomyza*-like properties.

In most cases, Kambyzellis' data parallel what Yang and Wheeler (1969) has found. Hybrid imagos were obtained in five instances where *grimshawi* provided the male gamete (*conspicua*, *hexachaetae*, *ochracea*, *hemipeza*, and *silvestris*). Imagos were also obtained from the cross *gradata* female  $\times$  *hawaiiensis* male. The most striking cases, of course, are the intergroup species hybrids between *grimshawi* and the two extra-vein species of the *planitibia* subgroup (*hemipeza* and *silvestris*). The production of such adults provides further evidence that these entities are congeneric. As in Yang and Wheeler's work, however, hybridization appears to be more successful within the cytological subgroups than between them.

These hybridization studies confirm in main outline the conclusions reached from the strictly cytological approach. Although they show many individual morphological and ethological peculiarities, Hawaiian *Drosophila* species show extremely close genetic affinity. One can only conclude that these startling peculiarities are, in a sense, superficial.

### *Evolution, Speciation, and Migration*

#### *Chromosomal Evidence for the Ultimate Origin of the Picture-Winged Species from Certain Continental Forms*

As has been pointed out previously, chromosomal data on Hawaiian *Scaptomyza* are confined to metaphase studies. These, furthermore, provide no clue to the origin of these flies. On the other hand, both metaphase- and salivary-gland chromosomes have now been obtained for 69 species of the picture-winged flies, a most striking and conspicuous element of the



TABLE XVIII

## Single Island Endemism Among Picture-winged Hawaiian Drosophilidae

Island	Number of species found on this island only				Species present on this island and others (cytologically studied)		
	No. cytologically studied	Estimated no. not yet studied					
Kauai	9	3		<i>D. crucigera</i> , <i>D. grimshawi</i>			
Oahu	11	17		<i>D. crucigera</i> , <i>D. grimshawi</i>			
Maui	24	8		<i>D. adiastrata</i> , <i>D. balioptera</i> , <i>D. grimshawi</i> , <i>D. spectabilis</i>			
Molokai	3	3		<i>D. balioptera</i> , <i>D. grimshawi</i> , <i>D. spectabilis</i>			
Lanai	0	1		<i>D. adiastrata</i> , <i>D. balioptera</i> , <i>D. grimshawi</i>			
Hawaii	17	3		—			
Total	64	+	35	+	5	=	104

Hawaiian Drosophilidae. The distribution of these cytologically well-known species by island of origin (Table XVIII) reveals a high degree of single-island endemism. Thus, on the six major islands, there are only 5 species out of the 69 that occur on more than one island. This tendency is all the more striking when one recalls the fact that Maui, Molokai, and Lanai have been fused into a single land mass several times because of fluctuations in sea level. If the data in Table XVIII are recalculated considering Maui-Molokai-Lanai as a single island, this unit ("the Maui complex") has a total of 30 endemic species. Thus, a total of 67 out of 69 species (all but *D. crucigera* and *D. grimshawi*) qualify as single-island endemics.

In placing value on the data from these 69 species, the question arises as to how representative of the entire picture-winged fauna the cytologically studied sample is. In order to approximate an answer to this, the writer has searched the collecting records, both published and unpublished, and has made a crude estimate of the number of endemic species probably belonging to this general group that are known but have not yet been studied cytologically. These numbers are given in the second column in Table XVIII.

Except for Oahu, the fauna appears to be well known, with the 69 species studied coming from all islands and being based on an apparent total of 104 species. It is clear that Oahu has had, at least until recent years, a quite substantial fauna of these large flies. Rediscovery of species taken on Oahu 10 to 20 years ago has proved difficult, and this appears to be related to the rapid and irretrievable disappearance of natural

habitats under the impact of human exploitation and the inexorable inroads of exotic forest elements. The only recourse appears to be to attempt to collect in the more remote valleys, which are difficult of access.

With regard to the ceiling number of 10+ species, suggested above, another note of caution should be sounded. From the beginning of *Drosophila* collections on the Hawaiian Islands, the number of species has been frequently underestimated. Accordingly, the existence of a number of as yet undescribed species remains a strong probability.

Among the eleven species studied from Kauai are represented members of all five cytologically recognized subgroups (Fig. 8 and Table XVIII). This includes two species, *D. primaeva* and *attigua*, which comprise all of the cytologically known members of subgroup V, the *D. primaeva* subgroup. Morphologically and cytologically (see Table IX) these species are distinctive in the Hawaiian fauna.

Accordingly, it is of very great interest that, in a preliminary study, Stalker (1968) has shown that certain "runs" of polytene-chromosome-banding in *D. primaeva* can be homologized with comparable sequences in certain mainland species. These homologies, moreover, relate the Hawaiian species more clearly to the *robusta* group rather than, for example, to the *virilis*, *repleta*, or *melanica* groups. All these groups, of course, belong to the subgenus *Drosophila*.

The *robusta* group appears to have its center of origin in Asia. Thus, on the northern Japanese island of Hokkaido alone there are six species (Okada, 1956; Takada, 1959; Kaneko et al., 1964; Kaneko and Takada, 1966), whereas in the whole of continental North America only two species, including *D. robusta* (*sensu stricto*), are found.

Although detailed comparisons are as yet unpublished, the precision of some of the banding comparisons may be exemplified by the following case (Stalker, personal communication). The basic sequence of bands in chromosome 5 in the *primaeva* subgroup is described within the Hawaiian lettering scheme as 5h (Fig. 7). It so happens that the arrangement represented by 5h is closer to the mainland forms than Standard 5; when the 5h inversion is made, producing Hawaiian Standard 5, the "run" of bands is broken up. These comparisons appear to be all the more significant because chromosome 5, in particular, is the least variable in Hawaii and the same element likewise is the most conservative among the mainland forms.

Accordingly, this type of evidence provides a valuable clue to the direction of the phylogeny. Intrinsicly, and without outside evidence, a chromosomal phylogeny is not directional. Any point in such a relationship diagram as Figure 8 can be considered ancestral and all others derived from it. Geological, geographical, behavioral, and morphological evidence, together with the cytological sequential evidence just cited, all converge

to indicate that, among the 69 cytologically known species, the *primaeva* subgroup of Kauai is indeed the closest to a theoretical ancestor for the group.

This is not to say that further studies may not (1) disclose other mainland forms which are even closer to Hawaiian species, or (2) disclose other Hawaiian species that are even closer to mainland forms. That this dual search continues, however, does not affect the basic outlines of the scheme given above.

**Migration and Speciation of the Picture-Winged Species within the Hawaiian Islands**

The establishment of Kauai as the probable ancestral home of the present *Drosophila* of Hawaii makes possible the erection, using the basic data given in Figure 8, of interpretative theories of the origin of all species and species subgroups. These theories are presented in Figure 13 through 18, with supplementary data provided in Table XIX. These interpretations, especially as regards the early evolution of the five subgroups on Kauai, will almost certainly be subject to revision and alteration as more data accumulate. They should be regarded as tentative. On the other hand, the proposed species founders for the fauna of the late Pleistocene island of

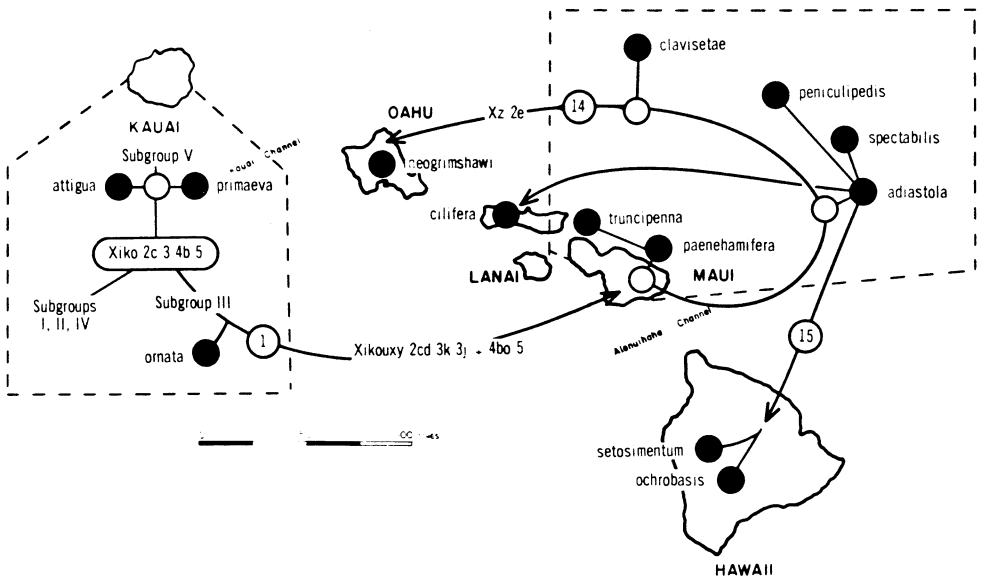


Fig. 13. Origin of *D. adiaetola* subgroup (III) on Kauai and its subsequent history. Founders nos. 1, 14, 15, cf. Fig. 8 and Table XIX; solid symbols: existing species; open circles and brackets: hypothetical populations.

TABLE XIX

## Chromosomal Composition of Interisland Founders

Found- No.	Island Donor	Recepi- ent	Chromosomal formula				Derived from a population:	Gives rise to:		
1	Kauai	Maui	Xikouxy	2cd	3k	3j/+	4bo	5	ancestral to <i>D. ornata</i> (subgroup III)	<i>D. adiaastola</i> sub- group (III) on all eastern islands
2	Kauai	Maui	Xijko	2	3d		4b	5	descended from the above	<i>planitibia</i> sub- group (II) on all eastern islands
3	Kauai	Hawaii	Xijko	2	3de		4bhij	5e	descended from the above and ancestral to <i>picticornis</i>	<i>setosifrons</i> of Hawaii only
4	Kauai	Oahu	X	2	3		4b	5	ancestral to <i>glabriapex</i>	<i>pilimana</i> and other "4b" species of <i>grimshawi</i> sub- group (I) on Oahu
5	Kauai	Oahu	X	2	3		4	5	of <i>grimshawi</i> (Kauai)	<i>grimshawi</i> (Oahu)
6	Oahu	Maui	X	2	3		4	5	from Founder no. 5	<i>grimshawi</i> of Maui complex and other Stan- dard 4 species of Maui
7	Kauai	Oahu	Xef	2	3z		4befg	5	ancestral to <i>ocellata</i>	<i>punalua</i> sub- group (IV) on Oahu
8	Oahu	Maui	X	2	3		4b	5	descended from Founder no. 4	"4b" species of <i>grimshawi</i> sub- group (I) on Maui
9	Oahu	Maui	X	2	3		4b	5d	descended from Founder no. 4	"5d" species on Maui (see Fig. 8)
10	Oahu	Hawaii	Xef	2	3z		4befg	5	descended from Founder no. 7	<i>punalua</i> sub- group (IV) of Hawaii
11	Maui	Oahu	X	2b	3g		4	5	descended from Founder no. 6	<i>gradata</i> (Oahu)

TABLE XIX (Continued)

Found- der No.	Island Donor	Recipi- ent	Chromosomal formula				Derived from a population:	Gives rise to:	
12	Maui	Kauai	Xa <sup>2</sup>	2b	3g	4u	5	similar to the above	<i>musaphilia</i> (Kauai)
13	Kauai	Maui	X	2	3	4	5a	ancestral to	<i>limitata</i> (Maui)
14	Maui	Oahu	Xikouvxyz	2cde	3fjk	4boq	5f	descended from Founder no. 1 (see Fig. 13)	<i>neogrimshawi</i> (Oahu)
15	Maui	Hawaii	Xikouvxy	2cd	3fjk	4bopq	5f	descended from Founder no. 1 (see Fig. 13)	<i>setosimentum</i> and <i>ochrobasis</i> (Hawaii)
16	Maui	Oahu	Xijkopqst	2m/+	3d	4b	5	descended from Founder no. 2 (see Fig. 14)	<i>planitibia</i> sub- group (II) on Oahu
17	Maui	Hawaii	Xijkopqrst	2	3d	4b	5	descended from Founder no. 2 (see Fig. 14)	<i>heteroneura</i> and <i>silvestris</i> of Hawaii
18	Maui	Hawaii	X	2	3	4b	5	descended from Founder no. 8	<i>conspicua</i> (Hawaii)
19	Maui	Hawaii	Xa <sup>2</sup>	2b	3g	4u	5	similar to that for Founder no. 12	<i>silvarentis</i> , <i>hawaiiensis</i>
20	Maui	Hawaii	Xg	2	3o/+	4	5	descended from Founder no. 6	<i>engyochracea</i> , <i>murphyi</i> , <i>ciliaticrus</i>
21	Maui	Hawaii	X	2	3	4	5	similar to Founder no. 6	<i>sproati</i>
22	Maui	Hawaii	X	2	3	4	5a	similar to Founder no. 13	<i>ochracea</i> <i>claytonae</i>

Hawaii, for example, appear to be unequivocal and should be regarded as firmly established.

THE PRIMA-EVA SUBGROUP (v). There are only two members of this subgroup and both have been found only on Kauai. *D. attigua* is a sympatric sibling species of *primaeva*; it has been described by Hardy and Kaneshiro (1969).

The two species differ by 13 fixed inversions; their hypothetical common ancestor differs from Standard *D. grimshawi* by 18 inversions, 14 of which are in chromosomes X and 4. These two chromosomes, it will be recalled, are the most variable among the five chromosomes (see Table XIII). In contrast, chromosome 3 of *primaeva* is identical to Standard, and 2 and 5 are not far away. If all of the inversions unique to the *primaeva* subgroup are made, the karyotype moves closer to the other subgroups, having the formula Xiko 2c 3 4b 5 (Fig. 13). The break-points of the inversions and chromosome maps are presented in Carson and Stalker (1969).

THE ADIASTOLA SUBGROUP (III). This subgroup, like the other three, apparently arose from the common ancestor just mentioned (Fig. 13). With the addition of inversions leading away from Standard (Xuxy 2d 3k 3j and 4o) a karyotype is reached which can be postulated as a common ancestor of *D. ornata* of Kauai and the rest of the *adiastola* subgroup. From this ancestor a migrant (Founder no. 1, Fig. 13) must have reached Maui directly, where, with the addition of a few more inversions (Xvw, 3f, 4p, 4q, 5f, and the fixation of 3j), the condition observed in present-day *D. adiaastola* could be reached.

Most of the Maui members of this group are chromosomally very close, despite the extraordinary curious morphologies found in *D. truncipenna*, *paenehamifera*, and *clavisetae*. The latter, for example, not only has a remarkable set of clavate hairs at the end of the abdomen of the male, but the species is characterized by the presence of an extra cross-vein in the R-5 cell of the wing. It is of great significance that the only other member of this subgroup showing this extra cross-vein is *D. neogrimshawi* of Oahu. Since this latter species has the Xz and 2e arrangements, found also in *clavisetae*, the evidence is strong that *neogrimshawi* is derived from a specialized and peculiar section of the *adiastola* subgroup, a northward migrant from Maui (Founder no. 14, Fig. 13).

The above situation indicates clearly that the initial southward migration (Founder no. 1) must have by-passed Oahu. Only later did a member of this group reach Oahu from Maui.

As has been mentioned several times, Maui, Molokai, and Lanai have previously been joined. Accordingly, the one species unique to Molokai, *D. cilifera*, may have reached there by simple allopatric speciation over a terrestrial route. Accordingly, this is not counted as an interisland founder despite the fact that this possibility cannot be excluded. Typical *D.*

*adiastola*, both chromosomally and morphologically, exists on Lanai. On the Big Island (Hawaii), however, there are two distinct species having the basic arrangement of *D. adiastola* with a series of additional unique inversions (*D. setosimentum* and *ochrobasis*: see Fig. 8). These two species appear to be derived from a Pleistocene founder (no. 15) deriving from an ancestral population having the same chromosome composition as *adiastola* (Fig. 13 and Table XVIII).

THE PLANITIBIA SUBGROUP (II). This subgroup comprises 14 species. Twelve of the 14 have an extra wing-vein in cell R-5. The single Kauai member, *D. picticornis*, and *D. setosifrons* of Hawaii, do not have the extra wing-vein, yet display two inversions, Xj and 3d, which are found in all other members of the group. The absence of extra-vein flies from Kauai, yet the presence there of *picticornis*, suggests that the latter is the modern descendent of an ancestral Kauai population from which a Founder (no. 2, Fig. 14) proceeded directly to Maui and produced the extra-vein flies on that island. *D. picticornis*, however, is not itself close to the primitive population from which the founder came. In fact, it appears somewhat specialized. A number of its special inversions, furthermore, are shared by the bizarre fly *D. setosifrons* of Hawaii. Although these two have

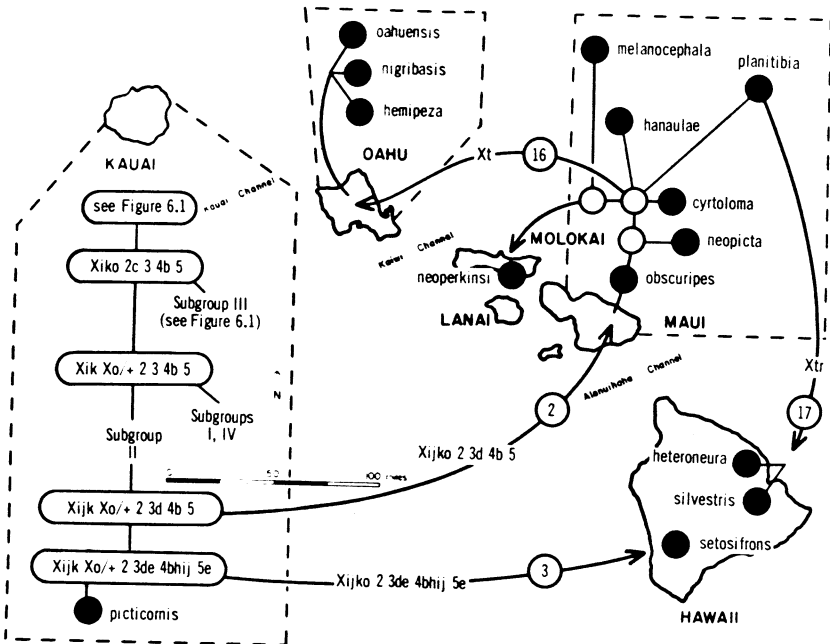


Fig. 14. Origin of *D. planitibia* subgroup (II) on Kauai and its subsequent history. Founder nos. 2, 3, 16, 17.

many inversion sequences in common, they are morphologically very different. *D. picticornis*, it will be noted, lacks X<sub>0</sub>, and inversion that is characteristic of all of the extra-vein flies and is also present in *setosifrons*. The latter is thus just one step closer to the extra-veined flies than *D. picticornis* is.

The interpretation that has been tentatively placed on this situation is given in Figure 14. Founder no. 2 must bypass Oahu because *D. obscuripes* of Maui is the closest of the extra-veined flies to *picticornis*, with *neopicta* one step further away. This latter species is heterozygous for X<sub>t</sub>, an inversion that is fixed in all others, including those of Oahu. This point is important because it proves that the Oahu flies are derived from a *neopicta*-like ancestor on Maui. Only three species have X<sub>r</sub>. These are *planitibia* and the two extra-veined flies of Hawaii, *heteroneura* and *silvestris*. Clearly, the latter two are derived from a *planitibia*-like ancestor on Maui (Founder no. 17). For reasons stated earlier, *neoperkinsi* of

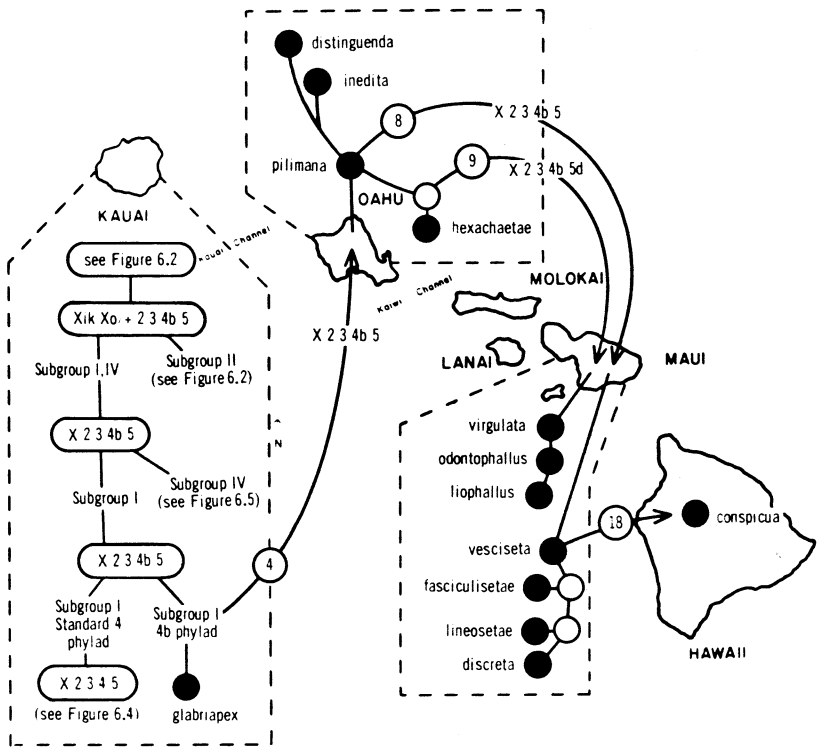


Fig. 15. Origin of the *D. glabriapex* section (gene arrangement "4b" phylad) of the *D. grimshawi* subgroup (I) on Kauai and its subsequent history. Founder nos. 4, 8, 9, 18.



Molokai is not considered a clear case of an interisland founder because of the possibility that it was formed by terrestrial allopatric speciation when the islands were joined.

**THE GRIMSHAWI SUBGROUP (1).** This subgroup is large and includes 36 species. Two phylads (or sections) are distinguishable on the basis of a fixed inversion difference, gene arrangement 4b. Thirteen of the species have 4b in fixed condition and 23 have the Standard Chromosome 4. These two groupings thus form separate phylads and it will be convenient to consider their history separately.

*The glabriapex section (4b phylad of Subgroup 1).* *D. glabriapex* is the sole member of this subgroup on Kauai. It can easily be derived from the ancestor of the *planitibia* subgroup with gene arrangements Xijk and 3d becoming Standard through the occurrence of these four inversions (Fig. 15). This chromosomal condition (X 2 3 4b 5) not only can give rise directly to *glabriapex* but can produce Founder no. 4, which can give rise to *pilimana* of Oahu, as well as the other "4b" species on that island. From Oahu, the large number of "4b species" on the Maui complex can be derived from two Founders (8 and 9, Fig. 15). Despite extensive speciation on Maui, these lineages have apparently resulted in only one invasion of Hawaii (Founder no. 18, giving rise to *conspicua*).

*The grimshawi section (Standard 4 phylad of Subgroup 1).* This group of 23 species, unlike the preceding, is well represented on Hawaii, and, furthermore, gives considerable evidence of reverse migration, with forms on Oahu and Kauai that are apparently derived from Maui.

The basic chromosomal formula of this section is easily derived by the "removal" of arrangement 4b, giving the Standard X 2 3 4 5 (see Fig. 16). This event, according to this theory, was accomplished on Kauai, with the evolution of *D. grimshawi* and *crucigera*. As has been mentioned previously, the latter 2 species are unique among the 69 species so far studied in that they are found, as apparently the same species, on more than one island. Details of the population structure of these two species were presented above (p. 511).

In Figure 16, three southward colonizations are postulated. Thus, *grimshawi* and *crucigera* of Oahu are apparently derived from migrants from Kauai. That migrant giving rise to *crucigera* is not considered a true evolutionary founder, in that the result has been apparently merely the simple colonization of Oahu from Kauai by this species. As was mentioned above (p. 529), there may have been two such colonizations of Oahu by *crucigera*.

A *D. grimshawi* migrant (Founder no. 5, Fig. 16) apparently gave rise to *D. grimshawi* of Oahu. The situation in this case, however, is different from that of *crucigera* since the Oahu populations of the former were apparently a stepping stone for the invasion of the Maui complex by *grim-*



Since *D. balioptera* has not so far been found to have  $3o_7+$ , or *orphnopeza* to have  $Xg_7$ , it appears that Founder no. 20 must have had the formula  $Xg_7 3o_7+$ . Such a founder could have arisen on Maui from an ancestral population having both  $Xg_7+$  and  $3o_7+$ . This same population could also have given rise to the three species *orphnopeza* ( $3o_7+$ ), *orthofascia* ( $Xg_7$ ), and *balioptera* ( $Xg_7$ ) on Maui. *D. engyochracea* of Hawaii, which has  $Xg_7$  but lacks  $3o_7$ , must represent a case wherein this species has refixed the Standard  $3+ / 3+$  from the  $3o_7+$  condition. These details may be gleaned from Figure 8.

**THE PUNALUA SUBGROUP (IV).** This rather small subgroup apparently also differentiated on Kauai from the hypothetical 4b ancestor of the *grimshawi* subgroup (Fig. 17). The sequential karyotype found in *ocellata* of Kauai occurs also in all other species of the subgroup; thus these species represent a very stable homosequential series. *D. basisetae* and *prolaticilia* of Hawaii each have an additional unique inversion, but stability characterizes the subgroup.

From an *ocellata*-like ancestor, a founder is believed to have reached Oahu (no. 7, Fig. 17) and then to have given rise to the two Oahu species that have a karyotype identical with it. It is noteworthy that no Maui species of this subgroup has been discovered. Accordingly, the facts sug-

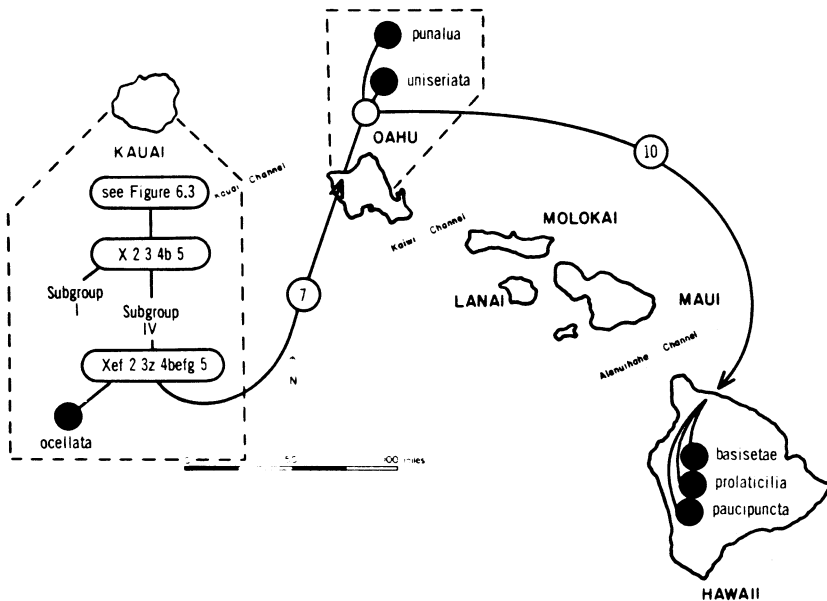


Fig. 17. Origin of the *D. punalua* subgroup (IV) on Kauai and its subsequent history. Founder nos. 7, 10.

gest that a founder (no. 10) went directly to Hawaii and gave rise to the three species found there.

#### *Colonization and Speciation Patterns in Antopocerus*

Among the Drosophilidae of Hawaii is a group of leaf-breeding species that have been placed in a separate genus, *Antopocerus*. The ecology and behavior of these species is well known (see above, p. 492). Spieth (1968b) recognizes three types of mating behavior among the nine species. What may be recognized as the most primitive type characterizes two species that are restricted to the Maui complex (Maui and Molokai). The three species found on Hawaii show derived types of behavior, and this is also true of the single species found on Oahu. Since no member of *Antopocerus* has ever been collected on Kauai, Spieth interprets this situation as indicating that *Antopocerus* evolved on the Maui complex, with both Hawaii and Oahu having been colonized from there. This view closely parallels that which ascribes a key role to the Maui complex in the evolution of the picture-winged species, especially the *D. adiantola* and *D. planitibia* subgroups. These also give evidence of colonization of Oahu from Maui.

So far, flies of this genus have not proved to be workable cytologically so that unfortunately it has not been possible to test Spieth's conclusions by polytene-chromosome mapping. It has likewise not been possible to obtain cytological confirmation that *A. diamphidiopodus* of the Maui complex and the island of Hawaii is indeed a single species throughout its range.

#### *Conclusion*

Reference to the papers of Carson and Stalker (1968a, b, c) will show that the *D. grimshawi* subgroup was previously supposed to have arisen on Maui. From there, it was thought to have spread southward to Hawaii, northward to Oahu, and thence to Kauai. Recent discoveries on Kauai, particularly the confirmation that *D. grimshawi* (*sensu stricto*) is found there, as well as *D. ornata* and *ocellata*, has led to revision of these ideas. Accordingly, it now seems much more likely that all five major subgroups of picture-winged flies existed on Kauai at an early time in the history of the Archipelago and spread to the other islands from there (Carson and Stalker, 1969).

The phylogenetic interpretations given in this chapter are summarized in Figure 18 and in Tables XIX and XX. In the preceding sections of this chapter, 22 interisland colonizations have been inferred from cytological information. For most of these, precise chromosomal formulas may be written (see Table XIX).

The island of Hawaii presents a case of special interest. It has been the recipient of nine founders and has apparently not been a donor in any

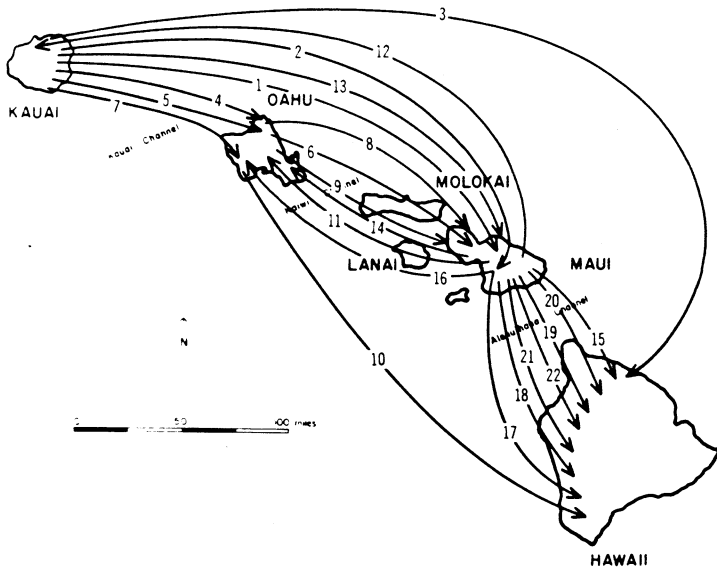


Fig. 18. Summary of the proposed minimum number (22) of interisland founders in the picture-winged *Drosophila* of Hawaii.

case in the present scheme. This is consistent with the fact that it is the youngest island.

Possibly because they have occurred in relatively recent geologic time, seven of the nine founder events involving the island of Hawaii can be traced with great clarity. For example, five sets of Hawaii species of the *D. grimshawi* subgroup can be traced to particular species known on Maui

TABLE XX

Minimum Number of Interisland Founders Among Hawaiian *Drosophila*

Recipient island	Donor island				Total receptions
	Kauai	Oahu	Maui complex	Hawaii	
Kauai	X	0	1	0	1
Oahu	3	X	3	0	6
Maui complex	3	3	X	0	6
Hawaii	1	1	7	X	9
Total donations	7	4	11	0	22

TABLE XXI  
 Five Sets of Species of the *D. grimshawi* Subgroup  
 on Hawaii that Have Close Relatives on Maui

Set No.	Island	Species	Chromosomal formula				
1	Hawaii	<i>silvarentis</i>	Xa <sup>2</sup>	2b	3g	4u	5
		<i>hawaiiensis</i>	Xa <sup>2</sup>	2b	3g	4u	5
	Maui	<i>recticilia</i>	Xa <sup>2</sup>	2b	3g	4u	5
2	Hawaii	<i>ochracea</i>	X	2	3	4	5a
		<i>claytonae</i>	X	2	3	4	5a
	Maui	<i>limitata</i>	X	2	3	4	5a
3	Hawaii	<i>engvochracea</i>	Xg	2	3	4l	5
		<i>ciliaticrus</i>	Xg	2	3o	4	5
		<i>murphyi</i>	Xg	2	3/3o	4	5
	Maui	<i>orphanopeza</i>	X	2	3/3o	4	5
		<i>balioptera</i>	Xg	2	3	4	5
		<i>orthofascia</i>	Xg	2	3n	4	5
4	Hawaii	<i>sproati</i>	Xb <sup>2</sup>	2	3	4	5
	Maui	<i>grimshawi</i>	X	2	3	4	5
5	Hawaii	<i>conspicua</i>	Xmm	2	3	4bm	5
	Maui	<i>vesciseta</i>	X	2	3	4b	5

(Table XXI). These facts serve as the basis for the erection of hypothetical Founders nos. 19, 22, 20, 21, and 18. In four of these five cases, the exact chromosomal formula necessary is known in an existing species on Maui. In the case of set no. 3 (Table XXI) the founder is apparently descended from a common ancestor of *orphanopeza* and *balioptera*, as discussed previously.

Two other sets of Hawaii-Maui species also show extremely close similarity. These are detailed on Table XXII and form the basis for the postulation of Founders nos. 17 and 15, discussed earlier.

Another interesting circumstance regarding the Maui-Hawaii similarities is the fact that there is as yet no known case of a "simple" colonization of the island of Hawaii by a species known from another island other than the *Antopocerus* case mentioned earlier. To put this another way, it may be said that each founding event has led to species formation and, in some cases at least, several new species have resulted on the recipient island. In fact, the rarity of simple colonizations, and, conversely, the extremely

high degree of single-island endemism, seem to be the most characteristic feature of the evolution and speciation of Hawaiian Drosophilidae.

Looking at the situation with regard to the oldest island, Kauai, it will be noted that, in contrast to Hawaii, it has apparently been the recipient of only one founder (no. 12, the ancestor of *D. musaphilia*). This circumstance, however, does not take into account the hypothetical one or more original colonizations of Kauai.

The very large number of species found on Maui suggests a flowering of speciation on that island following the arrival of six founders, three from Kauai and three from Oahu (Fig. 18). As has been pointed out previously, the case for the by-passing of Oahu by founders from Kauai is well documented for two of the three cases and the facts seem unequivocal. The writer is inclined to view this as a fortuitous event perhaps no more improbable than any other interisland movement. Nothing is known as to the nature of the propagules that have accomplished interisland movements. Nevertheless, it seems likely that adult flies are involved and that populations are established directly in high-altitude locations.

In the interpretation given, only the facts available to the present-day investigator have been used, that is, the concept of extinction has not been evoked. Thus, for example, it may be argued that founders like no. 1 or no. 2 could have gone from Kauai directly to Oahu, undergone some speciation and evolution on the latter island, and then sent further founders south to Maui. If this was the case, then one must assume that the intermediates have since become extinct or remain undiscovered. This cannot, of course, be disproved but nevertheless requires many more assumptions than the rather simple explanations that make use of the existing intermediates that have actually been found on Maui. In any event, the rule

TABLE XXII

## Two Additional Sets of Hawaii Species with Close Relatives on Maui

Set No.	Island	Species	Chromosomal formula				
6	Hawaii	<i>silvestris</i>	Xijkopqrst	2	3d	4b	5
		<i>heteroneura</i>	Xijkopqrst	2	3d	4b	5
	Maui	<i>planitibia</i>	Xijkopqrst	2	3d	4b	5
7	Hawaii	<i>setosimentum</i>	Xikouvwxym <sup>2</sup>	2cdl	3fjkl	4bopqb <sup>2</sup> c <sup>2</sup> d <sup>2</sup> e <sup>2</sup> f <sup>2</sup> n <sup>2</sup> o <sup>2</sup>	5f
		<i>ochrobasis</i>	Xikouvwxym <sup>2</sup>	2cdk	3fjk	4bopqb <sup>2</sup>	5f
	Maui	<i>adiastola</i>	Xikouvwx	2cd	3fjk	4bopq	5f

of parsimony has been adhered to in the interpretations made so far. The writer would thus not contend that alternate schemes based on multiple extinctions could not be constructed. On the other hand, the actual existence of intermediates and of species that are polymorphic for unique inversions that ultimately become fixed in other species suggests a simpler theory. Most existing species, furthermore, appear to be widely distributed on the island to which they are endemic. More detailed distribution data, however, are needed on this point but the data so far suggest that species confined to single valleys or kipukas are fewer than originally thought.

In most continental areas it is not possible to distinguish species that are newly formed in time from those which may have existed for many millions of years. Accordingly, it is of special interest that the geological newness of the island of Hawaii and the obvious derivation of the bulk of its fauna directly from Maui suggests that Hawaii's fauna is recent. Few studies have been made of the evolutionary biology of species selected for their historical newness. Accordingly, the endemic fauna of Hawaii appears to provide unique materials for this sort of study.

### Summary

The six largest islands of the Hawaiian chain are of volcanic origin and vary in age from about 5,000,000 years in the west (Kauai) to less than 1,000,000 years in the east (Hawaii), where two volcanos are still active. Maui, Molokai, and Lanai (the Maui complex) were joined and separated at least twice in recent geologic history. Thus, in the broad sense, the number of main islands with deep channels between them reduces to four: Kauai, Oahu, the Maui complex, and Hawaii. All islands receive heavy rainfall on windward slopes, due to the combination of persistent trade winds and high altitudes. These factors, coupled with the tropical latitude and rich volcanic soils support the growth of a number of forest types of which four serve as the habitat for the vast majority of drosophilids.

The oldest islands in the Hawaiian chain (the Leeward Islands: 15,000,000 years) are now worn down to the point that they probably no longer support a native fauna of Drosophilidae except possibly for a few species of *Scaptomyza*. The native species are now confined to the high main islands. Two of the main islands, however, i.e., Niihau and Kahoolawe, are low (1281 and 1415 feet, respectively); they have been greatly changed by man, and today probably support no native drosophilid fauna.

The family Drosophilidae on the islands comprises 650 to 700 species; all but 17 are endemic. A majority of species fall into two genera, *Drosophila* Fallén (324 species, 3 subgenera) and *Scaptomyza* Hardy (121



species, 7 subgenera). The remainder have been placed in endemic genera that are quite clearly either *Drosophila*-like (drosophiloid) or *Scaptomyza*-like (scaptomyzoid). Some species seem to combine certain characters of the two genera; this conclusion is based on internal anatomy, external morphology, and behavior. Structural peculiarities are extraordinarily diverse, particularly in males. Characters include protruding eyes, modified mouth parts, and elaborate foretarsi, including bristle-like, spoon-like, or forked adornments. Tibiae are sometimes knobbed; sets of bristles are sometimes missing; wings may have dark spots, an extra crossvein, or show arcuate or truncate shapes. Some *Drosophila* have extremely large body sizes; many species groups are informally recognized. The Maui complex has the greatest number of known species of drosophiloids (160), followed by Hawaii (98), Oahu (84), and Kauai (44); most are endemic to the specific islands from which they have been recorded.

Most drosophiloids are found at high altitudes in areas of high humidity and breed on fermenting vegetable materials. Nine oviposition sites on endemic plants are recognized, of which leaves of *Cheirodendron* (46 species reared) and stems of *Clermontia* (18 species reared) predominate. In all, 32 endemic genera of plants are used. The leaf-breeding species are difficult to rear in the laboratory. Ecological niches include species that breed on spider eggs, decaying flowers, and nondecaying plant hairs.

Scaptomyzoid males demonstrate a lunging, direct courtship, whereas most of the drosophiloids engage in elaborate ritualized displays, during which the secondary sexual characteristics are brought into play. Unlike drosophiloids in the rest of the world, courtship does not occur on the food, but males manifest lek behavior; they defend specific, localized territories.

Metaphase chromosomal data support the general concept of the dichotomy of scaptomyzoids and drosophiloids. The great preponderance of the latter have a karyotype that has been considered primitive for continental forms (five rods; one dot). Heterochromatin has frequently been added to this basic set. Polytene-chromosome mapping has been carried out for 69 large drosophiloids (the picture-winged species group); all can be described in terms of a single set of arbitrary Standard gene orders despite morphological diversity of the species. Except for one deletion, the chromosomes differ only by paracentric inversions, of which 115 are fixed and 63 are polymorphic; two thirds of the species lack chromosomal polymorphism. Species sometimes share a common polymorphism; within several species sharp local variations in gene arrangement frequencies occur, even within an island. This confirms the impression that population sizes are small and that inbreeding prevails. Only one case of sibling species has been recognized; on the contrary 10 series (31 species) are known in which morphological divergence has not been accompanied by any change in

sequential karyotype (homosequential species). The great biological unity of this group is underscored by the ease with which morphologically diverse species may be hybridized in the laboratory; no natural hybrids have been found. Compatability in ovary transplantation is high. Cytological subgroupings generally conform to groupings by behavior and genitalia.

The Hawaiian fauna apparently developed on the older Leeward Islands and migrated down the chain as the islands weathered down. The ancestors of the tremendous present-day fauna were doubtlessly from Kauai, as the cytological and behavioral evidence indicates. On the other hand, one would expect that, since this is the oldest of the main islands, it would support the greatest number of species of native plants and animals. At least with the Drosophilidae, this is not the case. Of 44 species known from Kauai, 5 have also been recorded from other islands; thus apparently 39 species are restricted to the island. It is highly significant in the study of the evolution of this group in Hawaii that of the drosophiloids, only the genus *Drosophila* occurs on Kauai; *Antopocerus*, *Celidosoma*, and *Nudidrosophila* are unknown from there. The extra-vein flies ("idiomyias") are also not found there, nor is the *D. haleakalae* complex. It appears that most of these groups and species complexes have evolved on the Maui complex and radiated out from there to the other main islands. This idea is supported by the cytological data on the picture-winged species and the ethological facts on *Antopocerus*.

*D. primaeva* of Kauai has some polytene-banding sequences that are recognizable in certain mainland forms, especially the predominantly Palearctic *robusta* group. Since such sequences are altered by inversions in other picture-winged species, it appears that *primaeva* is chromosomally closer to the ancestral stock than any other Hawaiian species for which full information is available. With this fact and the polytene chromosome relationships, theoretical evolutionary pathways for the subgroups of picture-winged flies have been constructed. After Pliocene speciation episodes on Kauai, a minimum of 22 interisland founder events are proposed. These include two direct invasions of the Maui complex from Kauai; Oahu is bypassed and is colonized later from the east. Nine colonizations of Hawaii took place in the late Pleistocene. Five of these cases show with great clarity that the ancestors came from Maui and that speciation resulted following the founder events.

The original ancestral stock (or stocks) that invaded the Hawaiian archipelago must have found a habitat completely empty of similar flies. There is a high probability that this ancestral stock was a species that utilized rotting bark as a substrate for its larval development. Significantly, the *virilis* group, which originated from the base of the phylogenetic stem that gave rise to the subgenus *Drosophila*, is exclusively restricted to rotting bark for its developmental stages. In any case, the Hawaiian habitat offered only limited suitable substrate for larval development and adult

nutrition. The native forests have an extreme paucity of fleshy fruits that are so important for many species of *Drosophila* in other parts of the world. Today the lobeliads of the genus *Clermontia* are the only segment of native flora that possesses such fruits, and even these are of modest size. Furthermore, the substrates that were available for the new immigrants must have been spartan in food value, i.e., the available food resources per given volume of substrate.

If only one immigrant was introduced, then this stock early split into the scaptomyzoids and drosophiloids. If there were separate introductions for each of these major groups, then they were constrained by the poverty of food sources to partially overlap in their use of substrates. Both groups radiated into various ecological niches, the scaptomyzoids evolving as physically small species, able to tolerate exposure to higher light intensities and thus able to use substrates such as the flowers of morning-glories and certain nonrotting vegetation as well as the same fermenting materials that the drosophiloids use. The drosophiloid stock evolved species that are in general larger than the scaptomyzoids and more restricted to the areas of low-light intensity in the dense forest.

All the species groups of drosophiloids with the exception of *anomalipes* and *quasianomalipes* appear to display lek behavior. It thus seems reasonable to assume that the ancestors of the major substrates that were used, i.e., the lobeliads, *Cheirodendron* and its relatives, were established on the islands before the drosophiloid immigrants arrived. Likewise, the agents responsible for the selection pressure that resulted in the evolution of the lek behavior must have also preceded the drosophiloids.

A number of factors appear to be responsible for the evolution of extraordinary numbers of species found today in the Hawaiian Islands. The major factors would appear to be the spartan nature of the food supply, which resulted in the evolution of a low reproductive rate and consequently small population sizes; the infrequent but repeated migrations from each island to adjacent islands, which resulted in effective isolating barriers; the added effects of volcanic and meteorological action, which further isolated small areas such as the kipukas; the evolution of lek behavior; and invasion of specialized food sources such as the leaves of a number of plants and the eggs of spiders.

Hawaii appears to be the only place in the world where the otherwise sharp distinctions between *Scaptomyza* and *Drosophila* tend to disappear. The number of species of *Scaptomyza* in Hawaii is twice that for the rest of the world. These facts provide evidence that the entire family in Hawaii could have stemmed from one ancestral introduction, with the genus *Scaptomyza* originating in Hawaii. From data presently available, however, definite espousal of the "one-introduction" over the "two-introduction" hypothesis seems premature.

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