



Rapid adaptive radiation and host plant conservation in the Hawaiian picture wing *Drosophila* (Diptera: Drosophilidae) [☆]



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ABSTRACT

The Hawaiian picture wing *Drosophila* are a striking example of adaptive radiation in specialist saprophages on an island system. We use DNA sequences from five nuclear genes with a total of 4260 nucleotides to provide a comprehensive phylogeny and biogeographic analysis of 90 species in the Hawaiian *Drosophila picture wing* clade. The current analysis indicates that the evolution of the *picture wing* clade took place more recently than previously suggested. The relationships of several morphologically anomalous taxa are resolved with strong support. Biogeography and host plant analyses show two periods of rapid divergence occurred when Kauai and Oahu were the main high islands, indicating that a combination of complex topographical features of islands and development of novel host plant associations was key to the rapid diversification of these lineages. For the past 2 million years, host associations within lineages have been largely stable, and speciation has occurred primarily due to the establishment of populations on newer islands as they arose followed by divergence by isolation. The existence of several apparently relictual taxa suggests that extinction has also played a major role in assembly of the present Hawaiian *Drosophila* fauna.

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1. Introduction

The Hawaiian picture wing *Drosophila* are often cited as a prime example of ecological and evolutionary diversification (Carson and Kaneshiro, 1976; Carson and Yoon, 1982; Craddock, 2000; Ringo, 1977; Templeton, 1979). Their diversity in chromosomal banding (Carson, 1992), wing pattern (Edwards et al., 2007), larval breeding ecology (Magnacca et al., 2008; Montgomery, 1975), and courtship behavior (Hoy et al., 1988; Spieth, 1982) have been extensively studied. However, relatively few cladistic phylogenetic analyses exist for the group. The Hawaiian Drosophilidae as a whole have been the target of a number of studies (e.g. O'Grady and DeSalle, 2008; O'Grady et al., 2011; Thomas and Hunt, 1993; Throckmorton, 1966), but only one (Kambysellis et al., 1995) has specifically focused on the *picture wing* clade in its entirety. The latter is useful as a general outline, but suffers from weak sampling within the large *grimshawi* group, absence of any *nudidrosophila* species, and few outgroup taxa.

The most comprehensive phylogenetic hypothesis regarding the *picture wing* clade is based on Carson and Yoon's (1992; 1982)

chromosome inversion studies, which include 107 of the 116 species. However, these data lack resolution due to the highly apomorphic nature of inversion state changes – species may retain relatively primitive configurations while related ones have numerous differences, especially when extinct ancestral populations may have been polymorphic for inversions. Homoplasy and retention of ancestral polymorphisms also evidently occur for chromosome inversions (as with inversion Xg in *D. balioptera* and members of the *orphnopeza* subgroup; Carson, 1992), but are not well understood in this context and difficult to account for with a small number of characters.

Several hypotheses about the Hawaiian *picture wing* clade are ripe for testing in a molecular phylogenetic context. First is the timing and mode of diversification. The standard model for the biogeographic evolution of Hawaiian taxa is a progression rule pattern, with the most basal species on Kauai and each lineage dispersing to younger islands as they arise (Funk and Wagner, 1995). Several Hawaiian insect groups have evidently evolved this way, including *Laupala* crickets (Shaw, 2002) and *Orthotylus* leaf bugs (Polhemus, 2002). Previous studies have dated the Hawaiian Drosophilidae at 23–27 million years, based on the *Drosophila-Scaptomyza* split (Russo et al., 1995; Thomas and Hunt, 1993), but these used outdated methods (e.g. strict clock substitution dating) and did not have the internal taxon sampling or resolution

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necessary to date the ages of the major extant clades or their splits from each other. The dispersal scenarios of Carson (1992), summarizing the chromosomal inversion data, assume a pre-Kauai ancestry for all *picture wing* species groups. He proposed 8 ancestral dispersals from Kauai and only two back-dispersals, and estimated the age of the entire clade to be considerably older than Kauai. However, the large water gap between Gardner Island and Kauai that existed between these two high islands around 5 Mya due to the smaller (and probably dry) intervening islands (Price and Clague, 2002) meant that relatively few groups of both insects and plants from that earlier period were likely able to reach Kauai. The fact that only a few Hawaiian plant and insect groups have been dated as older than 5 million years (Haines et al., 2014; Jordan et al., 2003; Price and Clague, 2002) supports this hypothesis.

Second is the importance of host plant usage in evolution of the *picture wing* species. The ecology of the group has been broadly studied, and most species appear to be host-specific or at least have a strongly-favored primary host plant (Magnacca et al., 2008). All breed almost exclusively in decaying bark of native trees, with the exception of the *hawaiiensis* subgroup and *D. picticornis*, which breed in the related habitat of fermenting sap fluxes, and a few generalists that occasionally utilize alien plants (Magnacca et al., 2008). Host plant switching among specialists is often considered a factor in adaptive radiation in other *Drosophila* groups on continental regions (Morales-Hojas and Vieira, 2012; Oliveira et al., 2012) and on islands with open niches for various insect taxa (Asquith, 1995; Percy, 2003; Roderick and Gillespie, 1998; Shaw, 1995), but previous examination of the extent of switching for Hawaiian *Drosophila* has been hampered by uneven taxon sampling within the *picture wing* clade (Kambysellis et al., 1995; O'Grady et al., 2011). Study of yeasts in *Drosophila* host plants has revealed that there is almost no overlap in yeast species among either substrates or plant taxa (O'Connor et al., 2014; Ort et al., 2012), which is presumably the basis of host specificity in Hawaiian *Drosophila*. However, a significant proportion of *Drosophila* species (44%, rising to 57% among better-sampled species) are at least occasionally reared from hosts other than their primary one (Magnacca et al., 2008), indicating flexibility that could allow for selection for host switching under certain conditions.

In this study, we used phylogenetic reconstruction to test hypotheses about (1) the origin and diversification of the *picture wing* clade in relation to the geologic history of the Hawaiian Islands; (2) the role of adaptation, in the form of host plant specialization and switching, in the evolution of the clade; (3) the sub-clade structure as it relates to the first two subjects and to pre-existing informal classifications (species groups and subgroups); and (4) the relationships of supposed "relictual" taxa, single species or low-diversity clades that show little clear similarity to others. Traditionally, the *picture wing* clade has been split into a number of species groups and subgroups, based on male genitalia (Kaneshiro, 1969), courtship behavior (Spieth, 1982), and chromosomal banding patterns (Kaneshiro et al., 1995), the precise composition of which vary depending on the delineation method used and the degree of splitting. Morphologically and behaviorally anomalous taxa such as *D. anomalipes* and *D. primaeva* have been moved around as different pieces of evidence were examined, sometimes being considered remnants of early radiations that never spread beyond Kauai (Spieth, 1975, 1981). Here, we test Carson's (1992) hypothesis of dispersal and diversification; the monophyly of species group and subgroup classifications, with a revised phylogeny-based structure from our results; and the influence of host switching on diversification. We examine these results in the context of ecological speciation models proposed for other animal groups (Kocher, 2004; Oliveira et al., 2012; Schluter, 2001; Strelman and Danley, 2003).

2. Methods

2.1. Taxon sampling

We included as many species as possible in order to provide a complete picture of the evolutionary history of the *picture wing* clade (Table 1). We were able to obtain 80 of the 117 species traditionally considered part of the *picture wing* clade sensu Kaneshiro et al. (1995) (including one undescribed species, *D. nr. alsophila* from Maui). In addition, the two members of the *anomalipes* group and eight representatives of the *nudidrosophila*-*ateledrosophila* group (two each from the *hirtitibia*, *nudidrosophila*, and *velata* subgroups, and one each from the *ateledrosophila* and *kuhao* subgroups) were included. Both of these groups, although morphologically highly divergent, are now recognized as part of the *picture wing* clade (Magnacca and O'Grady, 2008; O'Grady et al., 2011). All species groups and subgroups are represented except for the *macrothrix* subgroup; the *crucigera*, *discreta*, *distinguenda*, and *picticornis* subgroups have all known species included (Kaneshiro et al., 1995; O'Grady et al., 2010).

Multiple individuals were sequenced for multi-island species, isolated populations occurring on different volcanoes (e.g. East and West Maui), or where mtDNA sequences showed high intraspecific divergence (>1%), but in most cases these were not significantly different in nDNA, and their inclusion did not affect the analyses. They were excluded from the final analyses, except for *D. fasciculisetae*, *D. grimshawi*, *D. odontophallus*, and *D. orphnopeza* where genetically divergent Moloka'i and Maui samples were included, for a total of 93 ingroup terminals.

As outgroup taxa, we sequenced representatives from the other major groups of Hawaiian Drosophilidae. These included 14 members of the *antopocerus*-*modified tarsus*-*ciliated tarsus* (AMC) clade, five of the *modified mouthparts* group, and four of the *haleakalae* group. The isolated Kauai species *D. primaeva* and *D. adventitia* were also included. Finally, 14 *Scaptomyza*, representing all of the Hawaiian subgenera except *Alloscaphomyza* and *Rosenwaldia*, were included in order to root the basal node. Regardless of the closest non-Hawaiian relative among Drosophilidae, it is clear that *Scaptomyza* is the closest extant relative of the Hawaiian *Drosophila* (O'Grady and DeSalle, 2008; Remsen and O'Grady, 2002; Tatarenkov et al., 2001; Thomas and Hunt, 1991).

2.2. Gene selection, PCR, and sequencing

We selected five nuclear genes – elongation factor 1-gamma (EF1 g), yolk protein 2 (Yp2), *frizzled* 4 (Fz4), phosphoglucose isomerase (Pgi), and glycerol-3-phosphate dehydrogenase (Gpdh). The first was developed for phylogenetics for this study, as a single-copy nuclear gene with the appropriate level of divergence among the target taxa; unlike the commonly used EF1-alpha, it does not occur in multiple copies (at least in *Drosophila*), and evolves at a faster rate in the target taxa (it has not been used in other studies for more general comparison). The other genes have been used previously in phylogenetics of *Drosophila* and other Diptera (Barrio and Ayala, 1997; Bonacum et al., 2005; Ho et al., 1996; Kopp, 2006; Lapoint et al., 2011). Several additional genes (CAD, Marf, ITS-1, and Dip3) were screened on a representative subsample of the ingroup, but did not provide enough informative characters to be useful at this level of phylogenetic analysis. These sequences have been deposited in GenBank under accession numbers KR269947–KR269994, KR269995–KR270041, KR270042–KR270076, and KR270077–KR270109 respectively.

Total genomic DNA was extracted from specimens preserved in 95% EtOH using the DNeasy Blood & Tissue extraction kit (Qiagen, Inc.). DNA was obtained from either the abdomen (primarily the

Table 1
Specimens used in the analysis, with collection and extraction information and GenBank accession numbers.

Species group Species subgroup Species	Extraction	Island	Locality	Sex	Tissue	EF1 g	Fz4	Gpdh	Pgi	Yp2
<i>Scaptomyza</i>										
<i>Bunostoma</i>										
<i>S. anomala</i>	M09244	Kauai	Nualolo Trail, 22 Jun 2009	Male	Abdomen	JN815559	JN815713	JN815877	JN816040	JN816244
<i>Elmomyza</i>										
<i>S. cyrtandrae</i>	M09120	Hawaii	Army Rd. makai, 8 Dec 2009	Male	Body soak	JN815467	JN815636	JN815794	JN815958	JN816160
<i>S. n. sp.</i>	M09016	Kauai	Nualolo Trail, 23 Jun 2009	Female	Body soak	JN815364	JN815569	JN815725	JN815889	JN816057
<i>S. scoloplichas</i>	M09232	Hawaii	Kilohana enclosure, 25 Nov 2009	Male	Body soak	JN815550		JN815868	JN816031	JN816234
<i>Engiscaptomyza</i>										
<i>S. amplilobus</i>	M09121	Kauai	Nualolo Trail, 24 Jan 2010	Male	Body soak	JN815468	JN815637	JN815795	JN815959	JN816161
<i>S. crassifemur</i>	M09124	Maui	Piinaau Ridge east, 2 Jun 2009	Male	Body soak	JN815471		JN815798	JN815962	JN816164
<i>S. inflatus</i>	M09123	Oahu	Paliaka, 23 Sep 2009	Male	Body soak	JN815470		JN815797	JN815961	JN816163
<i>S. reducta</i>	M09014	Hawaii	Kukuioape, 5 Aug 2009	Male	Body soak	JN815362		JN815723	JN815887	JN816055
<i>Exalloscaptomyza</i>										
<i>S. caliginosa</i>	M09245	Hawaii	Kipuka Puauulu, 27 Aug 2011	Male	Abdomen	JN815560	JN815714	JN815878	JN816041	JN816245
<i>Grimshawomyia</i>										
<i>S. nasalis</i>	M09125	Maui	Piinaau Ridge east, 2 Jun 2009	Female	Body soak	JN815472	JN815639	JN815799	JN815963	JN816165
<i>S. undulata</i>	M09015	Hawaii	Kilohana enclosure, 3 Aug 2009	Female	Body soak	JN815363	JN815568	JN815724	JN815888	JN816056
<i>Tantalia</i>										
<i>S. nigrosignata</i>	M09233	Hawaii	Puu Pili, 20 Jun 2009	Male	Body soak	JN815551	JN815705	JN815869	JN816032	JN816235
<i>Titanochaeta</i>										
<i>S. chauliodon</i>	M09234	Maui	Piinaau Ridge east, 2 Jun 2009	Male	Body soak	JN815552	JN815706	JN815870	JN816033	JN816236
<i>Unplaced</i>										
<i>S. nr. lonchoptera</i>	M09128	Kauai	Alakai Swamp Trail, 24 Jun 2009	Male	Body soak	JN815475	JN815640	JN815802	JN815966	JN816168
<i>antopocerus-modified tarsus-ciliated tarsus</i>										
<i>antopocerus</i>										
<i>D. adunca</i>	M09067	Maui	Waikamoi, Haiku Uka, 20 Aug 2009	Male	Body soak	JN815414	JN815595	JN815752	JN815916	JN816107
<i>D. diamphidopoda</i>	M09068	Maui	Waikamoi, Haiku Uka, 19 Aug 2009	Male	Body soak	JN815415	JN815596	JN815753	JN815917	JN816108
<i>bristle tarsus</i>										
<i>D. nr. expansa #1</i>	M09065	Kauai	Kokee nursery, 24 Jan 2010	Male	Body soak	JN815412	JN815593	JN815750	JN815914	JN816105
<i>D. nr. perissopoda #4</i>	M09066	Kauai	Mahanaloa enclosure, 21 Jan 2010	Male	Body soak	JN815413	JN815594	JN815751	JN815915	JN816106
<i>D. quasiexpansa</i>	M09115	Maui	Piinaau Ridge east, 2 Jun 2009	Male	Body soak	JN815462	JN815632	JN815790	JN815954	JN816155
<i>ciliated tarsus</i>										
<i>D. atroscutellata</i>	M09075	Kauai	Nualolo Trail, 23 Jan 2010	Male	Body soak	JN815422	JN815603	JN815760	JN815924	JN816115
<i>D. brunneifrons</i>	M09069	Maui	Puu Kukui bog, 5 Jun 2009	Male	Body soak	JN815416	JN815597	JN815754	JN815918	JN816109
<i>D. imparisetae</i>	M09070	Hawaii	Puu Pili, 20 Jun 2009	Male	Body soak	JN815417	JN815598	JN815755	JN815919	JN816110
<i>D. medialis</i>	M09071	Hawaii	Stainback Highway, 21 Jul 2009	Male	Body soak	JN815418	JN815599	JN815756	JN815920	JN816111
<i>D. sp. 4</i>	M09072	Hawaii	Kukuioape, 29 Dec 2009	Male	Body soak	JN815419	JN815600	JN815757	JN815921	JN816112
<i>split tarsus</i>										
<i>D. ancyla</i>	M09073	Maui	Kahanaiki Valley, 18 Aug 2009	Male	Body soak	JN815420	JN815601	JN815758	JN815922	JN816113
<i>D. variabilis</i>	M09074	Maui	Maile Trail, 1 Jun 2009	Male	Body soak	JN815421	JN815602	JN815759	JN815923	JN816114
<i>spoon tarsus</i>										
<i>D. neutralis</i>	M09076	Hawaii	Tom's Trail upper forest, 5 Oct 2009	Male	Body soak	JN815423	JN815604	JN815761	JN815925	JN816116
<i>D. sordidapex</i>	M09077	Hawaii	Laupahoehoe, FR side, 14 Jul 2009	Male	Body soak	JN815424	JN815605	JN815762	JN815926	JN816117
<i>haleakalae</i>										
<i>D. iki</i>	M09222	Hawaii	Kukuioape, 11 Sep 2010	Male	Genitalia	JN815540	JN815695	JN815858	JN816022	JN816224
<i>D. insignita</i>	M09223	Oahu	Pahole Gulch, 27 Mar 2010	Male	Genitalia	JN815541	JN815696	JN815859	JN816023	JN816225
<i>D. melanoloma</i>	M09224	Molokai	Pepeopae trail, 14 Dec 2010	Male	Genitalia	JN815542	JN815697	JN815860	JN816024	JN816226
<i>D. ochropleura</i>	M09225	Hawaii	Kukuioape, 11 Sep 2010	Male	Genitalia	JN815543	JN815698	JN815861	JN816025	JN816227
<i>modified mouthparts</i>										
<i>D. larifuga</i>	M09226	Oahu	Puu Hapapa shelf, 24 Feb 2010	Male	Genitalia	JN815544	JN815699	JN815862	JN816026	JN816228
<i>D. hirtitarsus</i>	M09227	Maui	Kahana Valley, 4 Jun 2009	Male	Genitalia	JN815545	JN815700	JN815863	JN816027	JN816229
<i>D. nigrocirrus</i>	M09228	Hawaii	Powerline Road kipukas, 23 Aug 2009	Male	Genitalia	JN815546	JN815701	JN815864		JN816230
<i>D. nanella</i>	M09229	Kauai	Mahanaloa–Kuia Val. jct., 23 Jun 2009	Male	Genitalia	JN815547	JN815702	JN815865	JN816028	JN816231
<i>D. n. sp. "large spots"</i>	M09230	Hawaii	Army Rd. makai, 8 Dec 2009	Female	Genitalia	JN815548	JN815703	JN815866	JN816029	JN816232
<i>unplaced</i>										
<i>D. adventitia</i>	M09231	Kauai	Puu O Kila Rd., 25 Jul 2010	Male	Genitalia	JN815549	JN815704	JN815867	JN816030	JN816233
<i>D. primaeva</i>	M09157	Kauai	N. fork Wailua River, 19 Jul 2010	Male	Genitalia	JN815488	JN815652	JN815814	JN815978	JN816181
<i>Picture wing-nudidrosophila</i>										
<i>adiastola</i>										
<i>D. adiastola</i>	M09083	Maui	FBS transect 3, 3 Jun 2009	Female	Body soak	JN815430	JN815610	JN815767	JN815931	JN816123
<i>D. cilifera</i>	M09217	Molokai	Mokomoko Gulch, 15 Dec 2010	Male	Genitalia	JN815535	JN815690	JN815853	JN816017	JN816219
<i>D. clavisetae</i>	M09181	Maui	Waikamoi, Haiku Uka, 20 Aug 2009	Male	Genitalia	JN815508	JN815667	JN815830	JN815994	JN816197
<i>D. ochrobasis</i>	M09218	Hawaii	Kilohana enclosure, 25 Nov 2009	Male	Genitalia	JN815536	JN815691	JN815854	JN816018	JN816220
<i>D. setosimentum</i>	M09219	Hawaii	Hionamoia Gulch, 6 Aug 2009	Male	Genitalia	JN815537	JN815692	JN815855	JN816019	JN816221
<i>D. hamifera</i>	M09182	Maui	Waikamoi, Haiku Uka, 21 Dec 2010	Male	Genitalia	JN815509	JN815668	JN815831	JN815995	JN816198
<i>D. paenihamifera</i>	M09084	Maui	Puu Kukui trail, 5 Jun 2009	Female	Abdomen	JN815431	JN815611	JN815768	JN815932	JN816124
<i>D. truncipenna</i>	M09183	Maui	Waikamoi, Haiku Uka, 21 Dec 2010	Male	Genitalia	JN815510	JN815669	JN815832	JN815996	JN816199
<i>grimshawi</i>										
<i>aglaia</i>										
<i>D. conspicua</i>	M09085	Hawaii	Kukuioape, 29 Dec 2009	Male	Body soak	JN815432	JN815612	JN815769	JN815933	JN816125
<i>D. kikiko</i>	M09095	Kauai	Nualolo Trail, 23 Jan 2010	Female	Abdomen	JN815442	JN815621	JN815779	JN815943	JN816135
<i>D. nr. alsophila</i>	M09249	Maui	Waikapu Valley, 12 Oct 2011	Female	Abdomen	JQ845048	JQ845052	JQ845056	JQ845060	JQ845064

Table 1 (continued)

Species group Species subgroup Species	Extraction	Island	Locality	Sex	Tissue	EF1 g	Fz4	Gpdh	Pgi	Yp2
<i>crucigera</i>										
<i>D. affinisdisjuncta</i>	M09049	Maui	Kahanaiki Valley, 18 Aug 2009	Male	Body soak	JN815396	JN815580	JN815737	JN815901	JN816089
<i>D. balioptera</i>	M09099	Maui	Makawao FR nr. res., 27 Nov 2004	Male	Body soak	JN815446	JN815624	JN815782	JN815946	JN816139
<i>D. bostrycha</i>	M09163	Molokai	West Kawela Gulch, 14 Dec 2010	Male	Genitalia	JN815490	JN815654	JN815817	JN815981	JN816184
<i>D. craddockae</i>	M09050	Kauai	Alakai Swamp Trail, 24 Jun 2009	Male	Body soak	JN815397	JN815581	JN815738	JN815902	JN816090
<i>D. crucigera</i>	M09052	Oahu	Paliikea, 23 Sep 2009	Male	Body soak	JN815399	JN815583	JN815740	JN815904	JN816092
<i>D. disjuncta</i>	M09053	Maui	Waikamoi, Haiku Uka, 20 Aug 2009	Female	Body soak	JN815400	JN815584	JN815741	JN815905	JN816093
<i>D. grimshawi</i>	M09166	Maui	Waikamoi fence, 22 Dec 2010	Female	Genitalia	JN815493	JN815657	JN815820	JN815984	JN816187
<i>D. grimshawi</i>	M09165	Molokai	Huewai Gulch, 16 Dec 2010	Male	Genitalia	JN815492	JN815656	JN815819	JN815983	JN816186
<i>D. pullipes</i>	M09086	Hawaii	Stainback 3600' trail, 12 Jan 2010	Female	Abdomen	JN815433	JN815613	JN815770	JN815934	JN816126
<i>discreta</i>										
<i>D. discreta</i>	M09167	Maui	Waikamoi, Haiku Uka, 21 Dec 2010	Male	Genitalia	JN815494	JN815658	JN815821	JN815985	JN816188
<i>D. fasciculisetae</i>	M09008	Maui	Maile Trail, 1 Jun 2009	Male	Body soak	JN815356	JN815567	JN815721	JN815885	JN816049
<i>D. fasciculisetae</i>	M09169	Molokai	Puu Kolekole trail, 16 Dec 2010	Female	Genitalia	JN815496	JN815660	JN815823	JN815987	JN816190
<i>D. glabriapex</i>	M09033	Kauai	Pihea Trail, 22 Jun 2009	Female	Body soak	JN815381	JN815570	JN815726	JN815890	JN816073
<i>D. lineosetae</i>	M09235	Maui	Hanaula, Pohakea Gulch, 28 May 2011	Female	Genitalia	JN815553	JN815707	JN815871	JN816034	JN816237
<i>D. pilimana</i>	M09210	Oahu	Manuwai Gulch, 1 Mar 2011	Male	Genitalia	JN815529	JN815686	JN815849	JN816013	JN816215
<i>distinguenda</i>										
<i>D. distinguenda</i>	M09092	Oahu	Kaluaa Gulch, 26 Jan 2010	Female	Body soak	JN815439	JN815618	JN815776	JN815940	JN816132
<i>D. divaricata</i>	M09215	Oahu	Ekahanui Gulch, 2 Mar 2011	Male	Genitalia	JN815534	JN815689	JN815852	JN816016	JN816218
<i>D. inedita</i>	M09034	Oahu	Pia Valley, 26 Sep 2009	Male	Body soak	JN815382		JN815727	JN815891	JN816074
<i>hawaiiensis</i>										
<i>D. flexipes</i>	M09156	Oahu	Makaha Valley, 10 Aug 2010	Male	Genitalia	JN815487	JN815651	JN815813	JN815977	JN816180
<i>D. formella</i>	M09035	Hawaii	Kukuioepae, 5 Aug 2009	Female	Body soak	JN815383	JN815571	JN815728	JN815892	JN816075
<i>D. gradata</i>	M09036	Oahu	Paliikea, 23 Sep 2009	Male	Body soak	JN815384	JN815572	JN815729	JN815893	JN816076
<i>D. hawaiiensis</i>	M09037	Hawaii	Laupahoehoe FR, 24 Jul 2009	Female	Body soak	JN815385	JN815573	JN815730	JN815894	JN816077
<i>D. musaphilia</i>	M09088	Kauai	Nualolo Trail, 23 Jan 2010	Female	Body soak	JN815435	JN815614	JN815772	JN815936	JN816128
<i>D. recticilia</i>	M09038	Maui	Maile Trail, 1 Jun 2009	Male	Body soak	JN815386	JN815574	JN815731	JN815895	JN816078
<i>D. silvarentis</i>	M09089	Hawaii	Kukuioepae, 29 Dec 2009	Female	Body soak	JN815436	JN815615	JN815773	JN815937	JN816129
<i>D. turbata</i>	M09039	Oahu	above Nuuanu Pali lookout, 2 Aug 2009	Female	Body soak	JN815387	JN815575	JN815732	JN815896	JN816079
<i>lanaiensis</i>										
<i>D. digressa</i>	M09045	Hawaii	Manuka, olopuia kipuka, 7 Aug 2009	Male	Body soak	JN815392	JN815579	JN815736	JN815900	JN816085
<i>D. hexachaetae</i>	M09042	Oahu	Pia Valley, 26 Sep 2009	Male	Body soak	JN815389	JN815576	JN815733	JN815897	JN816082
<i>D. lanaiensis</i>	M09146	Lanai	Waiapaa Gulch, 29 Mar 2010	Male	Genitalia	JN815478	JN815643	JN815805	JN815969	JN816171
<i>D. moli</i>	M09044	Oahu	above Nuuanu Pali lookout, 2 Aug 2009	Female	Body soak	JN815391	JN815578	JN815735	JN815899	JN816084
<i>micromyia</i>										
<i>D. micromyia</i>	M09043	Kauai	Paaiki Valley, 23 Jun 2009	Male	Body soak	JN815390	JN815577	JN815734	JN815898	JN816083
<i>odontophallus</i>										
<i>D. odontophallus</i>	M09172	Maui	Makawao FR, 20 Dec 2010	Male	Genitalia	JN815499	JN815662	JN815825	JN815989	JN816192
<i>D. odontophallus</i>	M09170	Molokai	Makakupaia Gulch, 15 Dec 2010	Male	Genitalia	JN815497	JN815661	JN815824	JN815988	JN816191
<i>orphnopeza</i>										
<i>D. ciliaticrus</i>	M09056	Hawaii	Tom's Trail upper forest, 21 Jul 2009	Male	Body soak	JN815403	JN815586	JN815743	JN815907	JN816096
<i>D. claytonae</i>	M09159	Hawaii	Olaa Small Tract, 28 Jan 1998	Male	Body soak			JN815815	JN815979	JN816182
<i>D. engyochracea</i>	M09096	Hawaii	Kipuka Ki	Female	Body soak	JN815443	JN815622	JN815780	JN815944	JN816136
<i>D. limitata</i>	M09207	Molokai	Makakupaia Gulch, 15 Dec 2010	Female	Genitalia	JN815526	JN815684	JN815847	JN816011	JN816213
<i>D. murphyi</i>	M09059	Hawaii	Kilohana enclosure, 3 Aug 2009	Female	Body soak	JN815406	JN815588	JN815745	JN815909	JN816099
<i>D. obatai</i>	M09213	Oahu	Manuwai Gulch, 1 Mar 2011	Female	Genitalia	JN815532	JN815687	JN815850	JN816014	JN816216
<i>D. ochracea</i>	M09061	Hawaii	Hionamoa Gulch, 6 Aug 2009	Female	Body soak	JN815408	JN815590	JN815747	JN815911	JN816101
<i>D. orphnopeza</i>	M09079	Maui	Waikamoi, Haiku Uka, 19 Aug 2009	Male	Abdomen	JN815426	JN815606	JN815763	JN815927	JN816119
<i>D. orphnopeza</i>	M09177	Molokai	Hanalilolilo trail, 14 Dec 2010	Female	Genitalia	JN815504	JN815665	JN815828	JN815992	JN816195
<i>D. orthofascia</i>	M09237	Maui	Waikapu Valley, 1 May 2011	Female	Genitalia	JN815555	JN815709	JN815873	JN816036	JN816239
<i>D. sejuncta</i>	M09002	Kauai	Kuia Valley, 23 Jun 2009	Male	Body soak	JN815350	JN815562	JN815716	JN815880	JN816043
<i>D. sobrina</i>	M09214	Oahu	Manuwai Gulch, 1 Mar 2011	Female	Genitalia	JN815533	JN815688	JN815851	JN816015	JN816217
<i>D. sodomae</i>	M09175	Molokai	Makakupaia Gulch, 15 Dec 2010	Male	Genitalia	JN815502	JN815664	JN815827	JN815991	JN816194
<i>D. sproati</i>	M09062	Hawaii	Puu Pili, 20 Jun 2009	Female	Body soak	JN815409	JN815591	JN815748	JN815912	JN816102
<i>D. villosipedis</i>	M09003	Kauai	Kuia Valley, 23 Jun 2009	Male	Body soak	JN815351	JN815563	JN815717	JN815881	JN816044
<i>punalua</i>										
<i>D. basisetae</i>	M09004	Hawaii	Stainback Highway, 21 Jul 2009	Male	Body soak	JN815352	JN815564	JN815718	JN815882	JN816045
<i>D. paucipuncta</i>	M09090	Hawaii	Olaa Small Tract, 1 Jan 2010	Male	Body soak	JN815437	JN815616	JN815774	JN815938	JN816130
<i>D. prolaticilia</i>	M09091	Hawaii	Army R D. makai, 8 Dec 2009	Female	Body soak	JN815438	JN815617	JN815775	JN815939	JN816131
<i>D. prostopalpis</i>	M09063	Maui	Puu Kukui trail, 5 Jun 2009	Male	Body soak	JN815410	JN815592	JN815749	JN815913	JN816103
<i>D. punalua</i>	M09006	Oahu	above Nuuanu Pali lookout, 2 Aug 2009	Female	Body soak	JN815354	JN815566	JN815720	JN815884	JN816047
<i>vesciseta</i>										
<i>D. ambochila</i>	M09093	Oahu	Kaluaa Gulch, 26 Jan 2010	Male	Body soak	JN815440	JN815619	JN815777	JN815941	JN816133
<i>D. montgomeryi</i>	M09101	Oahu	Kaluaa Gulch, 26 Jan 2010	Male	Body soak	JN815448	JN815626	JN815784	JN815948	JN816141
<i>D. pihulu</i>	M09239	Maui	Waikapu Valley, 1 May 2011	Male	Genitalia	JN815557	JN815711	JN815875	JN816038	JN816241
<i>D. vesciseta</i>	M09102	Maui	Makawao FR nr. res., 11 Mar 2010	Female	Body soak	JN815449	JN815627	JN815785	JN815949	JN816142
<i>nudidrosophila</i>										
<i>ateledrosophila</i>										
<i>D. papala</i>	M09221	Hawaii	Kukuioepae, 11 Sep 2010	Male	Genitalia	JN815539	JN815694	JN815857	JN816021	JN816223
<i>hirtitibia</i>										
<i>D. hirtitibia</i>	M09191	Oahu	Makua Valley, 24 Mar 2010	Male	Genitalia	JN815518	JN815677	JN815840	JN816004	JN816207
<i>D. papaalai</i>	M09192	Kauai	Mahanaloa enclosure, 21 Jan 2010	Female	Genitalia	JN815519	JN815678	JN815841	JN816005	

(continued on next page)

Table 1 (continued)

Species group Species subgroup Species	Extraction	Island	Locality	Sex	Tissue	EF1 g	Fz4	Gpdh	Pgi	Yp2
<i>nudidrosophila</i>										
<i>D. eximia</i>	M09193	Maui	Kahanaiki Valley, 18 Aug 2009	Male	Genitalia	JN815520	JN815679	JN815842	JN816006	JN816208
<i>D. panoanoa</i>	M09194	Hawaii	Makaula-Ooma forest, 29 Aug 2010	Male	Genitalia	JN815521	JN815680	JN815843	JN816007	JN816209
<i>okala</i>										
<i>D. kuhao</i>	M09195	Oahu	Kahanaiki, 19 Feb 2010	Male	Genitalia	JN815522	JN815681	JN815844	JN816008	JN816210
<i>velata</i>										
<i>D. lauoho</i>	M09197	Molokai	Huewai Gulch, 16 Dec 2010	Male	Genitalia	JN815524	JN815682	JN815845	JN816009	JN816211
<i>D. pohaka</i>	M09198	Maui	Kahanaiki Valley, 18 Aug 2009	Male	Genitalia	JN815525	JN815683	JN815846	JN816010	JN816212
<i>picticornis</i>										
<i>D. picticornis</i>	M09153	Kauai	Nualolo Trail, 23 Jun 2009	female	genitalia	JN815484	JN815649	JN815811	JN815975	JN816177
<i>D. pilipa</i>	M09105	Kauai	Mahanaloa Valley, 23 Jun 2009	male	abdomen	JN815452	JN815630	JN815788	JN815952	JN816145
<i>D. setosifrons</i>	M09243	Hawaii	Cymbidium Acres, 7 Dec 1995	female	genitalia			AY006462		JN816243
<i>planitibia</i>										
<i>anomalipes</i>										
<i>D. anomalipes</i>	M09081	Kauai	Kokee nursery, 24 Jan 2010	Male	Body soak	JN815428	JN815608	JN815765	JN815929	JN816121
<i>D. quasianomalipes</i>	M09082	Kauai	Alakai Swamp Trail, 24 Jun 2009	Male	Body soak	JN815429	JN815609	JN815766	JN815930	JN816122
<i>cyrtoloma</i>										
<i>D. cyrtoloma</i>	M09148	Maui	Waikamoi, Haiku Uka, 20 Aug 2009	Male	Genitalia	JN815479	JN815644	JN815806	JN815970	JN816172
<i>D. hanaulae</i>	M09149	Maui	Puu Kukui trail, 5 Jun 2009	Male	Genitalia	JN815480	JN815645	JN815807	JN815971	JN816173
<i>D. ingens</i>	M09150	Maui	Puu Kukui trail, 5 Jun 2009	Male	Genitalia	JN815481	JN815646	JN815808	JN815972	JN816174
<i>D. melanocephala</i>	M09185	Maui	Waikamoi, Haiku Uka, 21 Dec 2010	Male	Genitalia	JN815512	JN815671	JN815834	JN815998	JN816201
<i>D. neoperkinsi</i>	M09186	Molokai	Pepeopae trail, 14 Dec 2010	Male	Genitalia	JN815513	JN815672	JN815835	JN815999	JN816202
<i>D. oahuensis</i>	M09246	Oahu	Waianae-Kaala Trail, 12 Oct 2011	Male	Genitalia	JQ845045	JQ845049	JQ845053	JQ845057	JQ845061
<i>neopicta</i>										
<i>D. neopicta</i>	M09187	Molokai	Pepeopae trail, 14 Dec 2010	Male	Genitalia	JN815514	JN815673	JN815836	JN816000	JN816203
<i>D. nigribasis</i>	M09247	Oahu	Waianae-Kaala Trail, 12 Oct 2011	Female	Genitalia	JQ845046	JQ845050	JQ845054	JQ845058	JQ845062
<i>D. substenoptera</i>	M09248	Oahu	Waianae-Kaala Trail, 12 Oct 2011	Female	Genitalia	JQ845047	JQ845051	JQ845055	JQ845059	JQ845063
<i>planitibia</i>										
<i>D. heteroneura</i>	M09190	Hawaii	UH-Manoa lab stock	Male	Genitalia	JN815517	JN815676	JN815839	JN816003	JN816206
<i>D. planitibia</i>	M09188	Maui	Waikamoi, Haiku Uka, 21 Dec 2010	Female	Genitalia	JN815515	JN815674	JN815837	JN816001	JN816204
<i>D. silvestris</i>	M09129	Hawaii	Kukuioepae, 29 Dec 2009	Male	Body soak	JN815476	JN815641	JN815803	JN815967	JN816169

reproductive organs and digestive tract) or by soaking the entire specimen in the lysis buffer for >8 h, depending on the size and rarity of the specimen (Table 1). PCR was performed at the University of Hawai'i, Hilo, on a BioRad C1000 thermocycler using the primer sequences and amplification conditions shown in Table 2. All fragments amplified cleanly without extraneous products; most of the primers work for most species at a wide range of temperatures. Amplification products were cleaned up using exonuclease I and shrimp alkaline phosphatase (USB Inc.). DNA was sequenced at the Genomics Core Facility of the John A. Burns School of Medicine, University of Hawai'i-Mānoa.

Chromatograms were edited in Sequencher 4.10.1 (GeneCodes Corp.). Alignment for each gene was performed with ClustalW2 (Larkin et al., 2007) and adjusted by eye to fix obvious errors from the algorithm. Fz4 contains a variable-length region of CAN repeats near the 3' end in the coding sequence, which cannot be aligned; this section was removed from the analysis. With this deleted, the data matrix consisted of 4260 bp of aligned sequence from the five genes. The full sequences (including excluded specimens) are deposited in GenBank, accession Nos. JN815349–JN816245 and JQ845045–JQ845064 (Table 1). There is very little missing data, with the exception of a few *Scaptomyza* outgroups that failed to amplify Fz4. The extraction for *D. setosifrons* was from an old pinned specimen and only Yp2 could be obtained from it; the Gpdh sequence comes from GenBank accession No. AY006462.

2.3. Phylogenetic analysis

The data matrix was analyzed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and RAxML 7.2.6 (Stamatakis, 2006), run on the Biportal at the University of Oslo (Kumar et al., 2009). Preliminary analyses were conducted comparing combined, fully partitioned by gene

and codon position, and intermediate partitioning schemes. These indicated that the five genes had significantly different rates of change in third codon positions, but that there were too few mutations in each gene's first and second positions to partition them separately by gene, resulting in failure of the parameters to converge. Therefore, eight partitions were defined in the data for final analysis: five for third codon positions of each gene, one for combined first codon positions, one for combined second positions, and one for the combined introns of Pgi, Fz4, and Gpdh. Models for MrBayes were chosen using MrModeltest 2.2 (Nylander, 2004). GTR + G + I was selected for the first and second codon position and intron partitions, and GTR + G for all of the third position partitions. Five independent MrBayes analyses were performed, each for 4.5 million generations, with default settings except the following: the substitution rate was allowed to vary among partitions (ratepr = variable); model parameters were unlinked across partitions; and the branch lengths prior set to unconstrained:exponential(100). All of these are necessary for correctly estimating tree length (Marshall et al., 2006). Parameter files were examined in Tracer 1.5 (Rambaut and Drummond, 2009, available at <http://beast.bio.ed.ac.uk>) for convergence, stationarity, and adequate effective sample sizes. Convergence was rapid; the first 500,000 generations of each were discarded as burnin, and the tree and parameter files concatenated. For RAxML, GTRGAMMA was used for all partitions. The program was run in rapid bootstrapping mode (-f a) with 100 bootstrap replicates.

Dating was performed using BEAST 1.7.4 using an uncorrelated lognormal relaxed clock model (Drummond et al., 2006; Drummond and Rambaut, 2007), unlinked GTR + G models for all partitions, and a Yule prior for branching, also run on Biportal. Four nodes across the ingroup taxa that exhibit progression rule divergence were used as calibration points – the *planitibia* group and the *lanaiensis* and *hawaiiensis* subgroups; the split between

D. sobrina and *D. orthofascia* + *D. ciliaticrus*; and the split between *D. silvestris* and *D. heteroneura*. Each was assigned a truncated normal distribution, with an upper cutoff at the age of the youngest island available for the basal split of each respective clade, but allowing for wide flexibility in time of dispersal and with no lower bound (Table 3). Maximum island ages are from measured dates in Clague (1996), with an upper bound set at the earliest measured date and mean at the end of shield-building for the first large volcano (the calculated date in Clague, 1996). This allows for dispersal early in the history of an island (e.g., at approximately the age of Mauna Loa today or slightly earlier), but with the highest probability after substantial forests have developed. The number of reliable calibration points is limited due to incomplete taxon sampling, absence of fossil specimens, and the existence of genetically divergent, apparently relictual lineages on young islands such as *D. conspiciua* and *D. engyochracea*. Therefore, we regard these as the best available in the tree, where there is a clear progression from older to younger islands among closely-related species. Furthermore, they should be regarded as maximum dates, since progressive colonization down the islands is possible after younger islands have arisen, but cannot occur before it has breached the ocean. Three independent runs of 20 million generations each were performed, recording trees every 1000 generations, with the log files analyzed in Tracer as above. Convergence was again rapid, and 2 million generations of each were discarded as burnin. The runs were concatenated and resampled at every 10,000 generations using LogCombiner for a total of 5400 trees, and the results summarized with TreeAnnotator.

2.4. Biogeography

To reconstruct the distribution of hypothetical ancestors, we used the dispersal-extinction-cladogenesis model (DEC; Ree et al., 2005; Ree and Smith, 2008) implemented in Lagrange (version 20110117, current code available at <https://code.google.com/p/lagrange/>). This allows for time constraints on location, an important factor in a data set such as this without clear progressions down the island chain and a high degree of back-migration (Ree and Smith, 2008). In addition, all taxa from Kauai and Oahu are missing from the basal *adiastola* group, increasing the probability of an incorrect basal node using an event-based method such as DIVA (Ronquist, 1997) which does not allow for time constraints.

The ultrametric tree obtained in BEAST was used as input for the Lagrange analysis. Since Molokai and Lanai were recently joined with Maui by a land bridge (Price and Elliott-Fisk, 2004) and share many species (Lanai has no island endemic picture wing *Drosophila*), they were combined with Maui as a single region. Thus, four areas were defined: Kauai (including older islands), Oahu, Maui Nui, and Hawaii. The maximum range size was set to 2, since most species are island endemics with rare exceptions occurring on two islands. Dispersal constraints were set based on the island ages of Clague (1996) as above. Nodes were marked if one reconstruction had greater than 50% likelihood, and all possible reconstructions within 2 likelihood units are shown.

2.5. Host plant usage

Evolution of host usage was evaluated using the function “trace character across trees” in Mesquite (Maddison and Maddison, 2011), using likelihood ancestral state reconstruction with the Mk1 model (Lewis, 2001). All 5400 trees from BEAST were used as input, and the results mapped onto the final BEAST tree. Each ingroup species was coded for the primary host (Magnacca et al., 2008), with the following states: Araliaceae (*Cheirodendron* and *Tetraplasandra*), Campanulaceae (primarily *Clermontia* and *Cyanea*), *Charpentiera/Pisonia*, *Urera* (Urticaceae), monocots

(*Chrysodracon* [=Pleomele] and *Freycinetia*), sap flux, minor hosts (*Wikstroemia*, *Sapindus*, *Hibiscus*), generalist, and unknown. *Charpentiera* (Amaranthaceae) and *Pisonia* (Nyctaginaceae) are grouped because, although not closely related, they are structurally very similar (both trees derived from herbaceous ancestors, with wood consisting of layers of anomalous secondary growth; Wagner et al., 1999), frequently occur in the same habitats, and are both used as hosts by several otherwise-specialist species. Three species, *D. silvestris*, *D. ciliaticrus*, and *D. limitata*, use alternate hosts relatively frequently (Magnacca et al., 2008). Because the character reconstruction does not allow for polymorphism in coding, they are coded the same as their group of closest relatives (which in all cases appear to be the most important host), and are considered to be examples of recent host adaptations.

3. Results

3.1. Phylogenetics

All analyses were nearly identical in the ingroup, differing primarily in arrangement of the *Drosophila* outgroup clades; none of these arrangements were well-supported (Fig. 1). These disagreements are caused by differing attachment of the *Scaptomyza* branch to the Hawaiian *Drosophila* lineage, a factor of the long distance between extant members of the two groups (see Discussion). Within the Hawaiian *Drosophila*, the major species groups and subgroups are all strongly supported as monophyletic, most with 100% Bayesian posterior probability (PP) and maximum likelihood bootstrap (BS) support. Relationships between them are fully resolved in all analyses but receive less support; some had > 0.9 PP, but all received < 50% BS (see supplementary figures for full trees with support values for all analyses). The only differences among analyses were that the *planitibia* group was moderately strongly supported as sister to the *nudidrosophila* + *picticornis* clade in the MrBayes and ML analysis, while BEAST put the *planitibia* group sister to the *grimshawi* group with very weak support; and the ML tree differed from the others in rooting the *grimshawi* group slightly differently (<50% BS support), and in placing *D. punalua* sister to the *paucipuncta* complex instead of *paucipuncta* + *distinguenda* (Fig. 1, Supplementary Data). In the last case, while the entire clade is strongly monophyletic in all, support for the position of *D. punalua* is always weak.

Several findings are notable. The close relationship between the picture wing and *nudidrosophila* species is confirmed. Moreover, the *ateledrosophila* complex (represented here by *D. papala*) is derived from well within the *nudidrosophila* group, and should be regarded as a subgroup of it. This clade is in turn most closely related to the *picticornis* group, three morphologically and ecologically divergent species that have long been considered members of the *planitibia* group (Bonacum et al., 2005; Carson, 1992). Most strikingly, the actual basal *planitibia* members are *D. anomalipes* and *D. quasianomalipes*, a pair of sympatric sibling species from Kauai that were formerly considered basal within the Hawaiian *Drosophila* due to their widely divergent morphology, courtship, and behavior (Spieth, 1975) and were only recently recognized as members of the picture wing clade (O’Grady et al., 2011; O’Grady et al., 2010). In contrast, *D. primaeva*, which has also been considered a basal member of the picture wing lineage (Carson, 1992; Kaneshiro et al., 1995; O’Grady et al., 2010), is not closely related – its position differs in the MrBayes and BEAST trees, but even in the latter where it is sister to the picture wing clade, *D. primaeva* diverges as part of the rapid basal radiation of Hawaiian *Drosophila*, and is isolated on a long branch. *Drosophila adventitia*, another Kauai species, is another relictual species with no close living relatives; it has previously been included in the *modified mouthparts* group due to its bizarre labellar appendage, but was

Table 2

Primers used in this study. PCR program as follows: initial denaturation for 3 min at 94°; 35 cycles of denaturation for 60 s at 94°, annealing for 60 s at 55° (Fz4), 62° (EF1 g, Pgi, Yp2), or 65° (Gpdh), and extension for 90 s (Fz4) or 60 s (all others); final extension for 4 min. Lengths include gaps in both coding and non-coding regions. Fz4 length does not include deleted CAN repeats (see Section 2.2).

Locus	Aligned bp	Primer	Direction	Sequence (5'-3')	Reference
EF1g	856	EF1g26F	Forward	GCTTWTGAGACCGCTGATGG	This study
		EF1g862R	Reverse	ATCTTRTCGAGACGCTGGAA	This study
Fz4	943	Fz4L	Forward	CGCTTTTCTATTGCGTACTAT	Lapoint et al. (2011)
		Fz4R	Reverse	GCTTGTACGGACTGCTGATTATT	Lapoint et al. (2011)
Gpdh	1071	GNLmod	Forward	CCCGACCTGGTTGAGGCTGCCAAGAATGC	Barrio and Ayala (1997)
		GNRmod	Reverse	ACATATGCTCAGGGTGATTGCGTATGCA	Barrio and Ayala (1997)
Pgi	633	PgiF1	Forward	GCCATGTTCTSYGGMCAGCAYAT	This study
		PgiR1	Reverse	TAACGACCTCCNACCCARTCCCA	This study
Yp2	757	YP2F	Forward	CAGCAGCGTTACAATCTCCAGCC	This study
		YP2R	Reverse	CCGAAGGGGCTCTTGAGITCAC	This study

already suspected of being outside that group (Magnacca and O'Grady, 2009).

Within the large *grimshawi* group, most of the species subgroups are as traditionally defined (Kaneshiro et al., 1995; O'Grady et al., 2010). However, the *conspicua* subgroup as previously defined is clearly polyphyletic and includes elements of several others, and the old definition of the *vesciseta* subgroup contained several species that converged on the small, all-yellow body form (e.g. *D. alsophila* and *D. hexachaetae*) but are not closely related to the main cluster, represented here by four of the seven known species. One of the former, *D. micromyia*, is sister to the entire *grimshawi* group. A new phylogenetically-based species group and subgroup classification is illustrated in Fig. 1, and presented for all species in Appendix A.

3.2. Dating and biogeography

Based on the combination of dating and ancestral reconstruction analysis, the origin and earliest split within the *picture wing* clade occurred prior to Kauai, with the separation of the basal *adiastola* group, followed by separation of the other major species groups on Kauai (Fig. 2). The main round of diversification then took place on Oahu, where the *grimshawi* subgroups evolved in rapid succession. Dating places all dates for the subgroup nodes – including nearly all 95% CIs – within the period when Oahu was mature but still the youngest island (median 2.32–3.10 Mya, 95% CI 1.82–3.82; Fig. 2). As a result, the majority of Kauai *picture wing* species (all except *D. anomalipes*, *D. quasianomalipes*, *D. picticornis*, *D. pilipa*, and the missing *D. ornata*) arrived there by back-colonization from Oahu or younger islands. The results from the biogeographic analysis are reflected in the topology – not only are there relatively few Kauai species in the *grimshawi* group, those that do occur are not basal within their subgroup (e.g. *D. craddockae*, *D. musaphilia*, *D. sejuncta*, *D. villosipedis*).

Most clades lack any indication of progression-rule dispersal and speciation, particularly in the *grimshawi* group. The *planitibia* group does show a complete example of this, as noted previously (Bonacum et al., 2005), with the *anomalipes* subgroup on Kauai, followed by a split into three lineages on Oahu and subsequent dispersal to Maui Nui and Hawaii (the Oahu relative of *D. planitibia*, *D. hemipeza*, is missing from our analysis). A few examples of progression starting on Oahu are evident, as with *D. moli*–*D. lanaiensis*–*D. digressa* and *D. sobrina*–*D. orthofascia*–*D. ciliaticrus* (Fig. 2). Overall, however, there is not a consistent biogeographic pattern within the *grimshawi* group, another indication of a more recent radiation. Since several lineages have not reached Hawaii or Kauai (e.g., the *odontophallus* and *cyrtoloma* subgroups), the general pattern appears stochastic (Funk and Wagner, 1995). However, this is influenced by missing species that might provide additional biogeographic resolution if they could be included.

Table 3

Calibration points for dating in BEAST. Dates are based on the age of the basal split of the clade indicated (i.e. not including the stem). Points are numbered as in Fig. 2.

Point	Clade	Upper bound	Mean	SD
1	<i>planitibia</i>	3.7	3.0	0.5
2	<i>lanaiensis</i>	3.7	3.0	0.5
3	<i>sobrina</i>	2.0	1.7	0.3
4	<i>heteroneura</i>	0.8	0.5	0.2

3.3. Host plant usage

Host plant use is generally conserved within lineages (Fig. 3). The *adiastola* and *planitibia* species groups each use two host types, with a single shift (Campanulaceae and Araliaceae, changing to Urticaceae and Campanulaceae, respectively; the Urticaceae-breeding *adiastola* species are rare and could not be included but are morphologically very similar to the derived *D. adiantola*). The *grimshawi* subgroups appear to have arisen largely as a result of host plant adaptation – there is a high degree of variation between the subgroups, but only a few major host shifts have definitely occurred since these lineages spread from Oahu. The *hawaiiensis*, *lanaiensis*, and *odontophallus* subgroups each breed in only a single host type, while the *vesciseta* and *orphanopeza* subgroups each utilize primarily two, with host changes occurring at or near the base of the group. The split in the *orphanopeza* subgroup between species that breed in Araliaceae and those that utilize monocots (*Chrysodracon* and *Freyinetia*) is ambiguous – the median date is 1.64 Mya, but the 95% CI extends back to 2.0 Mya, close to the origin of Maui Nui. The biogeography is unresolved at the node, and some other branches are reconstructed as Oahu at that time.

The *crucigera* subgroup contains the one definite recent host switch, the specialization of *D. craddockae* and *D. pullipes* on *Wikstroemia* (Fig. 3). This plant has unusual bark with fine, dense, silky fibers and which is not used by any other *picture wing* species. The remaining species of the subgroup are coded as generalists or primarily monocot-breeders according to the best available knowledge, which would indicate two origins of generalist breeding in the subgroup. However, there is only one rearing records each for *D. balioptera* and *D. bostrycha*, and none for *D. affinisdisjuncta*, so they may be generalists as well. The known generalist species, *D. crucigera* and *D. grimshawi*, appear to prefer the fibrous monocots along with fibrous dicot species (e.g. *Pisonia*, *Charpentiera*, and *Urera*), suggesting that the clade may have evolved with primarily a preference for physical characteristics of the breeding substrate rather than microbial, chemical, or other traits.

Notably, the *aglaia* and *discreta* subgroups, which consist almost entirely of species where the host associations are unknown, appear as sister taxa. While they do not share any obvious morphological affinity, it is one of the better-supported (if still tenuous)

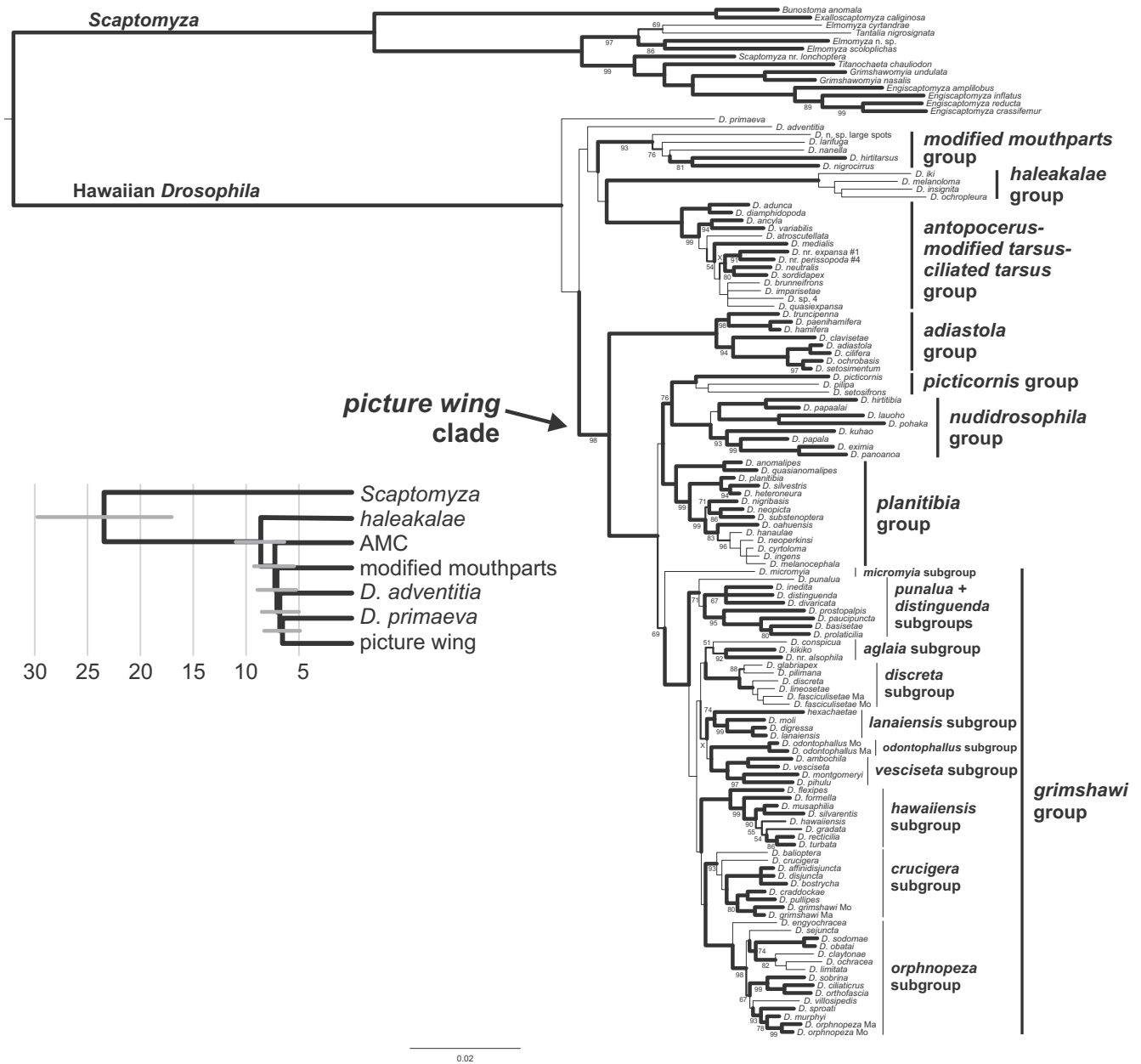


Fig. 1. Tree from MrBayes analysis, with species groups and subgroups indicated. Branch lengths shown are from MrBayes; the topology is identical to the RAxML analysis except for the position of *D. punalua* and the arrangement of the *grimshawi* subgroups (see Section 3.1). Thick branches have Bayesian posterior probability (PP) of 1; medium-width branches $0.95 \leq PP < 1$ for both Bayesian analyses, and are labelled with the bootstrap value only if less than 100%; thin branches denote $PP < 0.95$ for one or both Bayesian analyses and $< 65\%$ bootstrap support. Nodes marked with an X have high PP support in Bayesian analyses but low ML bootstrap. Inset shows the different arrangement of outgroup clades from BEAST, with 95% CI bars for divergence dates. The picture wing clade includes the *grimshawi*, *planitibia*, *nudidrosophila*, *adiaetola* and *picticornis* groups.

higher clades. *Drosophila* nr. *alsophila* may breed in *Ureca* based on its collection on the plant and similarity to *D. kinole* (Magnacca and Price, 2012), but all members of the *aglaia* subgroup are now extremely rare and conclusive determination of their hosts will be difficult. In contrast, the species of the *discreta* subgroup are among the most common on Maui Nui, yet have still never been reared.

Three clades do exhibit significant variance in host usage. The *punalua* subgroup is the most diverse in host usage, and appears to have undergone several host shifts (Fig. 3). However, the pattern of these changes is unclear because the host for many species is unknown, and three (all from Kauai and Oahu) could not be included. The three members of the *picticornis* group each have very different hosts, but the age of their divergence and their

morphological dissimilarity indicates that each represents a separate lineage which may have been larger in the past. Finally, the *nudidrosophila* group appears to be highly unstable in breeding hosts, but sampling is much thinner than in the rest of the tree and the status of many members is unclear – there are relatively few rearing records for many species, and at least some will freely utilize several types of mesic forest trees (Magnacca and O’Grady, 2008).

4. Discussion

The phylogenetic tree presented here resolves a number of the outstanding issues and anomalies in Hawaiian *Drosophila*. Our biogeographic analysis indicates that most of the evolution of the

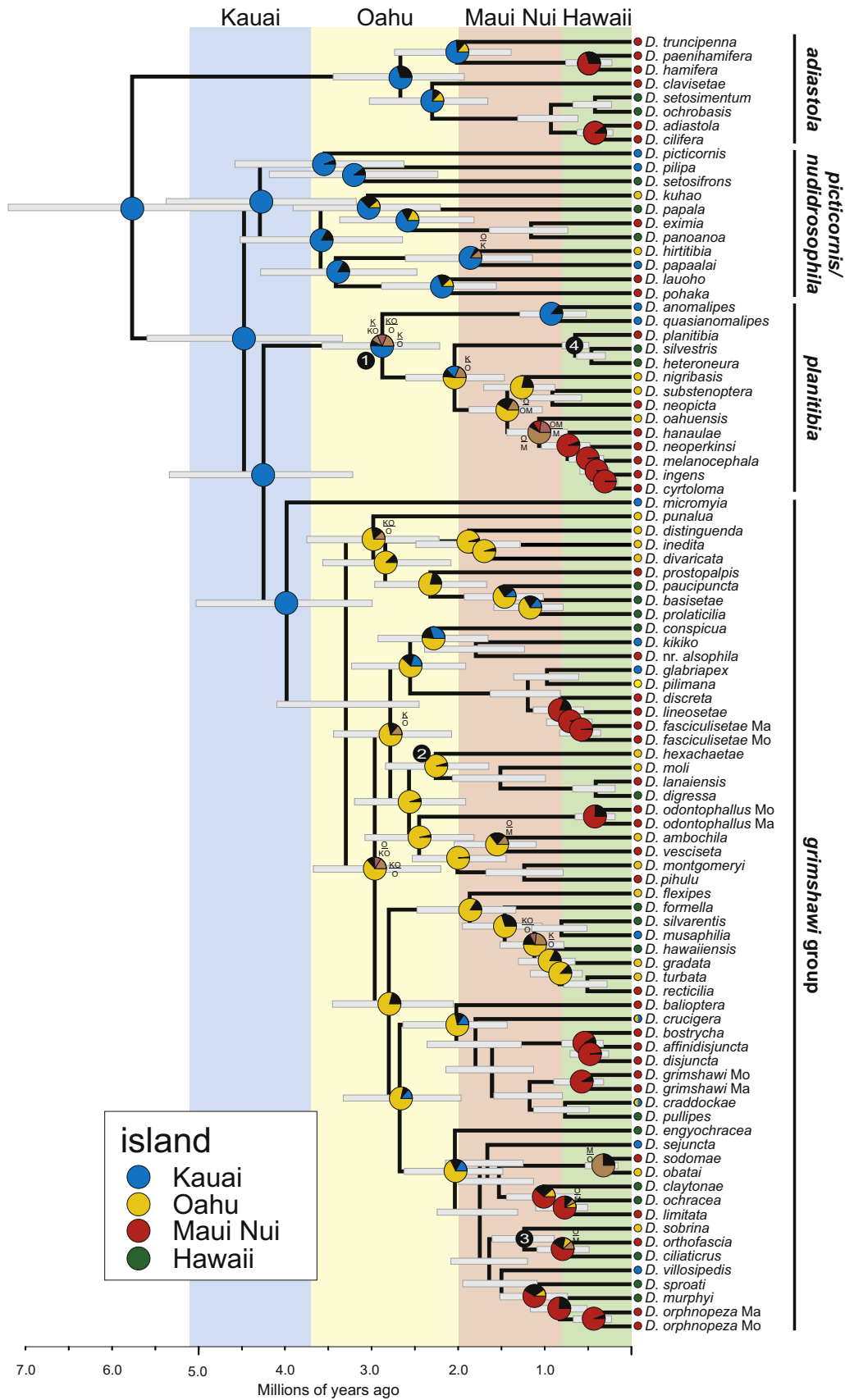


Fig. 2. BEAST chronogram, with nodes set at median divergence dates; bars are 95% confidence intervals. Numbered black dots indicate nodes used for calibration (see Table 3). Shaded background denotes the time periods when the labelled island was the youngest available for colonization. Results of biogeography reconstruction analysis indicated by pies on nodes. Pies show percent probability for island distributions within two log-likelihood units of the maximum; black denotes lower-probability alternatives. Data not shown for nodes where no alternative reached over 50% likelihood.

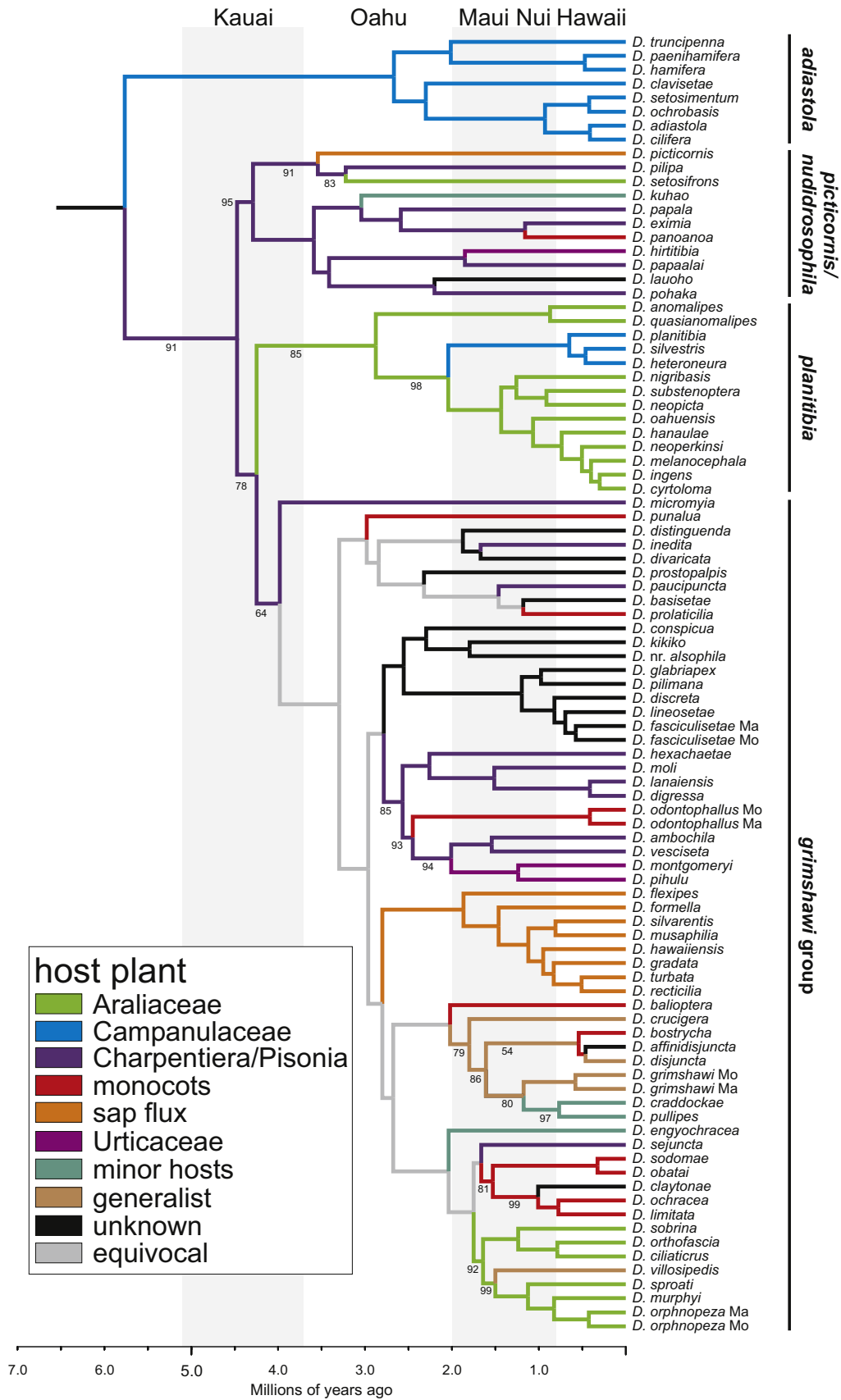


Fig. 3. Host plant reconstruction analyses mapped onto the BEAST chronogram, indicated by branch colors. Numbers below branches are percentage of trees where the ancestor shown was recovered; unlabelled colored branches are 100%, and branches with equivocal reconstruction (no alternative >50%) are gray. The “minor hosts,” each used by only one or two species, are *Hibiscus arnotianus* (*D. kuhao*), *Sapindus saponaria* (*D. engyochracea*), and *Wikstroemia* spp. (*D. craddockae* and *D. pullipes*). Background white and gray bars indicate periods when the labelled island (top) was the youngest available for colonization, as in Fig. 2.

picture wing clade took place more recently than previously thought, with the *grimshawi* subgroups radiating on Oahu between 2 and 3 Mya. Carson (1992) postulated 8 ancestors out of Kauai for picture wings, including four for the *grimshawi* group. Our data shows that there were only four total – one for the *adiastola* group, one for the *picticornis* group, one for the *planitibia* group, and one for all of the *grimshawi* group (plus an unknown number for the *nudidrosophila* group, possibly three, which Carson did not consider; our biogeographic reconstruction is weak in this group due to the large number of missing taxa). In addition to the evidence from the timing of splits and the biogeographic analysis, support for this hypothesis comes from the fact that, with the possible exception of *D. glabriapex*, all of the Kauai species (*D. craddockae*, *D. kikiko*, *D. musaphilia*, *D. sejuncta*, and *D. villosipedis*; *D. ocellata* and *D. opuhe* are not represented) are not basal within their subgroups, and no progression rule patterns starting with Kauai are evident within the *grimshawi* group.

Speciation appears to occur in two phases in the Hawaiian picture wing *Drosophila*: ecological diversification, with relatively rapid lineage splitting associated with host plant switching (Fig. 3); followed by geographical diversification, producing a larger but slower increase in total species number as dispersal to new islands and niche subdivision on older ones occurs. Two rounds of the first stage can be seen – the first at 3.8–4.7 Mya on Kauai, when the *picticornis*, *nudidrosophila*, *planitibia*, and proto-*grimshawi* lineages separate, and the second at 2.1–3.1 Mya on Oahu, when the *grimshawi* subgroups diverge into host plant-specific lineages. Within the past 2 million years, there have been very few clear examples of host switching.

What is the impetus for such changes? One interesting observation is that these two periods, both begin when the island in question (Kauai or Oahu) is about 0.6–1.0 million years old, which is in between the current ages of Haleakala and Kohala volcanoes on Maui and Hawaii respectively. That suggests that a mature island with at least some deeply eroded topography, and well-developed soil and forests may promote diversification of picture wing *Drosophila*. For species breeding primarily in trees of mesic gulches such as *Pisonia* and *Urera*, including the *grimshawi* and *nudidrosophila* groups, this geographical complexity may be important for divergence. Evidence for this can be seen on the island of Hawaii, where the incised valleys of Mauna Kea and Kohala are (or at least were, prior to human-mediated modification) inhabited by a far greater abundance of such mesic forest host trees than the younger surfaces of Mauna Loa and Kilauea.

The other prerequisite, and perhaps the most important one for why the picture wing groups diversified when they did, is the presence of suitable host plants. Among important *Drosophila* hosts, only the lobeliad radiation (Campanulaceae) has been dated, to ~13 Mya (Givnish et al., 2009). The majority of other Hawaiian plant groups that have been dated arrived ~4–5 Mya when Kauai was the primary high island, or more recently (Knape et al., 2012; Price and Clague, 2002; Sebastian et al., 2012; Willyard et al., 2011). It is notable that within the picture wing clade, the basal *adiastola* group is both the only one to branch off prior to the origin of Kauai, and almost exclusively utilizes lobeliads as hosts (Magnacca et al., 2008). Our results indicate that the majority of the plants used by the *grimshawi* group – particularly *Charpentiera*, *Chrysodracon*, *Pisonia*, and *Urera* – must have arrived in the Hawaiian Islands at least prior to the ecological expansion of the *grimshawi* group, around 3 Mya. It is also possible that arrival of the plants themselves triggered this burst of diversification, as the flies rapidly adapted to utilize new resources. Phylogenetic analysis of these plant groups would help understand the timing of the *grimshawi* group on the different host plants.

The divergence of the Hawaiian picture wing species groups and subgroups in association with host plant specialization is consistent with patterns seen in the diversification of other taxa.

Among *Drosophila*, the *repleta* group radiation occurred ~16 Mya in North and South America in association with the cactus host-plant radiation (Oliveira et al., 2012). Relatively rapid divergence in other animal groups (e.g. Darwin's finches, stickleback fish, cichlid fishes, anolis lizards) appears to occur soon after the invasion of new ecological niches, followed by or in conjunction with morphological and behavioral change (Kocher, 2004; Schluter, 2001; Streelman and Danley, 2003). The rapid splitting of Hawaiian picture wing groups appear to be due to localized evolutionary innovations on the islands of Kauai and Oahu, as a result of either colonization of the islands by new plant groups or the evolution of *Drosophila* to exploit and specialize on new hosts among the existing flora. These are accompanied by changes in characters such as wing pattern (Edwards et al., 2007) and courtship dance (Spieth, 1982) that serve as additional pre-mating reproductive barriers.

Within the large *grimshawi* group, our results clarify the relationships and composition of the subgroups. For example, Spieth (1982) used *D. assita*, *D. micromyia*, and *D. lanaiensis* (under the name *D. virgulata*) as exemplars of the *vesciseta* subgroup, noting that they had different behaviors and ascribing this to more primitive or derived states. However, it is clear from the phylogeny that these species are not closely related, and the behavioral differences observed are the product of much more distant evolutionary separation. Indeed, *D. micromyia* is an isolated lineage with no close relatives, and morphological similarities to both the *grimshawi* and *nudidrosophila* groups. With its relatively small size (similar to species in the *modified mouthparts* group), complete row of tibial cilia which are long and curved at the base, and faintly patterned wings, it is probably closest in appearance to the common ancestor of the picture wing clade. Likewise, *D. aglaia* and *D. conspicua* were included with the *discreta* subgroup, and Spieth (1982) noted that the behavior of the first two was significantly different. In our results, these two species are related to but separate from the tightly-knit *discreta* subgroup.

The placement of *D. engyochracea*, a Hawaii Island species, as the basal member of the *orphanopeza* group was surprising, but strongly supported in all analyses. It breeds in an unusual host, *Sapindus saponaria* (mane, Sapindaceae), in which the bark does not rot into a mush or slime like most typical picture wing hosts, and which has historically been restricted to only a few sites on the island of Hawaii. Its position as both a basal, young-island species and utilizing such an odd niche suggests that it may be a relic of a once-larger group, possibly one that all used *Sapindus* during a time when this tree may have had a wider distribution.

The positions of the enigmatic *anomalipes* and *picticornis* groups are particularly interesting, because our results lead to a reversal of how they were previously regarded. The two species of the former are little-studied and until recently (O'Grady et al., 2010) were not considered members of the picture wing lineage. They were never chromosomally analyzed and had not been included in any phylogenetic analysis until O'Grady et al. (2011), where they clustered with the largely-unresolved picture wing clade.¹ Based on their morphology (Throckmorton, 1966) and courtship and feeding behav-

¹ A YP1 gene sequence labelled as *D. quasianomalipes* (GenBank acc. No. U52361) was included in Kambysellis et al. (1995) and fell out between the *planitibia* and *grimshawi* groups; however, we sequenced a number of species for YP1 in preliminary work for this project, including both *D. quasianomalipes* and *D. anomalipes*, and their sequence is not similar to any we obtained. By doing pairwise comparisons of base differences, it is evident that the U52361 sequence is a composite of several fragments – one from *D. quasianomalipes* (1–270, determined from our data; also similar to *D. anomalipes*), two from *D. grimshawi* (310–445 & 700–985, sequences nearly identical in both datasets), and one that does not match any in either dataset (448–700; numbers are approximate positions in U52361 without gaps). This erroneous sequence is responsible for the difference in position of *D. quasianomalipes* in Kambysellis et al. (1995) compared to the present work.

ior (Spieth, 1975), they had been regarded as primitive and sharing many characteristics of continental *Drosophila*, possibly even representing relics of an early radiation (Spieth, 1975). Our results show that they are instead relatively derived – unambiguously the Kauai representatives of the *planitibia* group – without even long branches that would indicate ancient divergence or an unusual amount of genetic change. They serve as a striking example of how appearances can be deceptive, and evolution can cause rapid changes in isolated lineages.

It is also clear that the *picticornis* group, previously considered basal members of the *planitibia* group (Bonacum et al., 2005; Russo et al., 1995), is more closely related to the *nudidrosophila* clade than to other picture wings. The resemblance of the head setation of *D. setosifrons* and *D. pilipa* to various *nudidrosophila* (Hardy and Kaneshiro, 1968; Magnacca and O'Grady, 2008; Magnacca and Price, 2012) may be evidence of this relationship. However, in wing pattern and host usage, the three *picticornis* group species span the same diversity as the huge *grimshawi* group, and indeed they are nearly as genetically divergent from each other as any two species in that group, with no indication of unusually rapid genetic change (Fig. 1). This suggests that they may be relics of a slightly older radiation – originating on Kauai and formerly much more diverse, with members on other islands – that has since been largely replaced by species of the *grimshawi* group. All the known missing taxa are similar to those in the tree and can be placed with relative certainty, leaving no other potential members of the group. There is of course some possibility of discovering new species, but given that only five new picture wing species have been collected since 1975, it seems there are few left to find. Even if, for example, a representative of the *picticornis* group is discovered on Maui, the vast gap in morphology and breeding habit between the other three species (more comparable to the entire *grimshawi* group than to any other three sibling species) suggests that significant extinction has taken place.

Since we have no calibration points outside the *picture wing* clade, we expect our dating estimates to be increasingly unreliable deeper in the tree, particularly in *Scaptomyza* where generation times may be different (Obbard et al., 2012). However, it is noteworthy that the date for the Hawaiian *Drosophila*–*Scaptomyza* split comes out at a median of 23.4 Mya, similar to the estimates of Lapoint et al. (2013). The most *Drosophila*-like subgenera of *Scaptomyza* (i.e. those with large bodies and more than six rows of acrostichal setulae: *Engiscaptomyza*, *Grimshawomyia*, and *Titanochaeta*) are highly derived within the group, while those with more divergent traits, similar to continental *Scaptomyza* (*Bunostoma*, *Exalloscaptomyza*, and some *Elmomyza*) are basal, suggesting that the evolution of *Scaptomyza* has been much more complex than a gradual morphological divergence in Hawaii followed by dispersal of only some more distinct lineages to other areas. This matches previous results (Lapoint et al., 2013; O'Grady and DeSalle, 2008) and will no doubt be a productive area for future research.

Despite the new findings of this study discussed above, our tree is largely congruent with the chromosomal tree of Carson (1992, p. 414). Aside from the much better resolution of our tree, most conflicts arise from an evident tendency of species to retain chromosomal inversions as polymorphisms through several rounds of speciation, resulting in homoplasy. For example, inversion Xg is shared between *D. balioptera* and most Araliaceae-breeding members of the *orphnopeza* subgroup (except *D. sproati*). There is no question, however, that *D. balioptera* is closely affiliated with the *crucigera* subgroup based on its wing pattern, thoracic coloration, male genitalia, general habitus, and ecology, even without the present data; and that the

araliad-breeding and monocot-breeding members of the *orphnopeza* subgroup also belong together on the same basis (Kaneshiro, 1969). This indicates that inversion Xg likely originated as a polymorphism in the common ancestor of the *crucigera* and *orphnopeza* subgroups, and in the former was fixed in the basal *D. balioptera* and lost in the remaining species. In the latter, the inversion was retained as a polymorphism throughout the diversification of the clade, in various species becoming fixed (*D. engyochracea* and most araliad breeders) or lost (*D. orphnopeza*, *D. sejuncta*, *D. sproati*, *D. villosipedis*, and all monocot breeders). A similar situation can be traced in the *planitibia* group with inversions Xt, Xu², and 2m in the split between the *nigribasis* and *cyrtoloma* subgroups (Carson, 1992). Further research is needed to more fully understand the evolution of chromosomes and the importance of inversions within the picture wing *Drosophila*. Although gene sequencing data has largely supplanted the study of chromosome inversions for phylogenetics, it remains an interesting subject for genomic evolution.

While this study answers some of the questions about the evolution of the picture wing clade, we are unable to resolve the group's position among Hawaiian *Drosophila*. It has long been assumed to be highly derived, based on the larger size and divergent morphology of the species, and the closer resemblance of other Hawaiian species groups to typical continental *Drosophila* (Throckmorton, 1966). However, the possibility that it may be basal within the Hawaiian *Drosophila* was raised by O'Grady et al. (2011). Our MrBayes analysis found the same result, but BEAST resolved the same part of the tree in the traditional manner, with the *haleakalae* group basal. Neither was supported by strong PP values and the remainder of each tree was identical. These conflicting results and the lack of support means this issue will have to be revisited at another time. *Scaptomyza* is well established as the closest relative of the Hawaiian *Drosophila* (O'Grady and DeSalle, 2008; Remsen and O'Grady, 2002; Russo et al., 1995), but the long branch separating them indicates that a great deal of extinction has occurred since their divergence. The existence of relictual species such as *D. primaeva* and *D. adventitia*, and more recent taxa such as the *picticornis* subgroup with its few, highly divergent species that imply greater past diversity, indicates that extinction has played a major role in the formation of the Hawaiian *drosophilid* fauna. With the extant *Drosophila* lineages evidently diverging fairly rapidly from their common ancestor and on long branches relative to each other, their relationships may be very difficult to resolve.

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Appendix A. New species group and species subgroup classification for the *picture wing* clade

Species group Species subgroup Species	Authority	Island
adiastola		
adiastola		
<i>Drosophila adiaastola</i>	Hardy, 1965	Maui, Lanai
<i>Drosophila cilifera</i>	Hardy and Kaneshiro, 1968	Molokai
<i>Drosophila ochrobasis</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila ornata</i>	Hardy & Kaneshiro, 1969	Kauai
<i>Drosophila peniculipedis</i>	Hardy, 1965	Maui
<i>Drosophila setosimentum</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila touchardiaae</i>	Hardy & Kaneshiro, 1972	Oahu
<i>Drosophila toxochaeta</i>	Perreira & Kaneshiro, 1990	Molokai
clavisetae		
<i>Drosophila clavisetae</i>	(Hardy, 1966)	Maui
<i>Drosophila neoclavisetae</i>	Perreira & Kaneshiro, 1990	Maui
<i>Drosophila neogramshawi</i>	Hardy and Kaneshiro, 1968	Oahu
<i>Drosophila spectabilis</i>	Hardy, 1965	Maui, Molokai, Lanai
truncipenna		
<i>Drosophila hamifera</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila paenehamifera</i>	Hardy & Kaneshiro, 1969	Maui
<i>Drosophila truncipenna</i>	Hardy, 1965	Maui
<i>Drosophila varipennis</i>	(Grimshaw, 1901)	Molokai
<i>Drosophila nr. truncipenna</i>		Oahu
grimshawi		
aglaia		
<i>Drosophila aglaia</i>	Hardy, 1965	Oahu
<i>Drosophila alsophila</i>	Hardy & Kaneshiro, 1971	Hawaii
<i>Drosophila conspicua</i>	Grimshaw, 1901	Hawaii
<i>Drosophila kikiko</i>	Magnacca, 2012	Kauai
<i>Drosophila kinoole</i>	Magnacca, 2012	Oahu
<i>Drosophila nr. alsophila</i>		Maui
crucigera		
<i>Drosophila affinisdisjuncta</i>	Hardy, 1978	Maui
<i>Drosophila balioptera</i>	Hardy, 1965	Maui, Molokai
<i>Drosophila bostrycha</i>	Hardy, 1965	Molokai
<i>Drosophila craddockae</i>	Kambysellis & Kaneshiro, 1999	Oahu, Kauai
<i>Drosophila crucigera</i>	Grimshaw, 1902	Oahu, Kauai
<i>Drosophila disjuncta</i>	Hardy, 1965	Maui
<i>Drosophila grimshawi</i>	Oldenberg, 1914	Maui, Molokai, Lanai
<i>Drosophila pullipes</i>	Hardy & Kaneshiro, 1972	Hawaii
discreta		
<i>Drosophila discreta</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila fasciculisetae</i>	Hardy, 1965	Maui, Molokai
<i>Drosophila glabriapex</i>	Hardy and Kaneshiro, 1968	Kauai
<i>Drosophila lineosetae</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila pilimana</i>	Grimshaw, 1901	Oahu
distinguenda		
<i>Drosophila distinguenda</i>	Hardy, 1965	Oahu
<i>Drosophila divaricata</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila inedita</i>	Hardy, 1965	Oahu
hawaiiensis		
<i>Drosophila flexipes</i>	Hardy and Kaneshiro, 1968	Oahu
<i>Drosophila formella</i>	Hardy & Kaneshiro, 1972	Hawaii
<i>Drosophila gradata</i>	Hardy and Kaneshiro, 1968	Oahu
<i>Drosophila gymnobasis</i>	Hardy & Kaneshiro, 1971	Maui
<i>Drosophila hawaiiensis</i>	Grimshaw, 1901	Hawaii

Appendix A (continued)

Species group Species subgroup Species	Authority	Island
<i>Drosophila heedi</i>	Hardy & Kaneshiro, 1971	Hawaii
<i>Drosophila hirtipalpus</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila lasiopoda</i>	Hardy & Kaneshiro, 1975	Maui
<i>Drosophila musaphilia</i>	Hardy, 1965	Kauai
<i>Drosophila nukea</i>	Magnacca, 2012	Molokai
<i>Drosophila psilotarsalis</i>	Hardy & Kaneshiro, 1975	Hawaii
<i>Drosophila recticilia</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila silvarentis</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila turbata</i>	Hardy & Kaneshiro, 1969	Oahu
<i>Drosophila villitibia</i>	Hardy, 1965	Molokai
lanaiensis		
<i>Drosophila digressa</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila hexachaetae</i>	Hardy, 1965	Oahu
<i>Drosophila lanaiensis</i>	Grimshaw, 1901	Maui, Molokai, Lanai
<i>Drosophila moli</i>	Magnacca, 2012	Oahu
<i>Drosophila tarphytrichia</i>	Hardy, 1965	Oahu
macrothrix		
<i>Drosophila macrothrix</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila oreas</i>	Hardy, 1965	Maui
micromyia		
<i>Drosophila micromyia</i>	Hardy & Kaneshiro, 1975	Kauai
odontophallus		
<i>Drosophila gymnophallus</i>	Hardy & Kaneshiro, 1975	Oahu
<i>Drosophila liophallus</i>	Hardy and Kaneshiro, 1968	Maui, Molokai
<i>Drosophila odontophallus</i>	Hardy and Kaneshiro, 1968	Maui, Molokai
<i>Drosophila psilophallus</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila spaniothrix</i>	Hardy and Kaneshiro, 1968	Oahu
orhnopeza		
<i>Drosophila atrimentum</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila ciliaticrus</i>	Hardy, 1965	Hawaii
<i>Drosophila claytonae</i>	Hardy & Kaneshiro, 1969	Hawaii
<i>Drosophila engyochracea</i>	Hardy, 1965	Hawaii
<i>Drosophila limitata</i>	Hardy and Kaneshiro, 1968	Maui, Molokai, Lanai
<i>Drosophila murphyi</i>	Hardy & Kaneshiro, 1969	Hawaii
<i>Drosophila obatai</i>	Hardy & Kaneshiro, 1972	Oahu
<i>Drosophila ochracea</i>	Grimshaw, 1901	Hawaii
<i>Drosophila orhnopeza</i>	Hardy and Kaneshiro, 1968	Maui, Molokai
<i>Drosophila orthofascia</i>	Hardy and Kaneshiro, 1968	Maui, Molokai, Lanai
<i>Drosophila reynoldsiae</i>	Hardy & Kaneshiro, 1972	Oahu
<i>Drosophila sejuncta</i>	Hardy and Kaneshiro, 1968	Kauai
<i>Drosophila sobrina</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila sodomae</i>	Hardy and Kaneshiro, 1968	Maui, Molokai
<i>Drosophila sproati</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila villosipedis</i>	Hardy, 1965	Kauai
punalua		
<i>Drosophila basisetae</i>	Hardy and Kaneshiro, 1968	Hawaii
<i>Drosophila ocellata</i>	Hardy & Kaneshiro, 1969	Kauai
<i>Drosophila paucicilia</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila paucipuncta</i>	Grimshaw, 1901	Hawaii
<i>Drosophila prolaticilia</i>	Hardy, 1965	Hawaii
<i>Drosophila prostopalpis</i>	Hardy and Kaneshiro, 1968	Maui
<i>Drosophila punalua</i>	Bryan, 1934	Oahu
<i>Drosophila uniseriata</i>	Hardy and Kaneshiro, 1968	Oahu

(continued on next page)

Appendix A (continued)

Species group Species subgroup Species	Authority	Island
vesciseta		
<i>Drosophila ambochila</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila assita</i>	Hardy & Kaneshiro, 1969	Hawaii
<i>Drosophila montgomeryi</i>	Hardy & Kaneshiro, 1971	Oahu
<i>Drosophila opuhe</i>	Magnacca, 2012	Kauai
<i>Drosophila pihulu</i>	Magnacca, 2012	Maui, Molokai
<i>Drosophila pisonia</i>	Hardy & Kaneshiro, 1971	Hawaii
<i>Drosophila vesciseta</i>	Hardy and Kaneshiro, 1968	Maui
Unplaced		
<i>Drosophila mulli</i>	Perreira & Kaneshiro, 1990	Hawaii
<i>Drosophila pilatisetae</i>	Hardy and Kaneshiro, 1968	Maui
nudidrosophila		
ateledrosophila		
<i>Drosophila diamphidia</i>	(Hardy, 1965)	Hawaii
<i>Drosophila papala</i>	Magnacca & O'Grady, 2007	Hawaii
<i>Drosophila preapicula</i>	(Hardy, 1965)	Oahu
hirtitibia		
<i>Drosophila hirtitibia</i>	Hardy, 1965	Oahu
<i>Drosophila konaensis</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila mawaena</i>	Magnacca & O'Grady, 2008	Maui, Molokai, Lanai
<i>Drosophila papaalai</i>	Magnacca & O'Grady, 2008	Kauai
kahania		
<i>Drosophila kahania</i>	Magnacca & O'Grady, 2008	Oahu
<i>Drosophila longipalpus</i>	Magnacca & O'Grady, 2008	Hawaii
nudidrosophila		
<i>Drosophila aenicta</i>	(Hardy, 1966)	Oahu
<i>Drosophila amita</i>	(Hardy, 1965)	Hawaii
<i>Drosophila canavalia</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila eximia</i>	(Hardy, 1965)	Maui, Molokai
<i>Drosophila gemmula</i>	(Hardy, 1965)	Oahu
<i>Drosophila kualapa</i>	Magnacca & O'Grady, 2008	Kauai
<i>Drosophila lepidobregma</i>	(Hardy, 1965)	Hawaii
<i>Drosophila mahui</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila malele</i>	Magnacca & O'Grady, 2008	Kauai
<i>Drosophila panoanoa</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila poonia</i>	Magnacca & O'Grady, 2008	Kauai
okala		
<i>Drosophila akoko</i>	Magnacca & O'Grady, 2008	Oahu
<i>Drosophila kuhao</i>	Magnacca & O'Grady, 2008	Oahu
<i>Drosophila makawao</i>	Magnacca & O'Grady, 2008	Maui, Molokai
<i>Drosophila okala</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila panina</i>	Magnacca & O'Grady, 2008	Oahu
velata		
<i>Drosophila halapepe</i>	Magnacca & O'Grady, 2008	Hawaii
<i>Drosophila kauaiensis</i>	Magnacca & O'Grady, 2008	Kauai
<i>Drosophila lauoho</i>	Magnacca & O'Grady, 2008	Maui, Molokai
<i>Drosophila milolii</i>	Magnacca & O'Grady, 2008	Kauai
<i>Drosophila pohaka</i>	Magnacca & O'Grady, 2008	Hawaii, Maui
<i>Drosophila velata</i>	Hardy, 1965	Oahu
picticornis		
<i>Drosophila picticornis</i>	Grimshaw, 1901	Kauai
<i>Drosophila pilipa</i>	Magnacca, 2012	Kauai
<i>Drosophila setosifrons</i>	Hardy and Kaneshiro, 1968	Hawaii

Appendix A (continued)

Species group Species subgroup Species	Authority	Island
planitibia		
anomalipes		
<i>Drosophila anomalipes</i>	Grimshaw, 1901	Kauai
<i>Drosophila quasianomalipes</i>	Hardy, 1965	Kauai
cyrtoloma		
<i>Drosophila cyrtoloma</i>	Hardy, 1969	Maui
<i>Drosophila hanaulae</i>	Hardy, 1969	Maui
<i>Drosophila ingens</i>	Hardy & Kaneshiro, 1971	Maui
<i>Drosophila melanocephala</i>	(Hardy, 1966)	Maui
<i>Drosophila neoperkinsi</i>	Hardy and Kaneshiro, 1968	Molokai
<i>Drosophila oahuensis</i>	(Grimshaw, 1901)	Oahu
<i>Drosophila obscuripes</i>	(Grimshaw, 1901)	Maui
neopicta		
<i>Drosophila neopicta</i>	Hardy and Kaneshiro, 1968	Maui, Molokai
<i>Drosophila nigribasis</i>	Hardy, 1969	Oahu
<i>Drosophila substenoptera</i>	Hardy, 1969	Oahu
planitibia		
<i>Drosophila differens</i>	Hardy & Kaneshiro, 1975	Molokai
<i>Drosophila hemipeza</i>	(Hardy, 1965)	Oahu
<i>Drosophila heteroneura</i>	(Perkins, 1910)	Hawaii
<i>Drosophila planitibia</i>	(Hardy, 1966)	Maui
<i>Drosophila silvestris</i>	(Perkins, 1910)	Hawaii

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2015.06.014>.

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