Speciation in the Hawaiian 
Drosophila 

Sexual selection appears to play an important role 

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It has long been recognized that the Hawaiian Islands possess one of the most remarkable insect faunas on Earth and are indeed the ideal natural laboratories for the study of the processes of speciation and evolution (Hardy and Kaneshiro 1981). Nowhere else in the world do we find so many examples of explosive adaptive radiation—the evolution of a large number of divergent forms from a primitive ancestor. By far the most outstanding of these examples is the Hawaiian Drosophilidae (see reviews in Carson et al. 1970, Carson and Kaneshiro 1976, Hardy and Kaneshiro 1981, Kaneshiro 1983).

Here, in this isolated group of Pacific Basin islands situated more than 3500 kilometers from the nearest land mass, are found nearly one-fourth of the known species in the family Drosophilidae. More than 95% (484 out of 509 species in two genera) are endemic to Hawaii; they are found nowhere else in the world. Considering Hawaii’s small land mass—approximately 15,500 square kilometers—and the geological newness of the present-day high islands of the Hawaiian Archipelago, the Hawaiian Drosophilidae represents one of the most striking examples of adaptive radiation known for any group of animals anywhere, and these islands offer a unique opportunity for investigating dynamic stages of evolutionary phenomena.

The Hawaiian drosophilids are closely associated with vegetation in the native ecosystem. Many of the species are composed of extremely small populations with limited distribution. While it is not known how many species might have been lost due to perturbation of native habitats, there are clear indications of the fragility of these endemic flies. Within the last two decades of intensive evolutionary research on these flies, several species are known to have become extremely rare or extinct. A number of proposed and ongoing projects could have a major impact on a number of these rare and threatened drosophilid species. These projects include the geothermal development programs within native rain forests on the islands of Hawaii and Maui and a proposal to eradicate tephritid fruit fly species from the State of Hawaii by applying chemical pesticides, even in areas bordering native habitats. It will be important to pay close attention to potentially destructive projects in order to minimize their impact on Hawaii’s extremely fragile ecosystems.

Hardy’s (1963) taxonomic study of the Hawaiian drosophilids included 400 species. Since then, 89 additional species have been named and described (Hardy and Kaneshiro 1981). At least 200–250 more new species in this family are already present in the collection at the University of Hawaii but have not been described. More new species are still being collected as additional sites within the islands are sampled, and the total Hawaiian drosophilid fauna may exceed 800 species.

The Hawaiian drosophilids have not only developed a fantastic number of species, but also exhibit greater diversity of forms and habits than any other group in this family. The bodies and wings are often ornately patterned, and unusual modifications of the mouthparts and legs of the males frequently occur (Figure 1). Many of these features would be considered to signify different genera among species from other areas of the world, but in the Hawaiian drosophilids, these features have been shown to be of only species group importance. Spieth (1966, 1974) demonstrated that these structural peculiarities in the Hawaiian males are manifestations of oftentimes bizarre courtship patterns. The Hawaiian drosophilids are the birds-of-paradise of the insect world.

With the exception of the endemic genus Titanochaeta, which preys upon spider eggs, the Hawaiian drosophilids are predominantly saprophytic. The larvae feed as scavengers on decaying bark, leaves, and occasionally on flowers and fruits of native plants. They also feed on slime fluxes and fleshy fungi.
Heed (1968) lists nine major ovipositional substrates for these flies. Although a few species are generalists in their ovipositional behavior (e.g., Drosophila grimshawi from the Maui complex of islands will oviposit in more than ten different families of native plants), the majority are strict specialists, ovipositing in one or two plant species. It seems that, at least for the picture-winged species group of Hawaiian Drosophila, the degree of specialization in larval breeding site is associated with ovipositional behavior of the females rather than the nutritional requirements of the larval stages (Carson and Kaneshiro 1976). After a small piece of their natural substrate stimulates gravid females of specialist species to oviposit, the larvae will develop fully in standard laboratory medium.

In spite of their morphological, ecological, and behavioral diversities, the Hawaiian drosophilids constitute an extremely close-knit evolutionary group. From the data to date, it appears that the entire endemic fauna could have arisen from a single successful founder. Although there are two major lineages in Hawaii, the drosophiloids and the scaptomyzoids, several lines of evidence show distinct similarities in the two groups, and only in Hawaii do these two groups intergrade. Throckmorton (1966, p. 386), on the basis of comparative anatomy of internal structures of the two groups, states "... Hawaii must be considered to be the only place in the world where the otherwise sharp distinctions between Scaptomyza and Drosophila tend to disappear." In a major review of the family Drosophilidae, Throckmorton (1975, pp. 455-456) further states that although "... the problem of the origin of the drosophiloid and scaptomyzoid lineages of Hawaii remain unresolved... the parsimonious inference from the evidence, in its present state, still favors the origin of all the Hawaiian Drosophilids from a single introduction."

The ultimate goal of the systematist is to be able to arrange any group of organisms in a phylogenetic scheme that portrays evolutionary history. It is clear that such phylogenetic relationships must be based upon supportive information from many disciplines (e.g., morphology, genetics, ecology, behavior, physiology, and biochemical studies).

One of the most comprehensive methods of zoological classification has been developed during the last 25 years by the Hawaiian Drosophila Research Project, with support from the National Institutes of Health and the National Science Foundation. Investigators in Hawaii and in laboratories in the continental United States, Europe, Asia, and Australia are using a variety of genetic techniques, including isozyme analyses, DNA sequencing, chromosomal analyses, hybridization, and behavioral genetics, to obtain important information on the mechanism of speciation in this group of insects.

Nevertheless, some important questions remain. Did one introduction into the Hawaiian Islands really give rise to this large, endemic droso-

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philid fauna? Which particular non-Hawaiian group is the ancestor of the Hawaiian drosophilids? How old is the endemic drosophilid fauna (see Beverley and Wilson 1985)? How many drosophilid species (extant and extinct) have there been in the Hawaiian fauna? Why has there been such an explosion of species in the Hawaiian Islands, not just in the drosophilids but in other groups of animals as well?

In this article I present a brief overview of the Hawaiian Drosophila research group's results that illustrate mechanisms of species formation. I focus on the studies of the giant polytene chromosomes found in the salivary glands of Drosophila's last larval instar, and I discuss the recent analyses of molecular structures and patterns observed in these species. Finally, I briefly discuss what appears to be a powerful force in initiating the speciation process in the Hawaiian Drosophila, the dynamics of the sexual selection process.

Chromosomal data

The banding pattern of the giant polytene chromosomes found in the nuclei of salivary gland cells of certain dipteran groups reflect the sequence of genes on these chromosomes. With proper preparation and staining, the banding sequence of these polytene chromosomes lends itself to detailed comparisons among species. Such techniques have been especially useful for inferring phylogenetic relationships among species in the cosmopolitan genus Drosophila, but the picture-winged species group of the Hawaiian Drosophila provides an outstanding example of this technique's usefulness.

The banding pattern of the polytene chromosomes of more than a hundred picture-winged species have been analyzed in detail. Based on the similarity of banding sequences, each species has been positioned on a phylogenetic tree. Differences in the banding sequence are almost always due to inversions of the paracentric regions (i.e., a region of chromosome not including the centromere is rotated 180°, such that the gene sequence in the segment is reversed in relation to the rest of the chromosome; see Figure 2).

The chromosome studies on the picture-winged species of Hawaiian Drosophila conducted by Hampton L. Carson and his colleagues (Carson and Kaneshiro 1976, Carson and Yoon 1981) include detailed analyses of 213 inversions in 103 species. However, these inversions are unevenly distributed among the species; nearly two-thirds of the species have no fixed inversion differences.

Even among species whose banding patterns contain different inversions, the number of fixed inversion differences between the most closely related species is relatively small. For example, 25 species can be differentiated from their closest relatives by no more than two fixed inversions. In contrast, morphologically similar (sibling) species of Drosophila elsewhere in the world typically exhibit several fixed inversion differences in their polytene chromosomes. For example, Drosophila melanogaster and its sibling species Drosophila simulans, which are found in the continental United States, can only be distinguished morphologically by differences in the male genitalia, but they can be readily distinguished cytologically by more than a dozen inversion differences.

Despite the high degree of conservatism in the chromosomal banding sequences, closely related species in the Hawaiian drosophilids are morphologically distinguishable. In fact, the males of Hawaiian picture-winged species have striking secondary sexual structures that are easily distinguished. While in most cases, species sharing banding patterns (and therefore called homosequential species) are considered to be most closely related, there are situations where species identical in their chromosomal banding patterns are believed to belong to separate subgroups based on male genitalia characters (Kaneshiro 1968).

For example, the chromosomes of Drosophila pilimana, Drosophila glabriapex, Drosophila vescicata, and Drosophila aglaia are homosequential, but the species can be divided into three subgroups based on differences in male genitalia. In fact, morphological analysis indicates that D. pilimana and D. glabriapex are more closely related to three other species, from which they differ by at least two fixed inversion differences in their banding sequence, than they are to the others in the homosequential group. On the other hand, D. vescicata, is closely related morphologically to another group of species, which may differ from it by as many as four fixed inversions.

Species relationships based on morphology, especially that of male genitalia, is corroborated by analyses of courtship behavior and larval breeding-site ecology. Thus, it appears that similarity in chromosomal banding pattern does not necessarily indicate degree of relatedness in the Hawaiian species.

The contrast between these species' great morphological diversity and their conservative pattern of chromosomal banding indicates that chromosomal mutations via paracentric inversions have not been especially important in the dynamics of the speciation process. Considering the newness of the Hawaiian Islands, and thus the relatively brief evolutionary history of the endemic fauna, it is postulated that the initial stages of species formation are influenced by factors that do not correlate with chromosomal inversions. In contrast, evolutionarily older species outside Hawaii show large differences in the banding pattern that correlate with evolutionary distance between species.

Differentiation at the gene level?

The similarity in the banding sequences led investigators to look elsewhere for indicators of genetic divergence. In the heyday of starch-gel electrophoresis, which is a technique used to separate proteins on the basis of their molecular size and electrical charge, more than 25 loci representing genes that code for a number of protein and enzyme systems were analyzed for many of the Hawaiian picture-winged species (Craddock and Johnson 1979, Johnson et al. 1975, Sene and Carson 1977). Much to the disappointment of the molecular biologists conducting these studies, they found, as in the cytological studies, an extremely high degree of genetic similarity within groups of related species.

Indices of genetic similarity can be calculated from the electrophoretic
gene-frequency data (Nei 1972, Rogers 1972), and the amount of genetic differentiation between different levels of evolutionary divergence has been classified (Ayala et al. 1974). For example, populations of the same species in different geographic locations are expected to show an average similarity index (I) greater than 0.95. Sibling species give an index of approximately 0.56, while morphologically distinct species show an average index of about 0.35.

Based on these criteria, most Hawaiian picture-winged species would be considered subspecies rather than separate species. Even between species pairs that are so morphologically distinctive that even a taxonomist who does not specialize in flies could separate them without the aid of a microscope, the similarity indices can be as high as 0.90.

With the advancement of molecular technology, evolutionists have been investigating the detailed structures of the DNA molecule (DeSalle and Giddings 1986, DeSalle et al. 1986a, 1986b, Dickinson 1980, Hunt and Carson 1982, Hunt et al. 1981) looking for greater resolution of phylogenetic relationships among groups of species. For the Hawaiian drosophilids, these techniques have revealed so far only minor changes at the molecular level between closely related species. More sophisticated sequencing of various DNA molecules is now being conducted in hope of gaining the information needed to measure genetic divergence between closely related species.

Data from molecular studies have also been useful for determining for a variety of organisms the time since divergence of related species (i.e., the "molecular clock"). However, it is necessary to exercise caution in interpreting such data from single proteins. For example, in a recent study by Beverley and Wilson (1985), it was proposed that the ancestor of the Hawaiian drosophilids may have arrived in the islands as long as 40 million years ago—when only part of the Emperor Seamounts of the Hawaiian Chain was in existence and long before Kauai, the oldest of the present high islands, rose above sea level. This study was based on the analysis of a single protein, the larval hemolymph protein, in a few representative Hawaiian species. The phylogenetic relationships determined from this data do not agree with available morphological, behavioral, and ecological data. Therefore, the suggested age of the Hawaiian species is questionable. Where possible, data from as many different aspects of the biology of the organism need to be included in determining the evolutionary history of a group of species.

**Dynamics of sexual selection**

What is the driving force behind the speciation mechanism that has resulted in genetic divergence, but with only minor differentiation in gross chromosomal morphology and specific structural genes? For a clue, look once again at the morphological diversity in these species. Spieth (1966, 1968) first reported that the tremendous morphological diversity in these species is a manifestation of the males' elaborate mating dance. (Morphological differentiation is more evident in the males, while females of closely related species are, for the most part, nearly indistinguishable.) Spieth showed that all the sometimes bizarre secondary sexual characters found in the males are used in some way during their complex sexual displays. Sexual selection clearly plays an extremely important role in the speciation pattern of this remarkable group of organisms.

Only recently, however, have biologists begun to investigate the dynamics of sexual selection in the speciation process. About 10 years ago, mate-preference experiments between allopatric (geographically separate) pairs of closely related species (Kaneshiro 1976) resulted in data indicating asymmetrical sexual isolation (i.e., females from population A discriminate strongly against the males from population B, but, in the reciprocal direction, females from population B accept the courtship overtures of males from population A).

I studied the mate preference among four species of the planitibia complex of Hawaiian Drosophila and found that females of the species from geologically older islands were highly discriminant against males from geologically younger islands. On the other hand, females from the younger islands accepted males from the older islands oftentimes even better than males from their own population (Kaneshiro 1976). I proposed that courtship requirements are simplified during the early stages of a founder event, when the population size is small (Kaneshiro 1976, 1980, 1983). In the newly established population, there is strong selection for less discriminating females. Highly discriminating females may never encounter males that are able to satisfy their courtship requirements. Females that are less discriminating will be most likely to mate and leave progeny.

![Figure 2. Banding patterns of polytene chromosome 5 (top) and chromosome 3 (bottom) of D. obscuripes, a picture-wing species. Chromosome 5 shows the breakpoints of a single paracentric inversion e; while chromosome 3 shows the breakpoints of four paracentric inversions: m, e, r, and d. From Carson and Stalker (1968).](image-url)
cles (Carson 1968, 1971), there may be a shift in the distribution of female mating types towards those that are less discriminating. Such a shift in the mating system may become fixed in the new population. I have now extended my model to explore the role of sexual selection in the most dynamic stages of the speciation process (Kaneshiro in press, Kaneshiro and Giddings 1987).

Within a population, some males are highly successful in mating with the majority of the females, but other males are less successful in satisfying most females' courtship requirements. There are also females who show little discrimination in mate choice, while others are highly discriminant and they mate with only males of superior courtship ability. Classical sexual selection theory states that there should be strong selection against those individuals whose mating phenotypes deviate from the mean. But I suggest that instead, there is differential selection in the two sexes for the opposite ends of the mating type distribution.

I propose that sexual selection favors both males that are highly successful in mating and females that are not highly discriminant. It selects against males that are unsuccessful in mating and females that are highly discriminating in mate choice. This differential selection between the two sexes can act as the stabilizing agent for the sexual selection process. Matings between a male with superior behavioral qualities and a female that is less discriminant will result in offspring representing the normal distribution of mating types in the population. Unlike classical sexual selection theory, my hypothesis avoids runaway processes (Fisher 1930)—sexually selected characters that develop into features reducing the survivability of individuals. Thus, sexual selection itself maintains a balanced mate-recognition system in the population.

This differential selection model portrays sexual selection as a dynamic system. For example, when a population is small because the environment is hostile, the distribution of female mating types may shift in order that the population survive the stress. Selection, under these conditions, would even more strongly favor females that are less discriminating, because it is even less likely that highly discriminant females would encounter males able to satisfy their courtship requirements.

The differential selection model allows plasticity, permitting shifts in the distribution of mating types in the population. This characteristic is most important in the explanation of asymmetrical mating isolation between populations. Also, if other parts of the genome are affected pleiotropically by the sexual selection process, then sexual selection may be a mechanism for maintaining levels of genetic variability in the population.

While the Hawaiian Drosophila is an outstanding example of explosive adaptive radiation, the studies of the mating systems in these species suggest that adaptive shifts into novel environments may not be the primary mechanism by which speciation occurs in this group. Rather, shifts within the "sexual environment" play a dominant role in the initial stages of species formation. While genetic changes resulting from such shifts in the mating system can occur rapidly, I do not suggest that the speciation process is completed in a relatively short period of time, as in the punctuated equilibrium model of speciation. Changes within the sexual environment may be the entering "wedge" for the speciation process; other genetic changes within the population accumulate much more slowly.

Conclusions

As more than 20 years of research on the Hawaiian Drosophilidae, it is now apparent that comparatively small amounts of detectable genetic changes, as can be measured by cytological, electrophoretic, or molecular techniques, have occurred between species. Two explanations are possible for these observations. First, these species may be of such recent origin that their brief evolutionary history has not permitted the accumulation of significant genetic difference among related species. Second, factors other than those that involve chromosomal or allozymic changes may be involved in the dynamic stages of the speciation process in this fauna.

Studies of the elaborate mating system in these flies provide strong testimony to the significant role sexual selection has played in the speciation process. While the forces of natural selection are still considered to be important in directing the course of evolution in a population, adaptive shifts in response to changes in the external environment occur over a much longer period of time than adjustments to the mating system (i.e., the sexual environment), which are believed to occur during the initial, most dynamic stages of the speciation process.

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References cited


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