

Out of Hawaii: the origin and biogeography of the genus *Scaptomyza* (Diptera: Drosophilidae)

Patrick O'Grady^{1,*} and Rob DeSalle²

¹Department of Environmental Science, Policy and Management, 117 Hilgard Hall, University of California, Berkeley, Berkeley, CA 94720, USA

²Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA

*Author for correspondence (ogrady@nature.berkeley.edu).

The Hawaiian Archipelago is the most isolated island system on the planet and has been the subject of evolutionary research for over a century. The largest radiation of species in Hawaii is the Hawaiian Drosophilidae, a group of approximately 1000 species. Dispersal to isolated island systems like Hawaii is rare and the resultant flora and fauna shows high disharmony with mainland communities. The possibility that some lineages may have originated in Hawaii and subsequently 'escaped' to diversify on continental landmasses is expected to be rarer still. We present phylogenetic analysis of 134 partially sequenced mitochondrial genomes of Drosophilidae (approx. 1.3 Mb of sequence total) to address major aspects of adaptive radiation and dispersal in Hawaii. We show that the genus *Scaptomyza*, a group that accounts for approximately one-third of the species-level diversity of Drosophilidae in the Hawaiian Islands, originated in Hawaii, diversified there, and subsequently colonized a number of island and continental landmasses elsewhere on the globe. We propose that a combination of small body size, rapid generation time and unique ecological and physiological adaptations have allowed this genus to effectively disperse and diversify.

Keywords: biogeography; phylogenetics; Hawaii; *Scaptomyza*; *Drosophila*

1. INTRODUCTION

The Hawaiian Islands, the most isolated archipelago on Earth, are home to thousands of endemic taxa (Wagner *et al.* 1999; Liebherr & Zimmerman 2000). Although researchers have long studied adaptive radiation of the Hawaiian biota, few have considered the possibility that some lineages may have originated in Hawaii and subsequently 'escaped' to diversify on the mainland. First, the great distances involved make such a scenario implausible—traversing nearly 4000 km of open ocean once would appear to be very unlikely and a second migration across such a distance, especially after adapting to life in an island environment, would be hard to envision. Second, the phylogenetic placement of many Hawaiian lineages is poorly understood beyond the basic alpha taxonomic level, making tests of monophyly and phylogenetic relationships within various groups

difficult (Liebherr & Zimmerman 2000; Price & Clague 2002). Hawaiian taxa are generally assumed to be monophyletic and sister to morphologically similar mainland species. Two recent examples, one from migratory birds (Filardi & Moyle 2005) and another in the sandalwood family (Harbaugh & Baldwin 2007), suggest that long-distance migration from Hawaii is possible in some taxa.

Mayr (1954) considered remote island systems to be 'crucibles of evolution', owing to their associations with adaptation radiations. The largest radiation in Hawaii is the Hawaiian Drosophilidae, a group of approximately 1000 species (Kaneshiro 1997). There are two drosophilid genera present in Hawaii, *Drosophila* and *Scaptomyza*. Previous phylogenetic studies indicate these lineages are sister taxa, with the genus *Scaptomyza* embedded within a larger, paraphyletic *Drosophila* (Throckmorton 1966; Kambysellis *et al.* 1995; Russo *et al.* 1995; Remsen & O'Grady 2002). Throckmorton (1966) suggested that one or two colonizations of Hawaii by Drosophilidae could explain this pattern. However, owing to limited sampling within *Scaptomyza*, little could be said about evolutionary history within this genus.

The present study incorporates sequence information from 134 partial mitochondrial genomes to establish a robust phylogenetic hypothesis for the Drosophilidae, with a focus on the divergence of the endemic Hawaiian species. This work is of particular relevance owing to the recent publication of 12 complete *Drosophila* genomes (*Drosophila* 12 Genomes Consortium 2007) and the interest in expanding genetic and developmental information based on model organisms to related taxa (Markow & O'Grady 2007). Phylogenetic analyses suggest a single colonization of Hawaii by Drosophilidae, followed by divergence of the genera *Scaptomyza* and *Drosophila*, and the subsequent dispersal from Hawaii of some members of *Scaptomyza*. These results prompted us to further examine several anatomical and physiological characteristics of *Scaptomyza* and their Hawaiian relatives in the genus *Drosophila*, in the context of dispersal ability.

2. MATERIAL AND METHODS

(a) Taxon sampling

Taxa representative of drosophilid diversity were sampled, with particular emphasis on the large genus *Drosophila*. A total of 134 species placed in 10 genera were sampled. Twenty-five of the currently described species groups in the genus *Drosophila* were sequenced. Material was obtained from the Tucson *Drosophila* Stock Center and field collections made by the first author.

(b) DNA sequences and alignment

DNA was extracted from single flies (Qiagen). Polymerase chain reaction conditions were standard for mitochondrial DNA (e.g. Bonacum *et al.* 2005). Twenty-six primer pairs (Bonacum *et al.* 2001), some of which were overlapping, were used to amplify roughly 10 kb per taxon. The remainder of the mitochondrial genome was not amplified owing to lack of efficient priming sites or because it evolved too rapidly in the focal taxa to be aligned reliably. All sequences are deposited in GenBank (EU493462–EU494589). Trees generated in this study are deposited in TREEBASE (<http://www.treebase.org/treebase/index.html>).

(c) Phylogenetic analysis

Phylogenetic analyses were performed using maximum parsimony (MP) and Bayesian methods. Parsimony analyses used the heuristic algorithm in PAUP* (Swofford 2005) to conduct 1000 random addition replicates using a tree bisection–reconnection branch swapping. One thousand bootstrap replicates were run to assess support on the parsimony trees. Bayesian analyses included three separate character sets: all sites; protein coding loci only; and the first and second codon positions only. MODELTEST (Posada & Crandall 1998) indicated

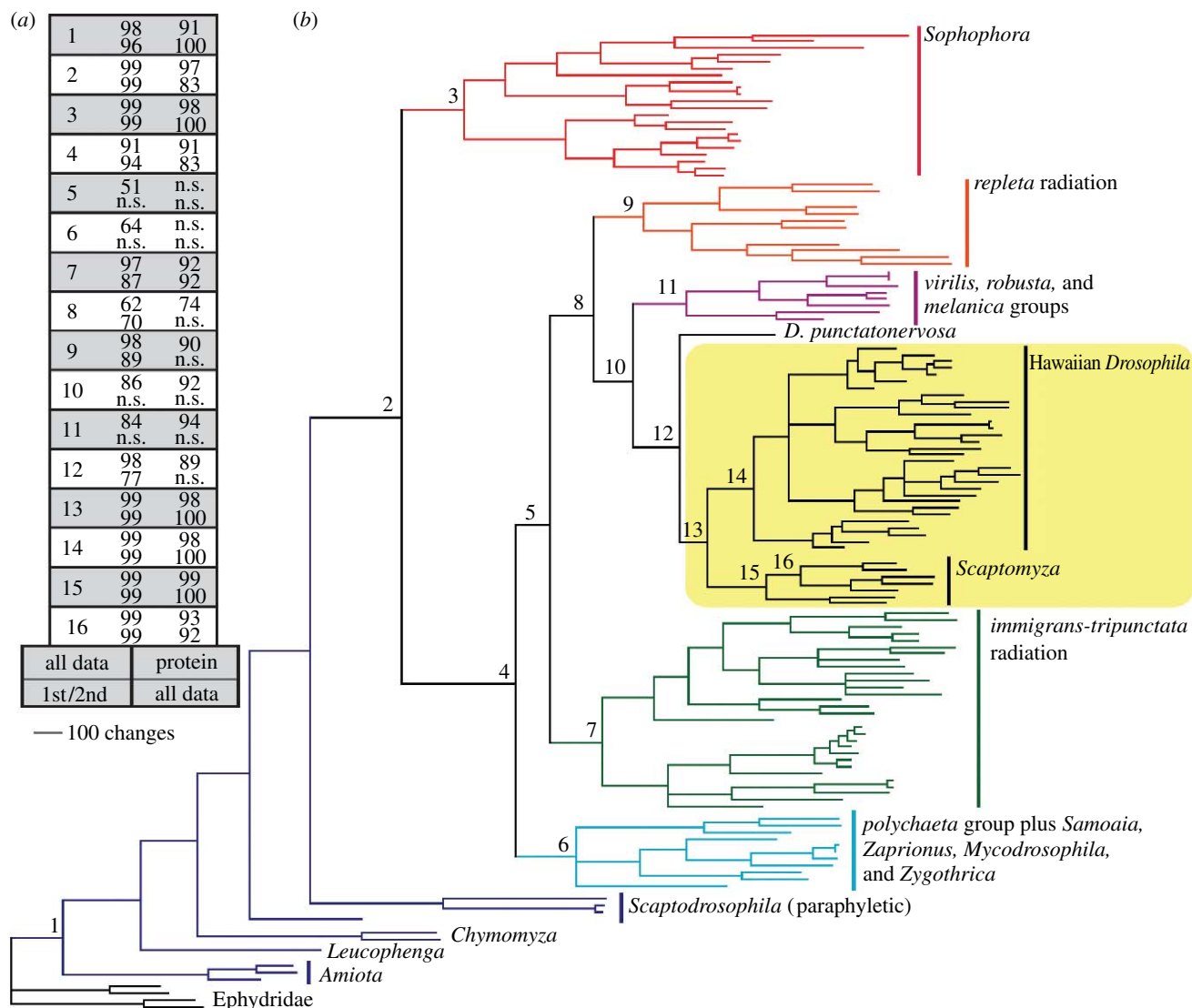


Figure 1. Phylogenetic relationships of the family Drosophilidae based on mitochondrial genome sequences. The Hawaiian Drosophilidae, Hawaiian *Drosophila* plus *Scaptomyza*, are shown in a yellow box. Other major lineages in this family are shown in red (the subgenus *Sophophora*), orange (*repleta* radiation, subgenus *Drosophila*), green (*immigrans-tripunctata* radiation, subgenus *Drosophila*) and shades of blue. Support values for numbered nodes are shown in the chart, (a); MP bootstrap for all sequenced positions (upper left), protein coding regions (upper right) and the first and second codon positions (lower left) are shown with Bayesian posterior probabilities for all data (lower right).

a GTR+G+I model as the best fit in all three data partitions. Two runs, each with four chains, were initiated simultaneously and sampled every 100 generations for 2 500 000 generations in MRBAYES v. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Generally, analyses with fewer characters (e.g. the first and second positions only) showed less support at nodes that were marginally supported in the MP analysis (figure 1).

(d) Measurement of flies and mapping of continuous data on the phylogeny

Males were used to estimate body size in millimetres (after Hardy 1965, 1966; Hardy & Kaneshiro 1968, 1969, 1971, 1975; O'Grady *et al.* 2003). Ancestral state reconstructions of mean body size were done in MACCLADE v. 4.0 (Maddison & Maddison 2005) using the Trace Continuous option and by minimizing the sum of squared changes on the phylogeny (figure 2). Data on egg to adult development times and temperature-dependent sterility in Hawaiian drosophilids (Markow & O'Grady 2006) was used to determine dispersal ability.

3. RESULTS

Phylogenetic analysis of partial mitochondrial genomes from 134 drosophilid species (over 1.3 Mb of sequence total) is largely congruent with the recently

published phylogeny based on 12 complete genome sequences (*Drosophila* 12 Genomes Consortium 2007). Our results indicate that the Hawaiian *Drosophila* and the genus *Scaptomyza* are strongly supported as sister taxa (figure 1), suggesting that a single ancestral colonist generated the impressive diversity of Drosophilidae in Hawaii. Sampling Hawaiian and mainland subgenera of *Scaptomyza* suggests a far more complex picture than that had previously been proposed. The genus *Scaptomyza* contains 272 described species, 161 of which are endemic to the Hawaiian Islands. The non-Hawaiian taxa are nested within the Hawaiian *Scaptomyza*, suggesting that at least one lineage escaped from Hawaii to found the continental taxa (figure 2).

Successful colonist species, such as *Drosophila melanogaster*, might be expected to share a suite of physiological characteristics including small body size, rapid development time and reliance on widely distributed host plant species. Mapping of body size onto the phylogeny (figure 2) indicates that the initial

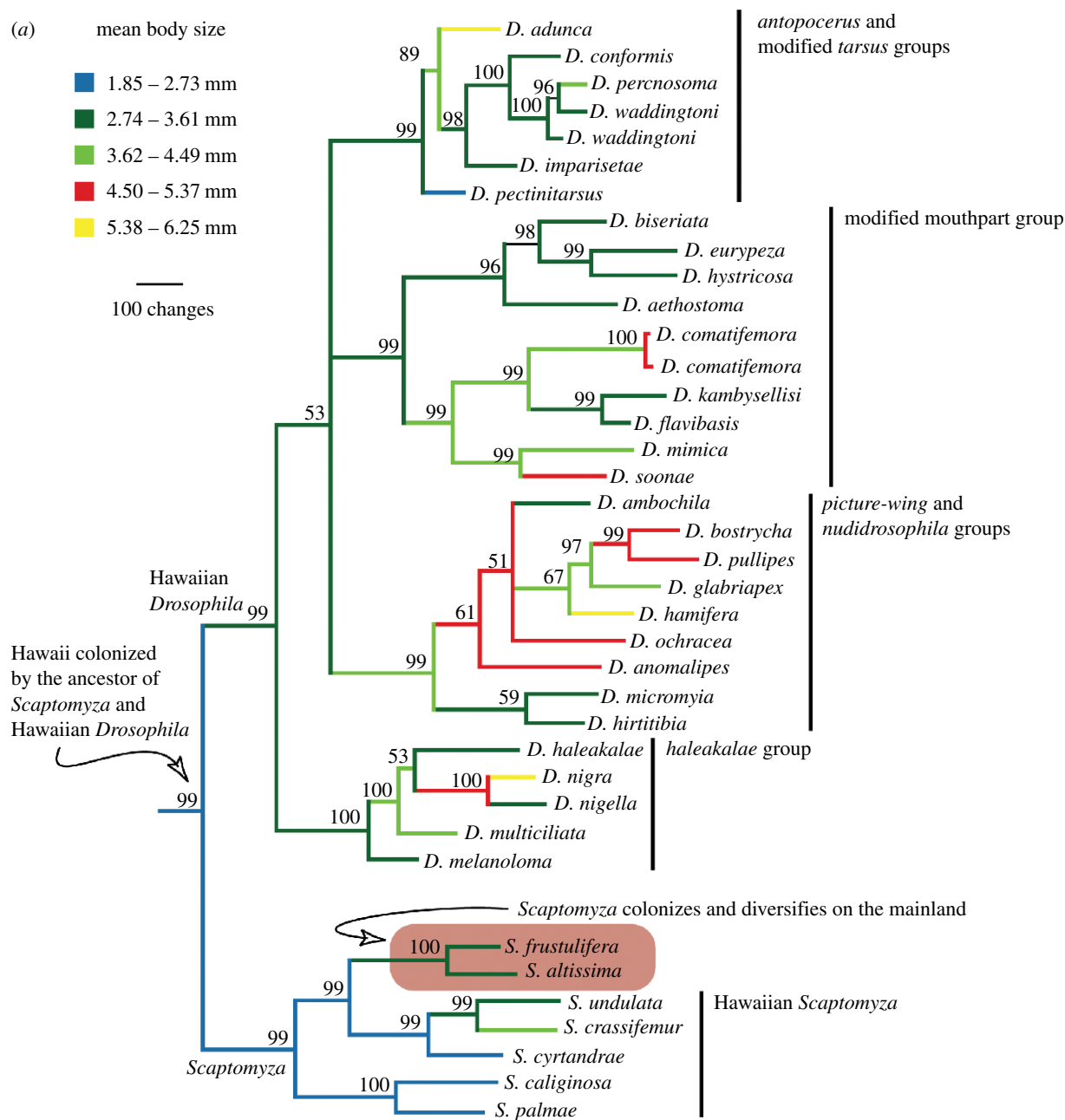


Figure 2. (a) Mean body size of Hawaiian *Drosophila* and *Scaptomyza* species mapped as a continuous character on the phylogenetic relationships of these taxa. Mainland *Scaptomyza* are highlighted in red. (b) Sticky fruit of *Pisonia*.

colonist to the Hawaiian Islands was much smaller (2.5 mm inferred at ancestral node) than many of the extant taxa in either the *Scaptomyza* (ancestral node = 2.5 mm) or Hawaiian *Drosophila* (ancestral node = 3.0 mm) lineages. Although the phylogeny shows multiple independent increases in body size among various lineages of both genera, these increases have taken place less frequently in *Scaptomyza* and most members of this genus remain smaller than Hawaiian *Drosophila* species.

The egg to adult development time in *Scaptomyza* is 15 days (18°C), compared with 23–29 days for Hawaiian *Drosophila* (Markow & O'Grady 2006). Owing to their smaller size and more rapid development time, *Scaptomyza* species are able to use smaller, more ephemeral hosts than members of the genus *Drosophila* (Heed 1968). Such substrates are found in strand habitats and might serve as stepping stones from Hawaii to a continental landmass.

4. DISCUSSION

The Hawaiian Drosophilidae (*Drosophila* and *Scaptomyza*) have undergone extensive diversification in the Hawaiian Islands. Phylogenetic analysis of 134 mitochondrial genome sequences indicate that the sister lineage of the Hawaiian *Drosophila* is the genus *Scaptomyza*, and that the biogeography of *Scaptomyza* is unique in that this group originated in Hawaii, diversified there and then dispersed to colonize a number of island and continental landmasses. Several independent lines of biogeographic, physiological and ecological evidence suggest that the genus *Scaptomyza* may be an excellent candidate for long-distance dispersal across oceans. Examining the geographical distribution of *Scaptomyza* (Wheeler 1982, 1986) reveals that 161 species (59%) are endemic to Hawaii and another 56 (21%) are present on remote island archipelagos (e.g. Marquesas, Tristan da Cunha). These numbers are underestimates, given that over 100 known, yet undescribed, species have been recorded from Hawaii. Representatives from 16 of the 21 *Scaptomyza* subgenera are restricted to remote island archipelagos and 13 consist entirely of island endemics.

Species that are able to develop quickly on widespread ephemeral substrates may be more effective colonists than those with specialized oviposition requirements and longer development times. Aside from requiring a smaller oviposition resource, some species of *Scaptomyza* use widespread host plants. For example, the fruit of endemic Hawaiian papala kepau (Nyctaginaceae: *Pisonia brunoniana*) has a sticky outer layer (figure 2b) that one species of *Scaptomyza* uses for oviposition, larval development and pupation (Heed 1968; Magnacca *et al.* in press). The genus *Pisonia* is found throughout the Pacific, even on remote seamounts and atolls. Many species of seabirds have been found far out at sea with these sticky *Pisonia* fruits tangled in their feathers (Whistler 1992). The genus *Pisonia* is thought to have colonized the Hawaiian Islands at least three, and possibly four, times (Wagner *et al.* 1999) most likely due to its ability to be carried great distances by migrating birds. These fruits were so effective at snaring birds that the ancient Hawaiians used them to trap forest birds to make their traditional feather capes (Ziegler 2002). It is possible that an ancestor of mainland *Scaptomyza* may have stowed away on a similar type of fruit.

It is clear that the history of colonization and diversification of Hawaiian Drosophilidae is far more complicated than the previously suggested single colonization and subsequent diversification *in situ* (O'Grady & Zilversmit 2004; Bonacum *et al.* 2005). Molecular phylogenetic studies that more thoroughly sample the diversity of species present in the islands, as well as multiple continental relatives, have given a much richer picture of the evolution of this group. As more detailed phylogenetic studies are performed on additional groups of Hawaiian species, it is probable that the histories of other lineages will also reflect multiple colonizations of, or even escapes from, the remote Hawaiian Archipelago.

- Bonacum, J., DeSalle, R., O'Grady, P., Oliveira, D., Wintermute, J. & Zilversmit, M. 2001 New nuclear and mitochondrial primers for systematics and comparative genomics in Drosophilidae. *Drosoph. Inf. Serv.* **84**, 201–204.
- Bonacum, J., O'Grady, P. M., Kambysellis, M. & DeSalle, R. 2005 Phylogeny and age of diversification of the *planitibia* species group of the Hawaiian *Drosophila*. *Mol. Phylogenet. Evol.* **37**, 73–82. (doi:10.1016/j.ympev.2005.03.008)
- Filardi, C. & Moyle, R. 2005 Single origin of a pan-Pacific bird group and upstream colonization of Australasia. *Nature* **438**, 216–219. (doi:10.1038/nature04057)
- Harbaugh, D. & Baldwin, B. 2007 Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersals throughout the Pacific. *Am. J. Bot.* **94**, 1030–1042.
- Hardy, D. (ed.) 1965 *Diptera: Cyclorrhapha II, series Schizophora, section Acalypterae I, family Drosophilidae. Insects of Hawaii*. Honolulu, HI: University of Hawaii Press.
- Hardy, D. 1966 Descriptions and notes on Hawaiian Drosophilidae (Diptera). *Univ. Tex. Publ.* **6615**, 195–244.
- Hardy, D. & Kaneshiro, K. 1968 New picture-winged *Drosophila* from Hawaii. *Univ. Tex. Publ. Stud. Genet.* **6818**, 171–262.
- Hardy, D. & Kaneshiro, K. 1969 Descriptions of new Hawaiian *Drosophila*. *Univ. Tex. Publ. Stud. Genet.* **6918**, 39–54.
- Hardy, D. & Kaneshiro, K. 1971 New picture-winged *Drosophila* from Hawaii. II. (Drosophilidae, Diptera). *Univ. Tex. Publ. Stud. Genet.* **7103**, 151–170.
- Hardy, D. & Kaneshiro, K. 1975 Studies on Hawaiian *Drosophila*, modified-mouthparts species no. 1 *mittelli* subgroup. *Proc. Hawaii. Entomol. Soc.* **22**, 51–55.
- Heed, W. B. 1968 Ecology of the Hawaiian Drosophilidae. *Univ. Tex. Publ.* **6818**, 387–419.
- Huelsenbeck, J. P. & Ronquist, F. 2001 MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755. (doi:10.1093/bioinformatics/17.8.754)
- Kambysellis, M. P., Ho, K. F., Craddock, E. M., Piano, F., Parisi, M. & Cohen, J. 1995 Pattern of ecological shifts in the diversification of Hawaiian *Drosophila* inferred from a molecular phylogeny. *Curr. Biol.* **5**, 1129–1139. (doi:10.1016/S0960-9822(95)00229-6)
- Kaneshiro, K. Y. 1997 R.C.L. Perkins' legacy to evolutionary research on Hawaiian Drosophilidae, Diptera. *Pac. Sci.* **51**, 450–461.
- Liebherr, J. K. & Zimmerman, E. C. 2000 *Hawaiian Carabidae (Coleoptera), part 1: introduction and tribe Platynini. Insects of Hawaii*. Honolulu, HI: University of Hawaii Press.
- Maddison, D. & Maddison, W. 2005 *MACCLADE: analysis of phylogeny and character evolution*, v. 4. Sunderland, MA: Sinauer Associates.
- Magnacca, K. N., Foote, D. & O'Grady, P. M. In press. A review of the endemic Hawaiian Drosophilidae and their host plants. *Zootaxa*.
- Markow, T. & O'Grady, P. M. 2006 *Drosophila: a guide to species identification and use*. London, UK: Academic Press.
- Markow, T. & O'Grady, P. M. 2007 *Drosophila* biology in the genomic age. *Genetics* **177**, 1269–1276. (doi:10.1534/genetics.107.074112)
- Mayr, E. 1954 Change of genetic environment and evolution. In *Evolution as a process* (eds J. Huxley, A. Hardy & E. Ford), pp. 157–180. London, UK: Allen & Unwin.

- O'Grady, P. & Zilversmit, M. 2004 Phylogenetic relationships within the *haleakalae* species group inferred by molecular and morphological characters (Diptera: Drosophilidae). *Bishop Mus. Bull. Entomol.* **10**, 117–134.
- O'Grady, P. M., Kam, M., Val, F. & Perreira, W. 2003 Revision of the *Drosophila mimica* subgroup, with descriptions of ten new species. *Ann. Entomol. Soc. Am.* **96**, 12–38. (doi:10.1603/0013-8746(2003)096[0012:ROTDMS]2.0.CO;2)
- Posada, D. & Crandall, K. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. (doi:10.1093/bioinformatics/14.9.817)
- Price, J. P. & Clague, D. A. 2002 How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proc. R. Soc. B* **269**, 2429–2435. (doi:10.1098/rspb.2002.2175)
- Remsen, J. & O'Grady, P. 2002 Phylogeny of Drosophilidae (Diptera), with comments on combined analysis and character support. *Mol. Phylogenet. Evol.* **24**, 248–263. (doi:10.1016/S1055-7903(02)00226-9)
- Ronquist, F. & Huelsenbeck, J. P. 2003 MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. (doi:10.1093/bioinformatics/btg180)
- Russo, C., Takezaki, N. & Nei, M. 1995 Molecular phylogeny and divergence times of drosophilid species. *Mol. Biol. Evol.* **12**, 391–404.
- Swofford, D. 2005 *Phylogenetic analysis using parsimony* (*and other methods)*. Sunderland, MA: Sinauer.
- Throckmorton, L. H. 1966 The relationships of the endemic Hawaiian Drosophilidae. *Univ. Tex. Publ.* **3**, 335–396.
- Wagner, W. L., Herbst, D. & Sohmer, S. 1999 *Manual of the flowering plants of Hawaii*, revised edn. Honolulu, HI: University of Hawaii Press.
- Wheeler, M. R. 1982 Drosophilidae: a taxonomic overview. In *The genetics and biology of Drosophila*, vol. 3a (eds M. Ashburner, J. N. Thompson & H. L. Carson), pp. 1–105. London, UK: Academic Press.
- Wheeler, M. R. 1986 Additions to the catalog of the world's Drosophilidae. In *The genetics and biology of Drosophila*, vol. 3e (eds M. Ashburner, J. N. Thompson & H. L. Carson), pp. 533–565. London, UK: Academic Press.
- Whistler, W. A. 1992 *Flowers of the Pacific island seashore*. Honolulu, HI: University of Hawaii Press.
- Ziegler, A. C. 2002 *Hawaiian natural history, ecology and evolution*. Honolulu, HI: University of Hawaii Press.