

Description of a New Allopatric Sibling Species of Hawaiian Picture-Winged *Drosophila*¹

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ABSTRACT: A new picture-winged Hawaiian *Drosophila* species from the islands of Kaua'i and O'ahu that is morphologically indistinguishable from *Drosophila grimshawi* Oldenberg from the Maui Nui islands is described, based on differentiation in ecological, behavioral, cytological, and molecular characters as well as ultrastructural features of the chorion. The new species, *D. craddockae*, and *D. grimshawi* represent the first clear case of an allopatric sibling species pair among Hawaiian Drosophilidae (i.e., there is strong evidence for a profound set of intrinsic, genetically determined differences that are not easily diagnosable by the usual morphological methods). Ecologically, *D. craddockae* is a strict specialist, with oviposition restricted to the decaying bark of *Wikstroemia*. *Drosophila grimshawi*, on the other hand, is a generalist that breeds in the decaying parts of 10 families of plants. Data from cytological, behavioral, and molecular analyses are consistent with the geological evidence that species on the older islands are usually more ancestral than those that evolved on the younger islands. Thus, although long-standing ecological theory states that specialization is a derived condition, the biological and genetic evidence all indicate that specialism in *D. craddockae* is the ancestral condition and that generalism evolved in *D. grimshawi* on Maui Nui as a derived trait.

BASED ON ANALYSES of data from the ecology; ultrastructural features of the chorion or eggshell; nucleotide sequences; jousting, oviposition, and mating behavior; and yolk protein molecular data, Piano et al. (1997) determined that the Hawaiian picture-winged *Drosophila* species *D. grimshawi* Oldenberg can be separated into two clades. One clade includes the Kaua'i and O'ahu populations of *D. grimshawi* and the closely related species *D. pullipes* from the island of Hawai'i. Ecologically, the populations in this clade are considered specialists with respect to their ovipositional behavior, utilizing a single endemic plant (Montgomery 1975). The second

clade includes the populations of *D. grimshawi* from the Maui Nui island complex (i.e., the islands of Moloka'i, Lāna'i, and Maui). These populations are considered to be ecological generalists, utilizing 10 families of plants as larval breeding substrates (Heed 1968, Montgomery 1975).

We agree with Piano et al. (1997) that the *D. grimshawi* populations from Kaua'i and O'ahu are ecologically and genetically distinct from *D. grimshawi* sensu stricto from Maui Nui (the type locality of this species is "Molokai Mts., 4,000 ft."), although morphological differences are not apparent. Also, Ohta's (1980) data on crosses among the Kaua'i, O'ahu, and Maui Nui populations of *D. grimshawi* as well as with *D. pullipes* from Hawai'i provide the strongest evidence for describing the Kaua'i and O'ahu populations as a distinct biological species. In this paper, we name and describe the Kaua'i and O'ahu populations of *D. grimshawi* as a new species and present a summary of the key differences that differentiate the Kaua'i and O'ahu pop-

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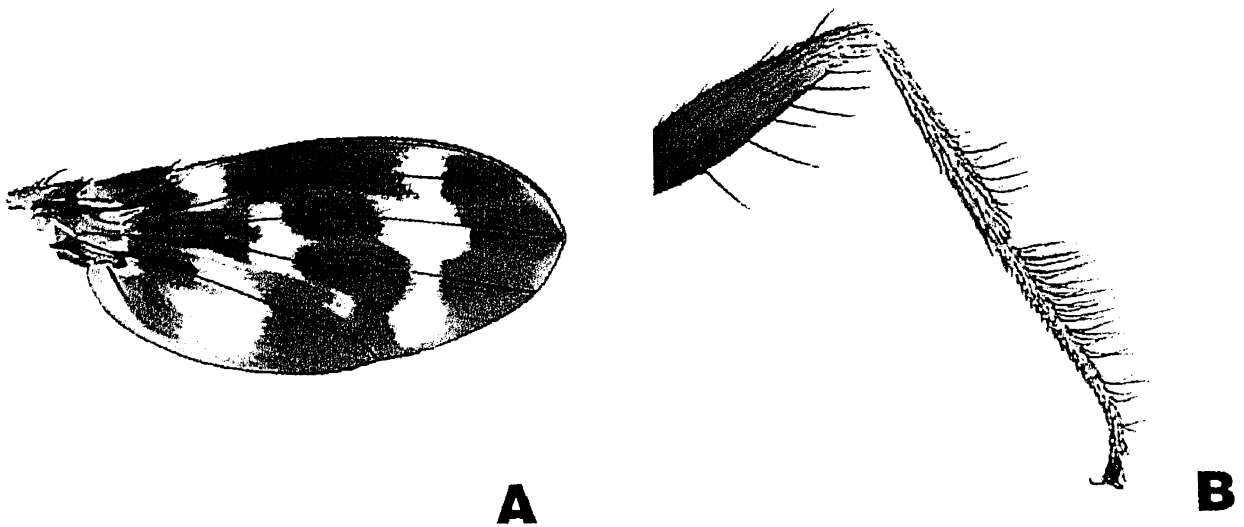


FIGURE 1. (A) Typical wing (male from Ka'au Crater, O'ahu) and (B) foreleg (male from Hanalilolilo Trail, Moloka'i) of *D. grimshawi* complex.

ulations from the Maui Nui populations as well as from *D. pullipes*.

Drosophila craddockae Kaneshiro & Kambysellis, n. sp.

There are no apparent morphological differences between adults of this species and *D. grimshawi* from the Maui Nui complex of islands. Morphologically, *D. grimshawi* sensu stricto, *D. craddockae*, and *D. pullipes* are essentially identical: refer to the description of *Drosophila grimshawi* Oldenberg in Hardy (1965:295–297). There appear to be some polymorphisms in the wing patterns and in the number of cilia on the front legs of the males (see Figure 1), but the differences observed do not reliably distinguish the different populations, although biometrical analyses have not been conducted. The only difference among the three species is in the coloration of the legs and pleurae, where *D. pullipes* is black rather than yellow. The other features that distinguish the three species are described below.

DISCUSSION: The following sections review the various attributes of these species that have led us to conclude that *D. craddockae* populations (Kaua'i, O'ahu) should be specifically distinguished from those of *D. grimshawi* of Maui Nui (Maui, Moloka'i, and Lāna'i). The extent of these morphologically

cryptic differences appears to justify our conclusion that the case at hand is one of allopatric sibling species.

The detailed differences among these species have further interest with regard to the question of the genetic origin of ovipositional generalism, as manifested in *D. grimshawi*. The origin of most of the picture-winged species on the younger Hawaiian islands can be chromosomally traced to ancestral populations that existed at one time on the islands of Kaua'i and O'ahu (Carson 1983). Thus, Carson and Ohta (1981) theorized that the generalism in this case evolved on the newer islands and was derived from an older population of specialist ancestors. Kambysellis and Craddock (1997) reviewed the host plant associations of the Hawaiian drosophilids and noted that only two of the 44 species analyzed were considered to be generalists (i.e., polyphagous in the use of more than five plant families as larval breeding substrates). They concluded that in both cases, generalism is the derived condition and that specialization on specific host plants is the ancestral trait. This contrasts with the commonly held notion that specialization is a derived trait (see, for example, Futuyma and Moreno 1988).

ECOLOGY: *Drosophila grimshawi* is polyphagous, having been reared from 13 genera

in 10 families of plants (Heed 1968, Montgomery 1975). In contrast, both *D. pullipes* and *D. craddockae* are monophagous and have been reared only from the decaying bark of *Wikstroemia* species. Under laboratory conditions, *D. grimshawi* readily oviposits in the culture medium, and the larvae can complete development without the addition of any of the natural plant material utilized as larval breeding substrate. Females of *D. pullipes*, however, will almost never oviposit in artificial medium, although when a small piece of decaying *Wikstroemia* bark is placed in the culture vials, the females generally react by ovipositing. After oviposition has taken place and the eggs hatch, the larvae readily move into the artificial medium and complete development. Females of *D. craddockae* when first brought into the laboratory behave in a manner similar to those of *D. pullipes* and require decaying *Wikstroemia* bark to stimulate oviposition. However, with some lines of *D. craddockae*, it has been possible to "wean" the females from requiring the natural substrate and the stock can eventually be maintained without the need to provide *Wikstroemia* bark as an oviposition stimulus. Clearly, the nutritional requirements for larval development of all three species are satisfied by the standard culture medium even though stimulation of oviposition in the two specialist species requires the presence of *Wikstroemia* in most cases.

BEHAVIOR: Ringo (1976) reported on a lek-associated communal display known as jousting among five species of Hawaiian *Drosophila* including *D. grimshawi* and the O'ahu population of *D. craddockae*. For the five species he studied, he observed one to 14 distinct postures and displays that may occur during jousting behavior. He determined that jousting was "frequent and elaborate" in *D. grimshawi*, but it was "brief, aggressive, simple, and infrequent" in *D. craddockae*, similar to what might be described for *D. pullipes*. Minor differences were observed between *D. craddockae* and *D. pullipes*, but these two species are clearly differentiated from *D. grimshawi* in this behavioral character.

ULTRASTRUCTURE OF CHORION: Piano et al. (1997) reported that the overall egg morphol-

ogy of all three species is similar, showing typical Hawaiian *Drosophila* egg pattern (Kambysellis 1993) with four respiratory filaments, a well-formed dorsal ridge, and a broad operculum. However, upon closer inspection using scanning electron microscopy (SEM), distinct and consistent differences were observed among the populations. Most important, Piano et al. (1997) showed that both Kaua'i and O'ahu *D. craddockae* had distinct follicle imprints with tall borders (Figure 2A,B), but that these borders were less prominent or in some cases completely absent in the Maui Nui *D. grimshawi* populations (Figure 2D). Furthermore, they showed that in *D. pullipes* the borders were well formed, similar to what is observed in *D. craddockae*, but the follicles are covered by small microvilli (Figure 2C), a feature that is absent in *D. craddockae* populations. Also, the eggs of *D. craddockae* have twice as many pores in the posterior pole as the eggs of *D. grimshawi* (Figure 2E,F). Thus the ultrastructure of the chorion appears to be a good character that differentiates all three species.

YOLK PROTEIN MOLECULAR DATA: E. M. Craddock and M.P.K. (unpubl. data) have found that the three species are also differentiated at the molecular level, specifically with respect to the size and composition of one of the three yolk proteins typically found in the eggs of *Drosophila* species. The YP1 protein shows distinct molecular weights when analyzed by SDS-gel electrophoresis. In *D. pullipes*, the YP-1 protein is the largest at 48.8, followed by *D. craddockae* at 47.9, and *D. grimshawi* at 46.0.

DNA SEQUENCE ANALYSES: A 915-nucleotide segment of the *Yp1* yolk protein gene was analyzed using maximum parsimony to generate a single most parsimonious tree using *D. bostrycha* and *D. disjuncta* as out-group species (Piano et al. 1997). The DNA tree differentiates the three species into two clades, with *D. pullipes* clustering with *D. craddockae* from Kaua'i and O'ahu and the Maui Nui *D. grimshawi* clustering together. This relationship corroborates the relationships based on the ecological data and those based on the ultrastructure of the chorion

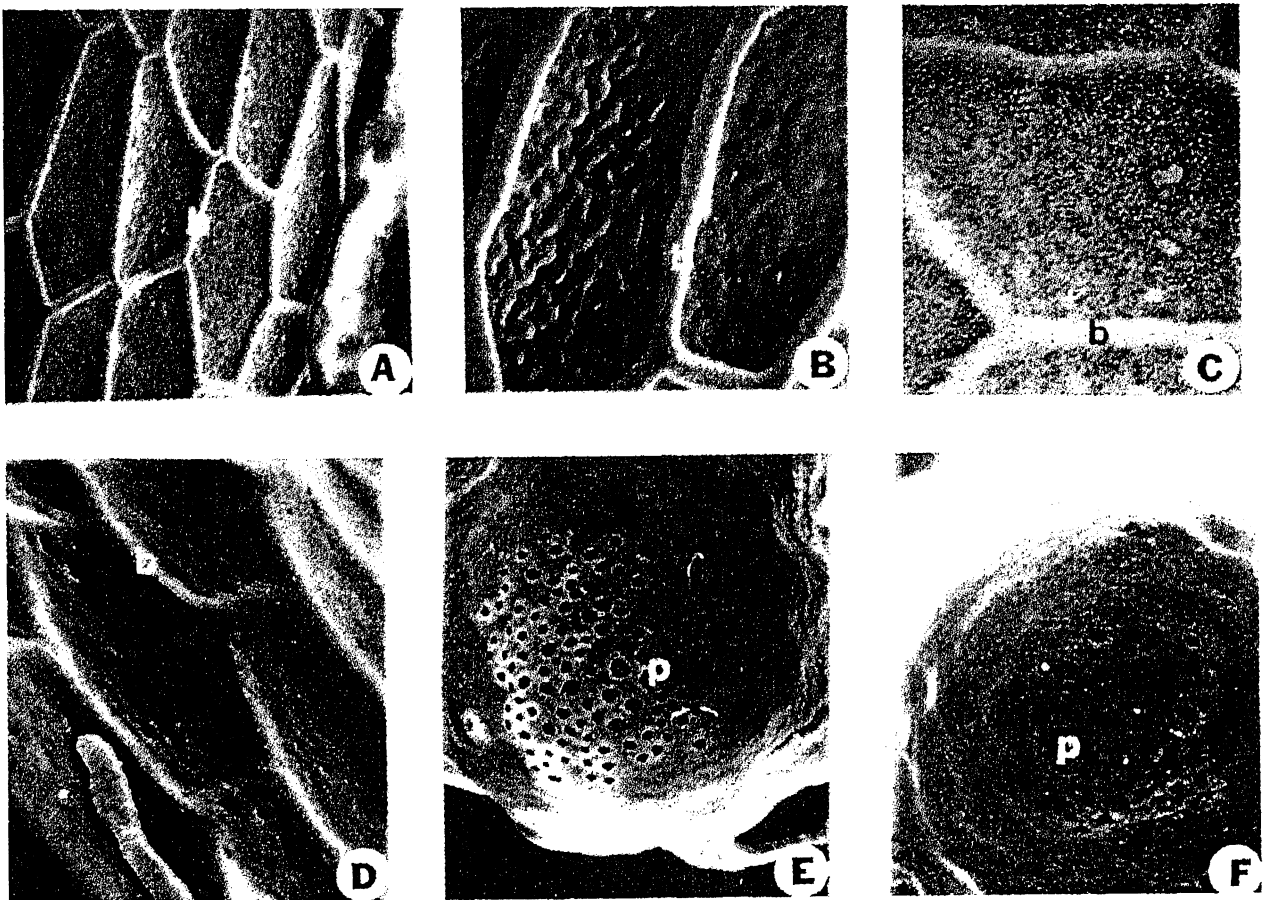


FIGURE 2. Morphological features of the eggshell of *D. grimshawi* complex species. Micrographs A–D show various views and magnifications of the follicular imprint patterns of the chorion: A ($\times 656$) and B ($\times 1235$) depict those of *D. craddockae* (Kaua'i); C, ($\times 1813$) *D. pullipes*; D, ($\times 1670$) *D. grimshawi* (Moloka'i). Micrographs E and F show the posterior pole region of *D. craddockae* (O'ahu) ($\times 442$) and *D. grimshawi* (Moloka'i) ($\times 279$), respectively (note difference in numbers of pores).

and clearly differentiates *D. craddockae* from *D. grimshawi*.

HYBRIDIZATION EXPERIMENTS: The most important data indicating that the populations from Kaua'i and O'ahu are clearly differentiated from *D. grimshawi* sensu stricto and that the former are biologically distinctive from the latter comes from Ohta (1980), in which reciprocal crosses among populations of *D. grimshawi* from Maui and Moloka'i, *D. craddockae* from O'ahu and Kaua'i, and *D. pullipes* from Hawai'i were conducted. Ohta (1980) showed that crosses between the *D. grimshawi* populations from Maui and Moloka'i (he did not have a Lāna'i strain available to conduct similar crosses) produced fully fertile progeny. Similarly, crosses between the two *D. craddockae* popu-

lations produced fully fertile F_1 , F_2 , and backcross progenies and he concluded that ecologically similar populations of Kaua'i/O'ahu and Maui/Moloka'i produced fertile offspring. However, crosses between *D. pullipes* and both *D. grimshawi* and the O'ahu *D. craddockae* populations produced some viable F_1 offspring but most were sterile or only a small number of males had motile sperm (criteria used to determine fertility). Even when F_1 s showed some fertility and F_2 or backcross progenies were obtained, examination of the male progeny from these crosses showed substantially reduced fertility. These data confirm the fact that *D. pullipes* is a biologically distinct species from both *D. grimshawi* and *D. craddockae* even though there are no morphological structures (only color differences on the legs and pleurae) to

differentiate *D. pullipes* from the other two species.

Ohta (1980) also conducted crosses between O'ahu *D. craddockae* and Maui and Moloka'i *D. grimshawi* populations. The results indicate that although there is a high degree of fertility in the F₁s of the different reciprocal crosses, F₂ and backcross progeny show substantially reduced fertility. This is certainly strong evidence for postmating breakdown and warrants describing the Kaua'i and O'ahu populations as a biologically distinct species from *D. grimshawi*.

CYTOLOGICAL STUDIES: Hybridization studies revealed a cytological peculiarity, which also distinguishes *D. craddockae* from *D. grimshawi*. When either *D. pullipes* or *D. craddockae* is crossed to *D. grimshawi*, a strikingly large and apparently redundant polytene chromosome band is invariably present in the heterozygous state in the F₁ (Stuart et al. 1981). This hypertrophied band marks the homologue derived from either the *D. pullipes* or *D. craddockae* parent and appears to be a unique fixed feature within these specialist species. Although labeled "3-18" in the Stuart et al. (1981) paper, on the newer maps of Hawaiian *Drosophila* chromosomes it is designated as 35A (Carson et al. 1992). Also, *D. craddockae* and *D. pullipes* are without intraspecific inversions, whereas most local populations of *D. grimshawi* are polymorphic for a long inversion in chromosome 4 (i.e., inversion 4a [see Table XVI in Carson et al. 1970]).

TYPE SPECIMENS: Holotype male from Halemanu Stream, Kōke'e, Kaua'i; collected by K. Y. Kaneshiro on 1 February 1980. Allotype female plus 4 paratype males and 7 paratype females from the same locality and collection data as holotype. Also, 1 male specimen labeled "Kokee, Kauai, 27-III-79" collected by D. E. Hardy is included as a paratype. All of the specimens from O'ahu are also designated as paratypes. There are 8 male and 3 female specimens (all labeled Y26T) that were reared from *Wikstroemia* collected from Ka'au Crater, O'ahu, on 29 June 1987 by A. T. Ohta. Other O'ahu specimens designated as paratypes are as follows:

1 male from Ka'au Crater labeled L93 collected on 15 July 1968 by J. P. Murphy; 2 males from "Hidden Valley" near Kahana Valley in the Ko'olau Mountains collected on 31 May 1970 by S. L. Montgomery; and 1 male from Castle Trail, Koolau Mountains, collected on 11 April 1970 by S. L. Montgomery.

ETYMOLOGY: It is with pleasure that we name this new species in honor of Dr. Elysse Craddock, who has made and continues to make significant contributions to our overall understanding of the evolutionary biology of the Hawaiian *Drosophilidae*.

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