Chromosomal Phylogenies and Population Genetics of the Picture-Winged Hawaiian Drosophilids: Impact on Evolutionary Biology

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Abstract

Using a single species as a standard, the banding orders of the 6 giant chromosomes of each of 107 species of large Hawaiian drosophilids have been determined. Most banding orders vary due to naturally occurring inversions. The data on inversion sharing between species yields a robust genetically-based phylogeny for these species on all of the high Hawaiian islands. Species on the newer islands can be traced chromosomally to 6 putative ancestral forms on Kaua'i. Population genetic studies of selected species reveals abundant intraspecific genetic variation involving female choice from among genetically variable males. The general value of these studies to some major concepts of evolutionary biology is discussed.

Introduction

About 40 years ago, a major project to study the evolutionary biology of the endemic Hawaiian Drosophilidae was launched by D. Elmo Hardy of the University of Hawaii and Wilson S. Stone of the University of Texas. The grant proposal approved by National Institutes of Health at the time stressed a multi-disciplinary approach. Accordingly, the principals were able to invite participation from a diverse set of biologists already having professional experience in various aspects of ecology, entomology, ethology, genetics, physiology and systematics. Following the tragic death of Stone at the height of his career and only a few years into the project, its realization fell on the shoulders of Hardy, who continued over many years to facilitate the project. He provided both basic systematics and field guidance, without which the work to understand the evolution of this extraordinary fauna would have been seriously hampered.

The first year of the project was 1963. By noteworthy coincidence, this was the same year that a brief paper by J. Tuzo Wilson, a Canadian geologist, appeared in the *Canadian Journal of Science*. Wilson proposed the stunning theory that the Hawaiian Islands are the sub-aerial tips of great volcanoes that have been formed in a strictly successive manner over a localized "hot-spot" under the moving Pacific tectonic plate. As the plate has moved slowly north and later northwestward, each new volcano was formed at the current southeast end of the archipelago.

Since Wilson's paper, data have accumulated that strongly support this theory (Clague & Dalrymple, 1987). Furthermore, the new data indicate that the Emperor Seamounts, a line of submerged extinct volcanoes dating back about 80 million years, have a similar but ancient origin. From this perspective, the present high islands are very new. The oldest, Kaua'i, was formed only about 5 million years ago. Much of the terrestrial biota may be descended from ancient ancestors that came from older islands now long submerged.

Wilson's discovery came at a crucial time for evolutionary biology. It has provided an interpretative background particularly for research on the evolution of the terrestrial fauna and flora of the Hawaiian archipelago.

After the basic systematics and geographical distributions had become clear, genetic and behavioral research on the Hawaiian drosophilids was concentrated in two main areas. These have been: 1) the detailed phylogenetic history of a special clade of about one hundred species of the subgenus *Drosophila* (the "picture-winged" flies) and 2) the population genetics and sexual behavior of one exemplary species, *Drosophila silvestris* (Perkins). This paper provides short reviews of the relevant findings and evaluates their impact on evolutionary biology as a whole.

Genetic Tracing of Phylogenetic Histories

Endemic Hawaiian Drosophilidae number about 1,000 species with over 400 species yet to be described (O'Grady *et al*., 2003). The job of cataloguing this immense fauna, begun by Hardy (1965) is still a long way from being completed. Despite great morphological diversity, the endemics are generally referable to only 2 genera, *Drosophil*a and *Scaptomyza* (Kaneshiro, 1976). Any member of this fauna can be easily distinguished morphologically from any of about 20 assorted drosophilid species that have been recently brought into the islands from the continents inadvertently through human activity.

These species are found only on the present high islands, 5 million years of age or less. Although no collections of drosophilid specimens have been made on the older, severely eroded islands, there is strong geological evidence that these islands were once high and presumably forested and thus could have harbored substantial biotas. Indeed, molecular genetic data suggest that the earliest founding event may have occurred about 26 million years ago (Russo *et al*., 1995).

Hawaiian endemics recognized as *Drosophila* fall into 7 groupings for which a preliminary phylogeny has been proposed (see a brief review in O'Grady *et al*., 2003). One of these groups, numbering about 120 species, is the "picture-winged" flies. These occur on all of the high islands from Kauaʻi southeastward, and are the focus of this paper. Studies of band sequences of the giant chromosomes show striking similarities to certain subgenus *Drosophila* species endemic to Hokkaido and the Pacific northwest of the United States (Stalker, 1972; Narayanan, 1973). The data indicate a boreal origin for the picture-winged species from the Bering Archipelago.

Characteristically, males of most of these species show elaborate secondary sexual characters that are both behavioral and structural. As in many other dipteran faunas, male secondary sexual characters have been prominently used for taxonomic purposes. Although morphological differences between the sexes are present in most drosophilids, the extent of elaborate male characters and territorial behavior is especially developed in Hawaiian *Drosophila*, prompting the late Professor Theodosius Dobzhansky to refer to them as the "birds of paradise of the *Drosophila* world".

With proper inducement, a single wild-caught picture-wing female will oviposit in the laboratory, yielding progeny that represent the natural insemination of the wild female. This "isofemale" procedure has made it possible to accurately match males and females of the same species, a process that is often difficult to do when sorting collections from the wild. Isofemale lines thus provide accurate systematic vouchers, especially for genetic studies that can only be done using larvae. In many cases, long-standing laboratory isofemale cultures of each species have been available for laboratory study.

Inversion Markers as Tracers of Relationships Between the Species of Picture-Winged *Drosophila*

Each of the 5 major giant chromosomes of the picture-winged *Drosophila* species displays about 500 chromatic bands in a linear order. Deletion of only a small number of these bands anywhere in these chromosomes is generally lethal, indicating that at the molecular level the bands are the physical sites of important genes. Although the chromosomes of each of the species studied display virtually all of these bands, the linear order of bands varies extensively within each chromosome. Changes in band order are caused by precise measurable chromosomal inversions that occur naturally. An inversion is the result of 2 simultaneous natural breaks in a chromosome, followed by a re-integration of the broken piece, creating a new linear order. The fact that 2 breaks occur simultaneously results in an easily readable chromosomal "marker" that is both unique and permanent, since the probability of a simultaneous occurrence of 2 identical breaks is very small.

Each inversion arises in the germ line of a single individual male or female at one time and place. If it survives and is passed on to progeny, it may be ultimately transmitted to some or all descendent members of the same species. Clearly it must begin as a heterozygote, after which it may come to replace the original order in certain populations. Thus, the probability of survival into future populations is assured if the new variant becomes homozygous, completely replacing the ancestral non-inverted sequence.

Table 1. Ancient theoretical *Drosophila* picture-winged founders from Kaua'i and their 95 descendents on the newer islands. The total number of species that have been chromosomally sequenced for this study is 107(see Carson, 1992).

ANCESTORS	DESCENDENTS
Six single founders that left Kaua'i, and a list of Kaua'i species*, to which each founder was closely related by one or more unique inversions	Lineages of species on the newer islands (number of species on each island)
"*ornata*-related" (1 species only)	*adiastola* group = 15 species (O'ahu - 2, Maui Nui - 11, Hawai'i - 2) [For a detailed phylogeny, see Fig. 1]
"*picticornis*-related" (1 species only)	*planitibia* group = 16 species (O'ahu - 4, Maui Nui - 9, Hawai'i - 3)
"*sejuncta*-related" (1 species only)	*sejuncta* group = 3 species (O'ahu - 0, Maui Nui - 1, Hawai'i - 2)
"*ocellata*-related" (1 species only)	*punalua* group = 5 species (O'ahu - 3. Maui Nui - 1, Hawai'i - 1)
"*glabriapex*-related" (2 species)	*glabriapex* group = 24 species (O'ahu - 12, Maui Nui - 7, Hawai'i - 5
"*craddockae*-related" (4 species**)	*grimshawi* group = 32 species (O'ahu -7 , Maui Nui -13, Hawai'i -12
Total: 6 ancestors	**Total: 95 descendent species** (O'ahu - 28, Maui Nui - 42, Hawai'i - 25)

*   Kaua'i has 12 endemic picture-winged species, including *D.attigua* and *D. primaeva*. The latter is judged to be the closest species to a hypothetical ancestor of all the picture-winged species.

**   The species shared by Kaua'i and O'ahu that morphologically resembles *D. grimshawi* of Maui Nui is now recognized as *D. craddockae* (Kaneshiro & Kambysellis, 1999)

Approximately 200 inversions have been discovered and given individual names in the order of discovery (Carson, 1992). For example, in chromosome 2, the first inversion found was named "2a", the second "2b" and so on. In some cases, the alphabet, when exhausted, has been used again employing superscripts such as: "$2a^2$", "$2a^3$", etc. The formulae are thus based on unique genetic differences arising in nature from an arbitrarily-chosen "standard" gene order. The essentially uniform sequences in all the chromosomes in one species, *Drosophila grimshawi* Oldenberg of Maui, serves this purpose. Some species groups are inversion-rich so that phylogenetic tracing is robust, as in the *adiastola* and *planitibia* groups. However, in the *glabriapex* and *grimshawi* groups a number of morphologically distinct species have identical inversion formulas. This suggests that the basis of the species differences must lie at the genic and not the gross chromosomal level. Many inversions have been found that are polymorphic, i.e., they coexist with the ancestral sequence in local populations.

When the chromosomal similarities are superimposed on the geographical distribution of each species, it is seen that on the oldest island of Kaua'i the relatively small number of endemic picture-wings fall into 6 inversion formulae. Each of these appears to represent a separate and unique ancestral source for a phylad of newer species present on the newer islands. Thus, each of the 95 species on the newer islands can be traced back by inversions to one of these 6 separate putative ancestors. This is based on the study of the geographical distribution of these chromosomal variants in species still existing on the island of Kaua'i (Table 1).
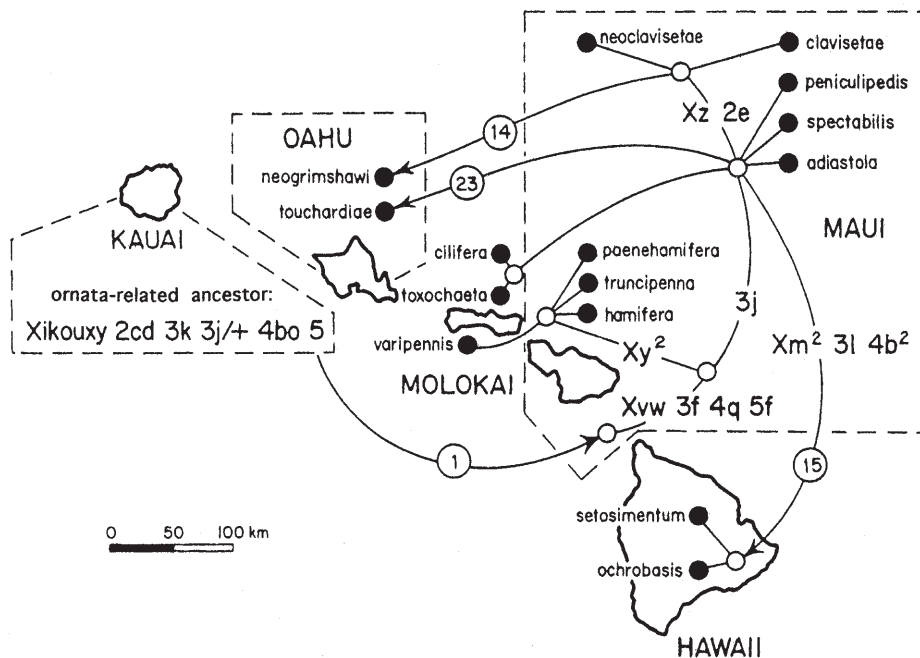
Figure 1. Inversion-sharing phylogeny for 15 species of the *adiastola* group, stemming from an *ornata*-related hypothetical ancestor on Kaua'i. Solid circles: existing species; open circles: hypothetical ancestral populations. Four hypothetical inter-island founder events are indicated (encircled numbers). Procedure: to trace ancestry, start from Kaua'i at the left of the figure where 12 inversions on chromosomes X,2,3,4, and 5 of modern *D. ornata* are listed. These variants were apparently carried by an ancestral founder (#1, encircled) to Maui. After arrival, forms descended from this founder added 5 new inversions in chromosomes X, 3, 4, and 5. After inversion Xy^2 is added, the invariable formula found in each of 4 distinctive modern species (*paenehamifera, truncipenna, hamifera* of Maui and *varipennis* of Moloka'i) is attained. In an alternate pathway, 3j, which has previously been retained in the polymorphic state 3j/+, becomes fixed. This leads to a common ancestor of the 3 species of the *adiastola* subgroup. Only 2 species of the group are found on O'ahu (*neogrimshawi* and *touchardiae*). These show distinctive formulae related to 2 different parts of the Maui phylogeny and are assumed to have arrived in O'ahu separately as "back migrants" from a younger island to an older one (founders 14 and 23). Only 2 species of the group are present on Hawai'i (founder 15). Illustration modified from Carson (1992).

These data are of special interest because of the genetic precision with which the picture-winged species may be grouped and the ancestry of each younger species determined by robust genetic data. A major fact emerging is that there are 95 descendent species that can be identified as endemic to one or more of the 3 newer island groups. This proliferation of species, moreover, is relatively new geologically, since all these species must be younger than the age of the Wai'anae range of O'ahu, i.e., 3.5 million years.

Figure 1 shows a proposed evolutionary scenario for one phylad of species, the *adiastola* group, listed in Table 1. Each species is endemic to the island on which its name appears. Island ages range from about 5 million years for Kaua'i to less than 400,000 years for Hawai'i. This hypothetical phylogeny is useful since the genetic data are superimposed on the geographical distribution of each species. This permits the proposal of intra- and inter-island phylogenies and founder events as explained in the caption. Similar phylogenies for the 5 other groups are given in Carson (1992) or can be located in references to earlier publications.

The phylogenetic differentiation of these species clearly proceeds primarily from older volca-

noes to newer ones. One may speculate that the relatively small number of species that have been formed on the 5 volcanoes of Hawaiʻi Island is directly related to the geological newness of this island.

New volcanoes appear to be especially open to colonization by waifs from an older volcano and surely the above step-wise pattern is the most prominent one; however, in some cases, the data suggest that richly-speciating groups may include species that have made the colonization from a newer volcano back to an older one. Two cases of this phenomenon involving Maui and Oʻahu are shown in Fig. 1.

Most of the inter-island movements of these drosophilids appear to be best explained by the "founder principle" (Giddings *et al.*, 1989). This suggests the inter-volcano and inter-island speciation events are strongly influenced by the constraints of the separation of populations by distance, which, in the case of new lava flows, may sometimes be very small. The above point brings us to a consideration of the data that exist on the dynamics of genetic change within individual populations of these island species.

Evolutionary Processes Within Populations

A large recent body of behavioral data from diverse animal and plant species strongly supports the conclusion that mate or gamete choice, especially by the female, is a significant driving force for genetic change in populations. I have recently pointed out that, if this theory is valid, it will be necessary to reinterpret the basic mode of selection in natural sexual populations. This will include reexamining the relationship between natural and sexual selection in wild populations (Carson, 2002, 2003).

Although little experimental work has been done, review of the data on mating patterns in insects strongly supports choice theory based on female choice of mate (Eberhard, 1996). The complex sexual behavior of the Hawaiian picture-winged species *Drosophila silvestris* has been shown to conform to this idea (Carson, 2002).

About 1970, when the phylogenetic tracing studies of the picture-winged Hawaiian *Drosophila* species had been largely competed, intensive population genetic work began on *Drosophila silvestris*. This species belongs to the *planitibia* group (Table 1) and is one of 3 species of this group that is endemic to the island of Hawaiʻi.

Drosophila silvestris is a useful choice for 2 reasons. First, it represents a genetically variable, recently-evolved endemic found on 5 successively younger volcanoes on the newest island, Hawaiʻi (Carson, 1982; Craddock & Carson, 1989). Secondly, it manifests a highly complex mating system involving female choice that gives evidence of being in a state of active genetic change in certain populations on Hawaiʻi (Carson, 1982, 2002).

Kohala volcano (age about 400,000 years), Hualalai, and Mauna Kea, like all the older volcanoes, appear to have ceased volcanic activity, whereas the 2 most recent volcanoes, Mauna Loa and Kīlauea, are currently active. A comparable range of geological activity is not found elsewhere in the archipelago. Phylogenetically, *D. silvestris* is particularly close to the chromosomally monomorphic species *D. planitibia* Hardy of Maui. These 2 species have a virtually identical basic inversion formula, although *silvestris* has many new added inversions that are polymorphic only within that species. Thus it has been hypothesized that the initial population of *silvestris* on the newer island was established from a founder individual (or individuals) arising from an ancient *planitibia*-like ancestor from Maui. In space and time, therefore, Hawaiʻi Island presents a very promising set of populations that may be in an active state of genetic differentiation from older to newer lava flows.

Within each local population, *silvestris* displays a number of specific inversion polymorphisms and electrophoretic variations that differ in frequency from one population to the next. These can be used to make a set of hypotheses relating to the pattern of its intraspecific phylogenetic differentiation from volcano to volcano stressing novel genetic change that has occurred very recently within a single species (Kaneshiro & Kurihara, 1981; Craddock & Carson, 1989).

Sexual Behavior and Mate Choice

Like the ancestral Maui species of this group of picture-wings, sexual behavior at maturity is high-ly complex. Individuals mature slowly and have substantial longevity in the cool upland environ-ments. Mature males seek out and apparently accumulate on the scapes of single tree ferns (*Cibotium* spp.) in groupings of 5–10 individuals. Each male patrols his own individual lek, aggressively defending it from all intruders. Females visit these leks, stimulating the initiation of courtship by the male. Despite this, copulation is very rarely observed in natural leks. The implication of these rarely-observed matings is that females move between leks but only very rarely is a male accepted.

In the laboratory, courtship between pairs of flies placed in chambers is often long and com-plicated, with the female frequently decamping to the degree possible in the artificial chamber. This may extend over many hours and "pair-matings" frequently fail to be consummated. The male approach to the female begins with persistent frontal displays and circling by the male from some distance away. In group experiments, many individual males are rejected.

While imperfectly mimicking the natural leks, quantitative studies of mating behavior in small plastic cages have yielded valuable information on copulation patterns. Tree fern leks are simulated by placing 10 to 12 mature, healthy, individually marked, virgin males into a clear plastic cage with perches and available food. After a period of several days for male environmental adjustment, a sin-gle mature virgin female is introduced for a one-half hour period. If there is no copulation during this time, the female is removed and replaced by another one. A copulating pair is covered with a glass vial until separation, after which the female is removed and her offspring reared. Each male is returned to the cage following separation. Each experiment is repeated with the same males over 6 days

A striking result is obtained (Carson, 2002). One-third of the males in the cages are rejected by all introduced females and remain un-mated over the week of testing. These may be designated as the "duds". In contrast, a separate one-third of the males are repeatedly accepted by a newly intro-duced female, often on a daily basis ("studs"). The final one-third consists of males accepted only a few times during the week of tests. All males are mature, healthy and court actively under these con-ditions.

The restrictive complications of sexual selection in *silvestris* appear not to be confined to that species. Although the behaviors of individuals in only a few other species of Hawaiian *Drosophila* have been studied quantitatively, the general importance of female choice can be inferred. Indeed, female choice may be characteristic of other drosophilids and possibly many other animals and plants.

Some Theoretical Questions and Conclusions

Why do these insular populations of Hawaiian *Drosophila* show such a continuous formation of new species? Why are the mating systems so profoundly exaggerated by constantly changing sexual selection as these new species are formed in this serial fashion? Why should behavioral sexual selec-tion have so much strength as these species evolve allopatrically on what are basically new sub-strates provided by emerging linear, spatially separated shield volcanoes? What is the relationship between natural and sexual selection? I suggest that the findings in Hawaiian *Drosophila* may pro-vide the basis for a few tentative working hypotheses.

Natural Selection Subsumes Sexual Selection

The Darwinian view that natural selection is ubiquitous is adopted here as the guiding principle in all populations. Eliminative natural selection takes a continually heavy toll, so that relatively few individuals of a natural population survive to the reproductive phase. As sexual maturity is reached in the populations of a species, the general pressure on the selection system shifts from survival to mechanisms that place those individuals that have survived to adulthood through a series of tests that force differential reproduction among them.

In the above process, choice of mate appears to be an all-pervasive, but often cryptic guide to this process (Carson, 2003). In my view, sexual selection emerges, not a separate process, but one that functions as a greatly refined extension of natural selection that serves to optimize the passage of adaptively superior DNA to the ensuing generation. This is the essence of Darwinian fitness.

Nature provides the female with a choice system that will enable the diagnosis of fitness of the male through various devices, such as male-to-male combat, lek displays, resistance to parasites and other environmental hazards.

This is where the subject of genetic variability among males must be addressed. Recombining sexual gene pools are far more complex than has been realized. The presence of genome-wide systems of balanced polygenic polymorphism undergoing recombination, especially in the female, is able to create fields made up of unique genetically variable males. This genetic variability confronts the choice mechanism of females.

Choosing females appear to be assessing male fitness by using many clues. Most particularly, they may be able to use discriminatory devices to sort out genetically based fitness qualities of their prospective mates. Much is yet to be learned about how this is accomplished and I see this as a major future challenge to genetically based ethology.

Active genetic change and species formation comparable to that of *Drosophila* is also observed among the majority of the endemic Hawaiian terrestrial biota of other insects, birds, snails and flowering plants (Zimmerman, 1948; Hubbell, 1968; Kay, 1994; Wagner & Funk, 1995).

Is the evolutionary process that has allowed formation of these new forms uniform throughout the geography of the islands or is there a special geographical or geological area where evolutionary change is concentrated? I suggest the facts support the latter view. Active eruptive zones and formation of new sterile surface lava is now, and has been for at least 20 million years, confined to a very small zone of the archipelago that lies above the Hawaiian hotspot. As the Pacific plate moves northwestward, it carries the aging lava flows and their volcanoes away from the extremely narrow region where active volcanism is at work.

At the time of formation, the lava is sterile and forbidding to most life but will immediately see the arrival of propagules from adjacent older flows. As the plate moves, volcanism will slowly cease and the flow of successful colonists will facilitate the emergence of a new ecosystem. Such colonists will face maximal environmental challenges to both the survival and reproductive phases of natural selection. Thus the volcanic hotspot continually spawns a long-persisting "evolutionary hot-spot" in the area directly above it.

Such a diagrammatic situation is rarely if ever found elsewhere in the world although short-term volcanic challenges characterize many single isolated oceanic islands as well as oceanic archipelagos with irregular volcanism such as Galápagos, the Societies, and the Canary Islands (Carlquist, 1965). In most of these cases, however, the volcanic challenge may be of relatively short duration rather than continuous as in Hawai'i, but volcanism may challenge genetic systems to yield isolated bursts of species. These are likely to be briefer episodes than those occurring in the long-lasting Hawaiian case where some lineages have been through the process earlier.

Most continental biota have come to some degree of ecological stability, characterized by broad patterns of gradual geographic change. Nevertheless, exuberant sexually reproducing, cross-fertilizing systems can be intensified in some special geographic sites. For example, such areas are found on the high, immobile continental volcanoes of Africa (Hedberg, 1970).

A case of genetic systems even closer to what is found in Hawai'i occurs in association with the geological revolutions associated with the actively spreading east rift zone of Africa. The cichlid fish fauna in the geologically new African lakes is one of the evolutionary wonders of the world (Echelle & Kornfield, 1984; Goldschmidt, 1996). Hundreds of species in single lakes are not only strongly adapted locally but, like in Hawaiian *Drosophila* they have developed what appear to be novel sexual selection systems that parallel those found in Hawaiian drosophilids. Thus, the development of the reproductive systems in cichlids appears to be a process that reinforces mate choice and Darwinian fitness as an intra-population outcome of natural selection. In both faunas, natural selection drives genetic change that is based on selection for intra-population fitness rather than selection for inter-specific isolation.

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