

17 ECOLOGICAL AND REPRODUCTIVE SHIFTS IN
THE DIVERSIFICATION OF THE ENDEMIC
HAWAIIAN *DROSOPHILA*

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The *Drosophilidae* endemic to the isolated Hawaiian archipelago provide one of the most spectacular examples of insular diversification known to evolutionary biologists (Zimmerman 1958; Carson et al. 1970; Williamson 1981). From one or at most two original founders from a distant continental source (Throckmorton 1966; Thomas and Hunt 1991; DeSalle 1995; Kaneshiro et al. 1995), more than 800 contemporary species have evolved after an initial colonization more than 30 Mya (Beverley and Wilson 1985; DeSalle 1992). This explosive speciation has resulted in forms that vary widely in size and morphology, with different species displaying bizarre modifications of the head, forelegs, wings, or mouthparts, especially in males (Hardy 1965; Hardy and Kaneshiro 1981). Such morphological divergence largely reflects the development of extraordinary secondary sexual characteristics correlated with male mating behavior (Spieth 1966, 1974, 1982). Significantly, the morphological and behavioral diversity among Hawaiian taxa far exceeds that of drosophilids anywhere else in the world. Given the small area, remoteness, and comparative geological youth of the current Hawaiian Islands (< 5.3 My), it is astounding that ca. 25% of the world's species of *Drosophila* are found on the six major islands of this archipelago. Most species are single-island endemics, and are often restricted to a single volcano, or even a single "kipuka" (a patch of forest isolated by lava flows). The Hawaiian drosophilids form a spectacular sexual radiation, and have been a frequent target for studies of geographic speciation and the evolution of mating barriers (Carson 1983, 1986; Kaneshiro 1983, 1989; Kaneshiro and Boake 1987; Carson et al. 1990; DeSalle 1995; Kaneshiro et al. 1995).

Although most often noted for their morphological and behavioral diversity, the Hawaiian drosophilids are also ecologically quite diverse. They occupy a wide range of altitudes (50 to 3,000 m) and moisture regimes that range from dry open forests to rain forests receiving more 8,000 mm of annual precipitation. Within this broad spectrum of habitats, different drosophilid species exploit a diverse array of ecological substrates for breeding, including sap exudates (fluxes) and decaying parts of several different plant species (Heed 1968, 1971; Montgomery 1975). Previous treatments have placed little emphasis on the role of ecological characteristics in the evolution of the Hawaiian drosophilids, with much greater attention being given to the role of sexual selection (Carson 1978, 1986; Kaneshiro 1983, 1989) and founder events (Carson 1971; Carson and Templeton 1984) in driving divergence and species proliferation. This chapter instead focuses on ecological differentiation in the Hawaiian drosophilids and associated adaptive differentiation, particularly with respect to the female

reproductive system. Using a phylogenetic approach, we reconstruct the sequence of ecological shifts in the evolution of the group and show how a phylogenetic analysis of ecological, morphological, developmental, physiological, and behavioral aspects of female reproduction in the endemic Hawaiian *Drosophila* can illuminate our understanding of the adaptive radiation of this remarkable group of organisms.

Today, roughly 30 species groups and 55 subgroups of Hawaiian drosophilids are recognized, based largely on morphology (K. Kaneshiro, pers. comm.). Given that systematic studies are still incomplete, the number of these groups may well increase. Clearly, the Hawaiian drosophilid radiation has been complex, likely resulting from the operation of multiple forces rather than just a single one. What was the adaptive basis of divergence in this group? These flies have evolved adaptations to the intraspecific sexual milieu (Carson 1978) as well as to the external environment. In the first arena, the driving force is sexual selection; in the second, environmental adaptation is driven by natural selection.

The exaggerated development of a variety of male secondary sexual characteristics, many of which provide key characters for identifying species groups, is clear evidence for the significance of sexual selection in the evolution of the endemic Hawaiian drosophilids. These traits are generally directly involved in the elaborate courtship and mating behaviors of these flies (Spieth 1966, 1974, 1982). For example, during the "head-under-wing" stage of courtship the enlarged and erect antennae of males of the *antopocerus* species group articulate with the female wing vanes and protect the male's head during his vigorous lunging movements (Spieth 1968a). In the modified-mouthparts group, the male labellum is variously modified in shape and hairiness (Hardy and Kaneshiro 1975); males of this group use this structure to grasp the female's genital area during courtship (Spieth 1966). Although members of individual species groups share particular morphological and behavioral modifications indicative of their phylogenetic affinity, nonetheless each species displays a unique courtship pattern and unique morphology. Intraspecific coadaptation of male and female behaviors constitutes a specific mate recognition system distinct from that of related species.

Classical models of sexual selection accent the intermale competition for mates and the role of epigamic selection by females in a runaway process that generates extreme forms of particular male characters (Fisher 1930; Lande 1981). Given that the sexual system is dynamic and highly labile with a variety of mating types segregating among both males and females, any destabilization, as during a founder event, may be followed by a shift of balance and a readjustment of the intersexual interactions, leading to a novel mating system (Carson 1986; Kaneshiro 1989). Thus sexual selection may be pivotal to the speciation process and the radiation of a group of organisms such as the Hawaiian drosophilids. Although most models of sexual selection assume that female preferences for certain male secondary sexual characters are counterbalanced by natural selection constraining the runaway process, Kaneshiro (1987) suggests an alternative mechanism whereby secondary sexual characters are not necessarily subject to direct selection, but rather originate and are maintained pleiotropically or via genetic linkage to some other feature of the mate recognition system.

Whereas the divergent morphologies and mating behaviors of Hawaiian drosophilids are readily explained as a consequence of sexual selection, this force cannot account for the other prominent feature of the group, namely their ecological divergence. Drosophilids are saprophages and the Hawaiian species have radiated from the original founder to breed in a diverse array of substrates that includes fungi, rotting flowers, leaves, roots, stems, bark, and tree fluxes from some 40 families of endemic Hawaiian plants (Heed 1968, 1971; Montgomery 1975). A few Hawaiian drosophilids are even parasitic on spiders' eggs (Hardy 1965; Heed 1968). Exploitation of such diverse breeding substrates has succeeded because natural selection has molded various aspects of the female reproductive system to adapt each species to its particular breeding niche and its local environment. Herein lies the real basis of the adaptive radiation of the endemic Hawaiian drosophilids.

It can be envisaged that saturation of the initial breeding substrates and the accompanying competition, coupled with the availability of open niches as new islands and habitats were colonized, led rather quickly to an ecological radiation, with natural selection driving the adaptation of the flies to novel breeding niches. Breeding substrates of the Hawaiian flies vary widely in predictability and nutrient reserves for larval growth. At the same time, patterns of egg production and distribution can vary dramatically: some Hawaiian species produce just one egg at a time, whereas others mature and oviposit hundreds of eggs at a time (Kambysellis and Heed 1971). This wide range of female reproductive strategies and the concomitant ecological divergence raise the following questions which provide the focus of this chapter.

- What is the relationship of female reproductive traits to the nature of the larval substrate?
- How are these traits (e.g., ovariole number per ovary, number of mature eggs per ovariole, length of egg respiratory filaments) adapted to features of the breeding substrate?
- What was the ancestral larval substrate for the Hawaiian drosophilids, and what has been the subsequent pattern of shifts in substrate?
- Do species utilizing a particular kind of substrate (e.g., plant stems) form a monophyletic group? If not, how many times has each substrate been invaded?
- Where there have been multiple invasions of the same substrate, have the female reproductive traits shown correlated shifts? And
- What is the relationship, if any, between variation in ecology and female reproductive traits and variation in male mating behavior and sexual characteristics, and what are the implications of such a relationship for evolution and speciation in the Hawaiian *Drosophila*?

Before addressing these questions and analyzing the evolution of female reproductive biology in these flies in relation to their ecological diversification in breeding substrate and host plant use, we present some further background on the biology and taxonomy of the Hawaiian *Drosophila*. This information provides the necessary context for understanding the adaptive radiation of the group.

Natural History of the Hawaiian Drosophilidae

The endemic Hawaiian drosophilids comprise an extremely diverse and species-rich assemblage, quite distinct from continental forms and, as a paradigm of the process of adaptive radiation on oceanic islands, a group worthy of intensive research. Initial multi-disciplinary studies conducted under the auspices of the Hawaiian *Drosophila* Project (Spieth 1981) accumulated a wealth of information on the morphology and systematics of the group, their polytene chromosomal inversion differences, karyotypes, ecology, behavior, and allozymic differentiation (see reviews by Carson et al. 1970; Carson and Kaneshiro 1976). These early studies suggested that, despite their extreme morphological divergence, the Hawaiian flies are genetically quite closely related and form a cohesive group. More recent molecular analyses have, in fact, confirmed that the Hawaiian drosophilids are monophyletic (Thomas and Hunt 1991; DeSalle 1992, 1995; Kambysellis et al. 1995).

Systematics

Although the initial taxonomic treatment distinguished nine genera of Hawaiian drosophilids (Hardy 1965), it is now clear that there are only two evolutionary lineages, the drosophiloids and the scaptomyzoids, with the majority of species belonging to either *Drosophila* or *Scaptomyza* (Kaneshiro 1976). The existence of forms that are in some respects intermediate between drosophiloids and scaptomyzoids suggests that both lineages may have derived from one original founder, with the genus *Scaptomyza* evolving in Hawai'i, and spreading from there to the rest of the world (Throckmorton 1966; DeSalle 1992; Kambysellis et al. 1995).

Systematically, the Hawaiian *Drosophila* are more closely allied to subgenus *Drosophila* (Thomas and Hunt 1991; DeSalle 1992, 1995; Kambysellis et al. 1995) than subgenus *Sophophora*, to which the well-studied species *D. melanogaster* belongs. However, there is no single continental form that can be designated as the sister to the Hawaiian lineages (DeSalle 1995). Whereas the majority of Hawaiian *Drosophila* are placed in subgenus *Drosophila*, a group of six or more species are instead placed in subgenus *Engiscaptomyza* (Kaneshiro 1969; Hardy and Kaneshiro 1981). Members of this group (represented in this study by *D. crassifemur*) are somewhat intermediate between drosophiloids and scaptomyzoids; they were retained in the genus *Drosophila* on the basis of external morphology, but display *Scaptomyza*-like internal anatomy and mating behavior.

The Hawaiian scaptomyzoids alone include more than 200 species, more than in the rest of the world combined. Most belong to the genus *Scaptomyza*, but there are also three other scaptomyzoid genera with from one to eleven species (Hardy and Kaneshiro 1981). Overall, the scaptomyzoids are less diverse morphologically than the more numerous drosophiloids, although their male genitalia are well differentiated (Takada 1966). Remarkably, they appear to be ecologically more diverse than the drosophiloids, utilizing several novel breeding substrates in addition to those utilized by the drosophiloids (K. Kaneshiro, pers. comm.). Analysis of the evolution of ecological shifts in the adaptive radiation of the scaptomyzoids must, however, await systematic and phylogenetic analysis of this enigmatic group.

Male sexual behavior

Whereas the Hawaiian drosophiloids are characterized by extremely elaborate species-specific courtship patterns and marked morphological differentiation of the males, Hawaiian *Scaptomyza* have a more uniform morphology and simplified courtship behavior, with basically an "assault-type" mating (Spieth 1966). These traits correlate with a lack of lek behavior and suggest that sexual selection may be relatively unimportant in the scaptomyzoids. This contrasts with the morphological and behavioral divergence of the drosophiloids, and the prominent role of sexual selection in the majority of the members of this lineage. Intermale competition is especially marked in members of the picture-winged group where males display a high level of agonistic behavior and often engage in ritualized fighting (Spieth 1982). Furthermore, body size of these species is generally very large, and mature males advertise their sexual readiness by characteristic displays on their leks.

Significantly, courtship and mating in Hawaiian drosophiloids take place only on the leks, not on the feeding and oviposition sites (Spieth 1966, 1968b). This spatial separation between mating and oviposition sites contrasts with the usual drosophilid pattern and suggests that the processes of sexual selection among males and natural selection on female traits exerted by the breeding substrate may be independent.

Reproductive isolation and speciation

Sympatric species are behaviorally isolated by their unique courtship repertoires. Strong premating barriers essentially prevent interspecific hybridization in the field, although in a few exceptional instances limited hybridization between closely related species has been recorded (Kaneshiro and Val 1977; Carson et al. 1989). Allopatric species typically show lower levels of premating isolation; laboratory hybridizations can therefore be used to assess the extent of postmating barriers (Yang and Wheeler 1969; Craddock 1974a,b). These range from limited F_1 hybrid male sterility to complete hybrid inviability, depending on the genetic distance between species.

Clearly, much of the great proliferation of Hawaiian drosophilid species has been triggered by founder events (Carson 1971; Carson and Templeton 1984). Chance colonization of a new island or volcano by a single fertilized female has frequently resulted in genetic divergence from the ancestral population and subsequent speciation. Genetic drift in the initial founder population must be central to founder-effect speciation, but it should be recognized that the new population will usually find itself in a novel environment and selective regime, and will likely experience a shift in sexual selection as well (Kaneshiro 1989). Thus drift, natural selection, and sexual selection may jointly operate to lead to speciation following founder events.

Some speciation events in the radiation of the Hawaiian *Drosophila* have apparently occurred independently of inter- or intra-island dispersal events. In some instances, shifts in sexual selection may have been responsible (Carson 1986; Kaneshiro 1987). Such speciation might be considered non-adaptive with respect to the external environment, but adaptive with respect to the intraspecific sexual environment. In other cases, however, it is germane to ask what role the environment and natural selection may have played in speciation and subsequent adaptation.

Ecological diversification of the Hawaiian *Drosophila*

Colonization of the Hawaiian Islands by drosophilids has been accompanied by radiation into a wide variety of altitudes and ecological niches in both wet and dry montane forests that vary markedly in ecological complexity. Moreover, the habitats these flies occupy are floristically diverse, providing opportunities for the evolution of a range of fly-host plant interactions. The adult flies are quite mobile and feed at a variety of sites throughout the forest; however, the larvae of a particular species are extremely localized in their distribution, typically being restricted to one or a few specific substrates, chiefly in decaying plant material. This is due to the specificity of female behavior in selecting sites for oviposition. Presumably, their behavior is guided by olfactory and other chemosensory cues, as well as tactile cues perceived by sensory hairs on the ovipositor, which they use to probe prospective substrates prior to actual oviposition. Female behavior is thus a primary factor in the specificity of host plant use for breeding, and behavioral divergence and evolution of particular female preferences must be considered as one of the components in the ecological radiation of these flies.

Knowledge of the breeding sites of Hawaiian drosophilids began with the records of Perkins (1913) in the *Fauna Hawaiiensis*, but is mainly due to the careful field work of Heed (1968, 1971) and Montgomery (1975). Although host plants are only known for about 20% of the endemic drosophilids, some 40 of the 114 families of vascular plants represented in Hawai'i (Wagner 1991) have been recorded as substrates, including a number of native ferns. In addition, species in the white-tip scutellum group use fungi. Although relatively little is known of the chemistry of endemic Hawaiian plants (Kircher and Heed 1970), it can be surmised that the plant families utilized provide a chemical environment tolerated by the developing larvae.

Ecological divergence in the breeding niche is not restricted to the type of endemic Hawaiian plant used, but also involves the particular part of the plant selected for oviposition. Virtually all parts of a decaying plant may be used. Individual species are remarkably specific in their choice of substrate and will breed in decaying leaves, flowers, fruits, stems, bark, or roots, but usually not in several or all of these. Other Hawaiian drosophilids use fluxes or sap exudates of particular trees as their breeding substrate, while yet others use fungi. Notwithstanding the specificity of the interaction between a fly species and its particular host plant and the part of the plant utilized, it must be pointed out that the plant is not the primary nutritive source for the larvae or the adults. Drosophilids are saprophagous, and as the larvae mine the substrate, they feed on the microbial fauna associated with the decaying plant material. Although most continental *Drosophila* feed on yeasts (see Begon 1982), both larvae and adults of the Hawaiian species feed primarily on bacteria (Robertson et al. 1968; M. Kambysellis, unpubl. data). Thus the ecological interaction is quite complex with a three-way interplay between bacteria, plants, and flies. We have initiated a project on identification and analysis of the bacteria found in the gut and fecal material of *Drosophila* species from different substrates and different habitats, and the bacteria associated with various host plants, but the data are complex and will not be discussed further here. Suffice it to say that the microflora is an important component

of Hawaiian habitats and may be a critical factor in the ecological interactions between *Drosophila* and their decaying breeding substrates. Selection of a particular plant for oviposition may depend as much on the associated microflora as on the chemistry of the plant. It may well be the combined odors released by the plant and associated bacteria that make a particular substrate attractive to a searching female and trigger her oviposition behavior.

Methods

Molecular systematics

The 39 Hawaiian taxa examined in this study are included in Table 17.1. Species groups and subgroups are largely based on morphological traits, following the arrangement of Hardy and Kaneshiro (1981) and Kaneshiro et al. (1995). Five continental drosophilids were used as outgroups, including one scaptomyzoid (*Scaptomyza adusta*) and four drosophiloids, representing the subgenera *Drosophila* (*D. virilis* and *D. buzzatii*) and *Sophophora* (*D. birchii* and *D. melanogaster*).

DNA was extracted from adult flies of each species, and a 1 kb segment of the *Yp1* yolk protein gene was PCR-amplified and sequenced (Kambysellis et al. 1995). The sequenced region encompassed the two introns and 64% of the coding sequences of the *Yp1* gene. The sequence for *D. melanogaster* was provided by Hung and Wensink (1981).

Global parsimony was used to construct a phylogeny based on the *Yp1* nucleotide sequence data, using an unweighted analysis implemented in PAUP version 3.1.1 (Swofford 1993). Given the large number of taxa involved, we conducted heuristic searches, using the random stepwise addition option for generating a different tree to begin each of ten replicate searches. The resulting topology of the ingroup taxa was independent of whether the outgroup chosen was *D. melanogaster*, both *Sophophora*s, or all four continental *Drosophila*. The level of support for each branch of the single most parsimonious tree was assessed via bootstrap analysis (Felsenstein 1985, 1988) and decay analysis (Bremer 1988; Donoghue et al. 1992). Bootstrap values from 1,000 replications were calculated using the random-input file option of MEGA (Kumar et al. 1993), and decay indices obtained by recursively saving trees one step longer than the previous trees and calculating the strict consensus until all the branches collapsed.

Since our *Yp1* phylogeny focuses on the picture-winged species and includes few early-divergent Hawaiian drosophilids (only 5 species from two lineages), we made use of another molecular phylogeny of 15 Hawaiian drosophilids, kindly supplied by Rob DeSalle, that includes 7 additional species. This maximum parsimony tree includes five groups of non-picture-wings (represented by two species each) and five picture-winged species, and is based on nucleotide sequences of fragments of four mitochondrial and four nuclear genes, totalling ~2.5 kb in all (DeSalle 1992; Baker and DeSalle 1997).

Analysis of character evolution

The evolution of ecological and female reproductive characters pertinent to the adaptive radiation of the Hawaiian *Drosophilidae* was analyzed using MacClade 3.04

Table 17.1. Genera and species groups of Hawaiian Drosophilidae analyzed in this study and their distinguishing features.

Group	Number of species	Distinguishing features	Taxa analyzed
<i>Scaptomyza</i>	200+	assault-type mating	<i>S. albovittata</i>
<i>Drosophila</i>			
Subg. <i>Engiscaptomyza</i>	6	intermediate morphology	<i>D. crassifemur</i>
Subg. <i>Drosophila</i>			
White-tip scutellum	~80	slender iridescent body	<i>D. longipedis</i> <i>D. iki</i>
Modified tarsi	~80	modifications of first and/or second tarsomere of male foreleg	
Bristle tarsi		clump of heavy bristles on front basitarsus	<i>D. petalopeza</i>
Spoon tarsi		second tarsomere flattened and concave	<i>D. waddingtoni</i>
<i>antopocerus</i>	~17	large porrect antennae in males	<i>D. adunca</i> <i>D. tanythrix</i> <i>D. yooni</i>
Modified mouthparts	~110	dense hairs, bristles or spines on apical lobes of male labella	<i>D. mimica</i> <i>D. soonae</i> <i>D. infuscata</i>
Picture wings	110	maculations on wings	
<i>primaeva</i>	2		<i>D. primaeva</i>
<i>adiastola</i>	16		<i>D. ornata</i> <i>D. truncipenna</i> <i>D. spectabilis</i> <i>D. setosimentum</i> <i>D. adiaastola</i>
<i>planitibia</i>	17		<i>D. picticornis</i> <i>D. setosifrons</i> <i>D. substenoptera</i> <i>D. nigribasis</i> <i>D. oahuensis</i> <i>D. obscuripes</i> <i>D. melanocephala</i> <i>D. cyrtoloma</i> <i>D. hemipeza</i> <i>D. planitibia</i> <i>D. differens</i> <i>D. silvestris</i> <i>D. heteroneura</i>
<i>glabriapex</i>	34		<i>D. pilimana</i> <i>D. fasciculisetae</i> <i>D. lineosetae</i> <i>D. macrothrix</i> <i>D. punalua</i>
<i>grimshawi</i>	39		<i>D. mulli</i> <i>D. sproati</i> <i>D. silvarentis</i> <i>D. heedi</i> <i>D. hawaiiensis</i> <i>D. disjuncta</i> <i>D. bostrycha</i> <i>D. grimshawi</i> ¹ <i>D. pullipes</i>

¹The molecular phylogeny includes both the Kaua'i and Maui populations of *D. grimshawi*. These may be incipient species.

(Maddison and Maddison 1992) to overlay character-states on the molecular phylogeny and determine the most parsimonious scenario for the origin of those states. We applied both accelerated and delayed transformation optimization options; the character reconstructions presented, however, use ACCTRAN, which minimizes parallel evolution. Records of host plant use (Heed 1968, 1971; Montgomery 1975) formed the basis for mapping the ecological shifts from one plant family to another, from monophagy to oligophagy and polyphagy, and from one breeding substrate to another. The evolution of female reproductive types was analyzed using available data on the egg/ovarian types of Hawaiian drosophilids (Kambysellis and Heed 1971; M. Kambysellis and E. Craddock, unpubl. data). The island distributions of the Hawaiian species were used in a similar manner to display geographic patterns of speciation in the group.

Results and Discussion

Molecular phylogeny of the Hawaiian Drosophilidae

Figure 17.1A presents the single most parsimonious tree of 39 Hawaiian *Drosophila* species based on a cladistic analysis of *Yp1* DNA sequences. This phylogeny (length $L = 1,550$; $CI = 0.605$; $RI = 0.706$) includes 34 picture-winged species, representing all five recognized species groups and 12 of the 14 recognized species subgroups (Kaneshiro et al. 1995), as well as two members of the modified-mouthparts group, and three members of the *antopocerus* group. Most of the previously recognized species groups and subgroups are resolved as monophyletic clades with strong statistical support (Figure 17.1A). The placements of two species (*D. mulli* and *D. punalua*) in our molecular phylogeny differ from their previously recognized morphological affinities. *Drosophila mulli* had been classified with *D. sproati* in the *grimshawi* group (Kaneshiro et al. 1995), but aligns with the *glabriapex* group in the *Yp1* phylogeny. *Drosophila punalua* had been placed in the *glabriapex* group, but our molecular data place it in the *grimshawi* group. A more significant discrepancy is that the *picticornis* subgroup does not form a monophyletic clade with the two other recognized subgroups (*cyrtoloma* and *planitibia*) of the *planitibia* species group. Although resolution of the basal branches is weak, other data also suggest that the taxonomic affinity of the *picticornis* subgroup needs to be reevaluated (Russo et al. 1995).

Figure 17.1B shows another molecular phylogeny of the Hawaiian Drosophilidae, provided by Baker and DeSalle (1997). This phylogeny is the single most parsimonious tree based on nuclear and mitochondrial DNA sequences ($L = 1,946$; $CI = 0.50$; $RI = 0.49$) and is quite robust, as indicated by the bootstrap values and decay indices. It includes representatives of the more primitive Hawaiian groups (*antopocerus*, spoon tarsi, bristle tarsi, white-tip scutellum, modified-mouthparts), the more advanced picture-winged group (5 spp.), and the more divergent scaptomyzoids (represented by *D. crassifemur* [subgenus *Engiscaptomyza*] and *Scaptomyza albobittata*). The two outgroups used are the continental species *D. melanogaster* and *D. mulleri*. This tree of 15 species provides a broad overview of the relationships among some of the major species groups of Hawaiian flies.

The two independent molecular phylogenies (Figure 17.1A,B) are congruent in indicating that the Hawaiian drosophilids are monophyletic, that the genus *Scaptomyza* diverged between the continental and Hawaiian *Drosophila*, and that the picture-winged flies comprise the most recently derived of the Hawaiian species-groups. The trees differ, however, in their placement of the *antopocerus* and modified-mouthparts groups. Because the topology of these two groups is weak in the *Yp1* tree (Figure 17.1A; Kambysellis et al. 1995), we favor the systematic arrangement arising from DeSalle's data (Figure 17.1B), which places the *antopocerus* group as sister to the modified-mouthparts group, with the latter being sister to the picture-winged group. Morphological similarities in the mouthparts of modified-mouthparts species and some of the *adiastola* species group – one of the more primitive picture-winged groups (Hardy 1965) – provide further support for this topology.

There is considerable overlap in the taxa included in the two analyses: the five picture-winged species, the two *antopocerus* species, and one of the two modified-mouthpart species in the Baker-DeSalle tree are included in our *Yp1* tree. However, there is no overlap in the sequence data sets used to derive the two phylogenies, which precludes combining the sequences in a single analysis. So, for illustrative purposes only, we have simply inserted the phylogeny of the 10 primitive, non-picture-winged Hawaiian drosophilids resolved in the Baker-DeSalle analysis into our *Yp1* phylogeny of the 34 species of the more derived picture-winged group to form a composite phylogeny (Figure 17.1C). This sampling of 44 Hawaiian species represents the broad diversity of Hawaiian drosophilids, based on the best available molecular data. For efficiency, we use this composite phylogeny as the basis for subsequent analyses of the evolution of ecological and reproductive characters.

Some of the features which distinguish species groups, as well as the distributions of the Hawaiian species, are also shown in Figure 17.1C. For the better sampled groups of picture-wings, the composite phylogeny indicates that dispersal has generally

Figure 17.1. (Opposite) Molecular phylogenies of the endemic Hawaiian Drosophilidae. (A) The single most parsimonious tree of 39 Hawaiian *Drosophila* species and 5 continental drosophilids based on nucleotide sequences of the *Yp1* gene. Bootstrap values from 1,000 replications are shown above the branches; decay indices are shown below. The arrow indicates the base of the Hawaiian *Drosophila*; the asterisk indicates the base of the picture-winged group. (B) The single most parsimonious tree of 15 Hawaiian drosophilids with two continental outgroups based on nucleotide sequences from 4 mtDNA and 4 nDNA gene regions (Baker and DeSalle 1997). (C) Composite tree, showing the phylogenetic relationships of 10 non-picture-winged Hawaiian species from (B) and 34 picture-winged species from (A) (as arrowed). Abbreviations for the morphologically defined species groups (in bold) are as follows: **Scapt** – scaptomyzoids; **wts** – white-tip scutellum; **ant** – *antopocerus*; **mt** – modified tarsi; **mmp** – modified mouthparts; **p** – *primaeva*. Picture-winged species subgroups (italics) are as shown; *pict* represents the *picticornis* subgroup. Island distributions of the 44 species are overlaid on the tree assuming accelerated transformation. To the right of the tree are shown distinctive morphological features of representative species and species groups (drawings taken from Hardy 1965). These include (1–6) the wings of six species approximately to scale; (7) the head of a male *D. heteroncura*; (8) a male *D. hamifera*, with (9) *D. melanogaster* for scale; and representative species with modified (10) mouthparts, (11) antennae, (12, 13) foretarsi, and (14) femurs. The numbers also identify the species whose traits are illustrated in the molecular phylogeny. Species illustrated but not in the phylogeny are as follows: (3) *D. neoperkinsi* of the *cyrtoloma* subgroup; (8) *D. hamifera* of the *adiastola* group; (10) *D. scolostoma* of the modified-mouthparts group; (12) *D. clavata* and (13) *D. attenuata*, both of the modified-tarsi group.

proceeded from older to younger islands in the Hawaiian chain. For example, in the *adiastola* group the Kaua'i species *D. ornata* is basal, with subsequent evolution of the Maui species, and finally the species on the youngest island of Hawai'i. Carson (1970, 1981), using chromosomal banding patterns, was the first to trace inter-island migrations in *Drosophila* down the Hawaiian chain. Among the 106 picture-winged species analyzed, he inferred a minimum of 45 inter-island colonization events, with eight founders from the currently oldest high island (Kaua'i) serving as progenitors of the 94 species found on the younger, more southeasterly islands in the chain (Carson 1992). Similar patterns have been inferred more recently, based on both chromosomal banding patterns (Kaneshiro et al. 1995) and DNA sequences (DeSalle 1995). Our molecular phylogeny (Figure 17.1A), although incomplete, confirms the general pattern of island hopping down the chain from older to younger islands. However, our molecular data imply fewer instances of back-migrations than are suggested by the chromosomal data.

Ecological character analysis

Table 17.2 summarizes data on oviposition sites known for the endemic Hawaiian *Drosophila*; Figures 17.2 and 17.3 display the inferred evolutionary patterns of ecological shifts in these character-states.

HOST PLANT ASSOCIATION – With respect to the taxonomic breadth of plants used as breeding substrates, the majority of Hawaiian drosophilids (81%) are monophagous; each monophagous species uses plants belonging to a single plant family. Oligophagy (the use of two to four plant families) is restricted to some of the more derived species. Only two of the 44 species analyzed show polyphagy (use of ≥ 5 plant families); in both cases, polyphagy is recently and independently derived (Figure 17.2A). Our unambiguous finding that specialization on specific plant hosts is the ancestral condition in Hawaiian *Drosophila* (a conclusion consistent under both ACCTRAN and DELTRAN) is contrary to the longstanding idea that specialization is a derived condition (Futuyma and Moreno 1988). Even where there have been shifts from one plant family to another (Figure 17.2B), most such changes have been from specialist to specialist; in only a few cases have they involved increased generalization.

It is interesting that allopatric island populations of *D. grimshawi* include both specialists and generalists (Montgomery 1975). This species is exceptional, in that it is distributed on all of the high islands except Hawai'i, which is inhabited by a very closely related species (*D. pullipes*) that differs only in the color of the legs and pleurae (Hardy and Kaneshiro 1972). Whereas *D. pullipes* and its sister taxa, the Kaua'i (Figure 17.2A) and O'ahu populations of *D. grimshawi*, are restricted to breeding on *Wikstroemia* of the plant family Thymelaeaceae (Figure 17.2B), the separate clade of *D. grimshawi* populations from Maui Nui (Maui, Moloka'i and Lana'i, interconnected during the Pleistocene due to lower sea levels) are polyphagous, using Liliaceae, Urticaceae, and eight other endemic families (but not Thymelaeaceae), as well as two introduced plants. By analyzing segregation in crosses between specialist and generalist forms and selection for opposite ovipositional behaviors, Ohta (1989) showed that only a few genes may regulate this behavior, the genetic variance being highly

Table 17.2. Summary of recorded oviposition preferences of endemic Hawaiian *Drosophila* with respect to utilization of plant families and specific breeding substrates. Except for the first column, all values shown are percentages.

Species group or subgroup	Use of major plant families/genera ¹										Breeding Substrates ²					
	No. of species	% Mono-phagy	Araliaceae	<i>Chetredendron</i>	Campanulaceae	<i>Clermontia</i>	leaves	stems	bark	flux	fruit	flowers	fungi	ferns		
Non-picture-wings³																
<i>antopocerus</i>	11	91	91	91	0	0	100	0	0	0	0	0	0	0		
Modified-																
mouthparts	21	86	0	0	48	48	43	43	0	0	38	10	24	0		
Picture-wings^{3,4}																
<i>primaeva</i> group	1	100	100	100	0	0	0	0	100	0	0	0	0	0		
<i>adiastola</i> group	11	82	0	0	73	55	18	73	55	0	18	27	0	18		
<i>planitibia</i> group																
<i>picticornis</i> subgroup	2	50	50	50	0	0	0	0	50	50	0	0	0	0		
<i>cyrtoloma</i> subgroup	6	100	100	83	0	0	0	17	100	0	0	0	0	0		
<i>planitibia</i> subgroup	5	40	40	40	100	100	0	100	60	0	0	0	0	0		
<i>slabriapex</i> group	22	82	5	5	0	0	5	77	14	5	0	0	0	0		
<i>grimsliawai</i> group ⁵	26	85	54	8	19	19	15	46	46	19	19	4	8	0		
<i>hawaiiensis</i>	9	67	11	0	0	0	0	22	11	100	0	0	0	0		
Total picture-wings	82	79	32	15	22	20	8	52	38	19	8	5	2	2		
Total, others	90	83	54	51	18	17	79	13	0	0	13	2	7	2		
Grand total	172	81	44	34	20	18	45	32	17	9	11	3	5	2		

¹*Chetredendron* is a genus in the plant family Araliaceae; *Clermontia* belongs to the family Campanulaceae.

²Some groups sum to more than 100% because certain species use more than one substrate.

³Ecological data from Heed (1968).

⁴Ecological data from Montgomery (1975).

⁵The values shown do not include the *hawaiiensis* subgroup which is listed separately.

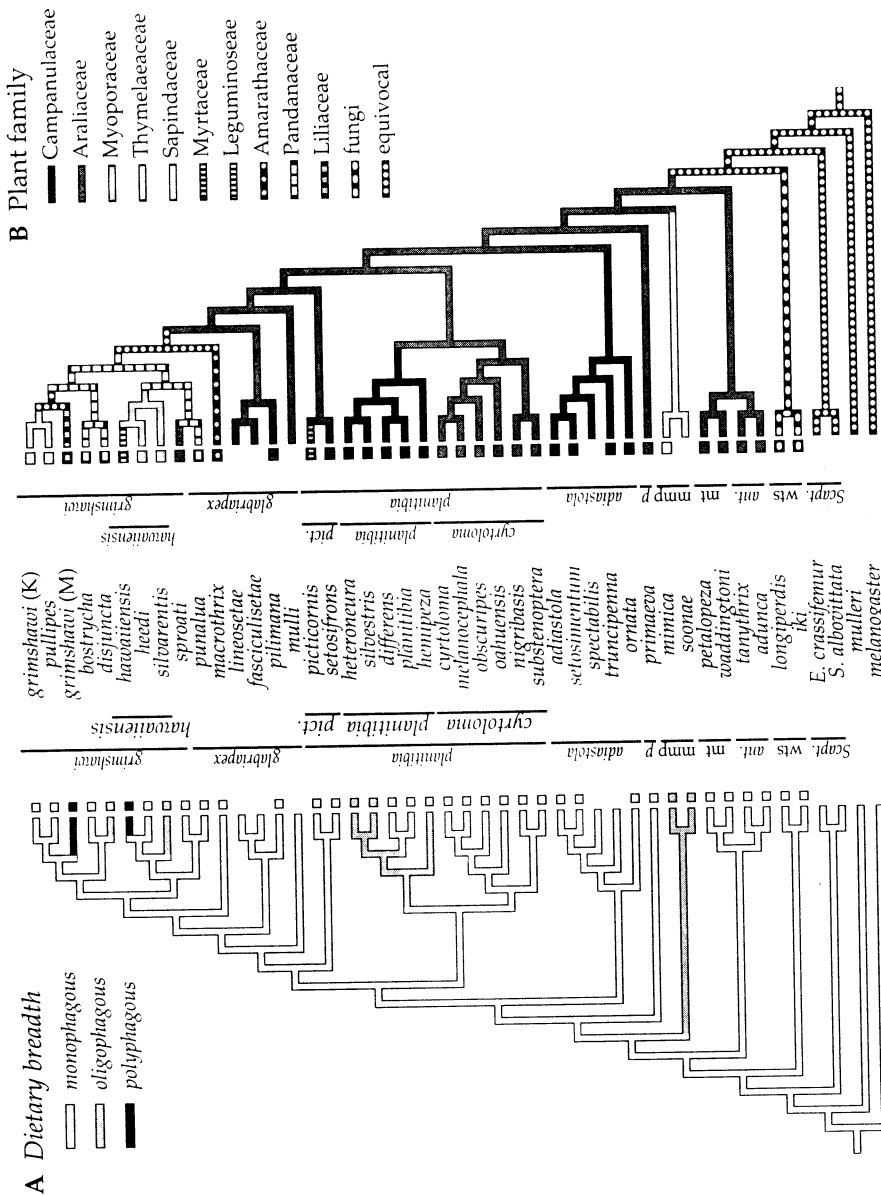


Figure 17.2. Evolution of host plant use by Hawaiian *Drosophila*, assuming ACCTRAN. Ecological data are from Heed (1968) and Montgomery (1975); no ecological data are available for the species that lack a coding box. (A) Degree of specialization in larval host plant. Most species are monophagous with respect to choice of the plant family used by the larvae; oligophagy and polyphagy are derived conditions. (B) Plant family used by larvae. Abbreviations for the drosophilid species groups and subgroups are those given in Figure 17.1.

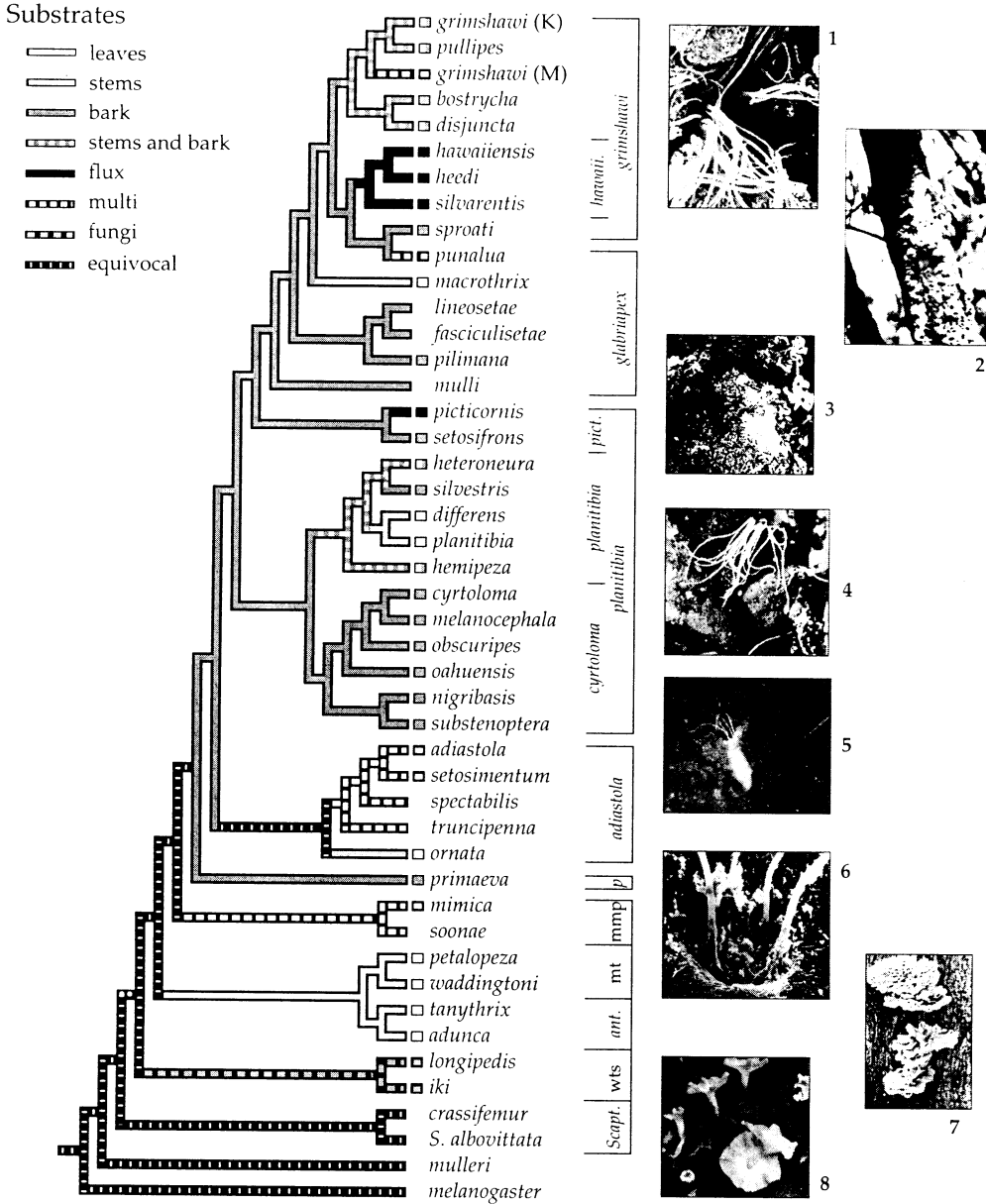


Figure 17.3. Evolution of larval substrate in Hawaiian *Drosophila*. Species are grouped into groups and subgroups as in Figure 17.1. To the right are shown representative oviposition substrates: (1) SEM of eggs of *D. silvarentis* oviposited in bark near a tree flux; (2) *Myoporum* tree flux; (3) *D. heedi* eggs oviposited in soil flux; (4) eggs oviposited in decaying *Sapindus* bark; (5) portion of decaying *Cheirodendron* leaf with a *D. adunca* egg; (6) SEM of *D. adunca* egg oviposited in *Cheirodendron* leaf; (7) fungi; (8) flowers of *Ipomoea*, a scaptomyzoid substrate.

additive. It appears that the dominant mutations favoring generalist ovipositional behavior arose in populations on Maui Nui, although the taxonomic relationships of these *grimshawi* taxa are ambiguous, and ecologically differentiated populations may well represent incipient species (Piano et al. 1997).

The family of host plants shown for oligo- and polyphagous species in Figure 17.2B is the one most frequently recorded for each drosophilid. Excluding the clade of fungus-breeding taxa, two plant families – Araliaceae and Campanulaceae – are used for breeding sites by a large majority of Hawaiian *Drosophila*. It is interesting that these two families are rather closely related (Chase et al. 1993; K. Sytsma, pers. comm.), which suggests that their members might bear some physical and chemical similarity. Two endemic genera in these families – *Cheirodendron* (Araliaceae) and *Clermontia* (Campanulaceae) – stand out as the predominant host plants, supporting the reproduction of 52% of the 172 *Drosophila* species reported (see Table 17.2). Although *Cheirodendron* is widespread in mesic to wet forests, there are only five species native to Hawai'i (Wagner et al. 1990). *Clermontia* is also widespread in moist lowland and montane forests, but has 22 endemic species and 9 heteronymic subspecies (Lammers 1995), making it the sixth largest of the 216 flowering plant genera endemic to Hawai'i (Wagner et al. 1990). The fact that larvae of so many Hawaiian drosophilids develop in the rotting tissues of *Cheirodendron* and *Clermontia* suggests that these genera may contain chemical attractants or that drosophilid larvae readily tolerate their secondary plant compounds. Kircher and Heed (1970) suggested that the physical characteristics of *Cheirodendron* leaves – which rot slowly under moist conditions – might make them a good breeding substrate.

Excluding the primitive white-tip scutellum group whose larvae develop in fungi, the primitive drosophiloids breed on Araliaceae (Figure 17.2B), primarily *Cheirodendron* (Table 17.2). The Araliaceae was apparently the first plant family invaded by Hawaiian drosophilids, as inferred using either ACCTRAN or DELTRAN reconstructions; this host is retained by many of the picture-winged species (Figure 17.2B). Based on our analysis, two independent shifts to the Campanulaceae appear to have occurred, both within the picture-winged group. The first involves the *adiastola* group; the second, the *planitibia* subgroup of the *planitibia* species group. In both instances, *Clermontia* is the principal genus utilized, but *Cyanea* – an even larger lobelioid genus of wet forest interiors and the largest plant genus endemic to Hawai'i (Givnish et al. 1995) – is also a frequent host. Shifts to the Campanulaceae have also occurred in some species of the modified-tarsi, modified-mouthparts, and *grimshawi* groups (Table 17.2), but the individual fly species involved were not included in our phylogenetic analysis.

The greatest host plant diversity is shown by the terminal *grimshawi* species group, which has undergone numerous host plant shifts into a taxonomically diverse group of plant families. Because of the frequency of oligophagy and polyphagy in this species group, the extreme diversity of host plants, and the fact that our molecular phylogeny includes only a partial sampling of *grimshawi* group species, we remain cautious as to the exact order and pattern of host plant shifts in this group. Members of this group evolved late and were among the last drosophilid lineages to colonize Hawaiian forests; thus, it is tempting to speculate that interspecific

competition and saturation of favored host plants may have favored the adoption of several new hosts by the *grimshawi* group. Alternatively, Carson and Ohta (1981) suggested that generalism in this group evolved in response to the presence of a series of unfilled niches.

BREEDING SUBSTRATE – An even more complex evolutionary pattern emerges when the actual substrates used for breeding are considered (Figure 17.3; Table 17.2), particularly those used by more recently derived groups. The scaptomyzoids, although poorly studied, are ecologically diverse (K. Kaneshiro, pers. comm.). Substrates for the two scaptomyzoids included in the molecular phylogeny are unknown, but similar species breed in flowers (Heed 1968), an ecological resource that is also utilized by a few of the modified-mouthpart species (Table 17.2). It is likely that the scaptomyzoids have undergone an ecological radiation in breeding substrate that is at least as broad as that of the drosophiloids. However, the poor representation of scaptomyzoids in our molecular phylogeny, as well as limited ecological data, precludes any meaningful analysis of the scaptomyzoid radiation at this time.

Confining our attention to the Hawaiian drosophiloids, the molecular phylogeny suggests that their ancestral breeding substrate was fungi, the substrate of the most primitive white-tip scutellum group – the so-called fungus feeders (Figure 17.3). A shift to breeding in decaying leaves then occurred, involving primarily the leaves of *Cheirodendron* (Heed 1968), which are widely used by the *antopocerus* and modified-tarsi groups (Table 17.2). Evolution of the modified-mouthparts group initiated diversification into a greater array of ecological substrates, including decaying stems and fruits (Table 17.2). This large group of over 100 species is poorly represented in the molecular phylogeny, so detailed analysis must await more data.

Evolution of the highly derived picture-winged species (represented by the branch to the ancestral Kaua'i species *D. primaeva*) was associated with a shift into another novel substrate, decaying bark. The relatively late invasion of this breeding site indicated by the phylogenetic mapping refutes Spieth's (1982) assertion that the original ancestor of the Hawaiian fauna was a bark-breeder. The shift to bark-breeding in the picture-winged species is by no means complete; many taxa are known to use decaying stems. Indeed, bark and stems are the predominant substrates used by 90% of the ecologically well-sampled picture-wings (Montgomery 1975). The distinction between breeding on bark vs. stems may be somewhat arbitrary; the term stem is applied to branches ca. 2 cm or less in diameter (Montgomery 1975). Nevertheless, there appears to be some evolutionary basis for this distinction, in that substrate choice is generally consistent within a species group. For example, although two species of the *adiastola* group are shown as using multiple substrates (Figure 17.3), the group as a whole is predominantly stem-breeding (Table 17.2), whereas the *cyrtolema* subgroup is predominantly bark-breeding. Because some species are reported to use both substrates, we coded substrate use as bark exclusively, stems exclusively, or stems as well as bark.

Another substrate used by picture-winged species is tree flux. Shifts to flux-breeding occurred twice in this group, in *D. picticornis* and the *hawaiiensis* subgroup of the *grimshawi* group. In each case, the shift was from breeding in decaying bark to

breeding in the moistened bark of living trees. In fact, some of these species can also use decaying bark; nonetheless, the use of fluxes is reported as a distinct ecological specialization. Adaptive aspects of this specialization and variation in this ecological way of life are discussed later.

Although use of a single substrate is the rule, one modified-mouthparts species and four of the 34 species of picture-wings included in our phylogeny are multi-substrate users; two of the latter are also oligophagous and one (*D. grimshawi* of Maui) is polyphagous. This broader array of breeding niches and generalist tendencies suggests that these species are opportunistic, or that competition from sympatric drosophilids has forced them to oviposit in substrates to which they are maladapted. Rearing only one or a few adults from some of the rarer substrates – as compared with these species' potential fecundity and the much larger numbers of adults reared from more typical substrates (Montgomery 1975) – suggests that oviposition is sometimes incidental in these polyphagous species. The assumption underlying the observations and interpretations of most evolutionary biologists is that endemic organisms are well (if not perfectly) adapted to their particular habitats. This assumption may need to be questioned in some instances. A possible case in point involves drosophilids found to breed in plants exotic to Hawai'i (e.g., the picture-winged *D. grimshawi* and *D. crucigera* [Montgomery 1975], and the *Exalloscaptomyza* that breed in flowers of the morning glory *Ipomoea*). On the other hand, adaptation in insular organisms can occur in a matter of a few generations (e.g., Grant and Grant 1993), so host shifts from endemic to introduced plants may have been rapidly followed by adaptation. The ecological niche of drosophilid larvae and adults includes the array of bacteria and yeasts associated with plant decomposition. It is premature to make any judgment about the complex interactions involved, but our current and future research may reveal the significance of the microflora in the ecological specificity of the endemic Hawaiian flies.

Female reproductive diversification in the Hawaiian *Drosophila*

Patterns of ovarian development in the endemic Hawaiian Drosophilidae reflect a diverse array of reproductive strategies (Kambyzellis and Heed 1971). Potential fecundity varies widely as a result of differences in the structure and function of the ovaries. In different species, these may produce just one egg or hundreds of eggs at a time. This variation is due to genetic differences specifying the anatomical structure of the ovaries, especially the number of ovarioles per ovary, which vary from one to more than 50 among the Hawaiian species. Control of the process of egg maturation within an ovariole is also variable, affecting the maximum number of mature eggs per ovariole, which varies from one to three or more. Species also differ in whether their ovaries show synchronous development (with all ovarioles maturing one or more eggs simultaneously) or asynchronous development. The number of functional ovarioles may vary from one to all of those present.

Egg morphology also differs among species at the ultrastructural level (Kambyzellis 1993) as well as the gross level (Throckmorton 1966). The size and shape of the egg, the absolute and relative length of the respiratory filaments, and their

number vary remarkably among the Hawaiian species. Whereas most continental *Drosophila* have short respiratory filaments, many of the Hawaiian species, particularly the bark breeders, have extraordinarily long filaments, up to three or four times the length of the egg. For example, in *D. sejuncta*, the egg is 0.97 ± 0.02 mm long, while the two posterior filaments are 3.80 ± 0.11 mm long (Kambysellis and Heed 1971). Yet other Hawaiian drosophilids possess only rudimentary filaments or lack them entirely.

Eggs of the Hawaiian species also vary in the structure and the thickness of the eggshell, or chorion (Kambysellis 1993). Whereas the outer endochorion is very thin in all the continental drosophilids studied thus far, in Hawaiian species the thickness varies from very thin in the scaptomyzoids to more than tenfold thicker in the picture-wings. It is interesting that there is some evidence that greater eggshell thickness is due to an increased level of chorion gene amplification during egg development (J. C. Martínez-Cruzado, pers. comm.). Structurally, there are two distinctive but variable chorion features in Hawaiian drosophilid eggs. The dorsal ridge – a structure absent from continental species, as well as the primitive scaptomyzoids and white-tip scutellum flies – is present in rudimentary form in the *antopocerus* and modified-tarsi groups, but is well developed in eggs of modified-mouthparts and picture-winged species. The collar, a structure at the anterior end of the egg near the micropyle, is absent from the scaptomyzoids and the white-tip scutellum groups but well formed in all the more derived Hawaiian lineages (Kambysellis 1993).

Another extraordinarily variable female character is the ovipositor (Throckmorton 1966), the structure at the posterior end of the abdomen used in egg laying. This structure varies enormously among the Hawaiian species in shape and in length, the longest ovipositors being found in the bark-breeding species (Franchi et al. 1997). It appears that the length of the ovipositor correlates with the depth to which the egg is inserted in the substrate during oviposition.

EGG/OVARIAN TYPES – Kambysellis and Heed (1971) recognized four discrete reproductive types among Hawaiian drosophilids, based on suites of ovarian and egg characters. Here we expand the number of these types, based on additional data; the characteristics of these types are summarized in Table 17.3. The number of ovarioles and number of mature eggs per fly provide a reliable indicator of potential lifetime fecundity (David 1970), because egg production is continuous throughout adult life once females become reproductively mature. Potential fecundity varies over an extraordinarily wide range in the Hawaiian drosophilids, from a very low value in species with Type Ia ovaries that mature only one egg at a time, to an extremely high value in species with Type IIIb ovaries, which are characterized by very high ovariole numbers and several mature eggs per ovariole.

Type Ia eggs have a smooth chorion but completely lack a dorsal ridge and respiratory filaments; Type Ib eggs have a chorion with a pattern of follicle imprints, a modified dorsal ridge in some species, and may possess rudimentary respiratory filaments (Figure 17.4). Type IIIb eggs differ from those of Type IIIa in having significantly longer respiratory filaments (Figure 17.4), a greater number of mature eggs per ovariole, and a clustered vs. solitary deposition of eggs in the substrate (Table 17.3).

Table 17.3. Characteristics of female reproductive types in endemic Hawaiian drosophilids.

Type	Ovarian traits			Egg traits			Chorion traits				Oviposition
	Ovarioles per fly	Functional ovarioles	Maximum # mature eggs per ovariole development	Number of respiratory filaments	Filament ¹ length	Length/width	Endochorion thickness	Dorsal ridge	Collar	Egg positioning	
Ia	2-7	1-2	1	0	—	1.4-2.9	very thin	absent	absent	absent	singly on surface
Ib ²	11	3-6	1	0	—	2.5	thin	modified	absent	absent	singly on surface
II	8-20	all	1	4	short (0.7-1.1)	3.2-3.8	thin	rudimentary	present	present	inserted singly
IIIa	24-101	all	2	4	short (0.7-1.2)	3.2-4.0	thick, solid	well-formed	present	present	inserted singly
IIIb	28-87	all	4	4	long (1.5-3.9)	3.4-4.6	thick, solid	well-formed	present	present	clusters into substrate
IV ³	38-45	all	1 rarely 2	2	very short (0.3)	5.3	?	rudimentary	present	present	singly (lab observation)
V ⁴	37-42	all	5	4	very short (0.2)	2.8	open, meshlike	absent	absent	absent	singly into substrate

¹The values in parentheses represent the range of ratios of the length of the posterior pair of filaments to the length of the egg.

²Description of reproductive traits based on the laboratory stock of *Scaptomyza albovitata*.

³Based on three individuals of the sole representative of this class, the endangered species *D. muli*.

⁴Based on one species, *D. migra*.

Female reproductive types IV and V are identified here for the first time. Type IV is distinguished by synchronous ovarian development and unusually elongate eggs with two very short filaments, the anterior pair being missing (Figure 17.4). Exceptionally short filaments also characterize Type V eggs; these have a more typical shape and the usual four filaments, but possess a quite differently structured chorion with a unique, open meshlike construction distinct from the solid chorion of all the other Hawaiian species (Kambysellis 1993).

EVOLUTION OF FEMALE REPRODUCTIVE TYPES – Overlaying the seven egg/ovarian types on our molecular tree (Figure 17.4) demonstrates that such types are generally conserved within a lineage, but that there has also been a series of reproductive shifts in the evolution of the Hawaiian drosophilids. Lineages of the more primitive, non-picture-winged species display greater diversity in female reproductive strategies (five egg/ovarian types represented) than the picture-winged species with three egg/ovarian types. The poorly sampled scaptomyzoids exhibit the lowest fecundity (Types Ia and Ib); further study, however, may reveal greater ovarian diversity. Among the non-picture-winged drosophilids, female reproduction shifts from Type V in the most primitive lineage, the white-tip scutellum flies, to Type II in the *antopocerus* and modified-tarsi groups, and then to Type IIIa in the modified-mouthparts group; the last type has a much higher reproductive potential than the others because it entails a greater number of ovarioles, all of which are functional.

The most primitive picture-winged species retain the Type IIIa pattern, but more derived species evolved types IIIb and IV. Significantly, species possessing Type IIIa ovaries and those bearing Type IIIb ovaries are each found in two disjunct portions of the tree (Figure 17.4). This implies that there must have been convergent evolution and/or evolutionary reversals in reproductive type. However, the exact pattern of female reproductive shifts and the inferred ancestral states in this portion of the phylogeny are sensitive to the assumptions made. Under ACCTRAN and disregarding the evolution of Type IV, there appear to have been four changes of reproductive type (Figure 17.4): two independent gains of Type IIIa (in the modified-mouthparts and *glabriapex* groups), and two “losses” of Type IIIa, with convergent shifts to Type IIIb occurring in the *planitibia* and the *grimshawi* groups. Under DELTRAN, Type IIIa arose only once (in the modified-mouthparts group) but there were three independent shifts to Type IIIb, on the branches leading to the *cyrtoloma* and *planitibia* subgroups, to the *picticornis* subgroup, and to the *grimshawi* species group, respectively. Although *D. punalua* may be misplaced in the molecular phylogeny, like other members of the *glabriapex* group it exhibits the Type IIIa pattern.

One picture-winged species (*D. mulli*) displays a unique egg/ovarian pattern, Type IV. Morphologically, *D. mulli* is somewhat anomalous in that it lacks the wing pigmentation characteristic of all the picture-winged species, yet external male genitalia and other traits clearly place it in the *grimshawi* species group (Kaneshiro et al. 1995). This phylogenetic affinity is confirmed by the molecular phylogeny (Figure 17.1A) although the subgroup designation does not agree with its morphological placement. Independent of this inconsistency, it is clear that there have been several discrete reproductive shifts in the evolution of the endemic Hawaiian drosophilids.

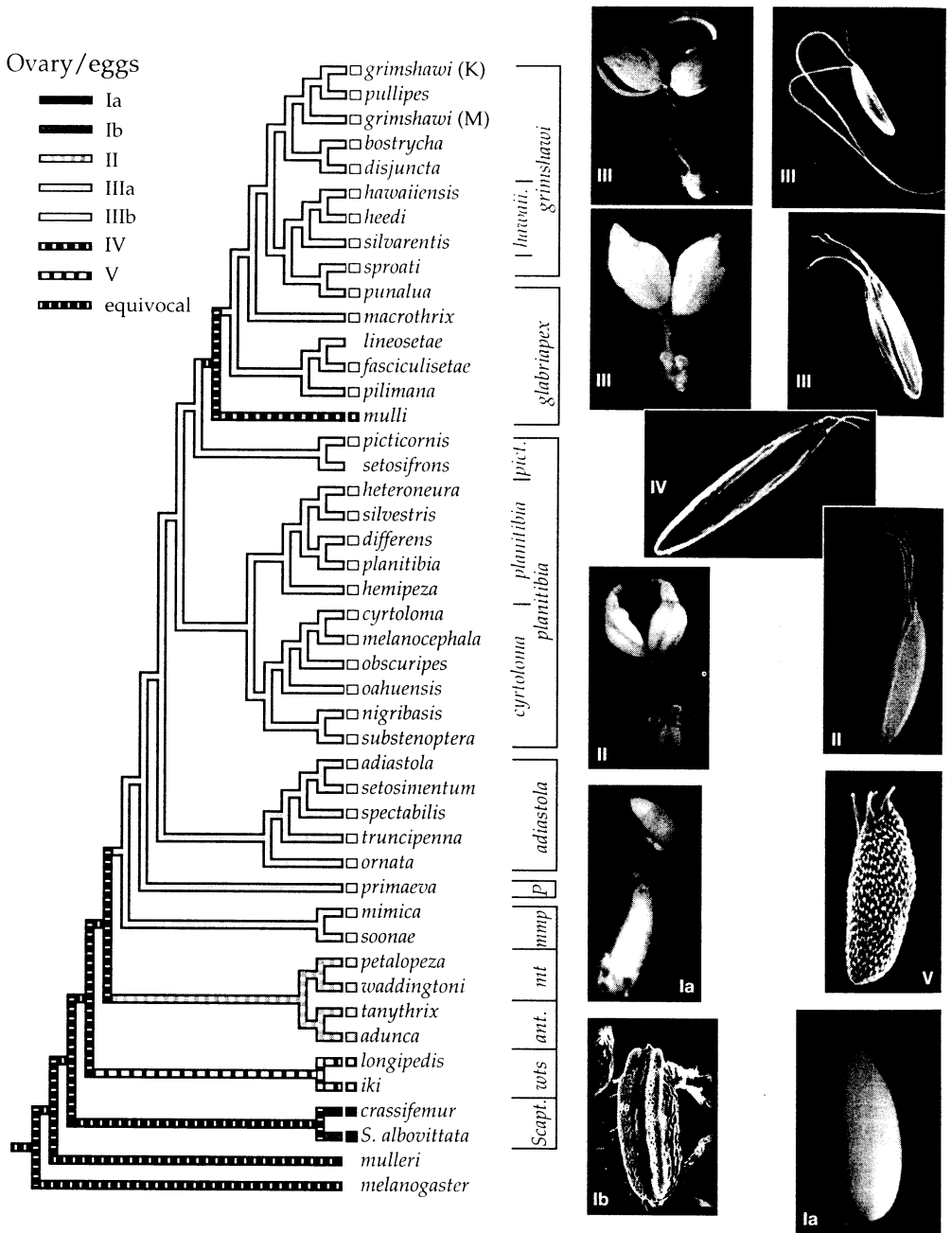


Figure 17.4. Evolution of ovarian types in Hawaiian drosophilids, assuming ACCTRAN. Specific egg/ovarian types generally characterize entire clades, indicating that reproductive shifts occurred early in their evolution. Ovaries of four types are shown in the first panel on the right, together with an egg of *S. albovittata* at the bottom. The ovaries are from *D. sejuncta* (Type IIIb), *D. punalua* (Type IIIa), *D. waddingtoni* (Type II), and *D. crassifemur* (subgenus *Engiscaptomyza*) (Type Ia). Note the larva in the vagina of *D. crassifemur*. To the far right are representative eggs of the various egg/ovarian types: *D. claytonae* (Type IIIb); *D. truncipenna* (Type IIIa); *D. mulli* (Type IV); *D. waddingtoni* (Type II); *D. longipedis* (Type V); and *D. crassifemur* (Type Ia).

The question is, what is the adaptive significance of the female reproductive differentiation observed among lineages of this group of flies?

RELATIONSHIP OF FEMALE REPRODUCTIVE STRATEGIES TO BREEDING SUBSTRATE – Although the patterns of evolutionary divergence in host plant and larval substrate (Figure 17.3) and in female reproductive strategy (Figure 17.4) are complex, comparison of these two patterns suggests that female reproductive strategies broadly correlate with host plants and larval substrates. Among the non-picture-winged drosophiloids, the early radiation into three successive breeding niches – fungi, leaves, and then a more diverse range of substrates including stems and fruits – was accompanied by evolution of three distinct reproductive patterns, involving ovarian types V, II, and IIIa, respectively. Larval substrates have not been recorded for the two scaptomyzoids included in the molecular phylogeny, but other scaptomyzoids of subgenus *Exalloscaphomyza* with the Type Ia pattern of low fecundity use decaying flowers (Kamysellis and Heed 1971).

The ecology of the picture-wings is considerably better analyzed (Montgomery 1975), but the breeding substrates of some of the sequenced species in the *adiastola* and *glabriapex* groups, and of *Drosophila mulli* remain unknown (Figure 17.3). The unique Type IV reproductive pattern of the latter species suggests that field studies may reveal a novel breeding substrate. Reproductive patterns of the remaining picture-winged species fall into either Types IIIa or IIIb. Type IIIa (moderate to high ovariole numbers, resulting in higher fecundity) appears to have first evolved in the modified-mouthparts lineage, and then was retained by the more primitive *primaeva* and *adiastola* groups of the picture-winged species; it also characterizes the more derived *glabriapex* group (Figure 17.4).

Unfortunately, the ecology of species with this reproductive type is rather poorly known. *Drosophila ornata* of the *adiastola* group and *D. macrothrix* of the *glabriapex* group are clearly stem breeders. Although they use other substrates as well, *D. punalua*, *D. adiaastola*, and *D. setosimentum* oviposit predominantly in stems. Based on preliminary data, ovarian Type IIIa thus appears to be associated with the stem-breeding habit. One of the two apparent exceptions to this relationship, the record of a single individual of *D. pilimana* reared from bark, may be misleading. The other exception, the bark-breeding habit of *D. primaeva*, is inconsistent with the general association between reproductive type and breeding substrate.

Egg/ovarian Type IIIb (with the highest fecundity) appears to have evolved several times in the picture-winged group with at least two independent reproductive shifts from Type IIIa to Type IIIb in the *planitibia* group and the *grimshawi* group (Figure 17.4). Type IIIb is well correlated with the bark-breeding habit (e.g., the *cyrtoloma* subgroup), but is also associated with the use of decaying stems. Multiple records of stem and bark-breeding in Type IIIb species from the *planitibia* subgroup and the *grimshawi* group raise the question of whether there is a significant distinction between decaying stems and bark for ovipositing females.

A few picture-winged species use tree fluxes as a breeding substrate; all have females with Type IIIb ovaries. In both evolutionary shifts to flux-breeding, the ancestor was a bark-breeder (Figure 17.3) with Type IIIb ovaries (Figure 17.4), suggesting that

this ovarian type was pre-adapted to breeding in fluxes. Although no major reproductive shifts took place in the adaptation to this novel niche, some changes in the female reproductive system are associated with different types of fluxes, as we discuss next.

ECOLOGICAL AND REPRODUCTIVE DIVERGENCE IN A PAIR OF SYMPATRIC SPECIES, *D. silvarentis* AND *D. heedi* – The preceding discussion has detailed the broad phylogenetic patterns of ecological and reproductive shifts in the evolution of the endemic Hawaiian *Drosophila*, but another perspective on evolution and adaptation can be gained by a close analysis of related but sympatric species, and ultimately, analysis of entire Hawaiian communities of endemic drosophilids, plants, and bacteria. Detailed studies of a pair of sympatric species endemic to the island of Hawai'i illustrate how a more careful analysis can lead to a fuller understanding of the ecological relationships among species and the adaptive aspects of their resource use. The two species, *D. silvarentis* and *D. heedi*, are closely related members of the *hawaiiensis* subgroup; both are flux breeders (Figure 17.3) and have Type IIIb ovaries (Figure 17.4). Nevertheless, they are ecologically and reproductively differentiated, demonstrating that the broad classifications of substrate and ovarian types may obscure some of the biologically significant aspects of the adaptation of these flies.

This pair of recently evolved sympatric species has been hailed as a striking example of precise niche partitioning (Kaneshiro et al. 1973). According to the chromosomal phylogeny of Carson (1981), they are sister species derived from a common founder from Maui. They are also the only picture-winged species that inhabit the arid, poorly vegetated area of the high-altitude saddle between Mauna Kea and Mauna Loa. They both depend on the flux drippings of one of the two trees in the area, *Myoporum sandwicense* (Myoporaceae). Based on cytological identification of larvae from natural substrates, Kaneshiro et al. (1973) determined that *D. silvarentis* oviposits only on tree fluxes, whereas *D. heedi* breeds exclusively on ground fluxes – that is, the soil moistened by the flux dripping from overhanging branches. Kaneshiro et al. (1973) argued that the few *D. silvarentis* larvae found in ground fluxes had dropped to the ground for pupation after having completed development in the branches overhead. Thus, the two species appeared to be significantly separated by the females' choice of oviposition site, the novel shift to breeding in soil fluxes presumably being favored because it reduced competition with the congener *D. silvarentis* (Kaneshiro et al. 1973).

Morphological differences in the eggs of these two species (Figure 17.5) allowed us to test more carefully the hypothesis that ecological divergence between these closely related species is actually due to a shift in the oviposition site. Eggs of *D. silvarentis* have the very long respiratory filaments (Figure 17.5B) typical of flies with Type IIIb ovaries. Although ovaries of *D. heedi* are also of the IIIb type, their eggs have shorter respiratory filaments and are much smaller than eggs of *D. silvarentis* (Figure 17.5E). Our field data (Table 17.4) confirm that *D. heedi* oviposits exclusively in soil fluxes, but show that *D. silvarentis* oviposits in both soil and tree fluxes, although preferentially in the tree fluxes. The oviposition sites of the two species are thus not completely separate, the overlap being due to the fact that *D. silvarentis* females will also oviposit in soil fluxes, particularly when they are fresh. This may have been expected given that the stimulus for oviposition is probably the odor arising from the flux drippings and/or from the

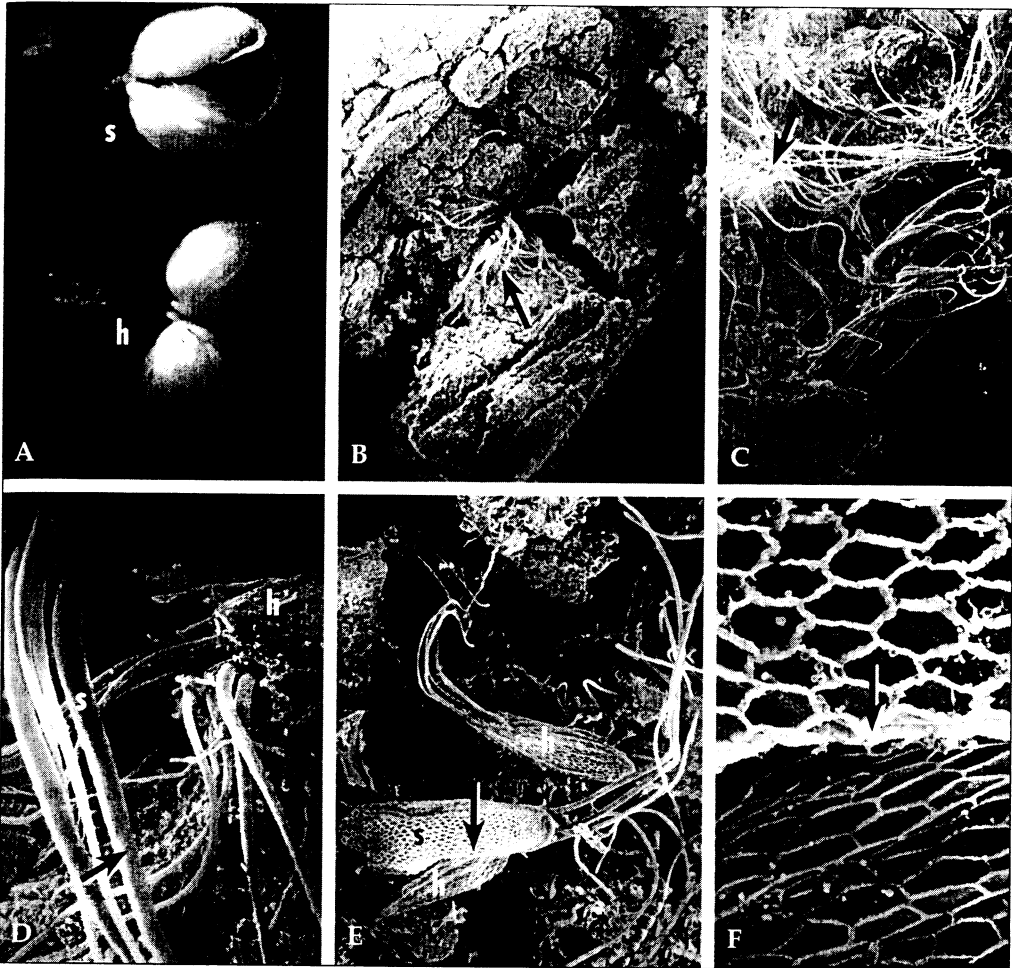


Figure 17.5. Reproductive differentiation between two sympatric flux-breeding species from Hawai'i. (A) Mature ovaries of *D. silvarentis* (s) and *D. heedi* (h) showing differences in ovariole numbers and filament length at the anterior end of each ovary (to the right). (B-F) SEMs of oviposited eggs in bark (B), and in soil fluxes (C-F). Arrows indicate respiratory filaments; eggs of *D. silvarentis* (s) and *D. heedi* (h) are identified. The arrow in (E) highlights a pair of overlapping eggs, which are shown at higher magnification in (F), emphasizing the distinctive chorion ultrastructures of the two species.

bacteria associated with the flux. The older, more established ground fluxes probably facilitate propagation of elements of the soil microflora, which then make the ground flux attractive to *D. heedi* females but less attractive to *D. silvarentis* females (Table 17.4). The "tree/soil fluxes" provide an intermediate niche suitable to both species, these substrates at the base of a vertical tree trunk being distinct from the soil fluxes which are moistened by the drippings from a horizontal branch overhead.

Although there is ovipositional overlap in the ground fluxes, the two species are not equally successful in ground fluxes. Table 17.5 shows that the relative success of *D. heedi* increases with time since oviposition; *D. heedi* clearly is better adapted to

Table 17.4. Reproductive divergence between two sympatric and ecologically similar species of *Drosophila* on the island of Hawai'i. Means and standard deviations of thorax length and ovariole number based on 32 field-collected females for *D. silvarentis*, and on 52 field-collected females for *D. heedi*.

Species	Thorax length (mm)	Ovarian development	Number of ovarioles per fly	Number of oviposited eggs in fluxes			
				tree	new soil	old soil	tree/soil
<i>D. silvarentis</i>	3.1 ± 0.03	asynchronous	39 ± 0.6	2,300	92	988	65
<i>D. heedi</i>	2.5 ± 0.04	synchronous	60 ± 0.7	0	2	3,136	48

conditions in the ground flux and appears to outcompete *D. silvarentis*, which shows high mortality. The reduced survival of *D. silvarentis* in ground fluxes may be due to the nutrient poverty of this substrate; the larvae of this species are much larger than those of *D. heedi* (see Table 17.4). Few *D. silvarentis* larvae apparently reach the critical size to enter pupation in ground fluxes. Larvae of the two species in the same ground flux would compete directly for nutrients, and it appears that selection against *D. silvarentis* is stronger, selecting against soil oviposition by this species.

From our molecular phylogeny (Figure 17.1A), it appears that *D. heedi* diverged from its sister-group more recently than *D. silvarentis*. Furthermore, *D. heedi* is the only member of the *hawaiiensis* subgroup to use soil fluxes – all others use tree fluxes. Regardless of the precise ancestor of *D. heedi*, it appears likely that this species diverged from breeding in tree fluxes to avoid competition, which can be quite severe on this substrate. Tree fluxes are quite rare, and eggs laid directly in the flux source usually die before completing embryogenesis, the only ones surviving being those laid in a restricted area surrounding the source (M. Kambysellis and E. Craddock, unpubl. data). Although soil fluxes are even rarer than tree fluxes, *D. heedi* has adapted to using this breeding resource effectively by making smaller eggs, by reducing the time required for larval development, and by reducing the length of the respiratory filaments; smaller eggs and smaller larvae at pupation result in smaller adults (Table 17.4). To optimize use of this unpredictable resource, *D. heedi* has evolved ovaries with more ovarioles and synchronous egg development (Table 17.4). When, after a prolonged search, a female does find one of the very rare soil fluxes in appropriate condition, she

Table 17.5. Proportions of *D. heedi* and *D. silvarentis* at different life stages in samples from soil fluxes. The fraction of *D. heedi* increases significantly ($P < 0.001$) between oviposition and the adult stage.

Life history stage	Sample size	Ratio of <i>heedi</i> / <i>silvarentis</i>	Reference
Oviposited eggs	4,124	3.2 : 1	Kambysellis and Craddock (unpubl.)
Third instar larvae	15	4.0 : 1	Kaneshiro et al. (1973)
Adults reared from flux	225	8.8 : 1	Kaneshiro et al. (1973)
Adults reared from flux	279	8.6 : 1	Kambysellis and Craddock (unpubl.)

can lay a large number of eggs on that flux simultaneously. The ecological and reproductive data presented here demonstrate that several reproductive traits have evolved in an adaptive direction in a relatively short time period (< 0.4 My), providing a clear example of natural selection resulting in character displacement. As demonstrated experimentally by Schluter (1994; see Chapter 18), interspecific competition can drive adaptive divergence. As the above example makes clear, resource competition is one of the forces that fostered the adaptive radiation of the Hawaiian *Drosophila* and led to the exploitation of novel niches for oviposition and breeding.

Adaptive significance of female reproductive strategies in Hawaiian *Drosophila*

Female reproductive potential in Hawaiian flies varies from the production and oviposition of one egg at a time to clusters of more than a hundred eggs. Coincident with this variation in fecundity is the exploitation of a variety of larval substrates. We believe that the differences in several features of female reproductive strategy – ovariole number, ovariole function, length of the ovipositor, length of the respiratory filaments, chorionic ultrastructure, and oviposition behavior – are adaptive and have arisen in response to strong selection exerted by the larval substrate. Most of these traits are largely genetically determined. Ovariole number is also affected by environmental factors, particularly larval nutrition (Kambysellis and Heed 1971). However, the genetic component of control is also strong, as evidenced by the retention of ovarian structure and function when larval imaginal discs are transplanted into species with very different ovarian types (Kambysellis 1970; Kambysellis and Heed 1971).

In species in which eggs are oviposited deeply within the breeding substrate – as in bark-breeders – ovipositors are very long; by contrast, scaptomyzoids that simply drop their eggs on the surface have extremely short ovipositors (Throckmorton 1966; Franchi et al. 1997). The length of the respiratory filaments of the egg also appears to be adaptive. The eggs of bark-breeding species (Type IIIb) have extraordinarily long respiratory filaments (1.5–3.9 mm) that are 1.5 to 4 times the length of the egg itself (Kambysellis and Heed 1971; Table 17.3; Figure 17.4). These long filaments act as “snorkels” facilitating respiratory exchange for the deeply buried developing embryo (Margaritis 1983) in the potentially anaerobic environment of the rotting substrate. The upper layers of bark substrates are generally dry and hard, and thus unsuitable for embryonic development, so the eggs must be inserted deeply. This has selected for long, heavily sclerotized ovipositors and eggs with long respiratory filaments (Type IIIb) in the invasion of the bark niche by picture-winged drosophilids of the *planitibia* and *grimshawi* groups (see Kambysellis and Heed 1971; Franchi et al. 1997).

Scaptomyzoid eggs (Types Ia and Ib), which are simply deposited on the surface of the breeding substrate, are devoid of respiratory filaments; the eggs of leaf-breeders (Type II) and stem-breeders (Type IIIa) are inserted just below the substrate surface (Figure 17.3), and have short filaments that are less than or equal to the length of the egg (see Figure 17.4). Among flux-breeding species (Type IIIb), the respiratory filaments are very long for those using tree fluxes, but considerably shorter for those

using soil fluxes (Figure 17.5). Thus, species differences in the lengths of ovipositors and egg respiratory filaments appear adapted to enhance embryo survival under different substrate conditions.

The thickness of the egg chorion also appears to show adaptive variation. Continental drosophilids – and the more primitive Hawaiian drosophilids that oviposit in decaying flowers and leaves – have a very thin chorion. The eggs of stem- and bark-breeders, however, have a thick solid chorion, probably to help withstand the mechanical pressure of the substrate (Kambyzellis 1993). In fungus-breeders, the chorion has a unique, scaffold-like structure with several open layers of interconnecting pillars, permitting respiration and extensive exchange of the ammoniacal gases that abound in this hostile breeding substrate.

Following embryogenesis and hatching, drosophilid larvae face a new set of selective pressures. They must obtain adequate nutrition to grow and develop, but must also withstand the chemical environment in which they find themselves. This may be quite noxious in fungal substrates (Jaenike et al. 1983) or even in rotting plant tissues. To survive, the larvae must be able to detoxify many of the chemicals they encounter. Shifts to breeding in new genera and families of plants that are chemically different probably required enzymatic adaptation to additional compounds.

The longevity of the substrate and its rate of deterioration also impose selective constraints on larval development – and hence, on adult size. For example, the ephemeral nature of decaying flowers puts a premium on brief development, selecting for rapid embryogenic and larval development; in the extreme case of *Exalloscaptomyza*, the females often larviposit (Figure 17.4) a first instar larva on morning-glory flowers (Kambyzellis and Heed 1971)! Rapid larval development of flower-breeding species results in very small flies. Slowly decaying bark, on the other hand, permits extended larval development, resulting in large larvae and adults; in fact, the Hawaiian bark-breeding picture-wings are the giants of the *Drosophila* world (Figure 17.1). The extreme range of variation in body size among the Hawaiian drosophilids is partly a consequence of their varied developmental histories which are related, in turn, to variation in oviposition site.

Perhaps the most remarkable outcome of varied selection regimes in the larval niche is the interspecific variation in fecundity, which ranges from one egg per day to several hundred. The phylogenetic trend in the Hawaiian drosophilids has been toward greater fecundity (Table 17.3; Figure 17.4), and appears to be driven by the exploitation of substrates that provide greater food resources. The increase in the physical volume of the substrate (from flowers, to leaves, to stems, branches, and tree trunks) should result in an increase in its larval carrying capacity. Females that responded to this opportunity by maturing and laying more eggs would have been selectively favored, leading to an increase in the frequency of alleles that underlie ovarian traits (e.g., ovariole number, number of mature eggs per ovariole) that contribute to higher fecundity.

Implications for Hawaiian *Drosophila* evolution

The preceding phylogenetic analysis of the reproductive ecology of the endemic Hawaiian Drosophilidae, taken with previously published accounts of the behavior

and biology of the group (Carson 1978, 1986; Carson et al. 1970; Speith 1982; Kaneshiro and Boake 1987), provides a new perspective on the evolution and adaptive radiation of this extraordinary group of organisms. The ecological differentiation of the group into lineages using different larval substrates (fungus-breeders, leaf-breeders, bark-breeders, flux-breeders) is a major component of the phylogenetic diversification of Hawaiian drosophilids (Figure 17.3). Given the long-standing emphasis on the role of sexual selection in the speciation of Hawaiian *Drosophila*, it is important to ask how natural selection (on female reproductive strategies) and sexual selection (on male secondary sexual characteristics, female choice) might interact (T. J. Givnish, Chapter 1). We are interested in the evolutionary diversification of the whole mating system, and therefore need to consider the interactions between males and females, as well as between both sexes and their environment.

Mating systems in *Drosophila* are highly variable (Markow 1996), but nowhere more than among the endemic Hawaiian species, with their varied male morphologies, male courtship repertoires, and patterns of female reproduction. What is the relationship between the reproductive ecology of various species groups and the mating systems they display? The main parameters to evaluate (Markow 1996) are age at reproductive maturity, remating frequency, number of sperm transferred and stored per mating, and ovariole number. Among Hawaiian species, there is great variation in all of these parameters, as well as in the incidence of lek behavior by males – and hence, in the intensity of sexual selection. Although male behavioral data are incomplete for the more primitive groups, the following extreme patterns are evident.

Among the majority of the picture-winged group, female reproductive maturation and the onset of receptivity to insemination are significantly delayed following adult eclosion, often for several weeks (Kambysellis and Craddock 1991), which implies that females will generally have dispersed far from their larval substrate by the time they mate. Males generally mature much more rapidly (Boake and Adkins 1994); the differential in maturation rates between sexes would promote outcrossing. Males of these species invest a great deal of time and effort in defending space on their leks and in courtship displays, but only rarely is a male successful in mating with a sexually receptive female who has been attracted to a lek. Sexual selection among males of these species is intense (Speith 1966, 1982). Remating is extremely rare in the picture-wings, although a few multiple inseminations have been detected (Craddock and Johnson 1978). Sperm remain viable in the female's sperm storage organs for months, and in a single copulation a male can transfer thousands of sperm (Kambysellis and Craddock 1991), enough to fertilize a female's eggs for almost a year (Carson et al. 1970) – that is, for her entire reproductive life. In such lek species, body sizes are large and ovariole numbers high (Types IIIa and IIIb), leading to females of high fecundity. Breeding substrates of these species – decaying stems, bark, and fluxes – are unpredictable, yet nutritionally rich and relatively long-lasting. Once located, these substrates provide abundant oviposition opportunities and nutritional support for large numbers of larvae.

Thus, in this reproductive strategy where the lekking males have limited opportunities to mate, there is a premium on male mating success, with strong selection for

increased size, and for unique combinations of behavioral and morphological traits, and in the females, selection for high fecundity, for a capacity to store abundant sperm, and to accurately select a productive substrate for oviposition, as well as selection on ovipositor and egg traits that will ensure embryonic survival and thus reproductive success. Thus sexual selection on male traits and natural selection on female traits operate concurrently to engender a successful reproductive strategy that may have first evolved in the modified-mouthparts group and then been retained (with modifications) by the more derived picture-wings.

At the other end of the spectrum, there is an alternative reproductive strategy characterized by a more rapid rate of reproductive maturation, lower fecundity, few sperm transferred per mating, and a high incidence of remating, with many more opportunities for males to mate and much less intense sexual selection. Such a strategy seems to apply to the scaptomyzoids and the fungus-breeders, where first insemination takes place early in the female's reproductive maturation, and only a few sperm (from 10 to about 200) are transferred per mating (Kambysellis and Craddock 1991). In most *Drosophila*, sperm are mainly stored in the paired spermathecae and only temporarily in the seminal receptacle, but among 170 field-collected females from seven species of fungus-breeders, sperm were found only in the seminal receptacle (Kambysellis and Craddock 1991), the spermathecae apparently being non-functional (Throckmorton 1966). It appears that each copulation provides only enough sperm to inseminate one synchronously matured batch of eggs (Table 17.3), and that following oviposition, the females must remate to fertilize the following clutch of eggs. Lek behavior has not been observed in the white-tip scutellum group, and is definitely absent from the scaptomyzoids which have an assault-type mating. It is unknown whether courtship takes place on the breeding substrate, but in any event, these groups display a very different mating system from the lekking picture-wings which utilize quite different breeding substrates. Further observations on all these reproductive and ecological aspects of the biology of the more primitive non-picture-winged lineages are required in order to correlate the environmental, behavioral, and physiological features of these flies with the evolutionary forces that have led to their diversification.

Although sexual selection may be less important in some drosophilid lineages than in the picture-wings, nonetheless the biology of all groups and all species has been molded by natural selection. Indeed, ecological constraints and competition for resources seem to have been primary in Hawaiian drosophilid evolution. The short branches at the base of the molecular phylogeny suggest an early radiation into separate evolutionary lineages that are ecologically distinct (Kambysellis et al. 1995). This initial ecological differentiation was rapidly followed by adaptation of the female reproductive system to each particular substrate, accomplishing the reproductive shifts and the correlations between reproductive type and breeding substrate detailed above. In certain lineages, sexual selection operating alone or in conjunction with founder events (Kaneshiro 1989) was instrumental in the species proliferation of each lineage that followed. Natural selection continued to hone the adaptation of each particular species to its individual niche, and in some instances directional selection

brought about subsequent ecological shifts, as have occurred in the two shifts to flux-breeding within the picture-winged group, in the shift of *D. heedi* to utilizing soil fluxes, and the shift of *D. mulli* to some other as yet unknown substrate. Thus natural selection has clearly been significant in the evolution and adaptive radiation of the Hawaiian drosophilid fauna, and in molding the rich array of ecological and female reproductive variations described in this chapter.

Conclusions

The adaptive radiation of the endemic Hawaiian *Drosophila* has been accompanied by divergence in multiple female reproductive traits (including ovipositor length, number of ovarioles per ovary, number of mature eggs per ovary, egg chorion thickness, and length of the egg respiratory filaments) that collectively enhance adaptation to the particular breeding substrate that characterizes each of the several evolutionary lineages. Tracing the evolution of several ecological and reproductive characters on an independently derived molecular phylogeny (based on mtDNA and nDNA sequences) demonstrates that female reproductive strategies broadly correlate with ecological divergence in host plant and larval substrate. Female reproductive Type I (low fecundity) is associated with flower-breeders; Type II, with leaf-breeders; Type IIIa, with stem-breeders; Type IIIb (highest fecundity), with bark- and flux-breeders; and Type V, with fungus-breeders. The breeding substrate of Type IV is unknown.

Our phylogenetic analysis has confirmed that the Hawaiian drosophilids are monophyletic, supporting their origin from a single ancestor, with subsequent divergence into two lineages of Hawaiian drosophilids, the scaptomyzoids and the drosophiloids, as previously indicated by the morphological analysis. Mapping substrate use on the molecular phylogeny indicates that the most primitive Hawaiian drosophiloids were fungus-breeders. Ecological shifts then occurred from fungi to decaying leaves, stems and fruit, bark, and finally to tree fluxes, with the latter two substrates being invaded by the most derived picture-winged group. There have been two independent shifts to flux-breeding, and apparently two or three shifts from stem-breeding to bark-breeding, although the distinction between these two substrates may depend mainly on the depth to which eggs are inserted during oviposition. Female reproductive traits have shown correlated shifts in the parallel shifts to new breeding substrates, validating the adaptive nature of the female reproductive variation.

Following the use of fungi by primitive Hawaiian drosophiloids, the first plant family invaded was Araliaceae. There have been several independent shifts to Campanulaceae, as well as shifts to numerous other endemic Hawaiian plant groups. Most Hawaiian *Drosophila* are monophagous at the family level; polyphagy is uncommon and seems to be a derived state. Natural selection exerted by the breeding substrate has been a major factor in Hawaiian *Drosophila* evolution, which, together with sexual selection on male behavior and morphology, has contributed to the evolution of a diversity of mating systems. In the early phyletic diversification of this group, adaptive shifts to new breeding substrates appear to have been most important, but such

shifts are also a feature of more recent speciation events; this is best exemplified by the adaptation of the species *D. heedi* to soil fluxes, in response to a relatively recent shift from the tree flux niche of its immediate ancestor.

Although our analyses of the adaptive radiation of the Hawaiian *Drosophila* should be considered preliminary because of the limited ecological and molecular data available, nonetheless, we believe that our findings reflect the major trends in this group, because the molecular phylogeny includes representatives of most morphologically defined groups, and because ecological and reproductive patterns are generally consistent within groups. It will, however, be important to investigate further the ecologically diverse but currently poorly analyzed scaptomyzoids and the modified-mouthparts and white-tip scutellum groups if we are to better understand the adaptive radiation of the remarkable Hawaiian Drosophilidae.

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