

Ecology and Evolution of *Drosophila ambochila*, A Rare Picture-Winged Species Endemic to the Wai'anae Range of O'ahu, Hawaiian Islands¹

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ABSTRACT: The rare O'ahu picture-winged fly *Drosophila ambochila* Hardy & Kaneshiro is endemic to two windward ravines in the Wai'anae Mountains that harbor its host plant. *Drosophila ambochila* is an ecological specialist that breeds on *Pisonia* stems and trunks in an intermediate stage of decay. By providing field-collected females with suitable substrate material, we have been able to observe the oviposition behavior of this species in the laboratory and obtain F₁ larvae. In nature, females oviposit each batch of mature eggs (~40–50) in a single cluster, by repeatedly inserting their long ovipositor into the same crack or beetle hole in the decaying *Pisonia* bark. Ovipositor, ovary, and egg morphology are characteristic of bark-breeding Hawaiian *Drosophila*, but SEM studies revealed a distinctive chorionic ultrastructure for the eggs of this species. Larval salivary chromosome analyses indicated that the O'ahu *D. ambochila* is most closely related to *D. alsophila* from the island of Hawai'i and have helped to resolve the phylogenetic relationships among six of the nine species belonging to the *vesciseta* subgroup of the *glabripapex* species group.

THE PICTURE-WINGED GROUP of Hawaiian *Drosophila* includes 111 named and several as yet undescribed species (Kaneshiro et al. 1995) and represents the best-studied species group in the Hawaiian drosophilid fauna of ~800 endemic taxa. This group of flies includes some of the largest *Drosophila* in the world, and, by contrast with the majority of smaller Hawaiian drosophilids of other species groups, picture-winged species are often amenable to laboratory culture on artificial medium. Phylogenetically, the picture-wings

are the most derived of all the Hawaiian drosophilid species, as demonstrated by the molecular phylogenies of DeSalle (1992) and Kambysellis et al. (1995). Relationships among 106 picture-winged species have been determined by cytological comparisons of inversions in the polytene chromosomes of the larval salivary glands (Carson 1983, 1992). For the most part, inferences based on the inversion data are congruent with those based on both morphological (Kaneshiro et al. 1995) and DNA sequence data (Kambysellis et al. 1995). Cytological analyses of interspecific relationships among the picture-wings have depended upon the capacity of field-collected females of these species to oviposit on artificial media and the successful laboratory rearing of F₁ larval progeny up to the late third instar. There are at least five picture-winged species that have not been included in the inversion phylogeny because they are very rare, or refractory to laboratory rearing, or both.

One such species is *Drosophila ambochila* Hardy & Kaneshiro of O'ahu. This species was described (Hardy and Kaneshiro 1971)

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from two male specimens collected by Montgomery in 1970 in the gulch between Pu'u Hāpapa and Pu'u Kānehoa in the Wai'anae Mountain Range. Subsequently, it was also found in nearby 'Ēkahanui Gulch below Pu'u Kaua. Montgomery (1975) recorded rearing 10 specimens from three different collections of decaying stems of *Pisonia* made at those sites in 1971 and 1972. Despite widespread collecting throughout Hawai'i over the past decades, *D. ambochila* has not been found elsewhere. This suggests that this species is endemic to possibly only a few gulches in the Wai'anae Range, one of about 15 species of picture-winged *Drosophila* that are similarly restricted in distribution, including the related O'ahu species *D. montgomeryi* Hardy & Kaneshiro. Collection of mature *D. ambochila* females from Pu'u Kaua in 1997 and 1998 along with successful rearing of larval progeny provided the opportunity to analyze this species' cytological affiliation to other members of the picture-winged group. Here we present chromosomal, morphological, ecological, and behavioral data on this rare species that are pertinent to understanding its phylogenetic relationships.

MATERIALS AND METHODS

Field Collections and Laboratory Rearing

Adult flies were collected at Pu'u Kaua from upper 'Ēkahanui Gulch in Honouliuli Preserve on 28 September 1997 (Z39 collection) and 2 August 1998 (Z70 collection), by visual collection from decaying *Pisonia*, by sweeping, and by use of fermenting mushroom and banana baits. In addition, samples of decaying vegetation were brought into the laboratory for rearing flies from the eggs oviposited in nature, as described previously (Montgomery 1975). Oviposited eggs were identified in the substrate material, using a dissecting microscope. The field-collected adult females were placed on laboratory medium (Wheeler and Clayton 1965) and stimulated to oviposit by including some decaying *Pisonia* branch in the vial (see below).

Oviposition Behavior

To observe oviposition in the laboratory, we first prepared the substrate material as follows. Pieces of fresh *Pisonia* stem from the field collecting locality were held, sealed in a clean plastic bag, for ~20–30 days in the *Drosophila* rearing room at the University of Hawai'i. During that time the naturally associated microbes promoted the progressive decay of these stems. A piece of decaying *Pisonia* bark (~10 × 30 mm) was placed into a shell vial containing Wheeler-Clayton medium, together with a wild-caught female, and the female's behavior was observed under a stereoscope. This was repeated using material from laboratory-held stems at various stages of decay until successful oviposition was observed. In addition, females were exposed to naturally decaying stems collected from the field, and their behavior was observed.

Morphological Analyses

The external male genitalia were prepared as previously described (Kaneshiro 1969) and observed by compound light microscopy. Using a stereoscope fitted with a micrometer, measurements were made of female thorax length, ovipositor length, egg length, and length of the anterior and posterior egg respiratory filaments. To prepare the female genitalia for scanning electron microscopy (SEM), the terminalia were dissected free of the eighth abdominal tergite, and the ovipositor and anal papilla were placed in phenol until the associated musculature was digested away. The specimen was then rinsed twice in ethanol and mounted on a stub to air dry before coating with carbon, followed by gold/palladium. Other specimens were mounted on stubs directly, without dissection and phenol treatment. Field- and laboratory-oviposited eggs, and mature eggs dissected from the ovaries were prepared for SEM as described previously (Kambysellis 1993) and the chorion ultrastructure was analyzed. The numbers of ovarioles and mature eggs in each ovary were also counted.

Chromosomal Analyses

Salivary glands were dissected from mature third instars resulting from the laboratory oviposition of a female from Pu'u Kaua (Z39P6) on a decaying *Pisonia* stem. Polytene chromosome squashes of 10 larvae were prepared and the banding patterns analyzed by comparisons with the standard chromosome sequences of *Drosophila grimshawi* Oldenberg (see Carson 1983).

RESULTS

Ecology

The species *D. ambochila* has been recollected recently from the windward side of the Wai'anae Range of O'ahu at an elevation of ~700 m. The 1997 adult collection included three females and one male from upper 'Ēkahanui Stream bank. In the summer of 1998, a second collection of eight adult females and six males was made in the main fork of 'Ēkahanui Stream below Pu'u Kaua. Although it is a rare and locally restricted species, there is a resident population of *D. ambochila* in at least two gulches of the Wai'anae Range of O'ahu.

The vegetation in these gulches can be characterized as a riparian forest (Gagné and Cuddihy 1990), currently dominated by *Pisonia* (pāpala kēpau) since the decline of the usually codominant *Charpentiera* (pāpala) in Wai'anae ravines, in part due to stem-boring native Lepidoptera (Sohmer 1973). *Urera* (ōpuhe) is also present. There are three species of *Pisonia* present in 'Ēkahanui Ravine: *P. sandwicensis* Hillebr., *P. brunoniana* Endl., and *P. umbellifera* (G. Forster) Seem. (Joel Lau, pers. comm.). *Pisonia sandwicensis* is endemic to the six larger Hawaiian Islands and grows as a small, openly branched tree, 12–15 m tall. The other two indigenous species grow as large shrubs or trees, up to 6 m and 10 m tall, respectively (Wagner et al. 1990). All have soft, brittle wood (Figure 1A) with rings of parenchymous tissue alternating with xylem tissues formed by anomalous

secondary thickening (C. H. Lamoureux, pers. comm.) (Figure 1B).

The *D. ambochila* population appears to be strictly associated with its host plant, *Pisonia*, which it uses as a breeding substrate. Adults of *D. ambochila* were reared out of the decaying *Pisonia* stems collected in both 1997 and 1998, confirming the earlier records of Montgomery (1975). No adults of this species were obtained from decaying *Pisonia* leaves. Specific identity of decaying trunks was often not determinable, but it is believed to be insignificant to usage by these flies. Eggs oviposited in *Pisonia* bark in the field were also identified as those of *D. ambochila*, because their chorion ultrastructure as revealed by SEM matched that of eggs taken from the ovaries of mature females (see Figure 3). In nature and in the laboratory, eggs are inserted in clusters of 40–50 in beetle holes (Figure 1C) or cracks in the papery bark, so that the only portions of the eggs visible from the surface are the clumps of long respiratory filaments (Figure 1C,D).

Oviposition Behavior

Drosophila ambochila females have been observed to oviposit in *Pisonia* bark in the laboratory, if in an appropriate state of decay. Stems that were still green and in very early stages of decay were totally ignored by mature females, as were stems that were in an advanced stage of decay. Material at an intermediate stage was attractive to *D. ambochila* in that females would visit and taste or feed on the substrate, which appears to be a prerequisite to oviposition.

Two successful ovipositions were observed in the laboratory, one stimulated by a laboratory-decayed stem (see Materials and Methods), and the other by moist bark from a naturally decaying tree trunk (10 in. [25 cm] in diameter) collected from the field. Upon being placed in the prepared vial, the first female immediately alighted on the laboratory-decayed *Pisonia* and commenced what appeared to be active feeding, with repeated extensions of the proboscis onto the substrate. The second female delayed for 10 min

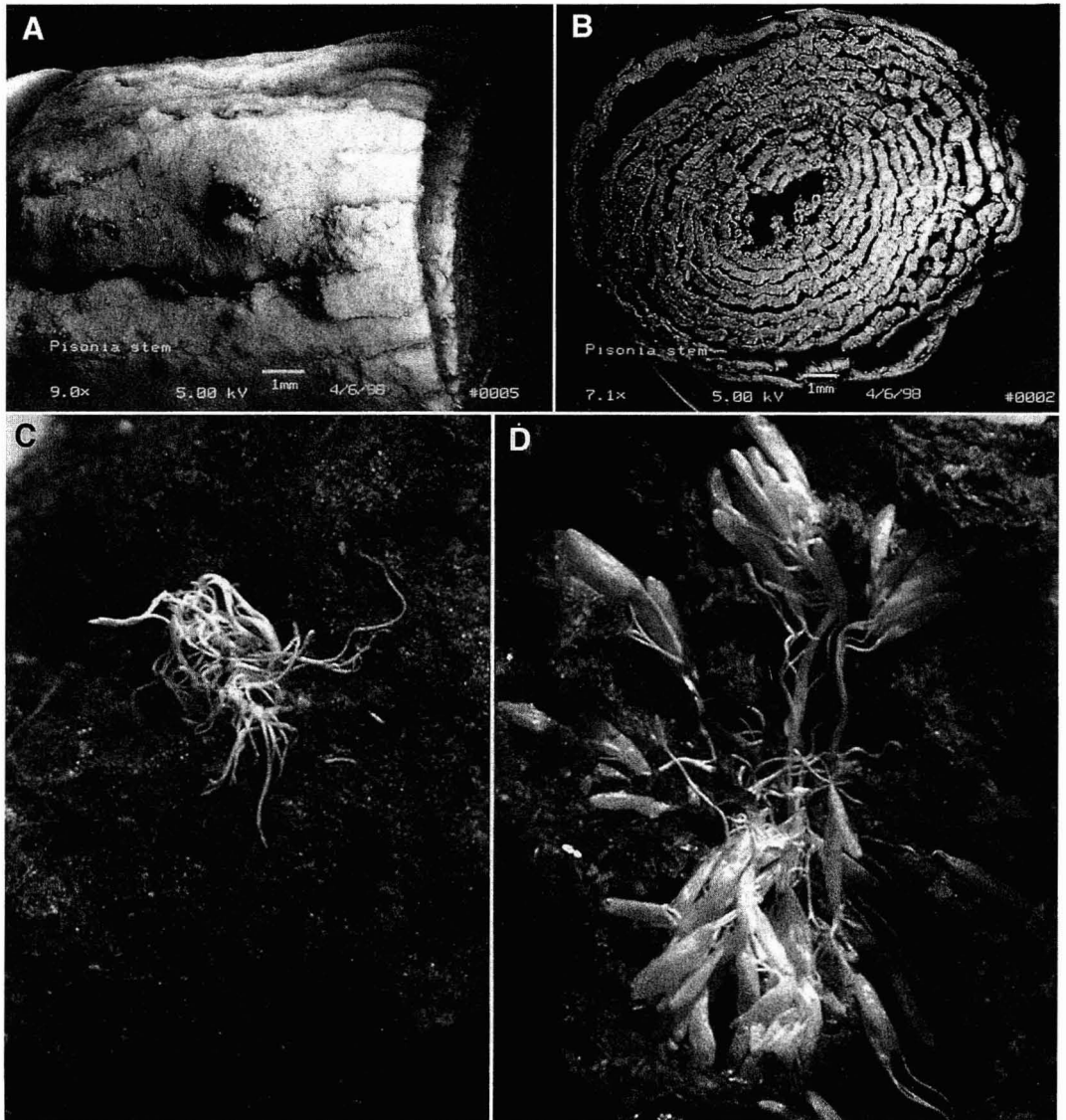


FIGURE 1. Breeding substrate and oviposition pattern of the O'ahu fly *D. ambochila*. *A, B*. SEM micrographs of field-collected *Pisonia* stem showing bark surface (*A*) and a stem cross section (*B*). Note the thin bark layers (see scale bar) formed by anomalous secondary thickening. *C, D*. Field-collected decaying *Pisonia* stem with *D. ambochila* eggs oviposited in a beetle hole; when viewed from the surface (*C*) only a clump of long respiratory filaments is visible. The same piece of bark viewed from inside (*D*) shows the protected cluster of ~ 50 eggs probably oviposited by a single female.

before walking onto the field-collected substrate. Both females fed at several sites on the bark surface and then stopped feeding and probed the substrate with their ovipositor. Having found a suitable oviposition site,

each female became immobile, extruded her ovipositor (Figure 2*A*), and subsequently everted the inner membranous lining of the ovipositor (the egg guide or egg canal) far beyond the ovipositor tip (Figure 2*C-H*).

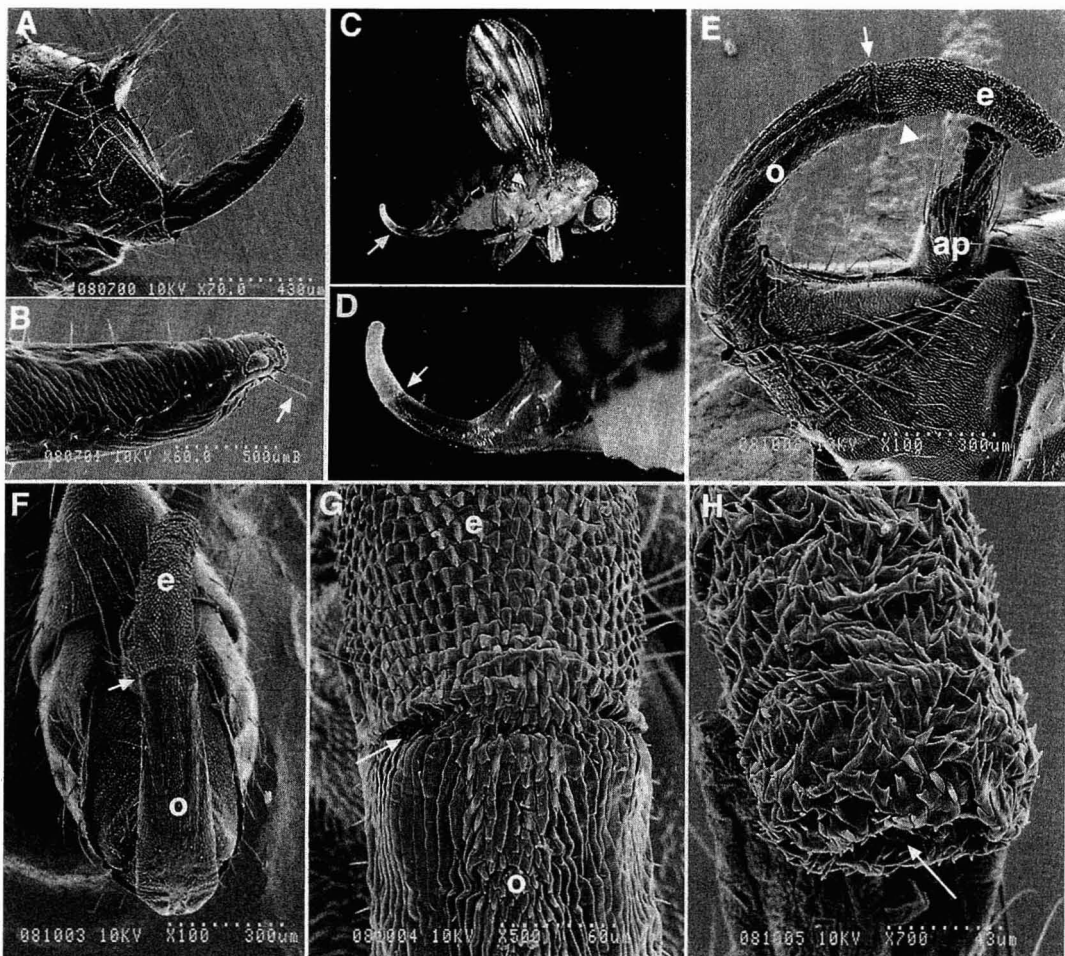


FIGURE 2. Ovipositor of *D. ambochila*. **A**. SEM of a lateral view of the female terminalia showing anal papilla dorsally and ventrally a portion of the long, slender ovipositor protruding from the eighth tergite. Here the ovipositor is partially extended from the resting state, as occurs when a female is preparing to begin oviposition. **B**. Higher magnification of tip of ovipositor showing texture of the valves and placement of the dorsal spinelike sensilla, and the apical and ventrolateral row of peglike sensilla. Arrow indicates the pair of prominent subapical sensilla on the ventral margins of the two valves. **C**. A dead adult female *D. ambochila* viewed under the dissecting microscope, showing fully extended ovipositor and completely everted egg guide, which practically doubles length of the ovipositor. This female had been frozen immediately following completion of oviposition, which induced extension of the ovipositor and eversion of the egg canal. The arrow in **C** and **D** indicates the tip of the ovipositor valves. Note that during oviposition the ovipositor is not in the position shown here or in Figure 2E, but is extended ventrally and anteriorly. **D**. Higher magnification of the fully extended ovipositor (compare with Figure 2A), showing its length relative to the anal papilla. **E–H**. SEM micrographs of fully extended ovipositor (o) and egg guide (e) as seen in lateral view (**E**) and from ventral surface of ovipositor (**F**). In **E**, the arrowhead to the left of the anal papilla (ap) indicates the dorsal tip of the right ovipositor valve, and the arrows in **E–G** show the transition between the ventral bridge between the two ovipositor valves and the usually internal egg guide. **G**. Higher magnification of the indicated region in **F**, showing in the lower portion the folded distal membranous bridge of the ovipositor, which expands during egg passage, and the transition to the everted egg canal showing its spiny inner surface. **H**. The flaccid terminus of the everted egg canal with the arrow indicating the opening. Magnifications of SEM micrographs are shown by scale bars.

With the ovipositor still fully extended, the egg guide was then retracted while the female remained stationary. This procedure was repeated eight times, with some movement visible in the ventral abdominal region each time.

While still stationary, the *D. ambochila* female lowered her abdomen toward the substrate, then brought her ovipositor under her abdomen and forward as far as the fifth sternite. In this position, she then attempted to insert the ovipositor into the substrate. If penetration was not achieved, the egg, which was already visible low in the reproductive tract, was not released. After the entire length of the ovipositor was successfully inserted into the substrate material, a single egg was released deep within the substrate. As the ovipositor was withdrawn, the egg respiratory filaments were exposed, with much of their length positioned above the substrate surface. This behavioral sequence was repeated over the course of the next hour, as the female inserted additional eggs into exactly the same position (see Figure 1C). Throughout this time she remained motionless, except for the oviposition attempts. The female then rested and cleaned her ovipositor with the hind legs for about 5 min. After moving to an adjacent site on the laboratory-decayed bark, the first female oviposited a few additional eggs before walking away.

In ovipositing on the field-collected substrate the second female behaved similarly, but after 15 min rest moved to the tissue paper included in the vial and commenced a second round of oviposition. As is standard practice in the handling and maintenance of

Hawaiian *Drosophila*, the paper had been previously saturated with the fermenting juice of the endemic Hawaiian plant *Clermontia* to maintain humidity in the vial; subsequently, it had also absorbed the juices of the decaying *Pisonia* bark placed into the vial. After an initial unsuccessful attempt to insert her ovipositor into the paper, the female succeeded in ovipositing under the edge of the folded paper, placing her eggs between the paper and the glass wall of the vial. Over the next 20 min she remained in the same position, while laying 25 eggs next to each other under the paper edge. The force she used was visible from the vibration of her front right leg before she steadied it on the glass, along with the other five. Throughout the oviposition process, the ovipositor was well extruded and quite separate from the more dorsal anal papilla. When oviposition was complete, the female walked away with her ovipositor retracted into the resting position (i.e., pointing more dorsally to almost touch the anal papilla). No more mature eggs were visible in the abdomen. In the course of 2 hr of oviposition, much of the time expended in unsuccessful attempts, this female laid a total of 34 eggs—9 eggs in the bark and 25 eggs under the paper.

Reproductive Morphology

Females of *D. ambochila* have a moderately high reproductive capacity, with synchronous development of one mature egg per ovariole (Table 1). Each egg has four respiratory filaments, comprising a pair of short anterior filaments and a pair of long poste-

TABLE 1
FEMALE BODY SIZE AND THE FEMALE REPRODUCTIVE SYSTEM OF *D. ambochila*

INDIV.	THORAX LENGTH (mm)	OVIPOSITOR LENGTH (mm)	OVARY 1		OVARY 2	
			NO. OF OVARIOLES	NO. OF MATURE EGGS	NO. OF OVARIOLES	NO. OF MATURE EGGS
1	1.45	0.775	20	0 ^a	19	0 ^a
2	1.69	0.860	23	23	23	23

^aThis female had been observed to oviposit 34 eggs just before dissection.

rior filaments, more than three times the length of the egg (Figure 3A). In a sample of 10 eggs, mean egg length was found to be 0.79 ± 0.007 mm; anterior filament length was 0.42 ± 0.007 mm and posterior filament length was 2.48 ± 0.026 mm. The eggs in this species are quite large relative to the body size of this rather small fly, being more than half the length of the thorax.

The ovipositor (Figure 2) is long and slender, and extends dorsally in the resting position (not shown). The anterior portion of the sclerotized valves is usually covered by the eighth tergite, but when extruded (Figure 2C–F) the ovipositor is approximately the length of the egg (Table 1). During oviposition, eggs can be placed at depths of almost 2 mm into the substrate. Each valve of the ovipositor (Figure 2B) bears a relatively long sensillum on the ventral side of the narrowed tip and a single row of short peg sensilla extending around the apex and ventrolaterally. As well, there are several longer and more sparsely distributed sensilla on the dorsal side of each valve. The two valves are connected dorsally and ventrally (Figure 2F,G) by a deeply folded membrane that unfolds during egg passage. The egg guide lining, which is in close contact with the chorion surface during oviposition, is covered with small spines (Figure 2G,H).

The anterior end of the *D. ambochila* egg has on its ventral side a prominent collar (Figure 3B), a chorionic structure that is typical of the eggs of all Hawaiian *Drosophila* species except members of the fungus-breeding white-tip scutellum group (Kambysellis 1993). The main body of the chorion is covered with small, spherical protrusions and lacks the prominent follicle imprints found in the majority of Hawaiian *Drosophila* species. At the posterior end of the egg where the pole cell precursors to the embryonic germ cells develop there is a cluster of more than 100 large aeropyles (Figure 3C). Unexpectedly, these aeropyles are located on the posterior ventral side of the chorion, rather than in the typical position on the posterior dorsal side of the egg. As well as these aeropyles, other chorionic features of the *D. ambochila* egg also provide for embryonic respiratory ex-

change. Anteriorly, the egg has a prominent operculum on the dorsal side (Figure 3D) that extends posteriorly beyond the bases of the two posterior respiratory filaments. The surface texture of the operculum (Figure 3E) provides for substantial plastron respiration (Kambysellis et al. 1998). The short anterior filaments have numerous large aeropyles, particularly in the basal two-thirds (Figure 3F,G), whereas on the posterior filaments much smaller aeropyles are only sparsely scattered (Figure 3G). The outer endochorion (Figure 3H) is rather thick, as is typical for eggs of the bark-breeding picture-winged species. The supporting pillars connecting the outer and inner endochorion are also typical of these species.

When we used SEM to examine field-oviposited eggs in decaying *Pisonia* bark collected from the same gulches as the adult flies (Figure 3I,J), we found that their chorionic ultrastructure matched that of *D. ambochila* in all respects, including the distinctive surface pattern of protrusions (Figure 3K).

The male genitalia of *D. ambochila*, and especially the shape of the aedeagus, are essentially similar to those of other members of the *vesciseta* subgroup as described by Kaneshiro (1969). The chaetotaxy on the ventral surface of the ninth tergum of the genitalia is somewhat different among species of the *vesciseta* subgroup and may be used to differentiate species. However, such detailed comparisons of the male genitalia are not required for differentiating between *D. ambochila* and the related *D. alsophila* Hardy & Kaneshiro, with which it shares a unique inversion (described below), because the wing markings are very distinctive between these two species.

Chromosomal Analyses

Favorable polytene chromosome spreads were obtained from the larvae resulting from the laboratory oviposition of female Z39P6 collected from the Pu'u Kaua locality, and the *D. ambochila* banding patterns were mapped onto the standard sequences of *D. grimshawi*. As observed in 10 favorable smears, the basic sequence formula relative to the standard

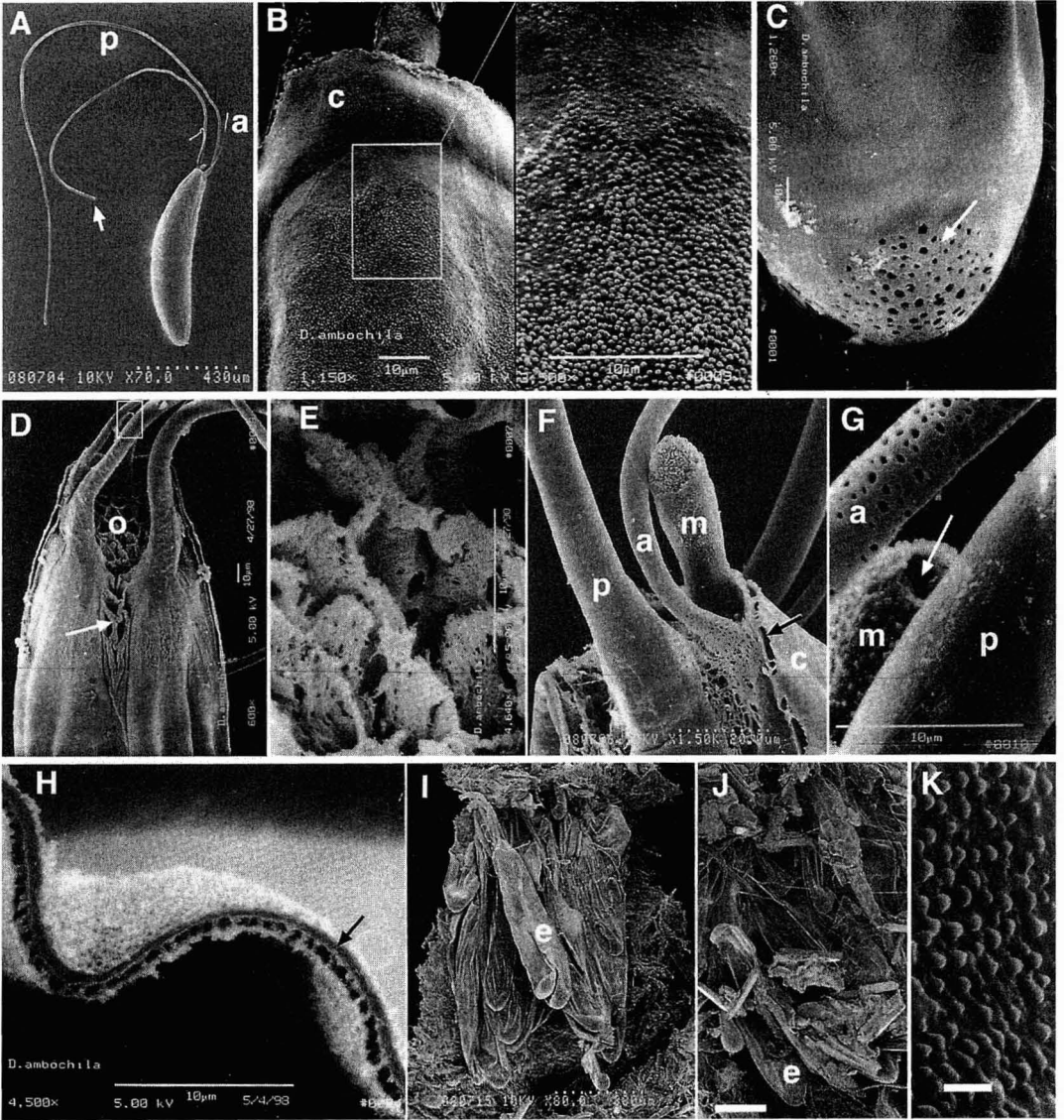


FIGURE 3. Egg and chorion ultrastructure of *D. ambochila* as revealed by SEM. Magnifications indicated by scale bars on each micrograph. **A.** Lateral view of a mature egg (dorsal side to the right) dissected from the ovaries showing the two short anterior filaments (a) and two long posterior respiratory filaments (p). One of the posterior filaments is broken (arrow). **B.** Ventral view of anterior end of egg showing the well-formed collar (c) and lack of prominent follicle imprint borders. Right panel shows a higher magnification of outlined chorionic region displaying densely packed surface protrusions. **C.** Posterior pole of egg showing extensive network of large aeropyles. Arrow points to one of the rows of minute aeropyles arrayed on the borders of the follicle imprints. **D.** Dorsal operculum (o) region at anterior end of egg, which in *D. ambochila* extends posteriorly (arrow) beyond bases of posterior pair of filaments. **E.** Higher magnification of operculum region, showing the well-formed and multiporous follicle imprint borders. **F.** Lateral view of anterior end of egg showing collar on ventral side (to the right), the hatching line along edge of the operculum (black arrow), the porous base of the anterior respiratory filament (a), the posterior filament (p), which lacks large aeropyles, and the micropyle (m) with short fibrils at its tip. **G.** Higher magnification of inset area in Figure 3D showing tip of micropyle (m) with terminal sperm entrance point (arrow), to the upper left the basal region of one of the anterior filaments (a) with a surface network of large aeropyles, and to the lower right one of the posterior filaments

was determined as X 2 3 4b 5. The chromosome 4b sequence characterizes the *D. glabripes* species group or 4b phylad of at least 25 species (Carson 1992). In *D. ambochila*, chromosome 4 was not found in the uninverted homokaryotypic state, as were the sequences of the other four chromosomes. Five larvae were heterozygous for the inversion configuration 4b/4bh³ and the other five were homozygous 4bh³/4bh³. The large 4h³ inversion was previously described and its breakpoints figured by Carson (1971) from *D. alsophila* collected at Moanuaheha, island of Hawai'i, in December 1970. The sequence readings of *D. alsophila* were reexamined using the polytene chromosome slides that had been preserved in a freezer, and the identity of the *D. ambochila* O'ahu inversion and the *D. alsophila* Hawai'i inversion was confirmed directly. As reported previously (Carson 1971), in *D. alsophila* all four larvae examined were homokaryotypic for 4bh³, with the uninverted 4b sequence not recorded, by contrast with the data from *D. ambochila*. All *D. alsophila* larvae also showed a unique X chromosome sequence, Xj³.

DISCUSSION

The species *D. ambochila* is one of the rare Hawaiian picture-winged flies, and one of 29 species that occur in the Wai'anae Mountains. Although it has been known to exist for more than 25 yr, its biology and relationships to other members of this group have remained somewhat obscure until now. The overall morphology, male genitalia, and the chromosomal data presented here clearly place this species in the *vesciseta* subgroup of the *glabripes* species group (the *grimshawi* 4b phylad).

As defined by external male genitalia, the

vesciseta subgroup contains eight other species (Kaneshiro et al. 1995). Three of these share the 5d inversion (Carson 1992) and can thus be considered more derived. The chromosomal relationships of the remaining five species and *D. ambochila* are depicted in Figure 4, arranged in order of geological age as inferred by their biogeography. The most ancestral species of this cluster of six species is *D. micromyia* of Kaua'i. The two O'ahu species, *D. montgomeryi* and *D. ambochila*, the Maui species *D. vesciseta*, and the Hawai'i species *D. assita* and *D. alsophila* are progressively younger and more derived, as indicated by their island of origin and the typical pattern of dispersal down the Hawaiian island chain from older to younger islands (Funk and Wagner 1995).

The discovery of the inversion 4h³ in *D. ambochila*, which is shared with one of the two species from the island of Hawai'i, *D. alsophila*, indicates the close phylogenetic relationship of these two species and confirms *D. ambochila* as a member of the *vesciseta* species subgroup. The fact that 4h³ is polymorphic in *D. ambochila* suggests that this inversion arose on O'ahu, rather than on the island of Hawai'i as previously inferred. Moreover, it appears that *D. alsophila* derived from an independent colonization of Hawai'i by an *ambochila*-like ancestral population on O'ahu. This finding appears to invalidate the previously suggested origin of *D. alsophila* from a *vesciseta*-like ancestor on Maui (see figure 13 of Carson and Yoon 1982).

The rearing of *D. ambochila* adults from field-collected *Pisonia*, the ultrastructural identification of field-oviposited eggs, and the laboratory observation of successful oviposition in *Pisonia* all confirm that the natural breeding substrate of this fly is decaying trunks of *Pisonia* (family Nyctaginaceae).

(p) with very fine aeropyles. H. Cross section of chorion showing thick outer endochorion (arrow) and pillars connecting outer endochorion and chorionic floor. Notice lack of distinct follicle imprint borders. I. Small portion of a field-collected decaying *Pisonia* branch showing some of the cluster of oviposited eggs (e). J. Another sample of field-oviposited eggs (e) in decaying *Pisonia*. Scale bar is 200 μ m. K. High magnification of chorionic surface of an egg in *J* showing the pattern of surface protrusions that is characteristic of *D. ambochila* (see Figure 3B, right panel). Scale bar is 1 μ m.

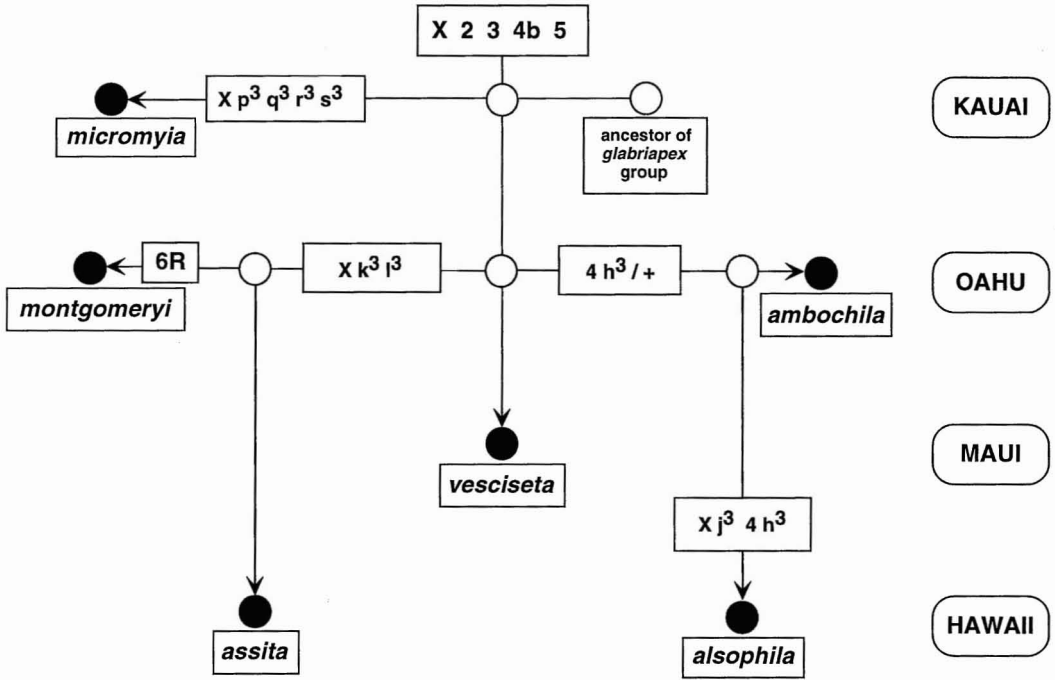


FIGURE 4. A proposed phylogenetic and geographic scheme for the evolutionary relationships of six of the species of the *Drosophila vesciseta* subgroup in the Hawaiian Islands. The polytene inversion formula of each species may be read cumulatively by starting from the top of the figure. (Note that these data represent additions to the chromosomal relationships of the picture-winged *Drosophila* proposed in fig. 2 of Carson 1992.) Solid circles represent extant species that have been analyzed chromosomally; open circles represent hypothetical ancestral populations. The island to which each species is endemic is shown to the right.

The bark of these small trees is paper thin, and stem decay produces a wet, fibrous matrix highly suitable for *Drosophila* larvae. Nonetheless, this host plant is used rather infrequently by members of the picture-winged group as a whole (Montgomery 1975), and in fact, the distribution of *Pisonia* on O'ahu is relatively patchy in ravines of both the Wai'anae and Ko'olau Ranges. *Drosophila ambochila* appears to be monophagous, which is typical of the majority (77%) of picture-winged species (Montgomery 1975, Kambysellis and Craddock 1997). The only other picture-winged species that are recorded as monophagous on *Pisonia* are the two Kaua'i species, *D. sejuncta* of the *orphnopeza* subgroup and *D. ocellata* of the *punalua* subgroup; the Maui species, *D. oreas* of undetermined affiliation (Heed 1968, Montgomery 1975); and the Kaua'i repre-

sentative of the *vesciseta* subgroup, *D. micromyia* (S.L.M., unpubl. data). Other picture-winged species that have been recorded as using *Pisonia* are not host-specific, being either oligophagous (*D. hexachaetae*, *D. pisonia*, *D. macrothrix*, *D. disjuncta*, and *D. inedita*) or polyphagous (*D. grimshawi* and *D. crucigera*) (Montgomery 1975).

The obligate requirement of *D. ambochila* for *Pisonia* restricts its distribution to those sites where this tropical tree is found, such as windward gulches with a riparian forest community. In these ravines where rainfall ranges from 1500 to 5000 mm per year, seasonal climatic changes are buffered. The genus *Pisonia* is often codominant with species of *Charpentiera*, another member of the Centrospermae that also has soft wood, but the demise of *Charpentiera* in Wai'anae ravines (Sohmer 1973) has left *Pisonia* as the

dominant plant. Nonetheless, the presence of *Pisonia* does not guarantee a large population of *D. ambochila*, because females are highly selective in their behavior, restricting oviposition to *Pisonia* bark that is at a critical stage of the decay process. The softness of the decaying wood and multilayered (onion-like) structure of *Pisonia* stems (Figure 1B) are quite favorable for larval development of this species. If there is sustained decay of damaged branches or trunks of *Pisonia*, a local population of *D. ambochila* may be able to persist. As far as is currently known, appropriate conditions are only found in two Wai'anae ravines: 'Ēkahanui Gulch below Pu'u Kaua and the Kalua'a Gulch between Pu'u Hāpapa and Pu'u Kānehoa. In Hawai'i, such a restricted distribution is not unique. Indeed, highly local endemism may be typical of relatively sessile animals that occupy a precise microniche. An outstanding example is the case of tree snails endemic to single gulches in the Wai'anae Mountains (Welch 1938).

Female reproductive traits of *D. ambochila*—most notably the high fecundity with synchronous maturation and oviposition of a large number of eggs (Figure 1C,D), the very long respiratory filaments of the egg (Figure 3A), and the long, slender ovipositor (Figure 2A)—are all consistent with its bark-breeding habit. Although it is a relatively small fly, the ovipositor length of *D. ambochila* (Table 1) is comparable with that of much larger-bodied bark-breeding species (Craddock and Kambysellis 1997). Its long ovipositor is well adapted to inserting the eggs into the decaying substrate, but this placement requires the eggs to have long filaments for embryonic respiratory exchange. Additional chorionic features of *D. ambochila* eggs such as the relatively thick endochorion (Figure 3H) and the distribution of aeropyles are also characteristic of the eggs of bark-breeding species (Kambysellis 1993) and together provide for the protection and respiration of the embryo during its development in the moist bark habitat (Kambysellis et al. 1998). The overall chorionic pattern is, however, unique to *D. ambochila*, which is consistent with previous observations that each Hawaiian *Drosophila*

species can generally be diagnosed by its distinctive combination of chorionic characters (Kambysellis 1993). The lack of prominent follicle imprint borders in the *D. ambochila* chorion may account in part for the frequently observed collapsing of these eggs during SEM preparation (see Figure 3I,J).

The preovipositional behavior of a series of eversions and retractions of the inner egg canal of the ovipositor (see Figure 2) has not been observed previously in any other Hawaiian drosophilid species. However, because oviposition has only been observed in the laboratory for a few species, we cannot say whether this behavior is unique to this species. We suggest that these muscular movements before oviposition in *D. ambochila* serve to achieve ovulation and transport of mature eggs from the ovarioles into the uterus ready for oviposition. Presumably, fertilization also occurs at this time, using the sperm stored in the spermathecae.

Information on the breeding substrates of related species in the *vesciseta* subgroup is incomplete. The Kaua'i species *D. micromyia* also uses *Pisonia* (S.L.M., unpubl. data), but the other O'ahu species, *D. montgomeryi*, uses stems and bark of the stingless nettle tree *Urera* of the family Urticaceae. This host plant is also used by the Hawai'i species *D. assita* (Montgomery 1975). This shared ecological trait is consistent with the sharing of two X chromosome inversions Xk^{313} by this species pair (Figure 4), confirming their close phylogenetic relationship. Unfortunately, there are no data on the breeding substrate of the Maui species *D. vesciseta*, nor the Hawai'i species *D. alsophila*, which, because of the shared $4h^3$ inversion, is putatively derived from an ancestral *D. ambochila*-like population on O'ahu. It could be predicted that *D. alsophila* has retained *Pisonia* as its breeding substrate, but this remains to be verified.

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LITERATURE CITED

- CARSON, H. L. 1971. Polytene chromosome relationships in Hawaiian species of *Drosophila*. V. Additions to the chromosomal phylogeny of the picture-winged species. Univ. Tex. Publ. 7103:183–191.
- . 1983. Chromosomal sequences and interisland colonizations in Hawaiian *Drosophila*. *Genetics* 103:465–482.
- . 1992. Inversions in Hawaiian *Drosophila*. Pages 408–439 in C. B. Krimbas and J. R. Powell, eds. *Drosophila* inversion polymorphism. CRC Press, London.
- CARSON, H. L., and J. S. YOON. 1982. Genetics and evolution of Hawaiian *Drosophila*. Pages 297–344 in M. Ashburner, H. L. Carson, and J. N. Thompson Jr., eds. *The genetics and biology of Drosophila*, Vol. 3b. Academic Press, London.
- CRADDOCK, E. M., and M. P. KAMBYSELLIS. 1997. Adaptive radiation in the Hawaiian *Drosophila* (Diptera: Drosophilidae): Ecological and reproductive character analyses. *Pac. Sci.* 51:475–489.
- DESALLE, R. 1992. The origin and possible time of divergence of the Hawaiian Drosophilidae: Evidence from DNA sequences. *Mol. Biol. Evol.* 9:905–916.
- FUNK, V. A., and W. L. WAGNER. 1995. Biogeographic patterns in the Hawaiian Islands. Pages 379–419 in W. L. Wagner and V. A. Funk, eds. *Hawaiian biogeography: Evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington.
- GAGNÉ, W. C., and L. W. CUDDIHY. 1990. Vegetation. Pages 45–114 in W. L. Wagner, D. R. Herbst, and S. H. Sohmer, *Manual of the flowering plants of Hawai'i*, Vol. 1. University of Hawai'i Press, Bishop Museum Press, Honolulu.
- HARDY, D. E., and K. Y. KANESHIRO. 1971. New picture-winged *Drosophila* from Hawaii, Part II. (Drosophilidae, Diptera). Univ. Tex. Publ. 7103:151–170.
- HEED, W. B. 1968. Ecology of the Hawaiian Drosophilidae. Univ. Tex. Publ. 6818:387–419.
- KAMBYSELLIS, M. P. 1993. Ultrastructural diversity in the egg chorion of Hawaiian *Drosophila* and *Scaptomyza*: Ecological and phylogenetic considerations. *Int. J. Insect Morphol. Embryol.* 22:417–446.
- KAMBYSELLIS, M. P., and E. M. CRADDOCK. 1997. Ecological and reproductive shifts in the diversification of the endemic Hawaiian *Drosophila*. Pages 475–509 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge University Press, Cambridge.
- KAMBYSELLIS, M. P., K.-F. HO, E. M. CRADDOCK, F. PIANO, M. PARISI, and J. COHEN. 1995. Pattern of ecological shifts in the diversification of Hawaiian *Drosophila* inferred from a molecular phylogeny. *Curr. Biol.* 5:1129–1139.
- KAMBYSELLIS, M. P., L. MARGARITIS, and E. M. CRADDOCK. 1998. Egg coverings, insects. Pages 971–990 in E. Knobil and J. D. Neill, eds. *Encyclopedia of reproduction*, Vol. 1. Academic Press, New York.
- KANESHIRO, K. Y. 1969. A study of the relationships of Hawaiian *Drosophila* species based on external male genitalia. Univ. Tex. Publ. 6918:55–70.
- KANESHIRO, K. Y., R. G. GILLESPIE, and H. L. CARSON. 1995. Chromosomes and male genitalia of Hawaiian *Drosophila*: Tools for interpreting phylogeny and geography. Pages 57–71 in W. L. Wagner and V. A. Funk, eds. *Hawaiian biogeography: Evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington.
- MONTGOMERY, S. L. 1975. Comparative breeding site ecology and the adaptive radiation of picture-winged *Drosophila* (Diptera: Drosophilidae) in Hawaii. *Proc. Hawaii. Entomol. Soc.* 22:65–103.
- SOHMER, S. H. 1973. A preliminary report of the biology of the genus *Charpentiera* (Amaranthaceae). *Pac. Sci.* 27:399–405.
- WAGNER, W. L., D. R. HERBST, and S. H.

- SOHMER. 1990. Manual of the flowering plants of Hawai'i, Vol. 1. University of Hawai'i Press, Bishop Museum Press, Honolulu.
- WELCH, D'A. 1938. Distribution and variation of *Achatinella mustelina* Mighels in the Waianae Mountains, Oahu. Bernice P. Bishop Mus. Bull. 152: 1-164.
- WHEELER, M. R., and F. E. CLAYTON. 1965. A new *Drosophila* culture technique. Dros. Inf. Serv. 40: 98.