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Multiple, independent colonizations of the Hawaiian Archipelago by the family Dolichopodidae (Diptera)

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The family Dolichopodidae forms two of the four largest evolutionary radiations in the Hawaiian Islands across all flies: *Campsicnemus* (183 spp) and the *Eurynogaster* complex (66 spp). They also include a small radiation of *Conchopus* (6 spp). A handful of other dolichopodid species are native to the islands in singleton lineages or small radiations. This study provides a phylogenetic perspective on the colonization history of the dolichopodid fauna in the islands. We generated a multi gene data set including representatives from 11 of the 14 endemic Hawaiian dolichopodid genera to examine the history of colonization to the islands, and analyzed it using Bayesian and maximum likelihood phylogenetic methods. We used a subset of the data that included *Conchopus* and the eight genera comprising the *Eurynogaster* complex to estimate the first phylogenetic hypothesis for these endemic groups, then used Beast to estimate their age of arrival to the archipelago. The *Eurynogaster* complex, *Campsicnemus* and *Conchopus* are clearly the result of independent colonizations. The results strongly support the *Eurynogaster* complex as a monophyletic group, and also supports the monophyly of 4 of the 8 described genera within the complex (*Adachia, Arciellia, Uropachys* and *Eurynogaster*). Members of the family Dolichopodidae have been dispersing over vast distances to colonize the Hawaiian Archipelago for millions of years, leading to multiple independent evolutionary diversification events. The *Eurynogaster* complex arrived in the Hawaiian Archipelago 11.8 Ma, well before the arrival of *Campsicnemus* (4.5 Ma), and the even more recent *Conchopus* (1.8 Ma). Data presented here demonstrate that the Hawaiian Dolichopodidae both disperse and diversify easily, a rare combination that lays the groundwork for field studies on the reproductive isolating mechanisms and ecological partitioning of this group.

Original Article

Title:

Multiple, Independent Colonizations of the Hawaiian Archipelago by the Family Dolichopodidae (Diptera)

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ABSTRACT

The family Dolichopodidae forms two of the four largest evolutionary radiations in the Hawaiian Islands across all flies: *Campsicnemus* (183 spp) and the *Eurynogaster* complex (66 spp). They also include a small radiation of *Conchopus* (6 spp). A handful of other dolichopodid species are native to the islands in singleton lineages or small radiations. This study provides a phylogenetic perspective on the colonization history of the dolichopodid fauna in the islands. We generated a multi gene data set including representatives from 11 of the 14 endemic Hawaiian dolichopodid genera to examine the history of colonization to the islands, and analyzed it using Bayesian and maximum likelihood phylogenetic methods. We used a subset of the data that included *Conchopus* and the eight genera comprising the *Eurynogaster* complex to estimate the first phylogenetic hypothesis for these endemic groups, then used BEAST to estimate their age of arrival to the archipelago. The *Eurynogaster* complex, *Campsicnemus* and *Conchopus* are clearly the result of independent colonizations. The results strongly support the *Eurynogaster* complex as a monophyletic group, and also supports the monophyly of 4 of the 8 described genera within the complex (*Adachia, Arciellia, Uropachys* and *Eurynogaster*). Members of the family Dolichopodidae have been dispersing over vast distances to colonize the Hawaiian Archipelago for millions of years, leading to multiple independent evolutionary diversification events. The *Eurynogaster* complex arrived in the Hawaiian Archipelago 11.8 Ma, well before the arrival of *Campsicnemus* (4.5 Ma), and the even more recent *Conchopus* (1.8 Ma). Data presented here demonstrate that the Hawaiian Dolichopodidae both disperse and diversify easily, a rare combination that lays the groundwork for field studies on the reproductive isolating mechanisms and ecological partitioning of this group.

Keywords

Colonization history, Diptera, Divergence dating, Dolichopodidae, Evolutionary radiation, Hawaiian Islands, Long distance dispersal

INTRODUCTION

Long distance dispersal from continental populations is critical to the formation of the Hawaiian flora and fauna (Carson & Kaneshiro, 1976; O'Grady *et al*., 2009), but is considered rare. This infrequent arrival and establishment has led to a flora and fauna that is disharmonic relative to those on the continents that served as sources (Gillespie & Roderick, 2002). Recently, several studies (reviewed in Heaney, 2007; Bellemain & Ricklefs, 2008) have shown that reverse colonization from Hawaii to continental landmasses is observed in birds (Filardi & Moyle, 2005), plants (Harbaugh & Baldwin, 2007) and insects (O'Grady & DeSalle, 2008; Lapoint *et al*., 2014), suggesting that dispersal plays a larger role than previously thought and evidence is accumulating to indicate that movement to and from island systems is more common, especially at geological time scales (Heaney, 2007; Cibois *et al.,* 2011; Hembry *et al.,* 2013; Casquet *et al.,* 2014). If a lineage is vagile enough to repeatedly colonize an area, there is a reduced chance that it will generate the reproductive isolation necessary to speciate and then radiate. Furthermore, if radiation does occur in a lineage and there is subsequent colonization of the area by close relatives, ecological theory would predict that the existing niches would be pre-empted (Hardin, 1960), rendering a second radiation unsuccessful. Thus, clear examples where a lineage colonizes and radiates repeatedly and substantially are rare.

The Hawaiian-Emperor Archipelago has a long and dynamic geological history, well isolated in the central Pacific Ocean far from any continental mass. It has been forming by the motion of the Pacific plate over a stationary hotspot (Wilson, 1963), generating an island chain that is at least 80 million years old (Clague & Dalrymple, 1987; Duncan & Keller, 2004; Sharp & Clague, 2006). Island formation during this long history has been episodic, with some periods characterized by only few, low elevation atolls and reduced species diversity and other times with multiple high islands capable of supporting a diverse flora and fauna (Price & Clague, 1992). Many of the older islands that are now submerged or heavily eroded to small land masses once provided the kind of high island habitat we are familiar with in the contemporary high islands (Niihau, Kauai, Oahu, Molokai, Lanai, Maui, Kahoolawe and Hawaii), which have been forming very recently – only over the past five million years (Clague & Dalrymple, 1987; Clague, 1996: Figure 1). The current high islands provide a rich array of habitats, ranging from low to high elevation and very dry to very wet vegetation types.

All of the flora and fauna arrived to this dynamic archipelago via long distance dispersal in an unlikely sequence of events in which taxa both managed to land on the islands and persist once there (Zimmerman, 2001; Gillespie *et al.,* 2012). Recent phylogenetic studies of Hawaiian insects (Jordan *et al*. 2003; Mendelson & Shaw, 2005; Shapiro *et al.* 2006; Medeiros *et al.* 2009; Lapoint *et al*., 2011; Medeiros & Gillespie, 2011; O'Grady *et al.,* 2011; Haines & Rubinoff, 2012; Bennett & O'Grady, 2013; Bess *et al.,* 2013; Goodman & O'Grady, 2013; Lapoint *et al.,* 2013; Goodman *et al*., 2014; Haines *et al.,* 2014; Lapoint *et al*., 2014), have begun to reveal the history of colonization to and diversification within the Hawaiian Archipelago, and it appears that history is somewhat idiosyncratic. Some large groups, such as Hawaiian Drosophilidae with an estimated 1,000 species, colonized the Hawaiian Islands tens of millions of years ago. Other diverse groups, such as *Nesophrosyne* leafhoppers, with 72 described and over 100 undescribed species (Bennett & O'Grady, 2011), and *Campsicnemus* flies with about 200 species (Goodman *et al*., 2014) are young, dating to only a few million years. One thing is clear, however – very few endemic Hawaiian plant or animal families have successfully colonized the islands multiple times (e.g., Araliaceae; Plunkett et al. 1997; Costello & Motley 2001) and in no case have any of these generated two radiations of with more than 50 species each.

Flies in the family Dolichopodidae are remarkable in that they have colonized the Hawaiian Islands multiple times and still have managed to generate two of the largest evolutionary radiations within the Hawaiian Diptera: *Campsicnemus* Haliday, 183 spp. (Goodman *et al.*, 2014), and the *Eurynogaster* complex, 66 spp. in eight genera (Evenhuis, 2005). In addition, they also generated a small radiation of 6 spp., *Conchopus* Takagi. In addition to these three radiations, four other dolichopodid genera contain endemic species: *Asyndetus* (1), *Hydrophorus* (2), *Paraliancalus* (2), and *Thinophilus* (1) (Table 1). Thus, the family Dolichopodidae offers a unique opportunity to examine the timing and frequency of long distance colonization events in the founding of the endemic Hawaiian fauna. While recent molecular phylogenies of Dolichopodidae (e.g., Lim *et al*., 2010; Bernasconi *et al*., 2007) have sampled some of these genera (e.g., *Campsicnemus, Hydrophorus, Thinophilus*), uneven sampling between studies and the lack of Hawaiian exemplars makes it difficult to infer the colonization history in detail. Furthermore, while the biogeography of *Campsicnemus* has been studied (Goodman *et al.* 2014), the evolutionary relationships among the three radiations and the monophyly and biogeography of the large *Eurynogaster* complex have never been examined.

The primary goal of this paper is to address the colonization history of the endemic Hawaiian Dolichopodidae and assess how many colonization events have generated the present-day diversity within this lineage. We sampled 11 of the 14 genera with endemic Hawaiian species and included samples from across the family Dolichopodidae. We sequenced a combination of five mitochondrial and two nuclear genes and used these data to estimate colonization times using the Bayesian algorithm implemented in BEAST to infer the colonization history of this family in Hawaii. With our sampling we also provide the first molecular phylogenetic analysis of the *Eurynogaster* complex, with which we assess the monophyly of this lineages and its constituent genera.

MATERIALS & METHODS

Taxonomic sampling

Specimens were collected from 2004 to 2012 from sites across the Hawaiian Islands. The bulk of Hawaiian Dolichopodidae species are endemic to high elevation (900 – 1700 m.) rain forest habitats, and thus collecting efforts were concentrated in these areas. Other habitats (*e.g.,* coastal strand, dry and mesic forests, alpine zone) were also sampled, including rocky beaches, the only known habitat of *Conchopus, Thinophilus, Asyndetus* and *Hydrophorus.* We succeeded in collecting specimens from 11 of the 14 Hawaiian dolichopodid genera with endemic species known from the islands (*Campsicnemus, Conchopus, Thinophilus* and eight genera from the *Eurynogaster* complex, Table S1a in Appendix S1 in Supporting Information). Data from the Hawaiian *Campsicnemus* are included here from a previous study from our group, and are described in Appendix A from Goodman *et al.* (2014). Material was collected by general sweeping of vegetation and leaf litter, pan and Malaise trapping, and hand collecting. To evaluate monophyly of and diversity within the *Eurynogaster* complex, we included representatives from each of its eight constituent genera (Table 1; Evenhuis, 2005). No *Eurynogaster* complex lineages were omitted from our sampling. All material was preserved in 95% ethanol.

All material was identified using the most recent key to species in Tenorio (1969) and Evenhuis (2005). Descriptions of new species from within the *Eurynogaster* complex discovered as a result of this project are in preparation. Unpublished new species included in the study were given letters (e.g., *Eurynogaster* n. sp. A, B, C, etc.). In addition to the extracted specimens, whenever possible, a series of conspecifics from the same site were also preserved in 95% ethanol. Voucher material has been deposited in the Bernice Pauahi Bishop Museum (Honolulu). In addition, new sequences were generated for outgroup specimens from the non-endemic Dolichopodidae: five specimens of *Dolichopus exsul*, two specimens of *Chrysotus longipalpis*, and one specimen each of *Condylostylus sp.* and *Tachytrechus angustipennis*. Finally, sequences from *Hercostomus indonesianus* were also downloaded from GenBank to include in the outgroup (see Table S1a in Appendix S1). Access and collection permits were granted by the State of Hawaii Department of Land and Natural Resources, the National Park Service (Hawaii Volcanoes and Haleakala National Parks), Maui Land and Pineapple, East Maui Irrigation, Parker Ranch, and The Nature Conservancy of Hawaii (Appendix S4).

Phylogenetic Analysis

Relationships within Dolichopodidae and colonization of the Hawaiian Islands

To address the question of whether the endemic dolichopodid fauna, including the three major radiations (*Campsicnemus*, the *Eurynogaster* complex and *Conchopus*) is the result of a single or multiple colonizations, new sequences were generated for the samples described above (and in Table S1a in Appendix S1) and were combined with the entire data matrix generated from the Goodman *et al*. (2014) *Campsicnemus* study. Extraction, amplification, sequencing, editing and alignment followed the same protocols described in Goodman *et al.* (2014). Loci used are described in Table 2. Eleven of the 14 dolichopodid genera with endemic species are represented. This yielded an alignment, referred to as dataset A, containing 183 individuals and seven loci containing 4763 base pairs that was used to assess deep temporal and biogeographic patterns within Hawaiian Dolichopodidae.

Phylogenetic Relationships within the Eurynogaster Complex

To assess the monophyly of the *Eurynogaster* complex and its component genera, seventeen described, four new, and five possible new species (labeled as “sp. nr.”) were included in the phylogenetic analysis (Table 1). This matrix was designated as dataset B. Phylogenetic analyses were performed on a data set consisting of 57 individuals (see Table S1 in Appendix S1) and seven loci containing 5,908 base pairs. Analyses were conducted on each gene individually using maximum likelihood (ML, see below). Dataset B was used to assess biogeographic patterns within the *Eurynogaster* complex of genera.

Datasets A and B were both analysed using ML and Bayesian inference (BI) optimality criteria. For each of the ML and the BI analyses, the optimum partitioning schemes were calculated in PartitionFinder (Lanfear *et al.*, 2012). The optimal partitioning scheme for the combined analysis of Hawaiian Dolichopodidae (dataset A), was calculated from 18 original data partitions (16S, 12S and 1st, 2nd, and 3rd codon positions for COI, COII, ND2, CAD, EF1α and one CAD intron region). Partitioning was calculated for the *Eurynogaster* complex dataset (dataset B) from 20 original data partitions (16S, 12S and 1st, 2nd, and 3rd codon positions for COI, COII, ND2, CAD, EF1αA and EF1αB, intron regions for CAD, EF1αA, EF1αB and ND2) and selected using Bayesian Information Criterion (Table S2b in Appendix 2). For both datasets, in the BI analyses, the best-fit model of sequence evolution for each data partition was also selected using PartitionFinder (Table S2b in Appendix S2: Lanfear *et al.*, 2012). Selection of models and partitions proceeded as described above and these are reported in Table S1b in Appendix S1. The ML analyses were performed on individual genes and on the concatenated data sets in RAxML 3.7.2 (Stamatakis, 2006) on CIPRES (Miller *et al.*, 2010) under the GTR GAMMA model with 1,000 bootstrap replicates and a final search for the best tree. The BI analyses were performed on the concatenated data sets using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) on CIPRES (Miller *et al.*, 2010), with each analyses run for 30,000,000 generations with 2 independent runs each.

MCMC convergence diagnostics: For the BI analyses, stationarity was assessed within and convergence among each of the runs using several complimentary approaches: (1) convergence metrics provided by MrBayes 3.1.2 were checked (Huelsenbeck & Ronquist, 2001) to ensure that the maximum standard deviation of split frequencies of any of the runs was under 0.05 and that the potential scale reduction factor for all parameters approached 1.0, and (2) the log-likelihood values for each run were plotted, the Effective Sample Sizes (ESS) were checked to ensure there were an adequate number of independent samples, and the posterior distributions of all parameters were examined using Tracer v.1.72 (Rambaut & Drummond, 2012). Tracer v.1.72 was also used to determine the burn-in phase by assessing each run's plot of log-likelihood values over generations –stationarity was assumed to have been reached when the log likelihood values reached a stable plateau. Finally, a 50% majority rule consensus trees was created from the resulting post burn-in trees.

Divergence Time Estimation in the *Eurynogaster* complex:

To estimate the age of the *Eurynogaster* complex lineage, divergence time estimation was performed on dataset B using a Bayesian relaxed-clock method implemented in BEAST 1.7.5 (Drummond *et al.*, 2012) on CIPRES (www.phylo.org: Miller *et al.*, 2010). The age of the *Eurynogaster* complex is unknown as representatives of the genus are not known outside of

Hawaii and biota in the Hawaiian Islands does not fossilize well. There is a fossil available for one genus that has an endemic species in the Hawaiian Islands (*Thinophilus* Wahlberg: subfamily Hydrophorinae), but the wide range in ages of the fossils (Baltic amber - Eocene/Oligocene; ca. 35–60 mya) compared with the very young ages of the islands make them unsuitable for use in this analysis. Instead, we used three biogeographic calibrations based on the island ages of Kauai, Maui and Hawaii (see Table S2a, Figure S2a in Supporting Information). We also ran two alternate analyses for comparison based on evolutionary rates, described in Appendix S2.

We selected two well-supported nodes for calibration from within a lineage of the genus *Eurynogaster* that exhibit a clear progression from older to younger islands (Oahu to Maui to Hawaii). We also performed a maximum likelihood ancestral state reconstruction in MESQUITE v.2.7.2 (Maddison & Maddison, 2009) to assign ancestral areas to all nodes in the phylogeny. We then selected a third well-supported node for calibration with a clear ancestral range reconstruction to the oldest island of Kauai. All three nodes were calibrated with island dates from Carson & Clague (1995) (Table S2a, Figure S2a). While island calibrations have been widely used for the estimation of divergence times in Hawaiian lineages (*e.g.,* (Rubinoff & Schmitz, 2010; Lerner *et al.*, 2011), it is plausible that divergence among populations occurred prior to island emergence and was thus unrelated, or that it occurred well after the emergence of the younger island (Heads, 2005). Standard deviations were chosen to accommodate some of this uncertainty, including a biologically relevant timeframe during which habitat was likely available on the islands, and the fact that the insects may have colonized the islands well before or after they reached their peak heights (Table S2a).

Divergence time estimation was performed on dataset B described above. The same seven gene concatenated data set (COI, COII, ND2, 12S, 16S, EF1α and CAD) was analysed in each of the analyses described here and in Appendix D. Partitions and the best fit models of evolution for each partition were selected using BIC in PARTITIONFINDER (Lanfear *et al.*, 2012). Initial analyses indicated that these models overparameterized the data in that the ESS values were extremely low for some parameters, despite being run with very long chains (beast-users Google group discussions). For the final runs, all GTR models were changed to HKY (Table C.2) and ESS increased significantly while divergence times and tree topology did not change. Base frequencies were estimated from the data. The partitioning scheme in the divergence rate analyses differed only slightly from the island calibration analyses in that COI was assigned its own partition (Table S1b). Site and clock models were unlinked and all partitions were analysed using an uncorrelated lognormal relaxed clock except for the partition comprised of CAD (positions 1 & 2) and the EF1α intron, for which a strict clock could not be rejected and was thus applied. The tree-shape prior was linked across partitions and the tree-shape prior was specified as a Yule Process. The xml file was hand edited to include a starting tree, generated using maximum likelihood in RAxML 3.7.2 (Stamatakis, 2006). Two independent MCMC searches were conducted, each running for 50 million generations and sampled every 1000 generations. The number of generations was selected to generate ESS values greater than 200 for each of the parameters (Drummond *et al.*, 2007). Convergence was assessed using TRACER v. 1.7.5 and trees were summarized to one Maximum Clade Credibility (MCC) tree using TREE ANNOTATOR v. 1.7.5 after removing a burn-in phase.

RESULTS & DISCUSSION

Phylogenetic relationships within the endemic Hawaiian Dolichopodidae

The family Dolichopodidae includes more than 6,800 described species (Yang *et al.*, 2006) in 232 genera worldwide (Pape & Thompson, 2013). A total of 29 genera are found in the Hawaiian Islands. Of these, fifteen have been introduced in the past 150 years, most likely through human activity, while the remaining fourteen genera present in the archipelago are known to contain endemic Hawaiian taxa (Table 1). The relationships between *Campsicnemus* and the *Eurynogaster* complex and the colonization history of these genera have remained an open question, largely due to the difficulty of placing both in a subfamilial context. While *Campsicnemus* is clearly placed in the subfamily Sympycninae, the placement of the *Eurynogaster* complex has been more difficult to ascertain (see Appendix S3 in Supporting Information). Individual taxa have previously been described as members of the subfamilies Sympycninae, Hydrophorinae, and Thinophilinae. Hardy & Kohn (1964) considered *Eurynogaster* and associated genera as part of the Sympycninae (see Figure S3a in Appendix 3). Later, Evenhuis (2005) transferred the entire *Eurynogaster* complex to the Hydrophorinae. If the current taxonomy placing these lineages in two separate subfamilies is correct, *Campsicnemus* and the *Eurynogaster* complex represent independent colonizations to the Hawaiian Islands.

Molecular evidence demonstrates that the endemic Hawaiian dolichopodid fauna is clearly the result of multiple colonizations to the archipelago (Figure 2, Figures S1a & S1b in Appendix S1). Several key nodes are well supported and allow us to infer the history of the Hawaiian Dolichopodidae. *Conchopus* (posterior probability (PP)=1, bootstrap (BS)=100: node A, Figure 2), the *Eurynogaster* complex (PP=1, BS=100: node B, Figure 2), and *Campsicnemus* (PP=1, BS=98: node C, Figure 2) are each strongly supported as monophyletic with respect to other dolichopodid genera. Another key node that is strongly supported in both analyses (PP=1, BS=99: node D, Figure 2) is the large clade that includes *Campsicnemus* and a number of non-Hawaiian genera in the subfamily Sympycninae (*e.g., Sympycnus*, *Teuchophorus*) and does not include the *Eurynogaster* complex. This demonstrates that there were at least three colonizations to Hawaii by the family Dolichopodidae, one each by the three radiations: *Campsicnemus*, the *Eurynogaster* complex, and *Conchopus.*

There is little support for the placement of *Thinophilus*, so its history of arrival to Hawaii remains enigmatic (Figure 2, Figures S1a & S1b in Appendix S1). This genus is known primarily from the Indo-Pacific, with one species each known from the Galapagos Islands and the Hawaiian Islands. Previously it has only been collected from rocky, wet sand on the south shores of Oahu (Carlton & Eldredge, 2009). The specimen included in this study represents the first record from Hawaii Island and suggests that directed collecting on the south shores of Maui and Kauai may turn up additional populations.

Three genera that contain endemic taxa were not included in this study because they are difficult to collect and we did not recover them in our sampling. While their placement must await future work, their omission here does not change the result that the Hawaiian Islands have been colonized multiple times. An additional issue is that support at many nodes in this phylogeny is poor, owing partially to the large divergences between the subfamilies and the incomplete taxon sampling with this enormous family. These issues are also seen in previously published phylogenetic studies of dolichopodid relationships (Lim *et al.,* 2010; Bernasconi *et al.*, 2007). The lack of support and long branches across most of the rest of this phylogeny preclude identifying the specific sister lineages to the Hawaiian taxa (Figure 2).

Phylogenetic Relationships in the *Eurynogaster* Complex

The *Eurynogaster* complex, with 66 described species (Yang *et al.,* 2006) and about a dozen awaiting description, comprises the fourth most species-rich radiation of Hawaiian flies, after the Drosophilidae, *Campsicnemus,* and *Lispocephala* (Muscidae). Although the *Eurynogaster* complex is one of the largest radiations of Diptera in Hawaii, phylogenetic relationships in this group have never been studied. This collection of genera are hypothesized to have derived from a single colonization to the Hawaiian Archipelago (Evenhuis, 2005). Little is known about the biology of these species, but collecting observations suggest that species found on the forest floor and on vegetation tend to be dull coloured, while species found in wet habitat, along seeps, streams and on wet banks tend to have shiny metallic thoraces and/or abdomens.

Molecular phylogenetic results presented here show strong support for Evenhuis's (2005) hypothesis of a monophyletic complex of related genera (PP=1, BS=100: Figure 2), as well as strong support for several of the genera within this radiation. We focused on the smaller dataset (dataset B) to address phylogenetic and biogeographic questions within the *Eurynogaster* genus complex. Analyses of individual genes are presented in Figures S1e-S1l in Appendix S1, and final data partitions and evolutionary models are reported in Table S1b in Appendix S1. Tree topologies generated using ML and BI approaches of the concatenated dataset B were very similar; at well-supported nodes, they are identical (Figures S1c & S1d).

In Figure 1, the maximum clade credibility tree from the Bayesian analysis performed in BEAST is used to display the patterns within the *Eurynogaster* complex, and the following PP and BS supports are from the BI performed in MrBayes and ML analysis performed in RAxML (shown in Figures S1c & S1d). The *Eurynogaster* complex is split into two clades: Clade A (*Adachia* + *Elmoia* + *Sigmatineurum* + *Major*, PP=1, BS=100) and Clade B (*Sweziella* + *Arciellia* + *Uropachys* + *Eurynogaster*, PP=1, BS=100). Current sampling indicates that the genus *Adachia* is monophyletic (PP=1, BS=100) and sister to a well-supported clade (PP=1, BS=100) composed of the genera *Elmoia, Sigmatineurum* and *Major* (ESM Clade). Sampling within the ESM clade is not extensive, with only a single representative each of *Sigmatineurum* and *Major*. Two representatives of the genus *Elmoia* were sampled and our results indicate that this genus is paraphyletic with respect to *Sigmatineurum* and *Major*. Denser sampling with the ESM clade will be necessary to resolve the placement of the *Elmoia* taxa.

Clade B includes the large genus *Eurynogaster*, along with *Arciellia*, *Uropachys* and *Sweziella*. *Sweziella*, represented by *S. tergoprolixa* from Maui, is the basal lineage within clade B and sister to the lineage formed by *Arciellia, Uropachys* and *Eurynogaster* (PP=1, BS=91: Figure 2). Current sampling indicates that the genus *Arciellia* and *Uropachys* are each monophyletic (PP = 1, BS = 100 and PP=1, BS=100, respectively) and sister to one another (PP=1, BS=100). *Eurynogaster* is strongly supported as monophyletic (PP=1, BS=100). This genus is confusing taxonomically and is in need of revision. There are three undescribed *Eurynogaster* species that were discovered as part of this work, *E.* n. spp. A–C. There are also a number of taxa that, while morphologically similar to named taxa, show significant sequence divergence from the described species. This sometimes corresponds to samples having been taken from different islands. For example, *E. maculata* from Oahu is quite different from the *E.* sp. nr. *maculata* samples collected from Maui (*E.* sp. nr. *maculata 141*) and Hawaii Island (*E.* sp. nr. *maculata 115* and *126*) – they are 3.9% and 3.6% divergent at COI, respectively. Furthermore, one exemplar of *E. maculata* from Maui is quite similar to *E.* sp. nr. *maculata 141* – it is identical at COI – suggesting that cryptic species may exist within the concept of what we currently recognize as *E. maculata*. This phenomenon is common in large evolutionary radiations in Hawaii (*e.g.,* Bennett & O'Grady, 2011). Another species we sampled, *E.*

cilifemorata, also seems to be a complex of species sampled from Maui and Oahu. Additional sampling within *Eurynogaster*, as well as thorough taxonomic revisions of the genera within this complex, will be necessary to better delineate species within this rapidly evolving clade.

Finally, four new species within the *Eurynogaster* complex were discovered as a result of this project, three within *Eurynogaster* and one within *Sigmatineurum*. An additional five possible new species (*Adachia* - 1 species; *Eurynogaster* - 4 species) were identified (labeled as "sp. nr.") and are in the process of examination to confirm their taxonomic status.

Arrival times and biogeography

We estimate that the *Eurynogaster* complex arrived in the Hawaiian Archipelago 11.83 (9.08-15.04) Ma, approximately within the timeframe that the Northwest Hawaiian Islands of La Perouse, Necker, and Gardner were providing substantial high island habitat (Price & Clague, 2002). This ancient lineage arrived well before the formation of the current high islands about 5 Ma and the arrival of *Campsicnemus*, which is estimated to have occurred approximately 4.6 Ma (Goodman *et al*., 2014). Early diversification into five of the eight contemporary genera took place in the older, now eroded, northwest Hawaiian Islands, and five colonizations of these ancestral lineages into the current main (high) islands are needed to explain the contemporary patterns of diversity. All of the diversification within the crown groups has occurred within the past 5 million years (Myr), the timeframe of the current high islands. The most speciose lineage within the *Eurynogaster* complex, the genus *Eurynogaster*, began diversifying approximately 2.6 (95% HPD: 1.94–3.26) Ma, about the time Oahu and Maui Nui were forming. We estimate that the small endemic dolichopodid genus *Conchopus* arrived quite recently – 1.77 (95% HPD: 1.09–2.6) Ma (Figure 1).

Within the *Eurynogaster* complex, a number of classic biogeographic patterns are evident, some of which are significantly different from what is observed in other large radiations. First, a progression rule pattern (Hennig, 1966) is common in hotspot archipelagos where islands appear along a chronosequence. The typical progression rule pattern seen in Hawaii occurs when the most basally branching taxon is present on Kauai, the oldest island, with more recently branching taxa present on the progressively younger islands of Oahu, Molokai, Maui and Hawaii (Wagner & Funk, 1995). While the progression rule is commonly observed in both the Hawaiian *Drosophila* (Bonacum *et al.,* 2005) and *Campsicnemus* (Goodman *et al*., 2014) lineages, it is less prevalent in *Eurynogaster*. Only a single lineage of the genus *Eurynogaster* shows a clear progression from Oahu to Maui to Hawaii (Figure 1).

Another phenomenon observed in Hawaiian lineages is within-island diversification, where species break up to diversify into new populations and eventually sibling species on the same island. This has been thought to be an uncommon occurrence, in part because it is fairly uncommon across the historically best-studied group in the islands, the Hawaiian *Drosophila* – for whom diversification primarily occurs following inter-island dispersal. However, even within this iconic group, there are examples and it has been very well studied in the sympatric sibling pair *D. silvestris* and *D. heteroneura* (Carson, 1982; DeSalle *et al.,* 1987; Price & Boake, 1995). Newer examples are now accumulating across taxonomic groups (*e.g.*: Goodman *et al*. 2012; Eldon *et al*. 2013; Bennett & O'Grady 2013; Liebherr, 2015), exposing how variable a process diversification can be, and how dependent it is on the dispersal capabilities of the groups studied (Price & Wagner, 2004). The *Eurynogaster* complex shows at least five clear instances of within-island diversification. *Uropachys* is a genus of 6 species only known from Kauai. Three *Uropachys* species were sampled for this study and are strongly supported as a monophyletic clade, indicating they diversified there. This pattern is also observed in *Adachia*, where *A. hispida* and *A. apicenigra* have both formed on Hawaii, and in several clades of the genus *Eurynogaster* where diversification has occurred on Oahu and Hawaii. Within-island diversification is also observed in some lineages of the other major dolichopodid radiation in Hawaii, the *Campsicnemus* (Goodman *et al*., 2014).

Colonization of and diversification within the Hawaiian Islands

It is clear that the endemic Dolichopodidae of Hawaii arrived to the archipelago in at least three successful colonization to radiation sequences over the last 12 Myr (*Eurynogaster* complex, 11.8 Ma; *Campsicnemus*, 4.6 Ma; *Conchopus*, 1.8 Ma) –demonstrating that dispersal to and establishment within this remote island group is more common than has been documented in other groups. This is fascinating because it means that three separate radiations occurred despite the excellent dispersal capabilities of these animals. In order to multiply into radiations, they must have been able to generate reproductive isolation rapidly enough to overcome gene flow from their highly vagile conspecifics. Members of this family are known to have complicated courtship behavior (Zimmer *et al.* 2003). Though this has never been studied in the Hawaiian fauna, it may be a contributing factor to the development of reproductive isolation as has been shown with the Hawaiian *Drosophilidae* (Kaneshiro, 1976; Price & Boake, 1995)*, Laupala* (Grace & Shaw, 2011) and *Nesosydne* (Goodman *et al.* 2015), and suggests fruitful research directions. There seems to be no correlation between the age of colonization and the diversity of each lineage.

MacArthur & Wilson (1967) stated that “an island is closed to a particular species when the species is excluded … by competitors already in residence…”. The Hawaiian Islands were clearly not closed to dolichopodid flies that arrived after the first wave 12 Ma. This suggests that, at the arrival of each new lineage, there was still plenty of ecological opportunity available or these insects are ecologically labile and able to adapt easily when faced with niches already occupied by competitors. Both statements may be true.

Very little is known about the ecology of the Dolichopodidae in Hawaii, but they are known to be predatory from observations elsewhere in the world (Ulrich, 2005). For the *Eurynogaster* complex (which only occur in Hawaii), there is only a single published account in the literature that includes ecological observations (Williams, 1938). Despite the dearth of ecological data available, we have some evidence to support the idea that the dolichopodids seem to adapt easily. In our 2014 study, we used morphological colouring together with field observations to infer that the Hawaiian *Campsicnemus* have rapidly diversified into three ecological types: (1) brown, low vegetation and litter dwellers, (2) black water skaters and (3) yellow canopy dwellers. Two of these are not known outside the Pacific, and one is not known outside Hawaii (Goodman *et al.* 2014). The Hawaiian *Conchopus* may have also undergone a shift in ecological type. This lineage can be traced back to East Asia (Takagi, 1965), where they are known primarily from barnacle colonies in the marine tidal zone, living in the interstices or in nearby cracks in the rocks and feeding on tiny invertebrates (Sunose & Sato, 1994). There are no native barnacles in the Hawaiian Islands, and *Conchopus* there are known from *puka* (holes) in beach rocks deriving from volcanic flows. Once established in this habitat, they radiated into six known species.

Prevailing dogma among Hawaiian evolutionary biologists in the past 30 years has been that colonization events to the archipelago are rare and colonization within the islands follow a few well-defined patterns, such as the progression rule (Wagner & Funk 1995). Recent molecular phylogenetic studies are beginning to overturn these overly simplified notions (Heaney 2007; Bellemain & Rickliffs 2008), finding that colonization and diversification are based on a combination of factors. These include characters linked to the dispersal and adaptability of the lineage in question and the ecological and environmental context of the islands when that lineage arrives. The current study highlighting the multiple colonizations that Dolicohpodidae have undergone in the past and the specific patterns of diversification within the *Eurynogaster* complex further demonstrate that there are no simple "rules" and each colonization event should be considered an independent event. The Hawaiian Dolichopodidae are an intriguing example of repeated, overlapping evolutionary radiations, ripe for field studies that can begin to untangle their propensity to speciate and ecological lability.

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REFERENCES

Bellemain, E. & Ricklefs, R.E. (2008) Are islands the end of the colonization road? *Trends in Ecology and Evolution*, **23**, 461-468.

Bennett, G.M. & O'Grady, P.M. (2011) Review of the native Hawaiian leafhopper genus *Nesophrosyne* (Hemiptera: Cicadellidae: Deltocephalinae) with descriptions of eight new species associated with *Broussaisia arguta*. *Zootaxa* **2805**, 1-25.

Bennett, G.M. & O'Grady, P.M. (2013) Historical biogeography and ecological opportunity in the adaptive radiation of native Hawaiian leafhoppers (Cicadellidae: *Nesophrosyne*). *Journal of Biogeography,* **40(8)**, 1512-1523.

Bernasconi, M.V., Pollet, M. & Ward, P.I. (2007) Molecular systematics of Dolichopodidae (Diptera) inferred from COI and 12S rDNA gene sequences based on European exemplars. *Invertebrate Systematics*, **21**, 453-470.

Bess, E.C., Catanach, T.A. & Johnson, K.P. (2013) The importance of molecular dating analyses for inferring Hawaiian biogeographical history: a case study with bark lice (Psocidae: *Ptycta*). *Journal of Biogeography*, **41**, 158-167.

Bonacum, J., DeSalle, R., O'Grady, P.M., Olivera, D.S.C.G., Wintermute, J. & Zilversmit, M. (2001) New nuclear and mitochondrial primers for systematics and comparative genomics in Drosophilidae. *Drosophila Information Service*, **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. Molecular Phylogenetics and Evolution*. **37**, 73–82.

Carlton, J.T. & L.G. Eldredge (2009). Marine Bioinvasions of Hawaiʻi: The Introduced and Cryptogenic Marine and Estuarine Animals and Plants of the Hawaiian Archipelago. *Bishop Museum Bulletin in Cultural and Environmental Studies* 4, pp. 202. Bernice P. Bishop Museum, Honolulu.

Carson, H. L. 1982. Evolution of *Drosophila* on the newer Hawaiian volcanoes. *Heredity,* **48**, 3–25.

Carson, H.L. & Kaneshiro, K.Y. (1976) Drosophila of Hawaii - systematics and ecological genetics. *Annual Review of Ecology and Systematics*, **7**, 311-345.

Carson, H.L. & Clague, D.A. (1995) Geology and biogeography of the Hawaiian Islands. *Hawaiian biogeography: evolution on a hotspot archipelago* (ed. by W.L. Wagner and V.A. Funk), pp. 14-29. Smithsonian Institution Press, Washington, D.C.

Casquet, J., Bourgeois, Y.X., Cruaud, C., Gavory, F., Gillespie, R.G. & Thebaud, C. (2015) Community assembly on remote islands: a comparison of Hawaiian and Mascarene spiders. *Journal of Biogeography*, **42**, 39-50.

Cibois, A., Beadell, J.S., Graves, G.R., Pasquet, E., Slikas, B., Sonsthagen, S.A., Thibault, J., & Fleischer, R.C. (2011) Charting the course of reed-warblers across the Pacific islands. *Journal of Biogeography*, **38**, 1963-1975.

Clague, D.A. (1996) The growth and subsidence of the Hawaiian-Emporer volcanic chain. *The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns and processes* (ed. by A. Keast and S.E. Miller), pp. 35-50. SPB Academic Publishing Amsterdam.

Clague, D.A. & Dalrymple, G.B. (1987) The Hawaiian-Emperor volcanic chain: part 1. Geologic evolution. *United States Geological Survey Professional Paper*, **1350**, 5-54.

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization and genetic code. *Journal of Molecular Evolution*, **22**, 252-271.

Collins, K.P. & Wiegmann, B.M. (2002) Phylogenetic relationships and placement of the Empidoidea (Diptera: Brachycera) based on 28S rDNA and EF-1alpha sequences. *Insect Systematics and Evolution*, **33**, 421-444.

Costello, A. & Motley, T. J. (2001) Molecular systematics of *Tetraplasandra, Munroidendron* and *Reynoldsia sandwicensis* (Araliaceae) and the evolution of superior ovaries in *Tetraplasandra*. *Edinburgh Journal of Botany,* **58**, 229–242.

DeSalle, R. (1992) The origin and possible time of divergence of the Hawaiian Drosophilidae - evidence from DNA-sequences. *Molecular Biology and Evolution*, **9**, 905.

DeSalle, R., Freedman, R., Prager, E.M. & Wilson, A.C. (1987) Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular Evolution*, **26**, 157-164.

Drummond, A.J., Ho, S.Y.W., Rawlence, N. & Rambaut, A. (2007) A Rough Guide to BEAST 1.4 (Program Manual). In:

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969-1973.

Duncan, R.A. & Keller, R.A. (2004) Radiometric ages for basement rocks from the Emperor Seamounts, ODP Leg 197. *Geochemistry Geophysics Geosystems* **5**, Q08L03, doi:10.1029/2004GC000704.

Eldon, J., Price, J.P., Magnacca, K. & Price, D.K. (2013). Patterns and processes in complex landscapes: testing alternative biogeographical hypotheses through intergrated analysis of phylogeography and community ecology in Hawaii. *Molecular Ecology*. **22**, 3613-3628.

Evenhuis, N.L. (2005) A review of the genera comprising species of the genus *Eurynogaster sensu* Hardy & Kohn, 1964 in Hawaii (Diptera: Dolichopodidae). *Zootaxa*, **1017**, 39-60.

Filardi, C.E. & Moyle, R.G. (2005) Single origin of a pan-Pacific bird group and upstream colonization of Australasia. *Nature*, **438**, 216-219.

Gillespie R.G. & Roderick G.K. (2002) Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology* **47**, 595-632.

Gillespie, R.G., Baldwin, B.G., Waters, J.M., Fraser, C., Nikula, R., & Roderick, G.K. (2012) Long-distance dispersal – a framework for hypothesis testing. *Trends in Ecology & Evolution*, **27(1)**, 47-56.

Goodman, K.R., Welter, S.C. & Roderick, G.K. (2012) Genetic divergence is decoupled from ecological diversification in the Hawaiian *Nesosydne* planthoppers. *Evolution*. **66**, 2798–2813.

Goodman, K.R. & O'Grady, P.M. (2013) Molecular phylogeny and biogeography of the Hawaiian craneflies *Dicranomyia* (Diptera: Limoniidae). *PLoS One*, **8(9)**:e73019.

Goodman, K.R., Evenhuis, N.L., Bartosoya-Sojkova, P. & O'Grady, P.M. (2014) Diversification in Hawaiian long-legged flies (Diptera: Dolichopodidae: *Campsicnemus*): Biogeographic isolation and ecological adaptation. *Molecular Phylogenetics and Evolution*, **81**, 232-241.

Goodman, K.R., Kelley, J.P., Welter, S.C., Roderick, G.K. & Elias, D.O. (2015). Rapid diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera: Delphacidae): the relative role of neutral and selective forces. *Journal of Evolutionary Biology*. **28(2)**, 415-427.

Grace, J. L. and Shaw, K. L. (2011) Coevolution of male signal and female preference during early lineage divergence of the Hawaiian cricket, *Laupala cerasina*. *Evolution.* **65**, 2184-2196.
Haines, W.P. & D. Rubinoff (2012) Molecular phylogenetics of the moth genus *Omiodes* Guenee (Crambidae: Spilomelinae), and the origins of the Hawaiian lineage. *Molecular Phylogenetics and Evolution*, **65**, 305-316.
Haines, W.P., Schmitz, P., Rubinoff, D. (2014) Ancient diversification of Hyposmocoma moths in Hawaii. *Naure Communications*, **5**, 3502, doi: 10.1038/ncomms4502.
Harbaugh, D. T. & Baldwin, B. G. (2007) Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceace): repeated dispersals throughout the Pacific. *American Journal of Botany*, **94**, 1028–1040.
Hardin, J. (1960) The Competitive Exclusion Principle. *Science*. **131**, 1292-1297.
Hardy, D.E. & Kohn, M.A. (1964) Dolichopodidae. *Insects of Hawaii*. University of Hawaii Press, Honolulu, HI, **vol 11**, 1-256.
Heads, M. (2005) Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics*, **21**, 62-78.
Heany, L.R. (2007) Is a new paradigm emerging for oceanic island biogeography? *Journal of Biogeography*, **34**, 753-757.
Hembry D.H., Kawakita A., Gurr N.E., Schmaedick M.A., Baldwin B.G., Gillespie R.G. (2013) Non-congruent colonizations and diversification in a coevolving pollination mutualism on oceanic islands. *Proceedings of the Royal Society B*, **280**, 20130361.
Hennig, W. (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana, Illinois.
Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. **17**, 754-5.
Jordan, S., Simon, C. & Polhemus, D. (2003) Molecular Systematics and Adaptive Radiation of Hawaii's Endemic Damselfly Genus *Megalagrion* (Odonata: Coenagrionidae). *Systematic Biology*. **52(1)**, 89-109.
Kaneshiro, K.Y. (1976) Ethological Isolation and Phylogeny in the Planitibia Subgroup of Hawaiian Drosophila. *Evolution*. **30(4)**, 740-745.
Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695-1701.
Lapoint, R.T., Gidaya, A., & O'Grady, P.M. (2011) Phylogenetic relationships in the spoon tarsus subgroup of Hawaiian *Drosophila*; Conflict and concordance between gene trees. *Molecular Phylogenetics and Evolution*, **58**, 492-501.
Lapoint, R.T., O'Grady, P.M. & Whiteman, N.K., (2013) Diversification and Dispersal of the Hawaiian Drosophilidae: the evolution of *Scaptomyza*. *Molecular Phylogenetics and Evolution*, **69(1)**, 95-108.
Lapoint, R.T., Magnacca, K.N., and O'Grady, P.M. (2014) Phylogenetics of the antopocerus-modified tarsus clade of Hawaiian *Drosophila*: Diversification across the Hawaiian Islands. *PLoS ONE,* **9(11)**, e113227.
Liebherr, J.K. (2015) The *Mecyclothorax* beetles (Coleoptera, Carabidae, Moriomorphini) of Haleakala, Maui: Keystone of a hyperdiverse Hawaiian radiation. *Zookeys*. **544**, 1-407.
Lim, G.S., Hwang, W.S., Kutty, S.N., Meier, R. & Grootaert, P. (2010) Mitochondrial and nuclear markers support the monophyly of Dolichopodidae and suggest a rapid origin of the subfamilies (Diptera: Empidoidea). *Systematic Entomology*, **35**, 59-70.

Lerner, H.R.L., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. (2011) Multilocus Resolution of Phylogeny and Timescale in the Extant Adaptive Radiation of Hawaiian Honeycreepers. *Current Biology*, **21**, 1-7.

MacArthur, R.H. & Wilson, E.O. (1967). The Theory of Island Biogeography. pp. 203. Princeton University Press, Princeton.

Maddison, W.P. & Maddison, D.R. (2009) *Mesquite: a modular system for evolutionary analysis*. Version 2.72. http://mesquiteproject.org.

Medeiros, M.J., Davis, D., Howarth, F.G. & Gillespie, R.G. (2009) Evolution of cave living in Hawaiian *Schrankia* (Lepidoptera: Noctuidae) with description of a remarkable new cave species. *Zoological Journal of the Linnean Society*. **156**, 114-139.

Medeiros, M.J. & Gillespie, R.G. (2011) Biogeography and the evolution of flightlessness in a radiation of Hawaiian moths (Xyloryctidae: *Thyrocopa*). *Journal of Biogeography*. **38**, 101-111.

Mendelson, T.C. & Shaw, K.L. (2005). Sexual Behavior: rapid speciation in an arthropod. *Nature,* **433**, 375-376.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010* (ed by, pp. 1-8. New Orleans, LA.

Moulton, J.K. & Weigmann, B.M. (2004) Evolution and phylogenetic utility of cad (rudimentary) amond Mesozoic-aged Eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution*, **31**, 363-378.

Nishida, G.N. (2002) Hawaiian terrestrial arthropod checklist. Fourth edition. *Bishop Museum Technical Report* **22**, 1-313.

O'Grady, P.M. and DeSalle, R. (2008) Out of Hawaii: The biogeographic history of the genus *Scaptomyza* (Diptera: Drosophilidae). *Biology Letters* **4(2)**, 195-199.

O'Grady, P.M., Magnacca, K.N. & Lapoint, R.T. (2009) Drosophila. *Encyclopedia of Islands* (ed. by R.G. Gillespie and D.A. Clague), pp. 232-235. University of California Press, Berkeley, CA.

O'Grady, P.M., Lapoint, R.T., Bonacum, J., Lasola, J., Owen, E., Wu, Y., & DeSalle, R. (2011) Phylogenetic and ecological relationships of the Hawaiian *Drosophila* inferred by mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution.* **58**, 244-256.

Pape, T. & Thompson, F.C. (editors). (2013) Systema Dipterorum. Version 1.5. *Available at: http://www.diptera.org/.* [Last accessed 16 October 2014].

Plunkett, G. M., Soltis, D. E. & Soltis, P. S. (1997) Clarification of the relationship between Apiaceae and Araliaceae based on matK and rbcL sequence data. *American Journal of Botany*. **84**, 565–580.

Price, D. K. & Boake, C. R. B. (1995). Behavioral reproductive isolation in *Drosophila silvestris, D. heteroneura* and their F1 hybrids (Diptera: Drosophilidae). *Journal of Insect Behaviour* **8**, 595–616.

Price, J.P. & Clague, D.A. (2002) How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London, B*, **269**, 2429-2435.

Price, J.P. & Wagner, W.L. (2004) Speciation in Hawaiian angiosperm lineages: cause, consequence, and mode. *Evolution*. **58**, 2185-2200.

Rambaut, A. & Drummond, A.J. (2012) *Tracer v1.7.2,* obtained from the "Workshop on Molecular Evolution", August 2011. *Tracer v1.5* is available from http://beastbioedacuk/Tracer.
Rubinoff, D. & Schmitz, P. (2010) Multiple aquatic invations by an endemic, terrestrial hawaiian moth radiation. *Proceedings of the National Academy of Sciences*, **107**, 5903-5906.
Shapiro, L.H., Strazanac, J.S. & Roderick, G.K. (2006) Molecular phylogeny of Banza (Orthoptera: Tettigoniidae), the endemic katydids of the Hawaiian Archipelago. *Molecular Phylogenetics and Evolution*. **41**, 53-63.
Sharp, W.D. & Clague, D.A. (2006) 50-Ma Initiation of Hawaiian-Emperor Bend Records Major Change in Pacific Plate Motion. *Science*, **313**, 1281-1284.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **86**, 651-701.
Stamatakis, A. (2006) RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models. *Bioinformatics*, **22**, 2688-2690.
Sunose, T. & Sato, M. (1994) Morphological and Ecological Studies on a Marine Shore Dolichopodid Fly, *Conchopus borealis* Takagi (Diptera, Dolichopodidae). *Japanese Journal of Entomology*. **62(4)**, 651-660.
Takagi, S. (1965) A contribution to the knowledge of the marine shore dolichopodidae of Japan (Diptera). Insecta Matsumaurana. **27(2)**, 49-84.
Tenorio, J.M., 1969. Supplement, Diptera: Dolichopodidae, Appendix (Phoridae), Volume 11. *Insects of Hawaii*, Honolulu, v + 73 pp.
Ulrich, H. (2005) Predation by adult Dolichopodidae (Diptera): a review of literature with an annotated prey-predator list. *Studia Dipterologica*, **11**, 369-403.
Williams, F.X. (1938) Biological studies in Hawaiian water-loving insects part III, Diptera or flies B. Asteiidae, Syrphidae and Dolichopodidae. *Proceedings of the Hawaiian Entomological Society*, **X**, 281-315.
Wagner, W.L. & Funk, V.A. (editors) (1995) *Hawaiian biogeography: evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington and London, 467.
Wilson, J.T. (1963) A Possible Origin of the Hawaiian Islands. *Canadian Journal of Physics*, **41**, 863-870.
Yang, D., Zhu, Y., Wang, M. & Shang, L. (2006) *World Catalog of Dolichopodidae (Insecta: Diptera)*. China Agricultural University Press, Beijing.
Zimmer, M., Diestelhorst, O. & Lunau, K. (2003) Courtship in long-legged flies (Diptera: Dolichopodidae): function and evolution of signals. *Behavioral Ecology*. **14**, 526-530.
Zimmerman, E.C. (2001) Volume 1 Reissue - Introduction with a new preface and dedication. *Insects of Hawaii*, Honolulu, 206 pp.

FIGURES

Figure Legends

Figure 1. Maximum clade credibility tree summarizing BEAST analysis of the *Eurynogaster* complex with geologic history of the archipelago. Node bars are the 95% highest posterior density intervals of the divergence time estimate – bars that are offset are not to scale. Islands that each specimen was collected from are shown next to each tip.

Figure 2. Majority rule consensus tree summarizing Bayesian analysis of the endemic Dolichopodidae, with the large radiations, *Eurynogaster* complex and *Campsicnemus* collapsed. Bayesian posterior probabilities (Mr. Bayes) and bootstrap supports from the maximum likelihood analysis (RAxML) are displayed as ovals.

Table 1(on next page)

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Composition and status of Dolichopodidae fauna of Hawaii. Genera with endemic species are **boldface.**

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Genus	Total spp. in Hawaii	Number of endemic spp.	Number of non-endemic spp.	Number of described spp, included in this study (undescribed spp.) [included from outside Hawaii]
Achradocera	2	0	2	0
Amblypsilopus	1	0	1	0
Asyndetus	1	1	0	0
Austrosciapus	1	0	1	0
Campsicnemus	183	183	0	70[14]
Chrysosoma	2	0	2	0
Chrysotus	1	0	1	1(1)[1]
Conchopus	6	6	0	3
Condylostylus	1	0	1	1
Dactylomyia	1	0	1	0
Diaphorus	1	0	1	0
Dolichopus	1	0	1	1
Eurynogaster complex				
Adachia	6	6	0	2 (1)
Arciellia	3	3	0	2
Elmoia	8	8	0	2
Eurynogaster	23	23	0	6 (7)
Major	1	1	0	1
Sigmatineurum	11	11	0	1
Sweziella	7	7	0	1
Uropachys	7	7	0	3
Hydrophorus	2	2	0	0
Krakatauia	1	0	1	0
Medetera	1	0	1	0
Paraliancalus	2	2	0	0
Pelastoneurus	1	0	1	0
Sympycnus	1	0	1	1[5]
Syntormon	1	0	1	1[5]
Tachytrechus	1	0	1	1
Thinophilus	1	1	0	1

Table 2(on next page)

Table 2

Primer names and references. Mitochondrial primer numbers correspond to the location in the *Drosophila yakuba* mitochondrial genome (Clary & Wolstenholme, 1985) . Sequences with no reference were designed as a part of this study.

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Primer name	Length	Genome	Reference or Sequence
Cytochrome Oxidase I (COI): 2183 or 2640 and 3041	829	mitochondrial	(Bonacum *et al.*, 2001)
Cytochrome Oxidase II (COII): 3037 and 3771	681	mitochondrial	(Bonacum *et al.*, 2001)
NADH Dehydrogenase 2 (ND2): 192 and 732	527	mitochondrial	(Bonacum *et al.*, 2001)
16S	530	mitochondrial	(DeSalle, 1992)
12S	559	mitochondrial	F14233, R14922 (Simon *et al.*, 1994) 12S_exF: 5'-TCC AGT ACA TCT ACT ATG TTA CG-3' 12S_inF: 5'-ATG TGT RCA TAT TTT AGA GC-3' 12S_inR: 5'-TAT TRG CTA AAT TTG TGC CAG C-3'
rudimentary (CAD), nested reaction: 320F and 843R, 338F and 680R	896	nuclear	(Moulton & Weigmann, 2004)
EF1αA	1036	nuclear	EF4 and EF5 (Collins & Wiegmann, 2002) EFF: 5'-CNC CTG GCC ATC GTG ATT TC-3' EFR: 5'-CAG CAT CTC CYG ATT TGA TGG C-3'
EF1αB	858	nuclear	EFF_B: 5'-GAT TAC TGG TAC ATC TCA AGC-3' EFR_B: 5'-TAG CAG CAT CYC CYG ATT-3'

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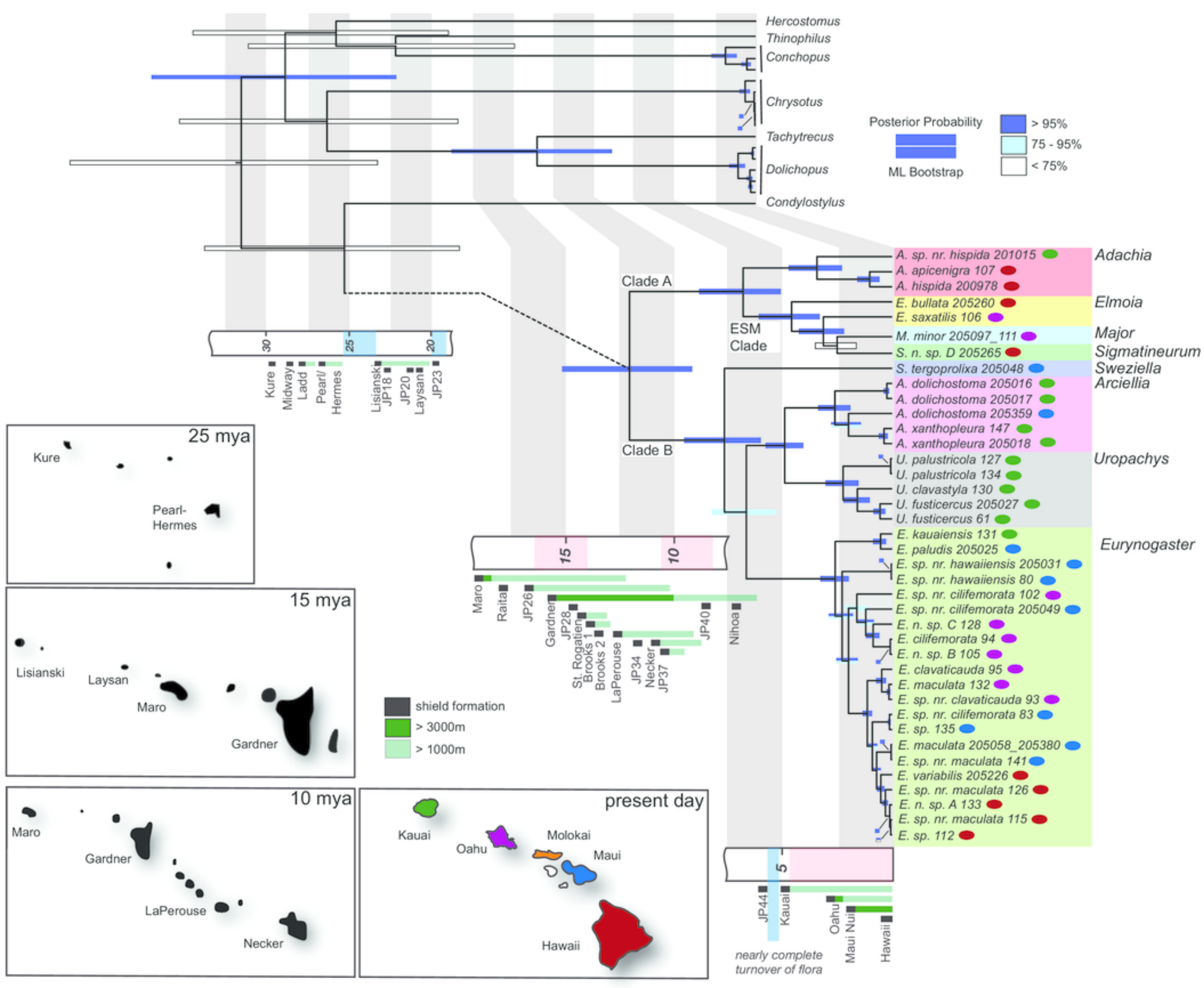

Figure 2

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Majority rule consensus tree summarizing Bayesian analysis of the endemic Dolichopodidae, with the large radiations, *Eurynogaster* complex and *Campsicnemus* collapsed. Bayesian posterior probabilities (Mr. Bayes) and bootstrap supports from the maximum likelihood analysis (RAxML) are displayed as ovals.