

INVITED REVIEW

**Dynamics of Sexual Selection in the Hawaiian Drosophilidae:
A Paradigm for Evolutionary Change**

Kenneth Y. Kaneshiro

Center for Conservation Research and Training, University of Hawaii at Manoa, 3050 Maile Way,
Gilmore Hall 406, Honolulu, Hawaii 96822. E-mail: kykanesh@hawaii.edu

Abstract. Research on the Hawaiian Drosophilidae has been ongoing for more than four decades and has served as a model system for understanding mechanisms of speciation not only for biota within the Hawaiian Archipelago but also for other groups elsewhere in the world. The multidisciplinary approach has been extremely productive and the results of the research have provided significant insights into the genetic mechanisms that are critical during the initial stages of species formation. While classical theories of evolutionary biology have been and continue to be tested using this group of insects, researchers who have been involved in the Hawaiian *Drosophila* project have also been able to formulate new ideas and new theories of evolutionary processes. In this paper, the dynamics of sexual selection, i.e. changes within the sexual environment of a population, are reviewed as an important driver of evolutionary change especially during the initial stages of species formation. Thus, while the Hawaiian Drosophilidae has often been cited as an excellent example of explosive adaptive radiation, sexual selection has played an even greater role in the evolution of this group.

Introduction

About 150 years ago, Charles Darwin, in his voyage of discovery on the H.M.S. *Beagle*, visited the Galapagos Islands, a group of isolated, volcanic islands in the western Pacific, off the coast of Ecuador. Darwin's observations of the biota of these islands were focused on two groups, the birds and the reptiles, and based on his studies, he formulated his theory of evolution by natural selection. One of the classic quotes from his diary states:

“Seeing every height crowned with its crater and the boundaries of most of the lava-streams still distinct, we are led to believe that within a period, geologically recent, the unbroken ocean was here spread out. Hence both in space and time, we seem to be brought somewhat near to that great fact – that mystery of mysteries – the first appearance of new beings on this earth.” (Darwin 1859).

Darwin certainly could have been describing the geology and biology of the Hawaiian Islands. Unfortunately, the H.M.S. *Beagle* did not stop in the Hawaiian Islands and Darwin did not have an opportunity to observe the endemic biota of these wondrous islands where evidence of island evolution clearly surpasses what he saw in the Galapagos.

Approximately half a century after Darwin's voyage to the Galapagos, an entomologist by the name of R.C.L. Perkins conducted an extensive survey of Hawaii's entomofauna during the period from 1893 to 1897. While Perkins collected many different groups of insects in Hawaii, he focused primarily on two groups, the *Megalagrion* damselflies and the platynine carabid beetles. Some of the insect groups presented logistical problems because of the remoteness and the difficult field conditions faced by entomologists during this pe-

riod. In the introduction to his book, *Fauna Hawaiiensis*, Perkins (1913) commented that "...the minute and obscure Diptera, the endemic species largely consisting of small Dolichopodidae, which shrink and distort on drying, and of infinite numbers of Drosophilidae, many of these also becoming distorted, have been little collected." Harsh field conditions deterred Perkins from making an extensive collection of these soft-bodied groups of species. Nevertheless, based on his "limited" sampling of the drosophilid fauna in Hawaii, he made the following prediction.

"*Drosophila* is represented by an assemblage of species, exhibiting great diversity in structure and appearance....To make an approximately complete collection and thorough study of the Hawaiian species would require the devotion of many years of special work. Not less than 250 species must exist in the islands, and double that number may very probably occur" (Perkins 1913:189).

Another 50 years passed before another entomologist, Elwood C. Zimmerman, published a paper in the journal *Evolution* describing the remarkable drosophilid fauna in Hawaii and challenged geneticists and evolutionists to take the opportunity to conduct "advanced research" on this group of insects. He wrote:

"Since I became aware, many years ago, of the astonishing development of *Drosophila* in Hawaii, I have tried to interest geneticists and evolutionists in the fauna, but I fear that it has been considered, at least by some workers, that my descriptions of the size and diversity of the fauna are exaggerated. I do not exaggerate. It is possible that the Hawaiian drosophilid fauna may be the most remarkable in the world. ...There may be as many as 300 species concentrated in an area smaller than the little state of Massachusetts or less than one-fifth the size of Ireland. Where else has such a drosophilid fauna developed? Is this fauna not worthy of detailed attention by those equipped to do advanced research on the genetics and evolution of this group of fascinating flies?" (Zimmerman 1958:557).

After Zimmerman's (1958) plea to investigate the Hawaiian drosophilids, a dipterist by the name of D. Elmo Hardy in the Department of Entomology at the University of Hawaii and a geneticist by the name of Wilson Stone, who served as Director of the Genetics Foundation at the University of Texas at Austin, agreed to a joint research project between the two institutions on the genetics and evolution of Hawaiian Drosophilidae. On July 1, 1963, an award from the National Institutes of Health (NIH) marked the beginning of the Hawaiian *Drosophila* Project. Stone and Hardy assembled the initial team of investigators to take a transdisciplinary approach toward understanding the explosive adaptive radiation of the endemic drosophilid fauna in Hawaii. The 1963 team consisted of: Hampton L. Carson and Harrison D. Stalker, cytogeneticists from Washington University (St. Louis, Missouri); Herman T. Spieth, behavioral ecologist from University of California (Riverside); Marshall R. Wheeler, taxonomist from University of Texas (Austin); Frances E. Clayton, cytogeneticist from the University of Arkansas (Fayetteville); William B. Heed, ecologist from the University of Arizona (Tucson); Lynn Throckmorton morphologist and developmental biologist from the University of Chicago; plus Hardy and Stone as Principal Investigators.

During the first year of the Hawaiian *Drosophila* Project, a key barrier to conducting sophisticated genetic and other evolutionary research in the laboratory was the ability to rear and maintain laboratory cultures. After trying numerous different ingredients including cereals with high protein and vitamin content, Wheeler and Clayton (1965) devised a culture medium, that opened the door for researchers to conduct a multidisciplinary study

toward understanding the evolutionary processes involved in the explosive speciation observed in this part of the endemic Hawaiian fauna. Over the past 40+ years, more than 80 senior scientists from around the world have participated in investigating the evolutionary biology of the Hawaiian Drosophilidae resulting in more than 400 papers being published in peer-reviewed journals.

Adaptive Radiation

Hardy's (1965) monograph, in which nearly 400 new species in the family Drosophilidae were named and described, provided the taxonomic tool for all future evolutionary studies on the group. Today, more than 500 species of Hawaiian Drosophilidae have been named and described and at least another 250 new and undescribed species are already in the collection at the University of Hawaii. It is estimated that there could be a thousand species in the endemic drosophilid fauna as new species continue to be collected from previously unsampled localities (Kaneshiro et al. 1995). Incredibly, the entire group may have descended from a single founder or at the most, two colonists that survived the trans-Pacific journey to the archipelago millions of years ago (Throckmorton 1966). The endemic species have radiated into an extremely diverse range of habitats from desert-like environments where the soil is powdery dry to wet rain forests with lush tree-fern jungles. For the most part, the larval stages have adapted to saprophytic niches in decaying parts of native plants, fungi and mushrooms, flowers, and slime fluxes. There is little doubt that the Hawaiian Drosophilidae is truly an amazing example of explosive adaptive radiation

Perkins' (1913) detailed observations of the breeding ecology of the Hawaiian drosophilids laid the foundation for future studies on the ecology of these species. The diversity of larval breeding substrates is clearly described in his works "...Some of the species are quite conspicuous, and are readily attracted by the sap oozing from a broken limb of a tree, or from exudations caused by decay or disease. Very many breed in stems of trees or plants, which, when decaying, yield abundant moisture, such as those of the arborescent lobelia, of banana, tree ferns, etc." (Perkins 1913:189). Based on Perkins' observations as a starting point, William B. Heed, an ecologist from the University of Arizona who had extensive field experience studying the ecology of drosophilids from the neotropical rain forests in Central America, began a systematic study of larval breeding ecology of the native Hawaiian drosophilids. He collected several hundreds of decaying leaves of nearly all of the plant species in the native Hawaiian forest, decaying branches and bark like those described by Perkins, slime flux (fermenting sap) that oozed from the trunks and branches of native trees and shrubs, different kinds of fungi and mushrooms that he found in the Hawaiian rainforests, and other substrates that appeared to be potential breeding sites for the Hawaiian drosophilids (Heed, 1968, 1971). The results of his studies indicated that for most of the drosophilid species, the larval breeding substrate was host specific and that females of each species oviposited in not only specific host plants but in only certain parts of the plant. That is, there are groups of species that lay their eggs only in decaying leaves (consequently often referred to as "leaf breeders"), while others oviposit only in decaying bark or branches (i.e. "bark breeders"). Others lay their eggs only in slime flux material in very specific host plants. These observations provided important baseline information and rationale for understanding how so many species can coexist in the same habitat without severe competition for larval breeding substrates.

The ecological studies of Heed and those of Steven L. Montgomery (1975) were critical for being able to culture those Hawaiian *Drosophila* species that refused to oviposit in the artificial laboratory medium. Kaneshiro (unpubl. obs. from the mid-1960s through the early 1970s) found that by placing pieces of bark or other plant material on which these species

oviposited in nature in a jar of sterile distilled water, it was possible to prepare a "tea" after a few days in ambient room temperature. Then, by placing a drop or two of the tea onto a folded piece of tissue inserted into a vial of mature adults, it was often possible to stimulate the females to lay eggs into the tissue. Once the eggs hatched, the larvae crawled down into the Wheeler-Clayton medium and quickly began to feed. By supplementing the yeastless Wheeler-Clayton medium with a cornmeal medium (containing yeast), the larvae were able to complete development, pupate and eclose as healthy adults. Many of the species that could not previously be reared in the laboratory because the females refused to lay any eggs, could now be reared and maintained in laboratory colonies for further investigation into their genetics, behavior and physiology.

While the ecological diversity of the Hawaiian Drosophilidae is not the primary thesis of this paper, it is important to note that the group provides an excellent example of adaptive radiation into a diverse range of macro- and microniches. However, the evolutionary research conducted on the Hawaiian Drosophilidae indicates that natural selection, while important in the evolution of the group, has not played the most important role during the initial stages of species formation (Kaneshiro 1993). Rather it has been suggested that sexual selection and shifts within the sexual environment have been much more important as the driver of evolutionary change (Kaneshiro 1989, Carson 1997).

Sexual Behavior

The studies of Herman T. Spieth on the elaborate courtship and mating behavior of the Hawaiian drosophilids were also a critical turning point in understanding the evolutionary processes responsible for the explosive speciation observed in this group of insects (Spieth 1966, 1968a, 1968b, 1974a, 1974b). His observations of the complex courtship displays showed that the oftentimes bizarre secondary sexual structures found in the males, were used as part of the mating ritual. In some cases, these secondary sexual characters were used to group species into separate genera. For example, species in the former genus *Antopocerus* were characterized by the males having a long, whip-like arista with shorter, dense hairs on the ventral and dorsal surfaces all of which is significantly different to what is characteristic of species in the genus *Drosophila*. Spieth observed that during the final stages of the courtship ritual, the male of *Antopocerus* species moves to the rear of the female, posture himself with his head under the females' wings, lift her wings using the specialized antennal features, and begin to vibrate his wings just prior to an attempt to copulate. In contrast, the females of the *Antopocerus* group, had antennal characteristics more typical of the genus *Drosophila*. Males of the former genus *Ateledrosophila* lack bristles on the dorsal surface of the head which are characteristic of the genus *Drosophila*, while the females of this group have the typical structures that characterize the genus *Drosophila*. The situation is similar for other groups of species such as the modified tarsus group and the modified mouthparts group, where the males are characterized by spectacular secondary sexual structures while the females lack such features. Although taxonomists, including Grimshaw (1901-1902) and Hardy (1965), used these characters to delineate species groups and even to separate species that shared such divergent characters into distinct genera, Spieth's studies provided evidence that led contemporary systematists to place species formerly described in four endemic Hawaiian genera (*Antopocerus*, *Ateledrosophila*, *Idiomyia*, and *Nudidrosophila*) in the genus *Drosophila* (Kaneshiro 1976).

Thus, Kaneshiro (1976), in a revision of generic concepts of the Hawaiian Drosophilidae, based on a detailed study of the external male genitalia and the extensive ecological (Heed 1968, 1971), behavioral (Spieth 1966, 1968a, 1968b), and cytogenetic data, synonymized three of the endemic genera with the genus *Drosophila*. It was shown that the key charac-

ters used by Hardy (1965) to differentiate species into the genera *Antopocerus*, *Nudidrosophila* and *Ateledrosophila* were not valid characters that warrant generic groupings. As shown by Spieth's studies, the characters used by the earlier taxonomists were secondary sexual characters used during the elaborate courtship sequence displayed by the males and that generic groupings based on these characters would provide a misleading interpretation of evolutionary divergence in the endemic Drosophilidae.

Spieth (1966, 1968a, 1968b, 1974a, 1974b, 1982) was also the first to observe and describe lek mating behavior in the Hawaiian *Drosophila*. While his descriptions of the physical and environmental characteristics of the lek sites (mating arenas) were mostly qualitative in nature, they were extremely important observations which enabled field researchers to find and collect many of the species which were not readily attracted to the standard baiting techniques. He observed that males of some of the picture-winged species, for example, took up station on horizontal branches or on the fronds of tree ferns and defended a section of the substrate against other males in competition for receptive females that may arrive at these territories. He found that males of other species occupied the underside of single leaves (usually of its larval host plant) as mating territories. Clearly, the lek mating system and the often fierce battles among males in defense of preferred territories within the mating arena as well as the elaborate courtship displays that take place on these territories indicate the intensification of the sexual selection system and the significant role that it plays in the evolutionary processes responsible for the explosive speciation of this incredible group of insects.

The Hawaiian Drosophilidae as a Model for Understanding Evolutionary Processes

In his book entitled *Progress and Prospects in Evolutionary Biology: The Drosophila Model*, Powell (1997:3) makes the statement:

Of the millions of species that inhabit the earth, biological researchers tend to concentrate on relatively few organisms that subsequently become "model systems." The reason is obvious: Research builds on past research. To advance the forefront of knowledge, the system one studies must be known up to that forefront.... Many organisms have been studied, and by a process not unlike natural selection, certain organisms come to the fore as popular models. Examples include the house mouse, yeast, *Escherichia coli*, corn and *Drosophila*.... Of all these models, it is arguable that none has received as much attention as has *Drosophila*.

In his book entitled *Island Populations*, Williamson (1981:168) made the following statement:

"Of all the groups of organisms, plants or animals that can be studied on islands the Hawaiian Drosophilidae are supreme. This is why. There are a great many species; their ecology is varied; and most important, a genetic analysis is possible..."

Williamson then proceeded to describe the multidisciplinary approach to the study of the Hawaiian Drosophilidae as being one of the most outstanding in the field of evolutionary biology. In the ensuing pages, I present a brief overview of how the research on this amazing group of insects has contributed not only toward testing classical concepts of evolutionary theory but also to the formulation of new ideas and concepts of evolutionary processes.

The Founder Principle of Speciation

The idea of the founder principle of speciation originated with Darwin's (1859) observations of the amazing organisms on the Galapagos Islands where he noted that the endemic fauna was derived from a few stray colonists from the South American continent a few hundred miles to the east. However, it was Mayr (1942) who first formulated the concept of the founder principle and defined it as "...the establishment of a new population by a few original founders (in an extreme case, by a single fertilized female) which carry only a small fraction of the total genetic variation of the parental population..." (Mayr 1963). Mayr also suggested that the severe bottleneck situation and the accompanying drift conditions resulted in a reduction in levels of genetic variability which in turn led to rearrangements of the coadapted gene complexes. He referred to this phenomenon as the "genetic revolution" that accompanies founder events.

Carson (1968, 1971a) proposed a modified version of Mayr's founder principle based on his chromosomal studies and the phylogenetic analyses of the picture-winged group which he called the "founder-flush" model. He suggested that while drift serves to perturb the coadapted genetic system of the founding population, the founder event itself is followed by rapid population growth due to relaxed selection pressures in the new environment/habitat. He referred to this as the "flush" phase of his model and that the rapid increase in population size minimizes the reduction in variability due to drift. Furthermore, Carson suggested that the relaxed selective conditions allowed new recombinants to persist in the population that would normally have been selected against in the ancestral population. In contrast to Mayr's model then, because of the increased levels of genetic variability following the flush phase of Carson's model, the population is better able to respond to the selective pressures present in changing environmental conditions.

There are other versions of the Founder Principle which are variations of Mayr and Carson's ideas. For example, Templeton (1980, and Carson and Templeton 1984) proposed the "genetic transience" model which stresses the importance of pleiotropic effects of the genetic environment while deemphasizing the significance of the external environment. Others such as Coyne and Orr (2004, p. 394) trivializes founder effect theories simply because "...they are not necessary: adaptive radiation can explain most or all of the patterns attributed by founder event theorists to genetic drift." They claim that speciation events do not require small population sizes as suggested by the Founder Principle and that larger populations should respond more readily to selection.

Powell (1978), Dodd and Powell (1985), Ringo (1977), and Meffert and Bryant (1991) conducted experiments to provide further evidence for the founder principle in the process of speciation. After subjecting *Drosophila* species (in the former three studies) and houseflies (in the Meffert and Bryant study) to a series of bottleneck events, several of the lines showed varying levels of reproductive isolation. Coyne and Orr (2004) claimed that these studies reported "weak and erratic results".

Sexual Selection as a Driver for Evolutionary Change

Spieth's qualitative descriptions of the complex mating behaviors displayed by the males of the Hawaiian *Drosophila* species laid the foundation for future studies of the sexual selection system in this group. Based on mate choice experiments among a small group of picture-winged species, Kaneshiro (1976b) reported on a peculiar, but not infrequent, outcome of these kinds of mate choice experiments — asymmetrical sexual isolation between reciprocal crosses. That is, females from one population (e.g. population A) may accept the courtship displays of males from another population (population B), resulting in successful matings. However, in the reciprocal combination, females of population B were less likely

to mate with males of population A. Kaneshiro (1976b) suggested that such shifts in behavior are a result of the severe drift conditions and the genetic revolution that accompany founder events. In subsequent papers, Kaneshiro (1980, 1983) hypothesized that the courtship requirements of females in more recently derived populations were simplified during the early stages of founder events and successful colonization, when the population size is very much reduced. A relaxation of sexual selection may accompany founder events because successful establishment of the new population is likely to be enhanced when the behavioral pattern is simplified.

Initially, Kaneshiro's (1976, 1980, 1983) observations of asymmetrical sexual isolation among related species led to the hypothesis that females of ancestral species discriminated against males of more derived species but that, conversely, females of derived species mated readily with males from an ancestral species. Thus, results of mate choice experiments using the protocol described in Kaneshiro's (1976, 1980) papers as well as other studies (e.g. Ahearn, 1980; Ahearn et.al., 1974; Ohta, 1978; Arita and Kaneshiro, 1979), provided a tool with which the "direction of evolution" among related species could be predicted.

It was also possible to trace the sequence of colonization/evolution among geographic populations within a species. Kaneshiro and Kurihara (1981) conducted mate choice experiments among six widely distributed populations of *Drosophila silvestris* from the Island of Hawaii; three from the Kona (southwest – south) side of the Island and three from the Hilo (northeast – east) side of the island (Figure 1). Results of mate preference experiments among laboratory strains established from these six populations enabled Kaneshiro and Kurihara to determine the most ancestral population of this species and the sequence of colonization to the other five regions of the Big Island. They showed that Mt. Hualalai, despite the fact that geologically it is not the oldest volcanic mass on the island, is the site where a Maui progenitor first colonized the island and successfully produced a founding population. From there, there were two lineages; one that established populations southward from Hualalai on the southwest and south slopes of Mauna Loa. The second lineage colonized the Kohala Mts. (which is older than Hualalai in geologic origin) and consequently the north and northeast slopes of Mauna Kea and finally the Kilauea Volcano, geologically, the youngest and volcanically most active area on the Island of Hawaii. The data from the mate preference studies of Kaneshiro and Kurihara (1981) strongly suggest that the populations from the Hilo (northeast) side of the island were more recently derived from the Hualalai population.

In a subsequent study, Carson and Bryant (1979) discussed the evolution of an incipient morphotype in certain populations on Hawai'i Island of *D. silvestris* which they believed to play an important role in the courtship behavior displayed by the males of this species. They showed that males from populations on the Kona (southwestern) side of the island has only two rows of cilia on the antero- and postero-dorsal surface of the foretibia. They reported that the males use the ciliated surface of the foretibia to "drum" on the dorsal surface of the female's abdomen during the final stages of the courtship display. They also discovered that the males from the Hilo (northeastern) side of the island had an extra row of cilia (three-row populations) on the dorsal surface in between the other two rows. The extra row of cilia in the populations on the Hilo side is believed to be a derived character since both of the putative ancestral species from Maui (*D. planitibia*) and Molokai (*D. differens*) have only two rows of cilia on the foretibia. The conclusions based on the mate preference studies (Kaneshiro and Kurihara 1981) are consistent with the conclusions of Carson and Bryant based on their comparative morphological studies.

In a related study, Carson and Teramoto (1984) conducted artificial selection experiments to select for high and low cilia number in the extra row of the foretibia. Interestingly, while they were successful in selecting for lines that had significantly different numbers of

cilia from the mean number, they were not able to maintain strains whose males had higher or lower than normal cilia number. They concluded their study by stating that sexual selection acts as a powerful stabilizing force in maintaining an optimal male phenotype and that "...selection in either direction away from the population mean produces dysgenic lines that cannot be maintained in the laboratory." They further suggested that "...females may favor males with cilia numbers near the mean of the population..." and that "...the sexual selection system may operate by selecting for high-fitness heterozygotes, thus providing for the maintenance of genetic variability for this character."

However, the mate preference experiments conducted by Kaneshiro and Kurihara (1981) between two-row and three-row populations indicate a different interpretation of the evolution of this incipient secondary sexual feature. They found that females from the three-row populations actually preferred to mate with males from the two-row populations rather than males from their own three-row populations. This observation refutes the suggestion by Carson and Teramoto (1984) that females may be favoring males with cilia number near the mean of the population and that the sexual selection system may be operating to maintain genetic variability by selecting for "high-fitness heterozygotes". Rather, Kaneshiro (1987) suggested that there may be other pleiotropic effects of the sexual selection system that might explain the polymorphism in secondary sexual characters. The observation that females may in fact prefer to mate with males of species that totally lack secondary sexual features typically found in males from their own species. Kaneshiro (1987) suggested that the extra row of cilia found in the Hilo side populations of *D. silvestris* is being maintained pleiotropically and not necessarily as a result of selection for that character. That is, males with higher mating success have higher cilia number and vice versa. Then, differential sexual selection for opposite ends of the mating distribution within a single interbreeding population and the genetic correlation between the behavioral phenotypes of the two sexes would explain the maintenance of the polymorphism in cilia number of the third row in the Hilo side populations without the sexual selection system having to select for high fitness heterozygotes as suggested by Carson and Teramoto (1984). Thus, the polymorphism seen in these secondary sexual characters is being maintained in pleiotropic balance through the differential selection for males with high mating ability and females with low discrimination.

In the Hawaiian *Drosophila*, Kaneshiro (1987) described another example of striking secondary sexual character which is being maintained in polymorphic balance due to pleiotropic effects of the sexual selection system, rather than selection for an optimal mean. Males of *D. planitibia* from the Island of Maui have a velvety black face while the females of this species have a yellow-brown face. Males of the related species from the Island of Molokai, *D. differens*, however, do not show a secondary sexual feature in the face having a yellow-brown coloration as do the females of this species. When mate preference experiments (Kaneshiro 1976b) are conducted between these two species, females of *D. planitibia* readily accept males of *D. differens*. In yet another example, males of *D. heteroneura* from the island of Hawaii are characterized by the striking hammer-head shape used during male-male aggressive behavior during lek behavior displays in defending mating territories. Males of *D. differens* completely lack such a feature and are characterized by having a normal drosophilid fly head shape. Yet, when mate preference experiments (Kaneshiro 1976b) are conducted between these two species, females of *D. heteroneura* prefer to mate with *D. differens* males over males of their own species despite the fact that *D. differens* males lack the hammer-head feature. Note that *D. differens*, being endemic to the Island of Molokai, is considered to be the putative ancestor of both *D. planitibia* and *D. heteroneura*. This pattern conforms to Kaneshiro's notion that results of mate preference experiments can in some cases be used as predictors of direction of evolution. For both of these species pairs, it is

suggested that the secondary sexual characters are being maintained as part of a balanced pleiotropic system involving other features of the sexual selection system rather than direct selection for the optimum mean of the character as suggested by Carson and Teramoto (1984).

The Dynamics of Sexual Selection as Synergists for Speciation

While observations of asymmetrical mating isolation between populations had been reported by early *Drosophila* researchers (e.g. Dobzhansky and Streisinger, 1944; Reed and Reed, 1950; Rendel, 1951; Bastock, 1956), it was not until the studies of Kaneshiro (1976b, 1980, 1983) that the significance of such asymmetries was better understood. Authors of the earlier studies suggested that asymmetrical mating isolation is attributable to differential mating propensities or perhaps that there were differences in the timing of sexual maturation between the two populations. Following the initial paper by Kaneshiro (1976b), a number of other studies (Kaneshiro 1980, 1983; Kaneshiro & Kurihara, 1981; Arita & Kaneshiro, 1979; Ahearn, 1980; Ohta, 1978; Powell, 1978; Dodd & Powell, 1986) reported on similar observations, which conformed with the suggestion that mating asymmetries could be used as a valuable tool for predicting the “direction of evolution”. However, results of other mate preference studies (Watanabe & Kawanishi, 1979; Wasserman & Koepfer, 1980; Markow, 1981) suggested that the direction of evolution could well be in the opposite direction predicted by the Kaneshiro (1976b, 1980, 1983) hypothesis. In an invited review paper in *Science*, Giddings & Templeton (1983) concluded that “...The Kaneshiro hypothesis has been extensively tested...; all these results are compatible with the Kaneshiro hypothesis...” and “...the application of this model in the future should expand our ability to resolve phylogenetic relationships.” DeSalle and Templeton (1987) state, “...we feel that these recent molecular studies confirm the validity of the Kaneshiro model when its assumptions are satisfied.” Shaw and Lugo (2001) conducted mate choice experiments among species of the native Hawaiian cricket genus *Laupala* and observed similar mating asymmetries between populations. They conclude that “The patterns of asymmetrical sexual isolation and mating asymmetry are consistent with those found in the native Hawaiian *Drosophila*”. Bradley et al. (1991) working on premating behavior of populations of pocket gophers in the genus *Geomys* observed similar mating asymmetries and concluded that “...data from stickleback fish (McPhail 1969), mole rats (Heth and Nevo 1981), and pocket gophers (this study) may prove that the prediction of the Kaneshiro hypothesis is more applicable to vertebrates than previously thought.”

In a paper by Kaneshiro & Giddings (1987), the authors put out a challenge to researchers interested in sexual selection theory:

“The challenge is not just to determine whether mating asymmetries exist within the groups of organisms being studied and whether the direction of evolution predicted by the various asymmetry models points to the correct direction based on other evidence. Rather we hope that investigators will ask the question of why such asymmetries exist and how they arose.”

Kaneshiro (1989) took on the challenge of Kaneshiro & Giddings (1987) and developed a model that provided an explanation for the observed mating asymmetries among related species pairs in the native drosophilid fauna. The mating experiments clearly indicated that there was a range of mating types segregating among both sexes. Among males, there are those that are extremely successful in satisfying the courtship requirements of most of the females in the population while others are less successful in mating and indeed some males

may not mate at all given the opportunity with several receptive females. Carson (1986) and also Kaneshiro (1989) observed that approximately 27% of the males mated with 64% of the females, while 39% of the males mated with approximately 36% of the females and 34% of the males did not mate at all even when provided with five receptive females on 5 successive days. Similarly, among females, there are those that are highly discriminating and those that are not so choosy in mate selection. Kaneshiro (1989) hypothesized that there is strong genetic correlation between male and female behavioral phenotype, i.e. male mating success and female choosiness. Experiments were conducted to select for a strain of choosy females (high line) and one in which the females were not so choosy (low line). Females that had not mated after being given the opportunity to mate with 10 different males over a period of 10 days were placed with males that were considered supermales having mated with several females over a 10 day period to establish a high line. To establish a low line, males which had not mated after having the opportunity to do so with 10 different females over a period of 10 days were placed with virgin females each day for 10 days; when these males were finally successful in mating with a female, it was rationalized that these females were of the non-choosy mating type. The results of the selection for both the high and low lines were astonishing, selecting for male and female behavioral phenotypes, i.e. high mating males with choosy females and low mating males with non-choosy females, simultaneously. The offspring of these lines were tested in mate preference tests against the standard laboratory strain of the species, the deviation from the mean distribution of the mating types segregating in the population was distinct even after a single generation of selection (Kaneshiro, unpubl. obs.). Considering that male mating success and female choosiness are most certainly polygenically controlled, the significant result of selection within a single generation suggests an extremely strong genetic correlation between the mating behavioral phenotypes in the two sexes.

Based on these observations, Kaneshiro (1989) proposed that within a single interbreeding population, the most likely matings are between the males that are most successful in satisfying the courtship requirements of the females and females that are not so choosy in mate selection, i.e. differential selection for opposite ends of the mating distribution in the two sexes. The strong genetic correlation between the behavioral phenotypes in the two sexes observed in the selection experiments would explain the maintenance of the entire range of mating types in the two sexes generation after generation. Thus, the differential sexual selection (DSS) model proposed by Kaneshiro (1989) states that sexual selection itself serves as a stabilizing mechanism in maintaining a balanced polymorphism in the mating system of a population. It avoids the need to invoke the forces of natural selection in maintaining a normal distribution of mating types in the population. Also, the DSS model avoids the problem of reduced genetic variability that results from the rapid coevolution of female preference of a male trait as described by the runaway sexual selection model (Fisher, 1930; O'Donald, 1977, 1980; Lande, 1981, 1982; Kirkpatrick, 1982).

Kaneshiro (1989) further developed the DSS model as a possible mechanism to explain founder event speciation (Mayr 1942; Carson 1971). He suggested that the most likely scenario is that a single fertilized female is carried by some extrinsic force (e.g. storm winds) to an adjacent island. Provided that the female is able to locate a suitable habitat and larval breeding substrate, a small number of offspring may be produced and a new colony established in a new habitat. This has occurred numerous times over the geological/evolutionary history of the Hawaiian Islands and all of the genetic, morphological, and behavioral evidence indicate more than 95% of the species are single island endemics. The closest relative of most of the species is on an adjacent island rather than one that is on the same island. If one considers the DSS model as a frequency-dependent process, during the initial stages of colonization when the population size is small, there would be strong selection for

less choosy females since choosy females, under small population conditions, may not even encounter males that are able to satisfy their courtship requirements. Over a few generations of small population size, and due to the strong genetic correlation between male and female behavioral phenotypes as suggested by the DSS model, there would be a shift in the distribution of mating types towards an increased frequency of less choosy females resulting in a corresponding shift in gene frequencies towards the genotypes of less choosy females. Such conditions serve as a catalyst for destabilization of the coadapted genetic system enabling the generation of novel genetic recombinants some of which may be better adapted to the new habitat or to changing environmental conditions. These new variants may be strongly selected and especially if they are linked or correlated with less choosy females, they could spread throughout the colonizing population very quickly. Thus, during the initial stages of colonization, the dynamics of sexual selection and the DSS model may be playing a critical role in generating a genetic milieu that is conducive to the formation of new species.

Recent Models of Sexual Conflict

Within the last decade, a number of studies report on and discuss the role of “sexual conflict” in speciation (Rice and Holland, 1997; Holland and Rice, 1998; Rice, 1998, Arnqvist et al., 2000, Gavrilets, 2000; Gavrilets et al., 2001; Gavrilets and Waxman, 2002; and many others). Gavrilets and Hayashi (2005) review these studies and refer to the factor known as sexual conflict as an important “engine of speciation”. Sexual conflict is used to describe the antagonistic roles played by the sexes in the process of reproduction. For example, it is argued (Rice and Holland, 1997; Holland and Rice, 1998; and Rice, 1998) that high mating rates have a deleterious effect on female fitness which results in continuous selection for increased “resistance” to mating attempts by males. As a consequence of the resistance in the females toward increased mating rates, the result is selection for males that are able to overcome such resistance. Therefore, the sexual conflict between the sexes over mating rate in turn results in continuous and rapid coevolution of male and female traits that determine mating rate. It is suggested that a by-product of this co-evolutionary process, what has also been referred to as the “arms race” between the sexes, is divergence between allopatric populations “...in these traits quickly losing mutual reproductive compatibility and, thus becoming different species” (Gavrilets and Hayashi, 2005).

One of the key issues of the sexual conflict models is that the empirical evidence is based on laboratory experiments that manipulate the selection process on one or the other sex. Parker and Partridge (1998) used game-theory methods to manipulate selection on males as a force for gene flow and on females to resist mating. Based on the results of their experiments, they concluded that “speciation will be more extensive in groups where females generally win mating conflicts than in those groups where males usually win” (Parker and Partridge, 1998, p. 266). Others (Gavrilets, 2000; Gavrilets et al. 2001; etc.) have developed mathematical treatments of the sexual conflict model and have attempted to refine the significance of the model by testing various assumptions that either enhance or reduce the strength of selection on either sex in the arms race for mating success rate. However, all of these models are laboratory constructs of the sexual selection system without much evidence of their evolutionary significance under natural conditions (for example, Rice, 1996). Carson (2003) describes these studies succinctly: “Behavior that has been variously described as ‘sexual conflict’ or ‘sexual antagonism’ between male and female may be confined to expendable individuals of relatively low Darwinian fitness, and thus are interesting laboratory constructions that have no evolutionary importance in nature.”

In contrast to the sexual conflict models which propose a continuously co-evolving arms

race between the sexes, the DSS model suggests that sexual selection is a stabilizing balanced polymorphic condition that when subjected to bottleneck situations either due to some kind of extrinsic stress condition or founder events, facilitates readjustment (i.e. reorganization) of the co-adapted genetic system via shifts within the sexual environment. The shift in gene frequencies toward the genotypes of less choosy females as suggested by the DSS model and by data collected from laboratory experiments (Kaneshiro and Val, unpublished results) as well as from natural populations (Arita and Kaneshiro, 1979), results in the break-up of the balanced co-adapted system which allows new recombinants to be generated. The novel recombinants that are better adapted to overcome the stress condition or the new habitat (in the case of founder events) will be strongly selected and spread through the population quickly — especially if the new genetic material is linked or correlated with the genotypes of the less choosy females. Thus, the DSS model views sexual selection as a dynamic, frequency-dependent process and as a driver for evolutionary change rather than as a “conflict” between the males and females in the population.

Natural Hybridization and its Role in the Evolutionary Process

Arnold (1997) in his book entitled, *Natural Hybridization in Evolution*, defines natural hybridization as “...successful matings in nature between individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters.” He goes on to say that “...botanists emphasized the evolutionary potential of hybrid genotypes to occupy novel habitats and thus act as progenitors of new clades. In contrast, zoologists championed the view that hybridization was maladaptive because the individuals involved produced fewer and/or less-fertile progeny.” Based on the differences in the way botanists and zoologists have thought of the role of hybridization in evolutionary processes, it is commonly believed that hybridization in plants is widespread while it has been thought that hybridization events between animal taxa are rare. Arnold’s (1997) book cites many examples of hybridization in nature between fully differentiated species or populations and he strongly suggests that natural hybridization events among plants as well as among animals are not as uncommon as previously believed. With the development of molecular tools, e.g. being able to assay maternally inherited mitochondrial DNA within single individuals using PCR techniques, has made it possible to better document cases of natural hybridization as well as to determine the direction of introgression.

It has been suggested that under certain conditions, the dynamics of sexual selection and the differential sexual selection model proposed by Kaneshiro (1989) may actually permit, and, perhaps, even facilitate natural hybridization between closely related species (Kaneshiro, 1990, 1995, 2004). In cases where there are two related species with overlapping distributions, which under normal conditions are strongly sexually isolated, such hybridization events are extremely rare. However, under certain environmental conditions which result in significant reduction in population size in one of the species, the DSS model would prescribe strong selection for less choosy females of the bottlenecked species. Within a few generations of reduced population size, there would be an increase in frequency of less choosy females enhancing the opportunity for hybridization with males from the related species. Thus, it is suggested that the dynamics of the sexual selection system provide opportunities for natural hybridization and permits the “leakage” of genetic material from a related species without compromising the integrity of the separate gene pools (Kaneshiro 1990, 1995). Some of the genes that are leaked across species barriers may be better adapted to the environmental conditions resulting in reduction in population size. Especially if such genes are being transmitted through the genetic system of the less choosy females, it is easy to imagine how selection would introgress such genes throughout the population very quickly.

Thus, it is suggested that natural hybridization which may be permitted by the DSS model may be playing a very important role in not only maintaining but also replenishing genetic variability that may have been lost due to drift during bottlenecked conditions.

The Role of the DSS Model in Invasion Biology

Invasive alien species have major impacts not only on agribusiness but also on native ecosystems. In order to develop more effective pest management programs against such alien species, it is important to understand the biology of “invasiveness”. It is not difficult to accept the notion that the invasion of an alien species, whether it be in the context of a pest of agricultural crops or one that impacts native ecosystems, is similar to the early stages of colonization during founder events. In the most likely case, small numbers of individuals of a foreign species finds their way into a new region whether autonomously, or accidentally or purposely transported by human activities. In many cases, these alien species are not considered to be pests in their native habitats from which they originated. However, during the early stages of colonization in a new habitat, the potential for novel genetic recombinants generated by DSS, can theoretically rapidly become invasive especially in habitats that may be lacking natural predators and parasites. An understanding of the DSS model and the role that DSS can play in the genetic revolution of founder events should encourage regulatory agencies to establish not only effective survey protocols to detect incipient populations of a newly invading alien species, but also to be able to implement and deploy control programs swiftly and effectively. Once an alien population has had the opportunity to colonize and become established, i.e. adapted to the new habitat of the region, control and/or eradication of the species will become exponentially more difficult.

DSS and the Potential for Resistance to Pesticides Used in Control Programs

In addition to the role that the DSS model can play in the invasion biology of alien species, it can also be a major factor in the evolutionary processes that result in insecticide resistance as well as the ability of pest populations to recover from population bottlenecks due to control/suppression programs that allowed residual populations to persist. Kaneshiro (1993a) described the situation with the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), in the Los Angeles area during the period 1986 through 1990. In 1986, a single wild medfly was trapped in the LA area. Because of the less than effective detection program, that single wild fly meant that either that single wild individual which had somehow made its way into the region was coincidentally trapped, or that the wild population had increased above the level of detection such that the single individual represented a small but now resident population. In the ensuing year, 45 wild flies were collected in the survey traps, indicating that the population had increased to an extremely high level. USDA fruit fly researchers (Lance & McGinnis, 1993) estimate that the 45 wild flies trapped in 1987 actually represented a population in the range of 500 – 2000 flies. A massive eradication effort involving both the aerial spraying of a protein bait spray laced with the insecticide malathion together with the sterile insect technique (SIT) was mounted during the peak fruit season and into early autumn when no more wild flies were detected in the survey traps. The eradication was declared a success! Yet in 1988, 54 wild medflies were collected in the survey traps and once again an eradication program was implemented. Similarly, the campaign to eradicate the “new” infestation of medflies that year was declared a success when no more wild flies were detected later in the Fall. However, in 1989-1990, nearly 300 wild individuals were trapped, most of which were collected in the same localities as the

previous three years, except that during this period, wild flies were being trapped throughout the winter months.

By applying the DSS model to the medfly infestation during the 1986-1990 period, it is possible that there would have been strong selection for less choosy females during periods when the population was small, i.e. below detection level. The shift in frequency of mating types accompanied by a corresponding shift in gene frequencies results in destabilization of the coadapted genetic system. This may have allowed the generation of novel recombinants some of which may be better adapted for the urban environment of southern California. By knocking down the population each season when the eradication program was implemented but allowing an incipient residual population to remain, the campaign may have inadvertently selected for a wild medfly population that became better and better adapted to the southern California environment. Thus, resulting in a population explosion during the 1989-1990 season, which even persisted over the winter months (Kaneshiro 1993a, 1993b).

In this case, then, the genetic revolution that accompanies founder events as a result of small population size and the DSS may have played an important role in the establishment of the medfly in the urban California environment (Kaneshiro 2000). In addition, the implementation of the control/eradication program, may have accelerated the adaptation of this species to the novel environment which contributed to the invasion of the medfly into a region where a multi-billion dollar fruit industry might have been severely impacted. Clearly, basic evolutionary research on pest species such as the medfly would further our understanding of the biology of these species. The results of such research would provide a solid foundation upon which more applied aspects of control can be developed. For example, if the DSS model is applied to the 1986 – 1990 medfly infestation in the Los Angeles area, the apparent increase in population size over the 4-5 year period can be easily explained. Furthermore, an understanding of the dynamics of the sexual selection system provided a clue on how to enhance the effectiveness of the control program. DSS suggests that when the wild population is reduced to a low level (i.e., below detection level), there would be strong selection for less-choosy females in the wild population. Under these conditions, the wild population would be most susceptible to the SIT control program, as the less-choosy females in the wild population would likely mate more readily with the released sterile males, and by simply continuing to release sterile flies for a few more generations beyond what was standard protocol at that time, it would be possible to eradicate totally any residual population before there can be further range expansion. In fact, this new protocol was implemented during the 1994 -1995 period and while the evidence is still circumstantial, it appears that the medfly has been totally eradicated from the Los Angeles area.

The Role of DSS in Conservation Biology

Recently, 12 species of Hawaiian *Drosophila* picture-winged species have been officially listed on the Federal Endangered Species List (11 as endangered and 1 as threatened) (Federal Register May 9, 2006). During the Science Panel Discussions as part of the fact finding aspect of the listing process, the natural history of the 12 Hawaiian *Drosophila* species was discussed in detail and the role of sexual selection in the evolution of these species was a major part of that discussion. The DSS model was also discussed in terms of its role in the conservation biology of rare and endangered species which are faced with small population sizes on the brink of extinction. Clearly, an understanding of the biology of small populations is crucial for addressing conservation issues since rare and endangered species are faced with extinction primarily as a result of drastically reduced population size. These population bottleneck conditions are no different than when populations are reduced in size due to environmental stress or during the early stages of colonization following a founder

event. The DSS model as described in this paper permits the generation of new genetic recombinants, some of which are better adapted to the stress conditions that caused the population to decline. The inference here is that if the habitat of species faced with small population size can be sustained by removing the threats, these populations have the capacity and potential to replenish genetic variability that may have been lost due to drift. In addition, if the dynamics of sexual selection permit and facilitate natural hybridization under certain conditions as described above, then when a population is faced with significant reduction in size such that there is strong selection for less choosy females, females of such populations may occasionally accept the mating rituals of males of a related species. Thus, natural hybridization may be playing an important role not only in *maintaining* but also *replenishing* genetic variability that may have been lost due to drift during population bottlenecks. When considering management of rare and endangered species, it may therefore be important to consider and understand the potential interaction with other related species within their distributional range; species from which genetic material may be “leaking” across species barriers to maintain adequate levels of genetic variability in the population.

Concluding Remarks

There is little doubt that the evolutionary research on Hawaiian Drosophilidae has made important contributions to basic and applied biological concepts. More than two decades ago, Mayr (1982) said that “Speciation...now appears as the key problem of evolution. It is remarkable how many problems of evolution cannot be fully understood until speciation is understood...”. During the ensuing two decades, there was renewed interest in researching and understanding the mechanisms of the speciation process. At least three books that address questions of speciation have been published (Giddings et. al. 1989; Otte and Endler, 1989; Lambert and Spencer, 1995). The research on the Hawaiian *Drosophila* is cited extensively in all three of these edited volumes.

There is also little doubt that adaptation is the central theme of the evolutionary process and that natural selection is the result of the interaction between heritable phenotypic characters within populations and the extrinsic environment in which such populations live. Most biologists acknowledge the fact that natural selection is indeed the most dominant force in the evolutionary process and even Darwin, in formulating his theory of sexual selection, acknowledged that “...sexual selection will also be dominated by natural selection tending towards the general welfare of species”. In recent years, there has been significant interest in the role of sexual selection and its influence on what Carson (1978) referred to as the “...mutual adjustment of the sexes to what may be called the intraspecific sexual environments.” The research of Kaneshiro (1980, 1989, 2004) and Carson (1986, 1997, 2003) has shown that sexual selection plays a significant role in the initial stages of species formation. In a paper presented at the 50th Anniversary of the Society for the Study of Evolution, Carson (1997) suggested that the evolution of novelty is even more evident when the population is subjected to bottlenecks and that the dynamics of sexual selection is especially important in the speciation process.

Nearly a quarter of a century has passed since Williamson declared the supremacy of the research on Hawaiian Drosophilidae in the study of evolution. The results of the research until then certainly have been nothing short of spectacular and only a few highlights have been presented in this review. Evolutionary research on the Hawaiian *Drosophila* has been a model system for understanding the biology, ecology and behavior of tephritid fruit fly species (Kaneshiro, 2000; Sivinski et. al., 2000) and has contributed to the development of more effective control/eradication programs (Kaneshiro 1993a, 1993b). The work on the Hawaiian Drosophilidae has been ongoing and has attracted interest among researchers

from the international arena. In 1995, a funding agency of the Japanese government provided more than \$3.5 Million to the Mitsubishi Kasei Institute of Life Sciences in Tokyo, Japan with Dr. Daisuke Yamamoto as the Principal Scientist to investigate the molecular genetics of sexual behavior in the Hawaiian *Drosophilidae*. One significant outcome from the Japanese project is the discovery of a sexually dimorphic feature in brain size with males of one group of picture-winged species having significantly larger ganglia than females (Kondoh et al. 2003). In another recent study, M. Kambysellis in collaboration with E. Craddock and K. Kaneshiro (unpublished data), discovered a large fauna of bacteria associated with the endemic Hawaiian *Drosophilidae*. This discovery and the characteristics of these bacteria have huge implications for medical research and the potential for their impact on emerging infectious diseases of humans. Another significant work on the Hawaiian *Drosophila* is the recent sequencing of the entire genome of *Drosophila grimshawi*, one of the picture-winged species (Patrick O'Grady, pers. commun.). The sequence can be accessed in the Flybase database (<http://flybase.bio.indiana.edu/blast/>) and will enable more sophisticated genomic analyses which will provide the foundation for future studies into the evolutionary biology of this amazing group of species.

Mark Williamson (1981) aptly concluded his chapter on the Hawaiian *Drosophilidae* with the statement:

“Any other study of evolution must surely seem inadequate after the study of the Hawaiian *Drosophilidae*.... There is an immense amount of work to be done on the group but the work carried out so far clearly established their supremacy.”

Acknowledgements

The research on the Hawaiian *Drosophilidae* has been supported by numerous grants from the National Institutes of Health and the National Science Foundation as well as from international sources. The author's time and effort in preparing this paper was supported in part by a National Science Foundation EPSCoR grant No. EPS02-37065.

References

- Ahearn, J.N.** 1980. Evolution of behavioral reproductive isolation in a laboratory stock of *Drosophila silvestris*. *Experientia* 36: 63–64.
- Ahearn, J.N., H.L. Carson, Th. Dobzhansky and K.Y. Kaneshiro.** 1974. Ethological isolation among three species of the planitibia subgroup of Hawaiian *Drosophila*. *Proc. Nat. Acad. Sci. USA* 71: 901–903.
- Arita, L.H. and K.Y. Kaneshiro.** 1979. Ethological isolation between two stocks of *Drosophila adiastola* Hardy. *Proc. Hawaii. Entomol. Soc.* 13: 31–34.
- Arnold, M.J.** 1997. Natural Hybridization and Evolution. In *Oxford Series of Ecology and Evolution*, eds. R.M. May and P.H. Harvey, pp. 1–215. Oxford University Press, Oxford.
- Arnqvist, G., M. Edvardsson, U. Friberg and T. Nilsson.** 2000. Sexual conflict promotes speciation in insects. *Proc. Nat. Acad. Sci. USA* 97: 10460–10464.
- Bastock, M.** 1956. A gene mutation which changes a behavior pattern. *Evolution* 10: 421–439.
- Bradley, R.D., S.K. Davis and R.J. Baker.** 1991. Genetic control of premating-isolation behavior: Kaneshiro's hypothesis and asymmetrical sexual selection in pocket gophers. *J. Heredity* 82: 192–196.
- Carson, H.L.** 1968. The population flush and its genetic consequences. In *Population Biology and Evolution*, ed. R.C. Lewontin, pp. 123–137, Syracuse Univ. Press, Syracuse, NY.
- Carson, H.L.** 1971. Speciation and the founder principle. University of Missouri, *Statler Symp.* 3: 51–70.
- Carson, H.L.** 1986. Sexual selection and speciation. In *Evolutionary Processes and Theory*, eds. S.

- Karlin and E. Nevo, pp. 391–409. Academic Press, London.
- Carson, H.L.** 1997. Sexual selection: a driver of genetic change in Hawaiian *Drosophila*. *J. Hered.* 88: 343–352.
- Carson, H.L.** 2003. Mate choice theory and the mode of selection in sexual populations. *Proc. Nat. Acad. Sci.* 100: 6584–6587.
- Carson, H.L.** and **P. Bryant.** 1979. Genetic variation in Hawaiian *Drosophila*. VI. Change in a secondary sexual character as evidence of incipient speciation in *Drosophila*. *Proc. Nat'l. Acad. Sci. USA* 76: 929–1932.
- Carson, H.L.** and **A.R. Templeton.** 1984. Genetic revolution in relation to speciation phenomena: the founding of new populations. *Annu. Rev. Ecol. Systematics* 15: 97–131.
- Carson, H.L.** and **L.T. Teramoto.** 1984. Artificial selection for a secondary sexual character in males of *Drosophila silvestris* from Hawaii. *Proc. Nat'l. Acad. Sci. USA* 81: 3915–1917.
- Coyne, J.A.** and **H.A. Orr.** 2004. *Speciation*. Sinauer Assoc. Inc., Sunderland, Mass. Publishers. 545 pp.
- Darwin, C.** 1859. *The Origin of Species*. Modern Library, New York.
- DeSalle, R.** and **A.R. Templeton.** 1987. Comments on “The Significance of Asymmetrical Sexual Isolation”. In *Evolutionary Biology*, Vol 12, eds. M.K. Hecht, B. Wallace and G.T. Prance, pp. 21–27. Plenum, New York
- Dobzhansky, Th.** and **G. Streisinger.** 1944. Experiments on sexual selection in *Drosophila*. II Geographic strains of *Drosophila prosaltans*. *Proc. Nat'l. Acad. Sci.* 30: 340–345.
- Dodd, D.M.B.** and **J.R. Powell.** 1986. Founder-flush speciation: an update of experimental results with *Drosophila*. *Evolution* 39: 1388–1392.
- Fisher, R.** 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Garilets, S.** 2000. Rapid evolution of reproductive isolation driven by sexual conflict. *Nature* 403: 886–889.
- Gavrilets, S.** and **T.I. Hayashi.** 2005. Speciation and sexual conflict. *Evolutionary Ecology* 19: 167–198.
- Gavrilets, S.** and **D. Waxman.** 2002. Sympatric speciation by sexual conflict. *Proc. Nat'l. Acad. Sci. USA* 99: 10533–10538.
- Gavrilets, S., G. Arnqvist** and **U. Friberg.** 2001. The evolution of female mate choice by sexual conflict. *Proc. Royal Soc. Lond. B* 268: 531–539.
- Giddings, L.V., K.Y. Kaneshiro,** and **W.W. Anderson.** 1989. *Genetics, Speciation and the Founder Principle*. Oxford Univ. Press, New York. 373 pp.
- Giddings, L.V.** and **A.R. Templeton.** 1983. Behavioral phylogenies and the direction of evolution. *Science* 220: 372–377.
- Grimshaw, P.H.** 1901-1902. *Fauna Hawaiensis*. 3: 51–73, 86.
- Hardy, D.E.** 1965. *Insects of Hawaii*, Vol. 12. Diptera: Cyclorrhapha II. Series Schizophora, Section Acalypterae I. Family Drosophilidae. Univ. of Hawaii Press, Honolulu, HI. 814 pp.
- Heed, W.B.** 1968. Ecology of the Hawaiian Drosophilidae. *Univ. Texas Publ.* 6818: 387–419.
- Heed, W.B.** 1971. Host plant specificity and speciation in Hawaiian *Drosophila*. *Taxon* 20: 115–121.
- Heth, G.** and **E. Nevo.** 1981. Origin and evolution of ethological isolation in subterranean mole rats. *Evolution* 35: 259–274.
- Holland, B.** and **W.R. Rice.** 1998. Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* 52: 1–7.
- Kaneshiro, K.Y.** 1976a. A revision of generic concepts in Hawaiian Drosophilidae. *Proc. Hawaii. Entomol. Soc.* 23: 255–278.
- Kaneshiro, K.Y.** 1976b. Ethological isolation and phylogeny in the planitibia subgroup of Hawaiian *Drosophila*. *Evolution* 30: 740–745.
- Kaneshiro, K.Y.** 1980. Sexual isolation, speciation and the direction of evolution. *Evolution* 34: 437–444.
- Kaneshiro, K.Y.** 1983. Sexual selection and direction of evolution in the biosystematics of Hawaiian Drosophilidae. *Ann. Rev. Entomol.* 28: 161–178.
- Kaneshiro, K.Y.** 1987. The dynamics of sexual selection and its pleiotropic effects. *Behav. Genet.* 17: 559-569.
- Kaneshiro, K.Y.** 1989. The dynamics of sexual selection and founder effects in species formation. In *Genetics, Speciation, and the Founder Principle*, eds. L.V. Giddings, K.Y. Kaneshiro, and W.W.

- Anderson, pp. 279-296. Oxford University Press, New York.
- Kaneshiro, K.Y.** 1990. Natural hybridization in *Drosophila*, with special reference to species from Hawaii. *Can. J. Zool.* 68: 1800-1805.
- Kaneshiro, K.Y.** 1993. Habitat-related variation and evolution by sexual selection. In *Evolution of Insect Pests*, K.C. Kim and B.A. McPherson (eds.), pp. 89-101. John Wiley & Sons, New York.
- Kaneshiro, K.Y.** 1995. Evolution, speciation, and the genetic structure of island populations. In *Ecological Studies, Vol. 115*, eds. P. Vitousek, L.L. Loope, and H. Adersen, pp. 23-33. Springer-Verlag, New York.
- Kaneshiro, K.Y.** 2000. Sexual selection and speciation in Hawaiian *Drosophila* (Drosophilidae): A model system for research in Tephritidae. In *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*, M. Aluja and A. L. Norrbom, eds. Pp. 861-877. CRC Press, New York.
- Kaneshiro, K.Y.** 2004. Sex, flies, and the genetic consequences of small population size. In *D.Elmo Hardy Memorial Volume, Contributions to the Systematics and Evolution of Diptera*, ed. N.L. Evenhuis and K.Y. Kaneshiro. Bishop Museum Bulletin in Entomol. 12: 43-51.
- Kaneshiro, K.Y.** and **L.V. Giddings.** 1987. The significance of asymmetrical sexual isolation. In *Evolutionary Biology*, Vol 12, eds. M.K. Hecht, B. Wallace and G.T. Prance, pp. 29-44. Plenum, New York.
- Kaneshiro, K.Y.** and **J.S. Kurihara.** 1981. Sequential differentiation of sexual isolation in populations of *Drosophila silvestris*. *Pac. Sci.* 35: 177-183.
- Kaneshiro, K.Y., R. G.Gillespie,** and **H.L. Carson.** 1995. Chromosomes and male genitalia of Hawaiian *Drosophila*: Tools for interpreting phylogeny and geography. In *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*, Wagner, W.L. and V.A. Funk (eds.) pp. 57-71. Smithsonian Institution Press, Washington DC.
- Kirkpatrick, M.** 1982. Sexual selection and the evolution of female choice. *Evolution* 36: 1-12.
- Kondoh, Y., K.Y. Kaneshiro, K. Kimura** and **D. Yamamoto.** 2003. Evolution of sexual dimorphism in the olfactory brain of Hawaiian *Drosophila*. *Proc. Royal Soc. Lond.* 270: 1005-1013.
- Lambert, D.L.** and **H.G. Spencer.** 1995. *Speciation and the Recognition Concept*. John Hopkins University Press, Baltimore. 502 pp.
- Lance, D.R.** and **D.O. McInnis.** 1993. Misrepresented reality? Letter to the Editor. *American Entomologist.* 41:196
- Lande, R.** 1981. Models of speciation by selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* 78: 3721-3725.
- Lande, R.** 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* 36: 213-223.
- Markow, T.A.** 1981. Mating preferences are not predictive of the direction of evolution in experimental populations of *Drosophila*. *Science* 213: 1405-1407.
- Mayr, E.** 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- Mayr, E.** 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.
- Mayr, E.** 1982. Processes of speciation of animals. In C. Barigozzi (ed.), *Mechanisms of Speciation*, pp. 1-19. Alan R. Liss, New York.
- Meffert, L.M.** and **E.H. Bryant.** 1991. Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. *Evolution* 45: 293-306.
- McPhail, J.D.** 1969. Predation and the evolution of a stickleback (*Gasterosteus*). *J. Fish Res. Bd. Can.* 26: 3183-3208.
- Montgomery, S.L.** 1975. Comparative breeding site ecology and the adaptive radiation of picture-winged *Drosophila* (Diptera: Drosophilidae) in Hawaii. *Proc. Hawaii. Entomol. Soc.* 22: 65-102.
- O'Donald, P.** 1977. Theoretical aspects of sexual selection. *Theor. Popul. Biol.* 12: 298-334.
- O'Donald, P.** 1980. *Genetic Models of Sexual Selection*. Cambridge University Press, Cambridge, MA.
- Ohta, A.T.** 1978. Ethological isolation and phylogeny in the grimshawi species complex of Hawaiian *Drosophila*. *Evolution* 32: 485-492.
- Otte, D.** and **J.A. Endler.** 1989. *Speciation and its Consequences*. Sinauer, Sunderland, Massachusetts. pp. 679.
- Parker, G.A.** and **L. Partridge.** 1998. Sexual conflict and speciation. *Philos. Trans. Royal Soc. Lond.* B 353: 261-274.
- Perkins, R.C.L.** 1913. In *Fauna Hawaiiensis*. Ed. D. Sharp, 1:CLXXX-CLXXXIX. Columbia Univ.

Press, New York.

- Powell, J.R.** 1978. The founder-flush speciation theory: an experimental approach. *Evolution* 32: 465–474.
- Powell, J.R.** 1997. *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press, New York. pp. 562.
- Reed, S.C.** and **E.W. Reed.** 1950. Natural selection in laboratory populations of *Drosophila*. II. Competition between white-eye gene and its wild type allele. *Evolution* 4:34–42.
- Rendel, J.M.** 1951. Mating of ebony, vestigial and wild type *Drosophila melanogaster* in light and dark. *Evolution* 5: 226–230.
- Rice, W.R.** 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381: 232–234.
- Rice, W.R.** 1998. Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In *Endless Forms: Species and Speciation*, D.J. Howard and S.H. Berlocher (eds.), pp. 261–270. Oxford University Press, New York.
- Rice, W.R.** and **B. Holland.** 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and interspecific Red Queen. *Behav. Ecol. Sociobiol* 41: 1–10.
- Shaw, K.L.** and **E. Lugo.** 2001. Mating asymmetry and the direction of evolution in the Hawaiian cricket genus *Laupala*. *Molecular Ecology* 10: 751–759.
- Sivinski, J., M. Aluja, G. Dodson, A. Freidberg, D. Hendrick, K.Y. Kaneshiro and P.J. Landolt.** 2000. Topics in the evolution of sexual behavior in the Tephritidae. In *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*, M. Aluja and A. L. Norrbom, eds. pp. 751–792. CRC Press, New York.
- Spieth, H.T.** 1966. Courtship behavior of endemic Hawaiian *Drosophila*. *Univ. Texas Publ.* 6615: 245–313.
- Spieth, H.T.** 1968a. Evolutionary implications of sexual behavior in *Drosophila*. *Evolutionary Biology*, pp. 157–193. Appleton Century Crofts, New York.
- Spieth, H.T.** 1968b. Evolutionary implications of the mating behavior of the species of *Antopocerus* (Drosophilidae) in Hawaii. *Univ. Texas Publ.* 6818: 319–333.
- Spieth, H.T.** 1974a. Courtship behavior in *Drosophila*. *Ann. Rev. Entomol.* 19: 385–405.
- Spieth, H.T.** 1974b. Mating behavior and evolution of the Hawaiian *Drosophila*. In *Genetic Mechanisms of Speciation in Insects*, ed. M.J.D. White, pp. 94–101. Australia & New Zealand Book Co., Australia.
- Spieth, H.T.** 1982. Behavioral biology and evolution of the Hawaiian picture-winged species group of *Drosophila*. In *Evolutionary Biology*, Vol. 14, eds. M.K. Hecht, B. Wallace, and G.T. Prance. pp. 351–437. Plenum, New York.
- Templeton, A.R.** 1980. The theory of speciation via the founder principle. *Genetics* 94: 1011–1038.
- Throckmorton, L.H.** 1966. The relationship of the endemic Hawaiian Drosophilidae. *Univ. Texas Publ.* 6615: 335–396.
- Wasserman, M.** and **H.R. Koepfer.** 1980. Does asymmetrical mating preference show the direction of evolution? *Evolution* 34: 1116–1124.
- Watanabe, T.K.** and **M. Kawanishi.** 1979. Mating preference and the direction of evolution in *Drosophila*. *Science* 205: 906–907.
- Wheeler, M.R.** and **F.E. Clayton.** 1965. A new *Drosophila* culture technique. *Dros. Info. Serv.* 40–98.
- Williamson, M.** 1981. *Island Populations*. Oxford University Press, London.
- Zimmerman, E.C.** 1958. Three hundred species of *Drosophila* in Hawaii? A challenge to geneticists and evolutionists. *Evolution* 12: 557–558.

