EVOLUTIONARY STUDIES OF TWO
DROSOPHILA SPECIES FROM HAWAII:
A MULTIDISCIPLINARY APPROACH

Kenneth Y. Kaneshiro
Department of Entomology
University of Hawaii at Manoa
Honolulu, Hawaii 96822

Various techniques of evolutionary biology are being utilized in an investigation of the phylogenetic relationships of two sympatric Drosophila species from the Island of Hawai'i and their counterparts on Maui Nui. The two species from Hawai'i are Drosophila heteroneura and D. silvestris while D. planitibia is from Maui and D. differens is from Moloka'i. Patterns of speciation and the evolutionary history of the four species in relation to one another can be inferred by pooling the data from various studies including morphology, hybridization experiments, ethology, cytology, electrophoresis, and ecology. Preliminary analyses of these data indicated that a single introduction into the Big Island of Hawai'i resulted in the speciation of both D. heteroneura and D. silvestris. However, further investigation into the morphology, electrophoresis, and behavior of the four species revealed a possibility that two separate introductions may have been involved in the speciation of the two Big Island species.

Background

The chromosomal relationships of all four species have been reported by Carson and Stalker (1968) and Craddock (1974). The two Maui Nui species are monomorphic and homosequential in the banding patterns of polytene chromosomes while D. heteroneura and D. silvestris share a unique polymorphic inversion in Chromosome III (3m/+). In addition, D. silvestris carries six other polymorphic inversions not found in the other three species. Thus, there are no fixed inversion differences in the karyotype of the four species.

Equally intriguing results have been obtained by measurements of genetic similarity based on allozymic variation (Johnson et al. 1975). Calculations of similarity indices among the four species revealed an unusually high level of genetic similarity ranging from 0.71 - 0.96. Ayala et al. (1974) described an observed relationship between genetic similarity indices and the categorization of taxa at and below the species level. If identity is taken at 1.0, local geographic populations of a species are shown to have mean similarity values of 0.97, subspecies and semispecies about 0.8, sibling species 0.52, and closely related but morphologically differentiated species 0.35. Despite striking morphological differences between D. heteroneura and D. silvestris, the similarity value of 0.96 reported by
Johnson et al. (1975) indicate an extremely close phylogenetic affinity between these two species.

Various programs of interspecific hybridization between these four species were undertaken to test the existence of postmating isolation barriers (e.g., hybrid inviability, sterility, etc.). Crosses involving *D. planitibia* and the other three species produced fertile female hybrids but sterile males. However, reciprocal crosses between the sympatric species *D. heteroneura* and *D. silvestris* produced vigorously fertile F₁ hybrid progeny of both sexes (Craddock, 1974 and Ahearn and Val, 1975). Furthermore, there appeared to be no obvious breakdown in hybrid fertility in the F₂ and backcross generations. Preliminary data on crosses involving *D. differens* and the two Big Island species indicate that F₁ hybrids are also fully fertile in both sexes.

Observations of the courtship behavior patterns of *D. heteroneura* and *D. silvestris* (Spieth, personal communication) indicate that there are no qualitative differences in the courtship behavior of these two species. However, Ahearn et al. (1974) and Kaneshiro (in press) report that sexual (behavioral) isolation between the two sympatric species is nearly complete which indicates that there may be quantitative differences in their courtship patterns.

Ecologically, Heed (1968) and Montgomery (1975) report that all four species utilize the decaying bark of *Clermontia* spp. (Lobeliaceae) as the primary substrate for the larval breeding site. In a recent collection, separate rotting branches of *Clermontia* yielded nearly equal proportions of both *D. heteroneura* and *D. silvestris* adults. Thus, despite the possibility of differences in larval nutritional requirements, ovipositional behavior of gravid females of both species is apparently stimulated by the rotting bark of *Clermontia*.

**Phylogenetic Interpretation**

By assessing the data accumulated from the various studies discussed above, Craddock (1974) proposed three possible patterns of speciation in the evolution of *D. heteroneura* and *D. silvestris*. Nevertheless, on the basis of the shared polymorphic inversion in Chromosome III, she favored the hypothesis that the two sympatric species arose from a single founder event from one of the Maui Nui populations. Since neither *D. differens* from Moloka'i nor *D. planitibia* from Maui carries the 3m inversion in their populations, derivation of the two Big Island species from two independent founders was not a parsimonious interpretation. However, subsequent morphological comparisons revealed an interesting phenotypic resemblance between *D. differens* and *D. heteroneura* and between *D. planitibia* and *D. silvestris* which provided conflicting evidence
for Craddock's single introduction hypothesis. The most obvious similarity between *D. planitibia* and *D. silvestris* is the velvety-black coloration of the face while those of *D. heteroneura* and *D. differens* are light brown to bright yellow. The marking in the costal margin of the wing also appears to relate *D. planitibia* with *D. silvestris* and *D. differens* with *D. heteroneura*. In *D. planitibia* and *D. silvestris*, this costal marking usually tapers and disappears at a level of the posterior crossvein while in the latter pair, the marking usually extends beyond the level of the posterior crossvein to the markings near the apex of the wing. On the basis of these phenotypic criteria, a second possible interpretation of the origin of the two Hawai'i species must be seriously considered. That is, two independent founders, one from Moloka'i (*D. differens* as progenitor) and one from Maui (*D. plantibia* as progenitor), may have given rise to *D. heteroneura* and *D. silvestris* respectively.

Further analyses of the behavioral relationships among the four species by Kaneshiro (in press) appear to support the "two-founder" hypothesis. Preliminary observations (F. Sene, personal communication) of one of the Esterase locus as determined by starch-gel electrophoresis indicate that *D. heteroneura* may be more closely related to *D. differens* than to *D. silvestris* or *D. planitibia* and conversely, *D. silvestris* may be more closely related to *D. planitibia* than to *D. heteroneura* or *D. differens*. These studies are continuing.

The shared polymorphic inversion in Chromosome III of the two Hawai'i species poses a major stumbling block to the two-founder hypothesis. The origin of an identical chromosomal polymorphism from two independent founders is highly improbable. Alternatively, the shared inversion may be explained by interspecific hybridization and a limited amount of genetic introgression between the two populations short of complete gene exchange. Natural hybridization between a pair of "good" biological species is, at best, a rare phenomenon. However, the discovery of fertile interspecific hybrids of *D. heteroneura* and *D. silvestris* (Kaneshiro and Val, in press) in the Kahuku Ranch area (S. Kona) permits invoking natural hybridization as a very possible mechanism by which the two species now share a polymorphic inversion.

Thus, inferences of phylogenetic relationships and evolutionary patterns of speciation is made possible by analyses of data from these various tools of evolutionary interpretation. We are continuing such studies by comparing the natural history of three sympatric populations of *D. heteroneura* and *D. silvestris*. One such population occurs at Pau-ahi, Kona, on the Greenwell Ranch where both species coexist with no evidence of interspecific hybridization. Another population being scrutinized occurs at Ka-huku Ranch, S. Kona, where approximately 2% of the specimens collected
thus far showed morphological properties reflecting inheritance from both species. The third population occurs in Ola'a Tract within the Hawaii Volcanoes National Park. Here, both species coexist with some evidence that genetic introgression may be occurring although not a single bona fide hybrid specimen has been collected. In the Pau-ahi and Ka-huku Ranch populations of *D. silvestris*, the abdominal tergites are jet-black while *D. heteroneura* displays a distinctive black-yellow contrasting pattern. However, in the Ōla'a Tract population, the black-yellow pattern of *D. heteroneura* appear to be segregating in the *D. silvestris* population. It is hoped that comparisons of the behavioral, morphological, ecological, and electrophoretic analyses of these three populations will enable us to better understand the genetic mechanisms involved in species divergence (e.g. "character displacement").

These studies will make possible inferences of phylogenetic relationships among four species of Hawaiian *Drosophila*. More important, it is hoped that these basic concepts of evolutionary theory can be applied to the understanding of the evolutionary strategies of other organisms in the Hawaiian biota.
Literature Cited


