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Diversification in the Hawaiian Drosophila

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Diversification in the Hawaiian *Drosophila*

By

Richard Thomas Lapoint

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Professor Patrick M. O'Grady, Chair
Professor George K. Roderick
Professor Craig Moritz

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Abstract

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The Hawaiian Islands have been recognized as an ideal place to study evolutionary processes due to their remote location, multitude of ecological niches and diverse biota. As the oldest and largest radiation in the Hawaiian Islands the Hawaiian Drosophilidae have been the focus of decades of evolutionary research and subsequently the basis for understanding how much of the diversity within these islands and other island systems have been generated. This dissertation revolves around the diversification of a large clade of Hawaiian *Drosophila*, and examines the molecular evolution of this group at several different temporal scales. The *antopocerus*, *modified tarsus*, *ciliated tarsus* (AMC) clade is a group of 90 described *Drosophila* species that utilize decaying leafs as a host substrate and are characterized by a set of diagnostic secondary sexual characters: modifications in either antennal or tarsal morphologies. This research uses both phylogenetic and population genetic methods to study how this clade has evolved at increasingly finer evolutionary scales, from lineage to population level.

The first chapter resolves the relationships within the AMC lineage, delimiting species group and subgroup relationships for the first time. This work complements recent phylogenetic studies focused on other lineages of Hawaiian *Drosophila*, most notably of the *picture wing* clade. This dissertation presents the most comprehensively sampled data set for the AMC in terms of both species and phylogenetic characters. The AMC clade is strongly supported as monophyletic and relationships among of the five major lineages in this radiation are proposed. Molecular dating analyses indicate a rapid radiation occurred about four million years ago, giving rise to all the extent lineages of AMC species. Interestingly, the AMC does not strictly follow the progression rule common to many other Hawaiian taxa. By comparing this lineage to other Hawaiian *Drosophila* lineages it is hypothesized that an initial burst of speciation occurred following colonization of the main Hawaiian Islands ands its magnitude was correlated with sexual selection within each group.

Focusing more specifically on the *spoon tarsus* subgroup within the AMC, I redescribe this subgroup in chapter two. The species boundaries in this group are discussed in light of diagnostic secondary sexual characters of males. *Drosophila septuosa* Hardy is regarded as a junior synonym of *Drosophila percnosoma* Hardy. A

new species, *Drosophila kikalaeleele*, is described. *Drosophila fastigata* Hardy, a species endemic to O‘ahu, is added to the species subgroup, bringing the total number of known species to 12. An updated key to species is provided to the *spoon tarsus* subgroup. I employ several methods to delimit relationships at the species/population interface to get a more refined view of the evolution of this subgroup in chapter three. Eight of the twelve species in this subgroup are found only on the Island of Hawaii, suggesting that they have diverged within the past 500,000 years. This rapid diversification has made determining the relationships within this group difficult. We find widespread agreement between phylogenetic estimates derived from different methods. Notably, our analyses suggest that the *spoon tarsus* subgroup, as currently defined, is not monophyletic.

The last chapter in this dissertation explores how biogeography influences microevolution within the spoon tarsus species, *Drosophila waddingtoni*, to understand the forces that drive macroevolution within the AMC clade. Previous chapters show that *D. waddingtoni* originated on the youngest island, Hawaii, and subsequently colonized the older islands of the Maui Nui complex, the opposite direction predicted by the progression rule. The recent origin of Hawaii suggests that this species will provide unique insight into the dynamics of recent island colonization events. Three nuclear and three mitochondrial genes are used to study gene flow and divergence following founder events. Biogeographic patterns, coupled with divergence time estimates suggest that the back colonization to Maui Nui occurred within the past 250,000 years and has since not led to significant population differentiation. These data also demonstrate that while migration between islands is possible and present in this species, it is not frequent enough to homogenize populations. Biogeography is identified as being a major driver in the diversification of this species.

The AMC clade is a useful tool in understanding diversification in the Hawaiian Islands and rapid radiations in general. By examining this lineage from several different evolutionary timescales this dissertation research elucidates the processes involved in generating and maintaining diversity at all taxonomic levels. This research is also useful in extrapolating how evolution drives diversity in other Hawaiian Drosophilidae clades and other native Hawaiian lineages as well.

Dedication

For my wife Crystal Lapoint, and to my family and friends whose help and support has been invaluable

Table of Contents

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Chapter 1: Phylogenetics of the *antopocerus*-modified tarsus clade of Hawaiian *Drosophila*: Diversification Across the Hawaiian Islands

Abstract

The Hawaiian *Drosophila* radiation has been characterized by rapid diversification in terms of species numbers, morphological diversity and ecological adaptations. Here I present a comprehensive phylogenetic treatment of the *antopocerus*, *modified tarsus* and *ciliated tarsus* (AMC) clade. This work enhances recent phylogenetic studies focused on other lineages of Hawaiian *Drosophila*, most notably of the *picture wing* clade. The AMC clade is a morphologically diverse clade of *Drosophila* endemic to the Hawaiian Islands that was initially proposed based on morphological and ecological characters. All males possess modifications to their forelegs, including *spoon* shaped structures, reduction in number of tarsal segments, or various ornamental *bristle*s. A matrix of 11 genes for 68 species was analyzed to improve phylogenetic inference within this clade. The AMC clade is strongly supported as monophyletic and relationships among of the five major lineages in this radiation are proposed. Molecular dating analyses indicate a rapid radiation occurred about 4 mya, giving rise to all the extant lineages of AMC species. Interestingly, the AMC does not strictly follow the progression rule common to many other Hawaiian taxa. Rather, this group seems to be much more vagile, moving freely around the island chain, back colonizing older islands and skipping some islands while traversing the archipelago.

Introduction

The extreme isolation and varied ecological habitats present in the Hawaiian Islands makes this archipelago home to some of the highest levels of endemism in the United States (Eldredge & Evenhuis, 2003) and a paradigmatic system for studying diversification. Among the most diverse of the endemic Hawaiian lineages is the Hawaiian Drosophilidae, a radiation derived from a single colonization event approximately 25 million years ago (Russo et al., 1995;Tamura et al., 2004). This clade has diversified into an estimated 1000 species (O'Grady et al., 2011), occupying a wide variety of ecological niches (Heed, 1968; Montgomery, 1975; Magnacca et al., 2008) and high morphological diversity (Hardy, 1965). Specifically, the different species groups display marked sexual dimorphism, with males possessing elaborate secondary characters, such as wing patterning, elongate antennae, tusk-like mouthparts and elaborate processes on the tarsi (e.g., Stark & O'Grady, 2009), that they use in copulation. Mating displays are likewise diverse (Spieth, 1966) and involve visual, auditory, tactile and chemosensory components (reviewed in Markow & O'Grady, 2006; 2008). A number of processes, including ecological adaptation (Kambysellis et al., 1995), mating behaviors (Carson, 1997) and founder events (Carson & Templeton, 1984), have been implicated in the generation of high species diversity in the Hawaiian Drosophila.

The biogeography of the Hawaiian Islands has also been hypothesized to drive the majority of diversification within the Hawaiian Drosophilidae (e.g., Funk & Wagner, 1995). The island chain is over 3500 km from the nearest continent making it the most isolated in the world (Carson & Clague, 1995). These volcanic islands rise out of the Pacific where lava seeps through a "hot spot" in the Earth's crust, and over time becomes large enough to sustain life. As the Pacific Plate moves, islands are carried northwest. When an island moves past the hotspot, it become dormant and gradually erodes back into the sea (Craddock, 2000). This leads to a linear progression of island ages with young islands in the southwest and sequentially older islands to the northwest (Figure 1). Within islands erosional forces and sporadic volcanic activity divide the landscape and create a diverse topography (Price & Clague, 2004). These factors all promote isolation between the islands and mainland, between the islands in the archipelago and within the islands themselves. Allopatric speciation and founder events have therefore been expected to be a major influence on the diversification of this clade (Carson & Templeton, 1984; Carson et al., 1990; Bonacum et al., 2005; Muir & Price, 2008).

To date most of this research has focused on the *picture wing* species group, a single large, charismatic clade within the Hawaiian *Drosophila*. The AMC clade, a lineage composed of the *antopocerus* species group, modified tarsus and *ciliated tarsus* subgroups, represents another large radiation of Hawaiian *Drosophila* species. This lineage is united by their habit of ovipositing in decaying leaf material, a behavior that has caused some authors (Throckmorton, 1966; Heed, 1968) to refer to these species as the "leaf breeders." In addition to oviposition preference, members of this lineage all share similar male genitalia, internal anatomy and mating behaviors (Hardy, 1977). They are also characterized extreme dimorphism in the male foreleg ornamentation, a secondary sexual character used in courtship. The AMC clade is divided into five major lineages, the *antopocerus*, *bristle tarsus*, *ciliated tarsus*, *split tarsus*, and *spoon tarsus* subgroups. The differences between these lineages are striking. For example, the

antopocerus species group is comprised of large flies (up to 6 mm long) with long whip-like antennae and was at one point considered to belong to a different genus (Hardy, 1977). The remaining four subgroups are placed in the modified tarsus section and are defined by an eponymous secondary sexual character on the male's forelegs (Hardy 1965; Hardy & Kaneshiro, 1979; Lapoint et al., 2009; Stark & O'Grady, 2009). Modified tarsus species are often abundant when present in an environment and males can be found lekking on the underside of leaves and displaying to females (Bell & Kipp, 1990; Shelly, 1987, 1988, 1990; Speith 1966). Together, these five lineages are comprised of 90 described species with several more yet to be described (O'Grady et al., 2010; Magnacca in prep) found throughout the main Hawaiian Islands (Hardy, 1965). This large clade is likely to be another example of a large adaptive radiation similar to the *picture wing* species group (Kambysellis et al., 1995).

The phylogenetic relationships within the AMC clade and its placement within the Hawaiian *Drosophila* have been studied indirectly for over four decades (Figure 1). Secondary sexual characters have been useful in defining several groups, some of which have subsequently been tested and supported by molecular phylogenetic analyses (Baker & DeSalle, 1997; Bonacum, 2001; Bonacum et al., 2005; Carson & Stalker, 1969; Kambysellis et al., 1995; O'Grady & Zilversmit, 2004; O'Grady et al., 2011). The earliest morphological analysis united *antopocerus* species group and the *modified tarsus* species group, and placed them sister to the *modified mouthpart* and *picture wing* clades (Figure 1A; Throckmorton, 1966). Genetic analyses have further strengthened support for the monophyly of the AMC clade (Figures 1B-C), although support for the group's placement as sister to the *modified mouthpart* and *picture wing* clades has been variable. For example, several studies analyzed representatives from one or two of AMC species subgroups (Kambysellis et al., 1995; Baker and DeSalle 1997; Bonacum, 2001), but did not include enough exemplars for a rigorous assessment of the monophyly of or evolutionary relationships within the AMC clade. O'Grady et al. (2011) recently generated a mitochondrial phylogeny focusing on multiple species from all of the AMC lineages that presented some novel findings. While the *antopocerus* species group, *split tarsus* subgroup and *spoon tarsus* subgroup were monophyletic in this study the *bristle* and *ciliated tarsus* subgroups were paraphyletic with respect to one another (O'Grady et al., 2011). Relationships among the major lineages of the AMC clade were not well supported, probably due to the rapid evolution of the mitochondrial markers used for the analyses. Interestingly, this study found some support for the sister relationship between the AMC clade and the *haleakalae* species group, rather than the traditional view of this group as sister to the *picture wing* and *modified mouthpart*s lineage. Though robust, the low support highlights the importance of a multilocus dataset. Saturation will cause a loss in phylogentic signal in older lineages and not likely in this case. However, incomplete coalescence is expected to be widespread in the case of recent radiations and, by chance alone, genealogies that support the wrong topology can be more common than those that support the true topology (Rokas et al., 2003; Pollard et al., 2006). Multilocus analyses, especially those that infer species tree rather than gene tree phylogenies can resolve this issue (Edwards et al., 2009; Knowles, 2009).

This current study improves on previous attempts to resolve the relationships within the AMC clade, by 1) including 68 AMC species, the largest number sampled to date and over 75% of the described species diversity, 2) sampling representative outgroup

taxa from all Hawaiian *Drosophila* species groups and 3) including sequence data from a total of 11 nuclear and mitochondrial loci. The Hawaiian *Drosophila* are known to be the oldest lineage on the Hawaiian Islands (Price & Clague, 2002), divergence times among the major lineages have not been estimated. This study examines timing of diversification in this group using biogeographic dates and external calibrations to estimate when and how rapidly the AMC, and other major lineages within the Hawaiian *Drosophila*, diversified. This research also explores how the different lineages within the AMC clade are related to each other and attempts to improve on support for relationships proposed by previous phylogenetic hypotheses. Combining the phylogenetic results with distributional information I tested if the progression rule pattern, common in other terrestrial Hawaiian species, was observed in the AMC clade. Consistent with geological history this rule predicts that more basal species in a phylogeny will be found on older islands and more recently divergent taxa will be found on younger islands (Funk & Wagner, 1995) and is borne out in several Hawaiian taxa (e.g. Baldwin & Sanderson, 1998; Jordan et al., 2003; Gillespie, 2004; Mendelson & Shaw, 2005; Bonacum et al., 2005). I also compared biogeographic patterns seen in this group to other Hawaiian taxa to determine whether they have followed similar diversification patterns within the archipelago.

Methods & Materials

Sampling, DNA amplification and Sequencing

Sixty-eight AMC clade species were collected from localities across the main Hawaiian Islands (Table 1). Specimens from all five AMC lineages (*antopocerus*, *split tarsus*, *spoon tarsus*, *ciliated tarsus* and *bristle tarsus*) were included. The 68 AMC taxa I sampled comprise about 75% of the known diversity of this clade. Of the described species 40% percent of the *antopocerus*, 55% percent of the *bristle tarsus*, 83% percent of the *spoon tarsus*, 58% percent of the *split tarsus* and 24% percent of the *ciliated tarsus* (O'Grady et al., 2010) were sampled. While not exhaustive, it is still a significant portion of the described diversity. Inferring from the number of as yet to be described species ("sp. nr."), there are many more AMC species to be described (Table 1). Outgroup specimens, included to provide a strong test of monophyly for the AMC clade and to facilitate dating, were selected from across the other major Hawaiian Drosophilidae clades: *Drosophila ochropleura* (*haleakalae* species group), *D. nigrocirrus* (*modified mouthpart* species group), *Drosophila grimshawi* (*picture wing* species group), *Scaptomyza varipicta* (genus *Scaptomyza*).

Collections were made by sweeping leaf litter and aspirating specimens directly from sponges soaked with fermenting banana or mushroom baits. Specimens were stored in 95% EtOH for identification and DNA extraction at UC, Berkeley. Species identifications were performed by the authors using published keys (Hardy, 1965; Hardy, 1977; Hardy & Kaneshiro, 1979; Lapoint et al., 2009). Several specimens were described as "near species" and were included in the analyses. These specimens were not identifiable using current keys though fit close to already described specimens, and await description (Magnacca, unpublished). Genomic DNA was extracted from individual flies using the Qiagen DNeasy DNA extraction kit (Qiagen, Inc). The only departure from this protocol was that some individuals were soaked in Proteinase K instead of being

macerated and subsequently preserved as point mounted vouchers. Other individuals were macerated according to manufacturers protocol when series were available to save in 95% EtOH. All voucher material has been deposited in either the B.P. Bishop Museum or the Essig Museum of Entomology at UC Berkeley. For details on collections and current deposition locations contact the author with the 6 digit barcodes listed for each specimen in Table 1.

All individuals were sequenced for 7 nuclear loci and 4 mitochondrial loci. The mitochondrial loci NADH dehydrogenase subunit 2, cytochrome oxidase I, cytochrome oxidase II, and 16S ribosomal RNA were amplified using universal mitochondrial primers (Simon et al., 1994). The nuclear loci included were *fz4*, *kl2*, *pds5,* (from Lapoint et al., 2011) and *snf, wee, ntid* and *boss* (O'Grady & Zilversmit, 2004; Zilversmit et al., 2002) (Table 2). PCR products were cleaned using standard ExoSAP-IT (USB) protocols. Cleaned products were sent to the UC Berkeley Sequencing Facility and sequenced in both directions on an ABI 3730 capillary sequencer. Contigs were assembled using Sequencher, ver. 4.7 (GeneCodes, Corp). Because of the recent divergence between the taxa in this study, alignment was trivial and performed by eye in MacClade, ver. 4.06 (Maddison & Maddison, 2002). Sequence alignments were translated to improve gap placement. The combined 11 aligned loci comprised a matrix made up of 6754 bp with 1121 parsimony informative characters (Table 2).

Models/Partitioning

The Akaike information criterion (AIC), implemented in MrModeltest, ver. 2.3 (Nylander, 2004), was used to estimate the best-fit model of substitution for each of the different partitions. To identify an optimal partitioning scheme, the dataset was partitioned in three ways: unpartitioned, with one model for the entire concatenated dataset; non coding, 3rd codon position and 1st and 2nd codon position combined were partitioned for each gene; and 1st codon position, 2nd codon position, 3rd codon position and non coding regions for each gene were given their own partition. Bayes factors were used to identify the most likely partitioning scheme (Table 2), by subtracting the harmonic mean of the -log likelihood of the different partitioning strategies. Acceptance or rejection of each partitioning strategy was based on the limits identified by Kass and Raftery (1995).

Phylogenetic Inference

Gene trees derived from individual analyses were estimated using MrBayes (Ronquist & Huelsenbeck, 2003). Since recombination is not expected to occur between genes located in the mitochondria and likewise genes located in the x-chromosome, the genes from these loci were concatenated into a mitochondrial and x-chromosome loci, respectively. Each recombining unit was used to estimate a gene tree for a total of 7 gene trees. Gene trees were run for 1 million generations and were sampled every 100 generations. Convergence in all Bayesian analyses was assessed by examining the cumulative split frequencies plot calculated by AWTY (Wilgenbusch et al., 2004) and identifying when the potential scale reduction factor (PSRF) approached 1 (Gelman & Rubin, 1992).

Sequences were concatenated, partitioned and analyzed in Bayesian (MrBayes,

ver. 3.1.2; Ronquist & Huelsenbeck, 2003) and maximum likelihood (RAxML, version 7.2.6; Stamatakis, 2006) frameworks. Both the Bayesian and maximum likelihood analyses were performed on the Abe Teragrid, accessed through the CIPRES portal (Miller et al., 2009). Likelihood searches were partitioned by locus and the GTRGAMMA model was used for each partition to estimate the tree, since some authors suggest against using proportion of invariant sites in conjunction with a gamma distributed rate matrix to avoid parameter conflict (Stamatakis, 2006). One thousand bootstrap replicates were performed to assess support for the inferred relationships. The concatenated analysis was partitioned and run in MrBayes for 30 million generations and was sampled every 100 generations. Chain temperatures were adjusted from a default of 0.2 to 0.1 to improve chain swap rates. Convergence was assessed by examining the cumulative split frequencies plot calculated by AWTY (Wilgenbusch et al., 2004) and identifying when the potential scale reduction factor (PSRF) approached 1 (Gelman & Rubin, 1992).

Since these species are expected to have diverged recently, there is a real chance of coalescent processes obscuring the relationships within the AMC. Incomplete lineage sorting can cause genealogies to conflict by chance and makes the consequent estimation of a species tree difficult. To deal with this issue the species tree was estimated in a coalescent framework via the program STEM v1.1 (Kubatko et al., 2009). Genealogies are estimated prior to the species tree estimation, which attempts to minimize the amount of conflict between these genealogies due to deep coalescences in a maximum likelihood framework. This method assumes that all loci evolve in clocklike manner, so to test for this each genealogy was analyzed in BEAST v1.6.1 (Drummond & Rambaut, 2007) using the AIC identified model of substitution and with an uncorrelated lognormal prior for the rate of molecular evolution. Tracer v1.5 (Rambaut & Drummond, 2007) was used to observe if the *ucld.stdev* parameter deviated from 0. Loci identified as evolving in a clock like manner were re-run with a strict clock model with a substitution rate set to one. This included all genes except for COI, COII and Boss. STEM is known to be sensitive to missing data, though the effects are not well characterized. To obviate this issue all taxa with missing data were removed from these analyses. Burn-in was calculated and these trees were summarized and included into the STEM analyses. STEM assumes that $\theta = 4N_e\mu$ is constant across lineages and that all trees are resolved. A θ of 0.0023, previously inferred from several *spoon tarsus* subgroup species (Lapoint et al., 2011) was used. The method of Yang (2002) was used to identify the relative evolutionary rates for each gene (r_i), and compared the average divergence of the ingroup to an outgroup. The values for r_i were scaled for the mitochondrial, Y linked and X linked loci to their appropriate inheritance scalars (0.25, 0.25 and 0.5 respectively).

Divergence Dating

Dating analyses were conducted using the program BEAST v1.6.1 (Drummond & Rambaut, 2007). The entire concatenated dataset was included and partitioned by gene using models of substitution identified in MrModeltest under the AIC. An uncorrelated lognormal model of rate variation and a birth-death speciation process for branching rates was used. The analysis was run for 50 million generations, sampling every 1000 generations. The analysis was rerun twice, to refine the tuning operators and weights for maximum efficiency. After all weights and operators were optimized the analysis was run

twice more and outputs were combined using LogCombiner v1.6.1. Tracer v1.5 (Rambaut & Drummond, 2007) was used to assess convergence and the effective sample size (ESS) for all parameters to identify that the posterior distribution for those parameters is effectively sampled.

Several nodes were calibrated using probabilistic priors. Due to a lack of fossil data multiple biogeographic and external calibrations were used. The oldest calibration point is the split between *Scaptomyza* and the Hawaiian *Drosophila*. This node is calibrated using the date identified by Russo et al., (1995) as 26.1 million years ago (MYA) (+/- 2.87). This node was calibrated using a normal prior centered on a mean of 26.1 with a 95% HPD from 23.23 to 28.97 mya (standard deviation of 1.75). I also used the biogeography of the Hawaiian Islands to inform several node ages. Since species endemic to the Island of Hawaii are not expected to be older than that island, the most likely time of divergence between Hawaii endemic lineages and their sister species on the next nearest island, Maui, is expected to be about 0.5 million years (Price & Clague, 2002). Since the Hawaiian lineage could have diverged before the formation of Hawaii and species on Maui subsequently gone extinct, or the island of Hawaii could have been colonized much later than the island's initial formation, I calibrated the time to most recent common ancestor (tmrca) of these groups with a normal distribution prior with a standard deviation of 0.15. This expects the most likely time of divergence to be 0.5 mya, but allows for divergence almost up to the current day and as far back as 0.9 mya. The Hawaiian *spoon tarsus* and the Hawaiian *antopocerus* species were calibrated using this prior.

To evaluate whether the AMC clade has undergone a rapid radiation I analyzed the dated phylogeny using several methods. First SymmeTREE v1.1 was used to identify if there has been a change in the diversification rate between lineages by comparing the amount of branching in the AMC tree to the expected amount of branching under a pure Yule model (Chan & Moore, 2005). Rate shifts were evaluated using the Δ_1 statistic under default conditions with the maximum clade credibility phylogeny obtained from the BEAST analysis with outgroups removed. A lineage through time plot was explored in LASER v2.3 with the same phylogeny. The γ statistic was calculated to identify if the rate of lineage accumulation is slowing compared to older bursts of speciation. Since incomplete taxon sampling is expected to simulate a slow down, I implement an MCCR method to test if the γ is still significant given the amount of missing species in the dataset (Pybus & Harvey, 2000). The number of species in this group was estimated at 90 after O'Grady et al. (2010).

Biogeography

Ancestral ranges of the species groups within the AMC clade were inferred using the present-day range of each species as a discrete character. Each species was coded as being from Hawaii, Maui Nui (including Maui, Molokai and Lanai), Oahu, or Kauai, or a combination thereof. Species from any of the islands of Maui Nui were treated as being from one island since the islands were connected in the very recent past, facilitating dispersal between islands (Price & Elliot-Fisk, 2004). The range of each species in these analyses was coded after known collection records described in the literature (Hardy, 1965; Hardy, 1977; Hardy & Kaneshiro, 1979; Lapoint et al., 2009).

First a dispersal, extinction and cladogenesis (DEC) model was implemented in the program Lagrange to infer the ancestral ranges of each species group (Ree & Smith, 2008). Lagrange employs a likelihood framework to infer geographical range evolution on phylogenetic trees, while inferring rates of dispersal and local extinction. Since this analysis requires the phylogeny to be time calibrated I used the phylogeny inferred via BEAST with outgroup taxa pruned from the tree. The input file was formatted using the Lagrange configurator (www.reelab.net/lagrange/configurator). Dispersal was modeled in two ways: 1) Migration was only allowed between adjacent islands, 2) no restrictions to movement between islands. I allowed for multiple island ranges since several species are now found on adjacent islands.

Stochastic mapping (SM), a Bayesian method for ancestral state reconstruction, was applied to range inference. This method infers the probability of a state change dependent on branch length and evolutionary rate. SM incorporates phylogenetic uncertainty into the reconstruction of the ancestral state. Indeed, due to the nature of island biogeography, this method is more robust than DEC when inferring the ancestral range of island taxa since there is such a low chance of vicariance (Ree & Smith, 2008). SM analysis was performed using SIMMAP 1.0b2 (Bollback, 2005) on a sub-sample of 1000 trees from the posterior distribution of trees generated in MrBayes.

Results

Phylogenetic Inference

MrModeltest2.3 (Nylander 2004) identified the most appropriate model of evolution for each individual gene region, as well as partitions within the concatenated data set (Table 3). Partitioning by codon position and noncoding region was identified as the optimal partitioning scheme for combined data. Models were implemented for each genealogy individually and as part of a concatenated, partitioned total dataset. Individual phylogenies generally display low resolution (Figures 2-9).

The partitioned concatenated analyses resolved the *antopocerus* species group, *split tarsus* subgroup and *spoon tarsus* subgroup, all of which were previously described based on morphology, as monophyletic groups (Figure 10). The *antopocerus* subgroup is basal and sister to the remaining AMC taxa, a group that will be referred to as the modified tarsus clade (Figure 10). The *split tarsus* subgroup is also well supported as monophyletic and is sister to a heterogeneous group of *spoon*, *bristle* and *ciliated tarsus* species. While the *spoon tarsus* subgroup is supported as monophyletic, it is shown as nested within a paraphyletic grade of taxa in the *bristle* and *ciliated tarsus* subgroups. I will refer to this clade as the *hirsute tarsus* clade (*spoon*, *bristle* and *ciliated tarsus* subgroups). The basal branching pattern within this large group is not well resolved.

The STEM analysis resulted in a species tree with a –ln L = -92770.917 (Figure 11). This species tree topology in this analysis is very similar to that obtained from the other analyses, and at the species group level relationships are well resolved and corroborate the concatenated partitioned phylogeny. The relationships within the *spoon tarsus* subgroup are qualitatively in conflict with those from the concatenated analysis while relationships in other subgroups are in concordance, though without support for a given topology this cannot be said with confidence. With the exception of a few taxa, the analyses are able to identify the relationships between all species groups, including the

ciliated and *bristle tarsus*. STEM does not estimate nodal support so relationships found in this analysis should be treated with a higher degree of uncertainty.

The *antopocerus* species group is monophyletic according to all methods, not an altogether surprising find considering the substantial morphological differences between the *antopocerus* and rest of the modified tarsus (Hardy, 1977). Despite the low resolution at the genealogical level making the identification of most species groups difficult, *antopocerus* is obviously monophyletic (Figure 2-9). Within the *antopocerus* most relationships are also well resolved and supported. Most of this group is endemic to the islands of Maui Nui, with the exception of *D. yooni* and *D. tanythrix* (Hawaii) and *D. arcuata* (Oahu, not included in these analyses). The Hawaiian species are identified as monophyletic within the rest of the clade (Figure 10). The analyses indicate that *antopocerus* is the most basal divergence within the AMC clade, which corroborates the relationship proposed based off of internal morphology (Hardy, 1977). Both of the Hawaiian Island species are the most recently derived lineages, which follows the progression rule of migration from older to younger islands.

The modified tarsus clade (Figure 10) is the largest grouping within the AMC clade, comprised of 75 species from the *spoon*, *bristle*, *ciliated* and *split tarsus* subgroups (Figure 10). The *split tarsus* subgroup in is the largest clade in the AMC with 24 described species and is found on all of the main Hawaiian Islands. This is a well-resolved clade that appears to have originated within the modified tarsus clade, and is sister to the *hirsute tarsus*. This clade is distinguished morphologically by the loss of a tarsomere on the foretarsi of the males and a long process developing from the apical end of the basitarsus (Hardy & Kaneshiro, 1979). Like the *antopocerus* species group, this subgroup is strongly supported as monophyletic in the concatenated analysis and in most of the genealogical analyses (Figure 2-10). There are two well-supported clades: a young island clade including the Hawaii and Maui Nui species and a clade comprised of species from Kauai. These two are found in all analyses as well, indicating a very old split.

The *hirsute tarsus* clade is comprised of the *spoon*, *bristle* and *ciliated tarsus* and is sister to the *split tarsus* clade. The ten true *spoon tarsus* species are united by having the second tarsal segment distinctly cuplike, with dense cilia within the concavity (Stark & O'Grady, 2010). The *spoon tarsus* subgroup was originally described as containing 12 species, but the analyses confirm that the inclusion of *Drosophila atroscutellata* and *Drosophila fastigata* in this group is not warranted. Excluding these two species the *spoon tarsus* subgroup is monophyletic, derived from within the modified tarsus clade. This is not a surprising find since the validity of the morphological characters that have previously included these species into this group has been questioned (Lapoint et al., 2011; Stark & O'Grady, 2010) and phylogenies containing these species have indicated similar results (Lapoint et al., 2011; O'Grady et al., 2011). While the *spoon tarsus* are now described as monophyletic, these results indicate that the *bristle* and *ciliated tarsus* form a paraphyletic grade at the base of the *hirsute tarsus*. The *bristle* and *ciliated tarsus* subgroups are comprised of species that share similar morphologies. The *bristle tarsus* subgroup displays a clump of stiff bristles at the apex of the basitarsus, while the *ciliated tarsus* subgroup is characterized by more diffuse bristles along the tarsal segments on the forelegs of the males. The characters that unite the *ciliated tarsus* species are also found to varying degrees throughout the other subgroups of the *hirsute tarsus*, and it is not surprising that these two clades are closely aligned. The initial and rapid burst of

radiation at the base of the modified tarsus clade presents a difficult phylogenetic issue that can not be adequately inferred using concatenation, though the STEM analysis infers the *bristle tarsus* to be basal, with both the *spoon* and *ciliated tarsus* subgroups to be derived within the *bristle tarsus*.

Divergence Dating

The entire analysis converged quickly as indicated by an ESS well over 1000 for most parameters and greater than 200 for all others. Convergence was difficult to reach due to an interaction between the I and Γ parameters used in the mitochondrial substitution models, but this was resolved by simplifying the substitution models to only include Γ rates. An empty alignment was run as well and found that the effects of the priors did not over influence the posterior distribution (Drummond & Rambaut, 2007). Based on these analyses, the AMC clade started diversifying about 4 million years ago when the ancestors of the *antopocerus* and modified tarsus clades diverged (Figure 12, Table 4). The *split tarsus* and the remainder of the modified tarsus taxa diverged about 3.3 million years ago (Figure 12, Table 4). Divergence times within in the remainder of the AMC clade are listed in Table 4.

SymmeTREE analysis indicates a significant Δ_1 ($p = 0.01$) at the base of the *hirsute tarsus* clade (Indicated on Figure 12), indicating a noticeable increase in the rate of diversification. Additionally, the γ statistic was found to be significant ($p = 0.001$), providing further indication that, while lineage formation may have been rapid in the past, the rate of diversification has slowed in the present. Growth is qualitatively identified in the lineage through time plot (Figure 13).

Biogeography

Lagrange was used to reconstruct ancestral distributions in the AMC clade. The stepping stone model that only allows movement between adjacent islands identifies a slightly more complex pattern similar to the progression rule, where species diverge as they colonize adjacent islands as they form. The ancestral range of the *spoon tarsus* clade is identified as Hawaii. The *split tarsus* and *antopocerus* ancestral range is estimated to both have diversified on the islands of Maui Nui. The ancestral range of the *ciliated* and *bristle tarsus* clade is inferred as ranging from Hawaii to Maui Nui. Again, the analyses suggest that the AMC clade originated in the islands of Maui Nui (Figure 14; Table 5). The less parameterized model detailed in the methods section inferred that both the *antopocerus*, *split, bristle* and *ciliated tarsus* clades had a common ancestor on Maui Nui. The ancestral range of the *spoon tarsus* is inferred as ranging from Hawaii to Maui Nui. Based on these analyses, the AMC may have originated in the islands of the Maui Nui complex. There is little evidence of the progression rule evident in these analyses except for the movement from Maui Nui to the island of Hawaii. The clades of *ciliated tarsus* endemic to Kauai and *bristle tarsus* found on Oahu are derived from within lineages that are strongly supported as evolving on Maui or Hawaii.

SIMMAP reconstructed the ancestral ranges of 1000 of the posterior trees produced by the MrBayes analysis (see Figure 15). Unlike Lagrange, SIMMAP integrates the ancestral states over the posterior distribution of trees and does not require complete resolution. Since Lagrange requires resolution of the phylogeny it reconstructs the ancestral distribution for, a poorly supported but resolved phylogeny can cause

unsupported inferences. The *antopocerus* are inferred as originating in Maui Nui, which is expected given the current distribution and age of this clade. However *D. arcuata*, an *antopocerus* species not included in this study, is endemic to Oahu and may change the reconstruction of this node. Despite the estimated age and high degree of dispersal apparent in the *split tarsus*, *bristle* and *ciliated tarsus*, and *spoon tarsus* clades, the ancestor of these nodes were found to originate in Hawaii with strong probability, evidently predating the origin of Hawaii. The entire AMC clade was reconstructed as originating in Maui Nui with high support. Again, the clades from older islands were found to have evolved within clades from younger islands, further indicating that the center of diversity for this group is Maui Nui and older island species have violated the progression rule.

Discussion

Dating Divergence in the Hawaiian Drosophila

The Hawaiian Drosophilidae are thought to have descended from a single colonization event to Hawaiian Archipelago approximately 25 million years ago (Russo et al., 1995; Tamura et al., 2004). Given the split between *Scaptomyza* and the Hawaiian *Drosophila* occurred soon after this colonization event, the Hawaiian *Drosophila* are estimated to have started diversifying into the major species groups about 9 million years ago (95% HPD 6.14/12.12 mya) (Figure 12). This is a time of high topographic diversity, when the now nearly submerged islands, Gardner and Necker, were both large in area with multiple islands in between them (Price & Clague, 2002). There may have been even greater diversity in Hawaiian *Drosophila* species groups, but a reduction in diversity is expected to have occurred between 10 and 5 million years when the archipelago was only composed of small and recently formed islands less than 1500 m in elevation (Price & Clague, 2002). From this it can be inferred that many lineages of Hawaiian *Drosophila* are likely to have gone extinct, and subsequently the few that survived radiated into the present day species groups found throughout the current High Islands. The AMC group itself appears to have started diversifying rapidly around 4 million years ago (95% HPD 2.99/5.28 mya), which corresponds to the time that current high islands started forming. Increased area and topographic diversity in islands is expected to drive high levels of diversification (Emerson & Gillespie, 2008), and given the dates that are estimated this is a possible explanation for the bursts in Hawaiian *Drosophila* speciation.

The different lineages within the AMC clade all diverged nearly simultaneously, but these dates coincide with island formation as well. The *antopocerus* species group diversified approximately 2.2 million years ago, the estimated earliest age of the Maui Nui islands. The *split tarsus* diverged from the rest of the *ciliated tarsus* subgroup about 3.5 million years ago, and both started diversifying about 3 million years ago, around the time of the formation of the island of Oahu. The youngest subgroup in the AMC, the *spoon tarsus*, started to diversify around 1.5 million years ago and the Hawaiian Island radiation of this group started to diversify before the island of Hawaii was habitable (~0.9 million years ago). Given these dates the phylogeography of these clades is expected to recapitulate the progression rule, where older lineages are found on older islands and younger lineages on younger islands, a paradigm in Hawaiian biogeography (Carson, 1983; Funk & Wagner, 1995; Bonacum et al., 2005). However, the ancestral state

reconstructions to do not corroborate this (Figure 14-15). While the oldest subgroup in the modified tarsus species group includes a large portion of species from Kauai, The oldest lineage in the AMC is found almost entirely on the islands of Maui Nui. Likewise, lineages endemic to Oahu or Kauai are found nested within Maui Nui clades.

Effects of a Rapid Radiation

This analysis greatly improves on the resolution and support of the topology of the AMC clade, but these methods still do not identify the relationships with strong support at the base of the *hirsute tarsus* clade. This study illustrates the issues with the using a single or few loci to identify species within a rapid radiation: due to the high degree of conflict and low resolution observed between and within genealogical topologies (Hickerson et al., 2006) there is low support for identifying consistent subgroups. This conflict is likely to be the result of very rapid divergences at the base of the AMC clade resulting in very short coalescent times. The currently large population size estimates for many of these species (Lapoint et al., 2011) and the rapid radiation in each major lineage (O'Grady et al., 2011) indicate that the majority of the Hawaiian *Drosophila* relationships probably fall within the anomaly zone, where incomplete coalescence will cause random conflict in genealogies (Knowles & Carstens, 2007). The lack of resolution at the base is therefore expected to be the result of incomplete lineage sorting, and the STEM analysis was expected to identify relationships at this level that other analyses did not. This expectation was justified since the STEM phylogeny identified more monophyletic subgroups than the concatenated analysis did. STEM suffers from some compromising assumptions such as a constant θ and that genealogies are known without error, but is capable of computationally accommodating the large amount of data and accounts for incomplete coalescence of the genealogies (Leache & Rannala, 2011).

The difficulty in resolving the relationships between the *bristle* and *ciliated tarsus* subgroups in spite of the use of multiple unlinked nuclear markers and methods that take into account genealogical and species tree conflicts indicates that a very rapid radiation occurred (Figure 10-12). In fact, most of the diversification within the AMC appears to have occurred in short time frame (Figure 12), which would explain previous difficulty in inferring the relationships between the different lineages. The rapid increase in the rate of speciation indicates that something in the environment or biology of the lineage changed. Based upon previous work on Hawaiian species, there are three candidate forces that are driving this rapid diversification: 1) The *hirsute tarsus* subgroup may have evolved a character that allowed them to exploit their environment more readily. 2) Around the time the *hirsute tarsus* clade started to diversify there was an increase in available landscape – the current high islands were forming (Price & Clague, 2002) and increased topographic diversity has been shown to increase genetic diversity in some Hawaiian lineages (Jordan, et al., 2005) and the availability of open niches may have caused a reduction in extinction rate. 3) Sexual selection may be driving divergence as isolated populations experience drift in mating preferences (Gavrilets, 2000). I consider each of these possibilities below.

The timing of these divergences appear to qualitatively match the history of island formation with bursts of speciation following the formation of new islands; however, the ancestral range reconstructions describe a much more dispersive pattern (Figure 14-15,

Table 5). Even though the timing of the AMC divergence is estimated as occurring on the older Hawaiian high islands (Kauai and Oahu), the ancestral range reconstructions under either the DEC model or SM model identify that most of the diversification occurred on the younger islands (Maui Nui and Hawaii). This is not possible, as these islands did not exist until much more recently. This pattern may result from two processes. First, extinctions may bias the ancestral range reconstructions. Most of the extant lineages in the AMC are from these younger islands (~80%) and the sampling and subsequent analyses reflect this present day distributional bias. Extinctions or incomplete species sampling on Kauai and Oahu are possible given the widespread habitat loss in recent and historic times, especially on Oahu. Alternatively this pattern may reflect a real history of diversification on the islands of Maui Nui. This is the more likely scenario given that the greatest amount of actual diversification occurred at approximately the time of the formation of the earliest volcanoes of Maui Nui and lineages on older islands appears to be the results of colonization back up the island chain. While extinction is no doubt a possible factor within the span of time the High Islands have existed, the reconstructions are robustly supported enough for us to expect this is a Maui Nui radiation. Maui Nui has been separated and connected multiple times in the islands approximately two million year history, most recently connected during the last glacial maxima (Price & Elliot-Fisk, 2004). This has alternately led to a high degree of topographic diversity and increased area, followed by periods of isolation between volcanic mountains, which are expected to promote speciation (Emerson & Gillespie, 2008). Maui Nui has shown to be a crucible of diversity in several Hawaiian lineages including *Tetragnatha* spiders (Gillespie, 2004) and other Hawaiian *Drosophila* species (Bonacum et al., 2005). Allopatry in combination with adaptation to a new environment could be expected to result in this large radiation of species.

The AMC radiation follows a pattern indicative of an adaptive radiation, with short internal nodes coalescing at the base of a speciose clade (Nee et al., 1992). Previous work done on the *picture wing* species group has shown that adaptation to different plants used as oviposition sites is a likely driver of diversification (Kambysellis et al., 1995) and this research has ensconced the Hawaiian *Drosophila* as a paradigm of adaptive radiation (Schluter, 2000). While this is true of the *picture wing Drosophila*, it is unlikely to have driven the diversification in the AMC clade. The AMC exploit a nearly uniform ecological niche across the Hawaiian Islands: almost all species oviposit in decaying leaves of species from the family Araliaceae (Magnacca et al., 2008; O'Grady et al., 2011), and all species are endemic to wet forests from 1000 m to 2000 m in elevation (Hardy, 1965).

The secondary sexual structures characteristic of each lineage appear to have evolved coincidentally with the radiation of each lineage, making them candidate drivers of the high rate of diversification. These characters are used in sexual displays and mating behaviors, and are highly variable between species groups (Spieth 1966; Hardy 1965). Geographic isolation followed by a random and slight change in the way these secondary sexual characters are used could cause pre-mating isolation when sister species came back into contact with each other. Further research needs to be done to test this hypothesis for this clade, but the work that has been done identified slight differences in how lekking sites are partitioned between closely related species (but see Spieth, 1966; Bell & Kipp, 1990; Shelly, 1987, 1988, 1990). This idea is also not without precedence in

the Hawaiian *Drosophila*: a verbal model of how this could have driven the Hawaiian *Drosophila* diversification has been proposed (Kaneshiro, 1976; 1983), and secondary sexual characters have been shown to be important in mate choice (Boake, 2005). Future research will benefit from augmenting the understanding of the mating behaviors and preferences of these species and exploring this radiation in light of sexual selection.

Comparison to Other Hawaiian Drosophila *Lineages*

The AMC clade shares several evolutionary similarities with other lineages in the diverse Hawaiian *Drosophila* radiation. The *haleakalae* species group and planitibia species group of the *picture wing* clade are estimated to have start to rapidly diversify around the time of the formation of the Main Hawaiian Islands, approximately five to six million years ago (Russo et al., 1995; O'Grady & Zilversmidt, 2004; Bonacum, et al., 2005). These analyses also indicate that older divergences between subgroups and species groups occurred between approximately eight and 10 million years ago, around the time that island habitats over 1000 m were still available on the Gardner Pinnacles and several other islands (Price & Clague, 2002). There were possible remnants of ideal rain forest habitat available when the island of Kauai formed, but it would have been geographically distant, and since climate models predict warmer temperatures at this time these habitats would have been at a higher elevation (Price & Clague, 2002). This corroborates the findings that diversification in the Hawaiian *Drosophila* went through multiple spurts following increases in topographic diversity and area and reductions in the formations of major lineages at periods of low and few islands. All molecular analyses qualitatively appear to be rapid radiations. These analyses have difficulty resolving the relationships at the base of most of the major species groups within the Hawaiian *Drosophila*: the *haleakalae*, *picture wing*, AMC and *modified mouthpart* all seem to diversify rapidly as indicated by low resolution and support at the base of the estimated phylogenies despite increasing taxonomic and molecular sampling (Baker & DeSalle, 1997; Bonacum, 2001; O'Grady & Zilversmidt, 2004; Bonacum, et al., 2005; O'Grady et al., 2011). While this may be due to an increase in speciation rate, given the widespread increase in diversification after what is generally predicted to be an increase is available environment, it is very possible this is a decrease in extinction rate. It has been predicted that any Hawaiian lineage older than the main Hawaiian Islands would have gone through a bottleneck as only a few lineages are expected to have traversed the wide oceanic gap between Kauai and Necker (Price & Clague, 2002).

The AMC share more in common with the *haleakalae* species group than the *picture wing* species group. Similar to the *haleakalae* species group, I do not find a pattern of progression down the island chains (O'Grady & Zilversmit, 2004; O'Grady et al., 2011). This conflicts with the *planitibia* and other *picture wing* species groups where this pattern is observed (Kambysellis et al., 1997; Bonacum et al., 2005). Like the *haleakalae* species group, the AMC is not diverse in its host substrate usage, and exploits on a single type of substrate. Meanwhile the *picture wing*, *ateledrosophila* and *nudidrosophila* (PNA) species group utilizes several families of plants (Magnacca et al., 2008; O'Grady et al., 2011). The AMC also display a wide range of secondary sexual characters and mating behaviors like the *modified mouthpart* and PNA species group and unlike the *haleakalae* species group (Spieth, 1966). Future work on the *modified mouthpart* species group is required for a complete comparison, but the AMC, PNA and

modified mouthpart clades are much larger (90, 157, 106 species, respectively) than the *haleakalae* species group (54) (O'Grady et al., 2010). This leads to the inference that sexual selection is indeed an important factor in increasing the rate of diversification following an expansion post colonization of the present day high islands. While cited as a paradigm of a sexually selected radiation (Boake, 2005; Ritchie et al., 2007) future work is needed to directly test the importance of sexual selection that is likely a major candidate driving an increase in diversification rates.

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Tables

Table 1. Individuals sampled and gene coverage. Barcode refers to O'Grady Lab bar-coding conventions and can be referred to identify full collection details. Species with an * were used in the STEM analysis. Locality refers to islands of collection, not entire range for the species.

Table 2. Gene details and diversity. Primer sequences from: [1] Lapoint et al., 2011; [2] Zilversmit et al., 2004; [3] O'Grady & Zilversmit, 2004; [4] new to this study; [5] Simon et al., 1994. [6] Number of parsimony informative characters.

Table 3. Optimal partitioning scheme. Results of Bayes Factors used to identify the partitioning scheme (Brown & Lemmon, 2007).

Table 4. Ages of major lineages, species groups and subgroups estimated by BEAST. Important ages with 95% Highest posterior densities. Refer to Figure 12 to identify nodes.

Table 5. Lagrange ancestral range reconstructions. Refer to Figure 14 to identify nodes. Value to the left of | indicates state of upper branch, values to the right indicates the state of the lower branch. –lnL and and relative probabilities of each reconstruction are provided.

Table 1. Individuals sampled and gene coverage

Genus	Spp Group	Species	Barcode	Locality	ND2	COI	COII	16s	boss	ntid	snf	wee	fz4	kl2	pds5	Total
Drosophila	antopocerus	*aduncа*	202546	Maui Nui	x	x	x	x	x	x	x			x	x	9
Drosophila	antopocerus	*diamphiodiopoda*	200785	Maui Nui	x	x	x	x	x	x	x		x	x	x	10
Drosophila	antopocerus	*orthoptera**	202540	Maui Nui	x	x	x	x		x	x	x	x	x	x	10
Drosophila	antopocerus	*stigma*	202548	Maui Nui	x	x	x	x		x	x	x		x	x	9
Drosophila	antopocerus	*tanythrix**	202520	Hawaii	x	x	x	x		x	x	x	x	x	x	10
Drosophila	antopocerus	*yooni*	202550	Hawaii	x	x	x	x		x	x	x		x	x	9
Drosophila	bristle tarsus	*apodasta*	201029	Kauai	x	x	x	x		x	x		x	x	x	9
Drosophila	bristle tarsus	*basimacula**	201030	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*brevitarsus*	201038	Oahu	x	x	x		x	x	x		x	x	x	9
Drosophila	bristle tarsus	*expansa**	201012	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*perissopoda*	201034	Kauai	x	x	x	x		x	x		x	x	x	9
Drosophila	bristle tarsus	*petalopeza**	202536	Maui Nui	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*prodita**	201010	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*quasiexpansa*	202532	Maui Nui	x	x	x	x		x	x	x			x	8
Drosophila	bristle tarsus	*redunca**	201884	Maui Nui	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*seclusa*	201011	Maui Nui	x	x	x	x	x	x	x	x		x	x	10
Drosophila	bristle tarsus	*sp. nr. basimacula 1*	201031	Kauai	x	x	x	x	x	x	x	x		x	x	10
Drosophila	bristle tarsus	*sp. nr. basimacula 2**	201032	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*sp. nr. basimacula 3*	201033	Kauai	x	x	x	x	x	x		x	x	x	x	10
Drosophila	bristle tarsus	*sp. nr. Brunneisetae**	201018	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*sp. nr. perissopoda 1'*	201035	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*sp. nr. perissopoda 2*	201036	Kauai	x	x	x	x	x	x	x		x	x	x	10
Drosophila	bristle tarsus	*sp. nr. perissopoda 3*	201037	Maui Nui	x	x	x	x	x	x	x		x	x	x	10
Drosophila	bristle tarsus	*torula*	202545	Maui Nui	x	x		x	x	x		x	x		x	8
Drosophila	bristle tarsus	*trichaetosa**	201013	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	bristle tarsus	*unicula*	200997	Hawaii	x	x	x	x		x	x			x		7
Drosophila	ciliated tarsus	*brunneifrons**	202547	Maui Nui		x	x	x	x	x	x	x	x	x	x	10
Drosophila	ciliated tarsus	*clavitibia*	201326	Maui Nui	x	x	x	x	x	x	x		x	x	x	10

Table 1. Individuals sampled and gene coverage (continued)

Genus	Spp Group	Species	Barcode	Locality	ND2	COI	COII	16s	boss	ntid	snf	wee	fz4	kl2	pds5	Total
Drosophila	ciliated tarsus	*imparisetae*	201014	Hawaii	x	x	x	x	x	x	x	x	x	x		10
Drosophila	ciliated tarsus	*kraussi*	202553	Oahu	x	x	x	x	x	x	x		x	x		9
Drosophila	ciliated tarsus	*latigena*	201015	Hawaii	x	x	x	x	x	x	x	x		x	x	10
Drosophila	ciliated tarsus	*medialis**	201017	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	ciliated tarsus	*nigritarsus**	202511	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	ciliated tarsus	*paucula*	202549	Oahu	x	x	x	x		x	x	x		x	x	9
Drosophila	ciliated tarsus	*sp. nr. Melanopedis**	201016	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	ciliated tarsus	*williamsi*	202551	Oahu	x	x	x	x		x	x	x		x	x	9
Drosophila	split tarsus	*ancyla*	201019	Maui Nui	x	x	x	x	x	x	x	x				8
Drosophila	split tarsus	*attenuata*	202552	Hawaii	x	x	x		x	x	x	x				7
Drosophila	split tarsus	*basisetosa*	201021	Hawaii	x	x	x	x	x	x	x		x	x	x	10
Drosophila	split tarsus	*capitata**	201050	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*chaetocephala*	201051	Oahu	x	x	x	x	x		x	x			x	8
Drosophila	split tarsus	*cneucopleura**	201022	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*cracens*	202538	Maui Nui	x	x	x	x	x	x	x	x	x		x	10
Drosophila	split tarsus	*forficata*	201053	Kauai	x	x	x	x	x	x	x	x	x		x	10
Drosophila	split tarsus	*fundita*	201023	Maui Nui	x	x	x	x	x	x	x	x				8
Drosophila	split tarsus	*furcatarsus**	201054	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*kokeensis**	201055	Kauai	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*paracracens*	202556	Hawaii	x	x	x	x		x	x	x			x	8
Drosophila	split tarsus	*paucitarsus**	201897	Maui Nui	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*pectinitarsus**	201025	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*procerisetae**	201880	Maui Nui	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*propiofacies**	201027	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*sp. nr. Ancyla**	201020	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*sp. nr. dorsigera*	201052	Kauai	x	x	x	x		x	x	x	x		x	9
Drosophila	split tarsus	*sp. nr. Fundita**	201028	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	split tarsus	*systenopeza**	201026	Maui Nui	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*atroscutellata**	070236	Kauai	x	x	x	x	x	x	x	x	x	x	x	11

Table 1. Individuals sampled and gene coverage (continued)

Genus	Spp Group	Species	Barcode	Locality	ND2	COI	COII	16s	boss	ntid	snf	wee	fz4	kl2	pds5	Total
Drosophila	spoon tarsus	*conformis**	201312	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*contorta*	200120	Maui Nui	x	x	x	x		x	x		x	x	x	9
Drosophila	spoon tarsus	*dasycnemia**	202326	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*fastigata**	070069	Oahu	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*incognita**	202333	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*kikalaeleele*	202385	Hawaii	x	x	x	x			x		x	x	x	8
Drosophila	spoon tarsus	*neutralis**	202319	Hawaii	x	x	x	x		x	x	x	x	x	x	10
Drosophila	spoon tarsus	*percnosoma**	200125	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*sordidapex**	202321	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Drosophila	spoon tarsus	*waddingtoni**	202431	Hawaii	x	x	x	x	x	x	x	x	x	x	x	11
Hawaiian Drosophilidae outgroups																
Drosophila	Haleakalae	*ochropleura*	202558			x	x	x		x	x		x	x	x	8
Drosophila	mod. mouthpart	*nigrocirrus*	202557		x	x	x	x		x	x	x	x	x	x	10
Drosophila	picture wing	*grimshawi*	FLYBASE		x	x	x	x	x	x	x	x	x	x	x	11
Scaptomyza	Scaptomyza	*varipicta*	201060		x	x	x	x		x	x					6

Table 2. Gene details and diversity

Locus	Primer Label	Primer Sequence	Locus Type	Aligned Length	PIC[6]	Individuals Sequenced
Kl-2[1]	Kl2L	5'-TAATACAGAACGGTGGTATGGGTAT-3'	Y	571	63	60
	kl2R	5'-GTTGCTTGGCTAATTCGTAAAGAGT-3'				
Fz4[1]	Fz4L	5'-GCGTCTTTCTATTGCGCTACTAT-3'	X	974	89	55
	Fz4R	5'-GCTTGTACGGACTGCTGATTATT-3'				
Snf[2]	snfL	5' GAAGATGCGGGGCCARGCNTTYGT 3'	X	395	81	69
	snfR	5' GAACAGCATGGACAGCATCATYTCRTT 3'				
Pds5[1]	Pds5L	5'-GGATACTTTGTGGACAATTCAGAGT-3'	autosomal	594	87	64
	Pds5R	5'-AGATATTTCACGAACTCTTCAGCAC-3'				
Boss[2]	BossF1	5' ACCAGATGCCCTGGGGNGARAA 3'	autosomal	726	136	53
	BossR1	5' TGGACAGGGAGCCGCKNARCCARTT 3'				
Ntid[3]	ntidF1	5' GGGCCGCATCTTCGARCAYAARTGG 3'	autosomal	567	98	69
	ntidR1	5' TGGAGGGGTAGGTGTTCCARCARTA 3'				
Wee[4]	weeL	5' GCCTGGGCCGAGGAYGAYCAYATG 3'	autosomal	297	40	56
	weeR	5' TCACGTGGCCCAGGTCNCCDATYTT 3'				
ND2[5]	192	5'-AGCTATTGGGTTCAGACCCC-3'	mito	523	132	69
	732	5'-GAAGTTTGGTTTAAACCTCC-3'				
COI[5]	2183	5'-CAACATTTATTTTGATTTTTTGG-3'	mito	831	201	71
	3037	5'-TYCATTGCACTAATCTGCCATATTAG-3'				
COII[5]	3041	5'-ATGGCAGATTAGTGCAATGG-3'	mito	765	167	70
	3791	5'-GTTTAAGAGACCAGTACTTG-3'				
16s[5]	16sF	5'-CCGGTTTGAACTCAGATCACGT-3'	mito	511	27	69
	16sR	5'-CGCCTGTTTAACAAAAACAT-3'				
Total				6754	1121	705

Table 3. Optimal partitioning scheme

Partitioning Schemes	# of partitions	-lnL harmonic mean (post burn in)	Bayes Factor	Support for Rejection
Partitioned by codon	38	-41031.737	-	-
Partitioned by codon (1st and second position combined)	28	-41091.747	60.01	Very Strong
No Partitions	0	-41481.187	389.44	Very Strong

Table 4. Ages of major lineages, species groups and subgroups estimated by BEAST

Node	Mean Age	95% Low HPD	95% High HPD
1	23.43	19.91	26.89
2	8.79	6.14	12.12
3	4.04	2.99	5.28
4	2.18	1.49	2.98
5	3.37	2.51	4.32
6	3.11	2.89	3.99
7	1.6	1.09	2.19
8	2.82	2.08	3.64

Table 5. Lagrange ancestral range reconstructions

	no constraints			stepping stone model		
Node	**Island**	**lnL**	**Rel. Prob.**	**Island**	**lnL**	**Rel. Prob.**
1	H|H	-123.7	0.9632	H|H	-161.6	0.7342
2	H|M	-123.9	0.7733	M|M	-161.3	0.9555
3	M|M	-123.8	0.854	M|M	-161.3	0.9859
4	M|M	-123.7	0.9259	M|M	-161.3	0.986
5	M|M	-123.7	0.9092	M|M	-161.3	0.9838
6	K|K	-123.7	0.9902	K|K	-161.3	0.9816
7	K|K	-123.7	0.9669	K|K	-161.3	0.9666
8	K|H	-124	0.7327	K|K	-161.5	0.8014
9	K|HK	-123.9	0.7399	K|K	-161.8	0.5981
10	H|H	-124.1	0.6413	M|M	-162.3	0.3612
11	H|H	-124.1	0.6544	M|M	-161.6	0.7633
12	H|H	-124	0.673	M|M	-161.3	0.987
13	H|H	-123.9	0.7584	M|M	-161.3	0.9864
14	H|HM	-123.9	0.7652	H|HM	-161.4	0.9318
15	M|M	-124.3	0.4991	M|M	-161.3	0.9961
16	M|M	-123.8	0.8211	M|M	-161.3	0.9888
17	M|M	-123.7	0.92	M|M	-161.3	0.9831
18	M|M	-123.7	0.9729	M|M	-161.3	0.9842
19	H|H	-123.7	0.9379	M|M	-161.4	0.8765
20	H|M	-123.8	0.8463	M|M	-161.4	0.9131
21	M|M	-124.1	0.6375	M|M	-161.3	0.9831
22	M|H	-124.2	0.5848	M|M	-161.3	0.9842
23	M|HM	-124.7	0.3396	M|M	-161.3	0.9925
24	HM|H	-124.3	0.5288	M|M	-161.3	0.9828
25	M|M	-123.7	0.9937	M|M	-161.3	0.9827
26	M|M	-123.7	0.9185	M|M	-161.3	0.9878
27	M|M	-123.8	0.8349	M|M	-161.4	0.9269
28	O|K	-124.2	0.5938	O|K	-162.4	0.3294
29	O|O	-124.3	0.5319	O|O	-162.3	0.3844
30	H|H	-124.8	0.3285	M|M	-161.8	0.6041
31	M|H	-124.7	0.3387	M|M	-161.4	0.8819
32	HM|H	-124.7	0.3313	M|M	-161.6	0.7653
33	K|K	-123.6	0.9986	K|K	-161.3	0.9946
34	K|K	-123.6	0.9991	K|K	-161.3	0.9977
35	K|K	-123.6	0.9991	K|K	-161.3	0.9979
36	K|K	-123.6	0.9952	K|K	-161.3	0.989
37	K|K	-123.7	0.9713	K|K	-161.4	0.9419
38	K|K	-123.6	0.9966	K|K	-161.3	0.9909
39	K|K	-123.7	0.9582	K|K	-161.4	0.9239

Table 5. Lagrange ancestral range reconstructions (continued)

	no constraints			stepping stone model		
Node	**Island**	**lnL**	**Rel. Prob.**	**Island**	**lnL**	**Rel. Prob.**
40	K|K	-123.8	0.873	M|M	-161.8	0.6291
41	HM|M	-124.1	0.6192	M|M	-161.8	0.6291
42	HM|M	-124.5	0.4095	M|M	-161.6	0.7165
43	H|HM	-124.1	0.6464	M|M	-161.4	0.9037
44	M|M	-124.1	0.6625	M|M	-161.4	0.9069
45	M|K	-124.1	0.6497	M|K	-161.9	0.5667
46	M|M	-124.4	0.4663	M|M	-161.7	0.6673
47	M|M	-124	0.6697	M|M	-161.4	0.9109
48	M|M	-124.1	0.6247	M|M	-161.4	0.938
49	M|M	-123.9	0.7973	M|M	-161.8	0.5857
50	H|H	-124.1	0.6366	H|H	-161.3	0.973
51	H|H	-123.7	0.9617	H|H	-161.3	0.9714
52	H|H	-123.9	0.7611	H|H	-161.5	0.7823
53	H|H	-123.7	0.9211	H|H	-161.4	0.9507
54	H|H	-123.7	0.9312	H|H	-161.3	0.9593
55	H|H	-123.6	0.9998	H|H	-161.3	0.9989
56	H|H	-123.6	0.9993	H|H	-161.3	0.9967
57	H|H	-123.6	0.9962	H|H	-161.3	0.9829
58	H|H	-123.7	0.9305	H|H	-161.3	0.9613
59	M|H	-123.9	0.7547	H|H	-161.9	0.559
60	HM|H	-124.1	0.6576	H|H	-161.9	0.5488
61	M|M	-124.2	0.557	HM|M	-162.2	0.4238
62	M|M	-123.8	0.8142	HM|M	-161.4	0.8974
63	M|M	-123.7	0.9104	HM|M	-161.3	0.9648
64	M|M	-123.7	0.9101	HM|M	-161.3	0.9779
65	M|M	-124.5	0.4385	HM|M	-161.3	0.9778
66	M|M	-124.3	0.5028	HM|M	-161.3	0.9826

Figures

Figure 1. Previous phylogenetic hypotheses regarding relationships within the Hawaiian *Drosophila*. Highlighted boxes include the placement of the AMC clade. A. Relationships based on internal morphology (Throckmorton, 1966). B. combined nuclear and mitochondrial gene sequences for 9 representative AMC species (Bonacum, 2001). C. Mitochondrial sequence data for 55 AMC species (O'Grady et al., 2011).

Figure 2-9. Genealogies. Bayesian topology shown. * indicates posterior probabilities > 0.9 and RAxML bootstrap values > 70. Identity of each gene indicated in top left of figure.

Figure 10. Concatenated, partitioned phylogeny of the AMC. Bayesian topology shown. Partitioned by gene and coding (by codon position) and noncoding. * indicates posterior probabilities > 0.9 and RAxML bootstrap values > 70. Branch to outgroup shortened for image clarity.

Figure 11. Phylogeny of the species tree inferred using STEM.

Figure 12. Chronogram of the AMC estimated in BEAST. Relaxed calibrations placed at nodes indicated by an *. Node bars indicates age range. Alternating grey and white bands indicate time when islands formed until next island formed. Nodal support similar to that of the concatenated partitioned analysis. Node of rate increase indicated by Δ.

Figure 13. Lineage through time plot.

Figure 14. Ancestral range reconstruction estimated under a DEC model. H refers to Hawaii, M refers to Maui Nui, O refers to Oahu and K refers to Kauai. Ranges below bar indicates ancestral range with age and stepping stone restrictions on DEC model. Ranges above bar indicates ancestral range without any additional restrictions. Value to the left of | indicates state of upper branch, values to the right indicates the state of the lower branch.

Figure 15. Ancestral range reconstruction estimated under a stochastic mapping model. Range reconstructions on nodes. Reconstructions with posterior probability > 0.90 indicated by an *. HI refers to Hawaii, MN refers to Maui Nui, OA refers to Oahu and KA refers to Kauai.

Figure 1. Previous phylogenetic hypotheses regarding relationships within the Hawaiian *Drosophila*

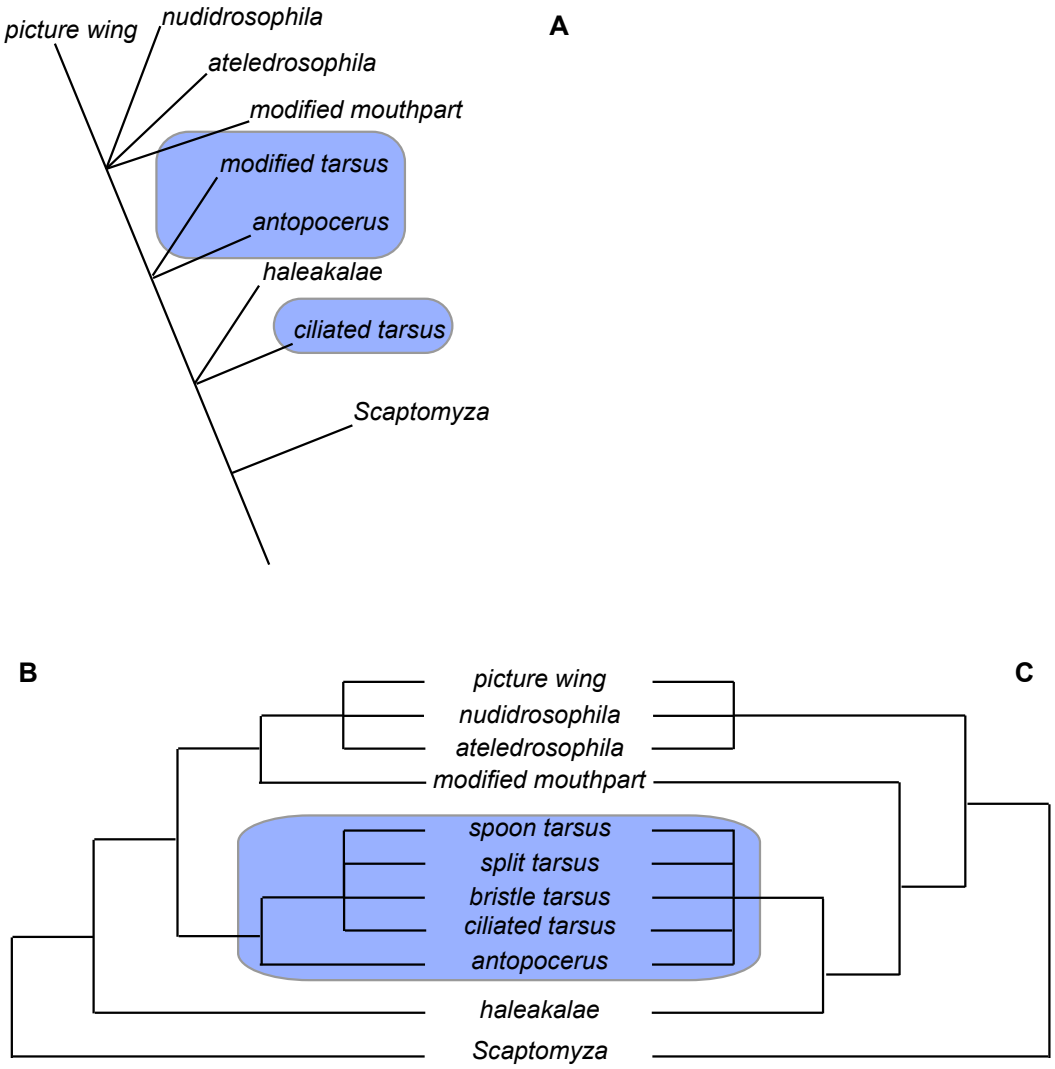


Figure 2. Boss Genealogy

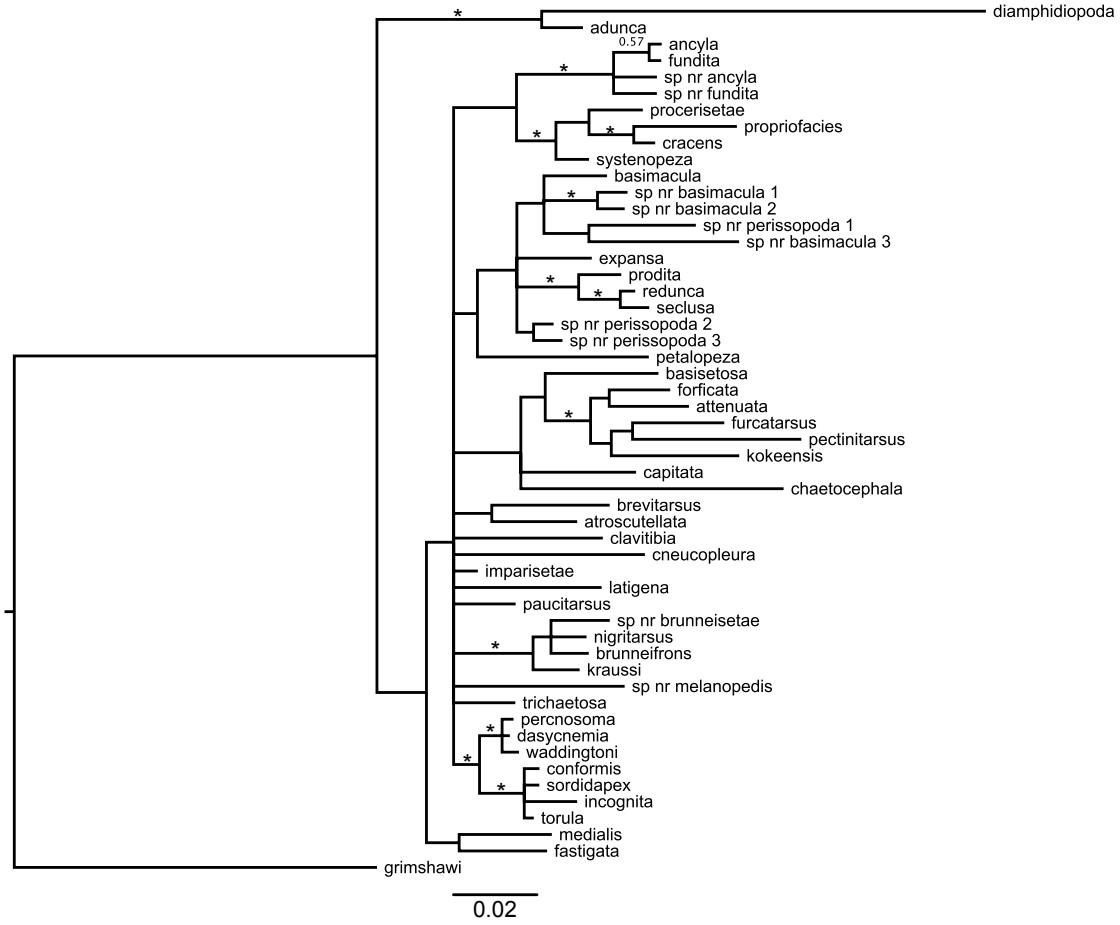


Figure 3. Fz4 Genealogy

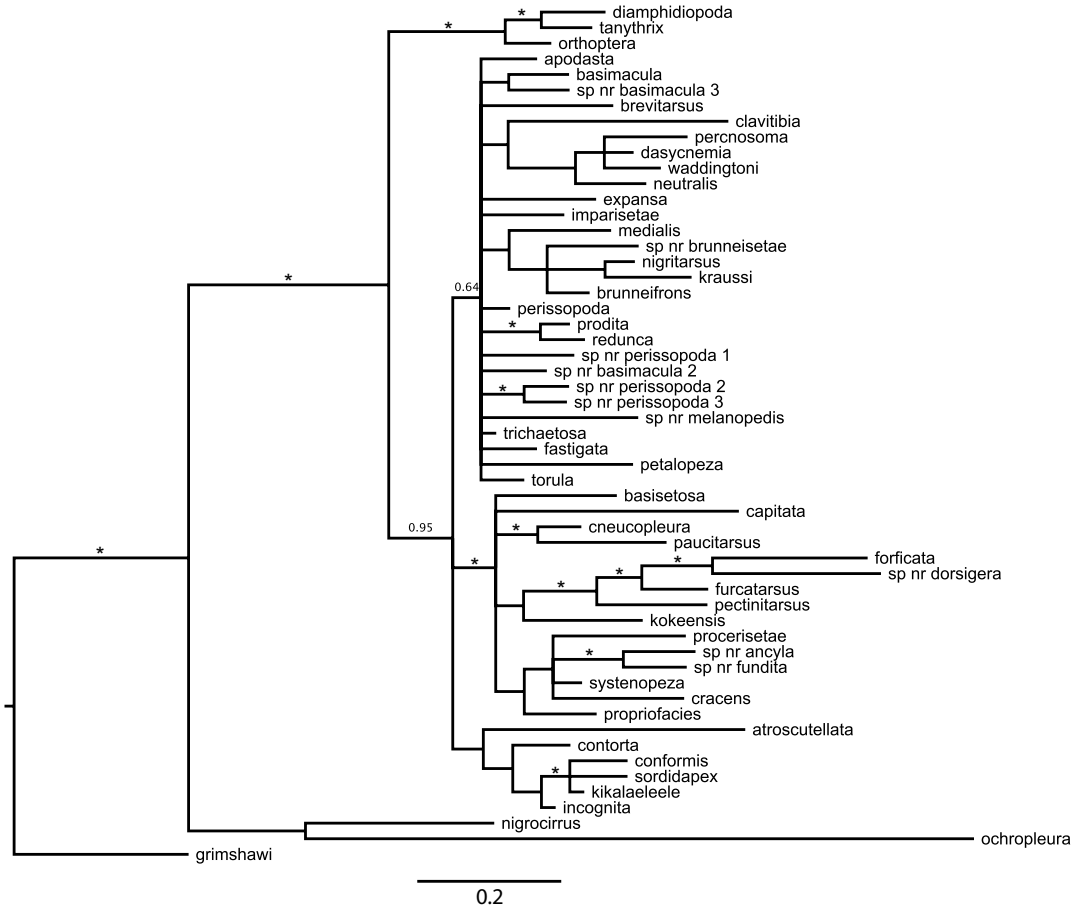


Figure 4. Kl2 Genealogy

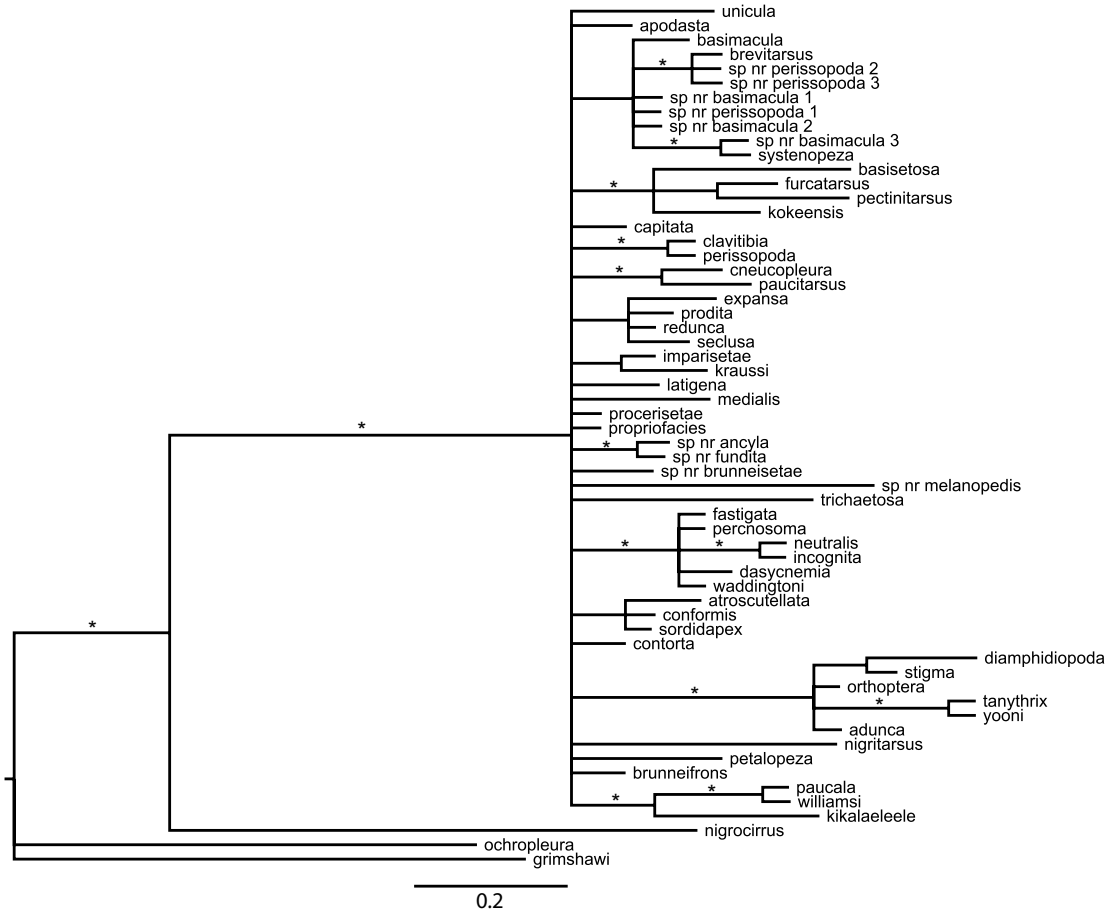


Figure 5. mtDNA genealogy

Figure 6. Ntid genealogy

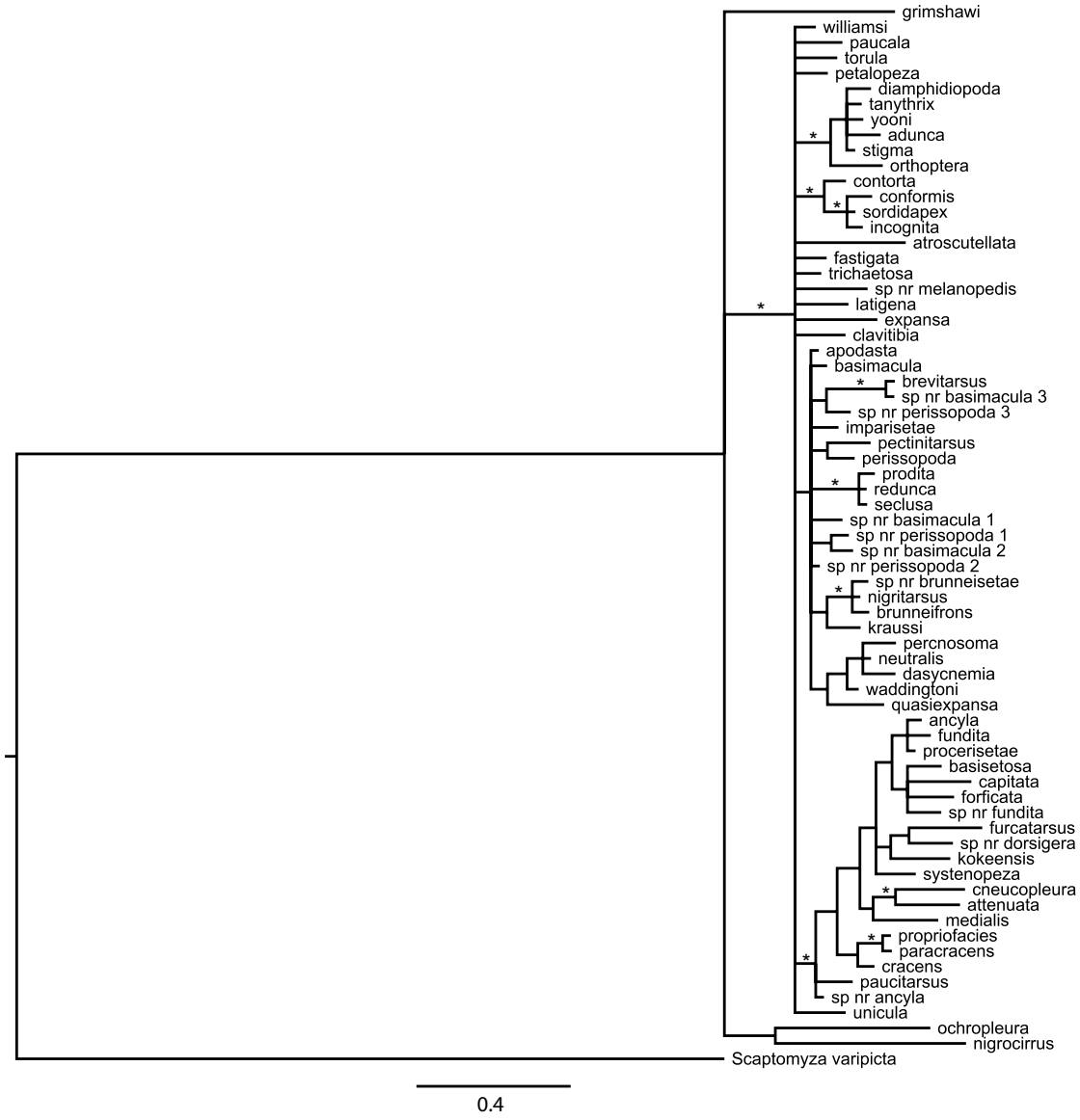


Figure 7. Pds5 genealogy

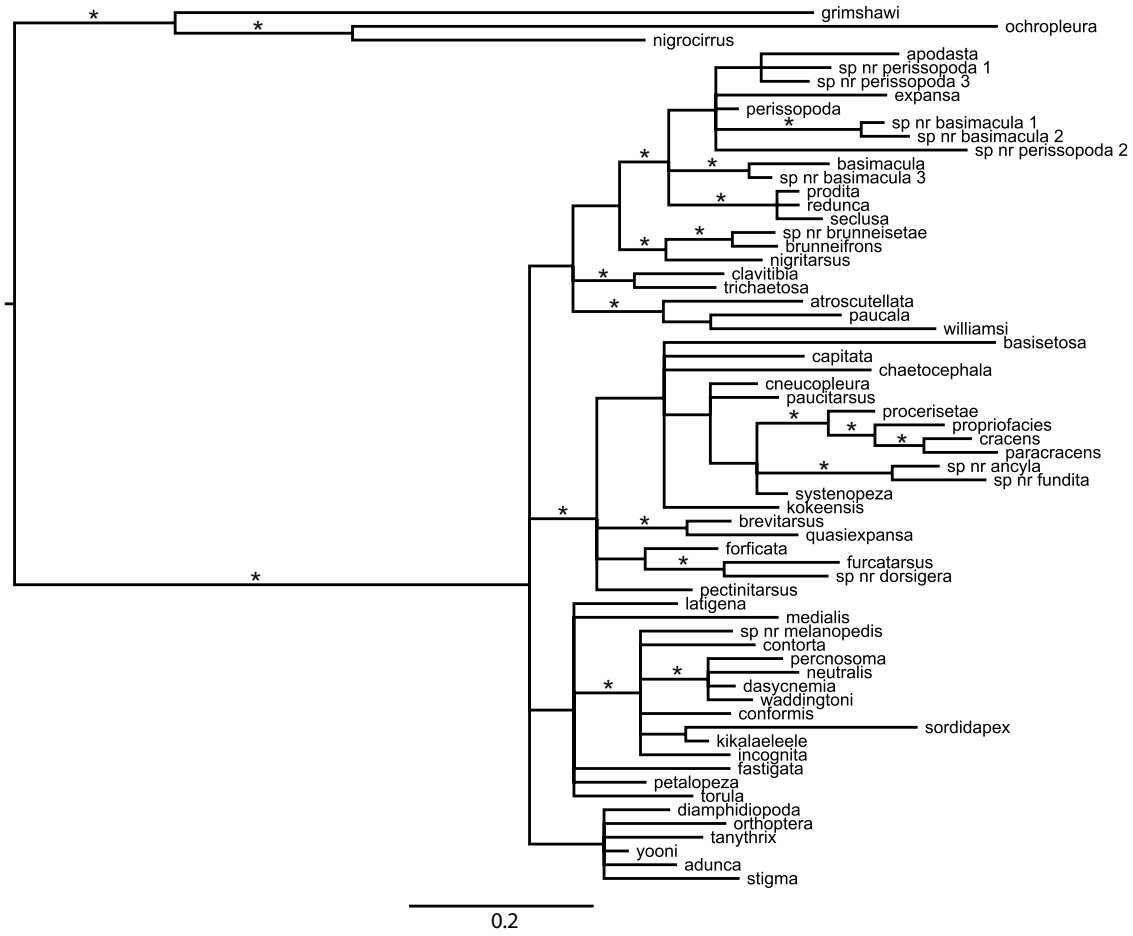


Figure 8. Snf genealogy

Figure 9. Wee genealogy

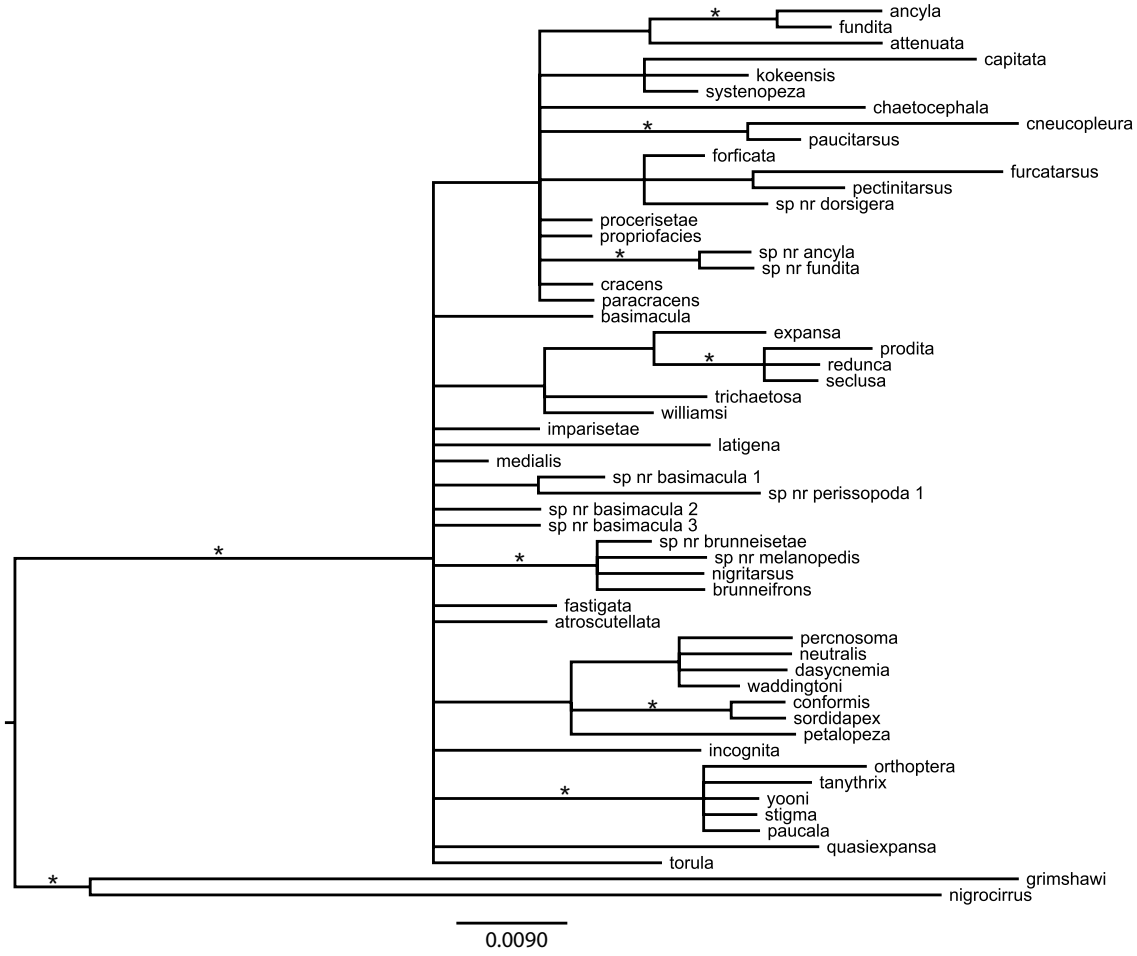


Figure 10. Concatenated, partitioned phylogeny of the AMC

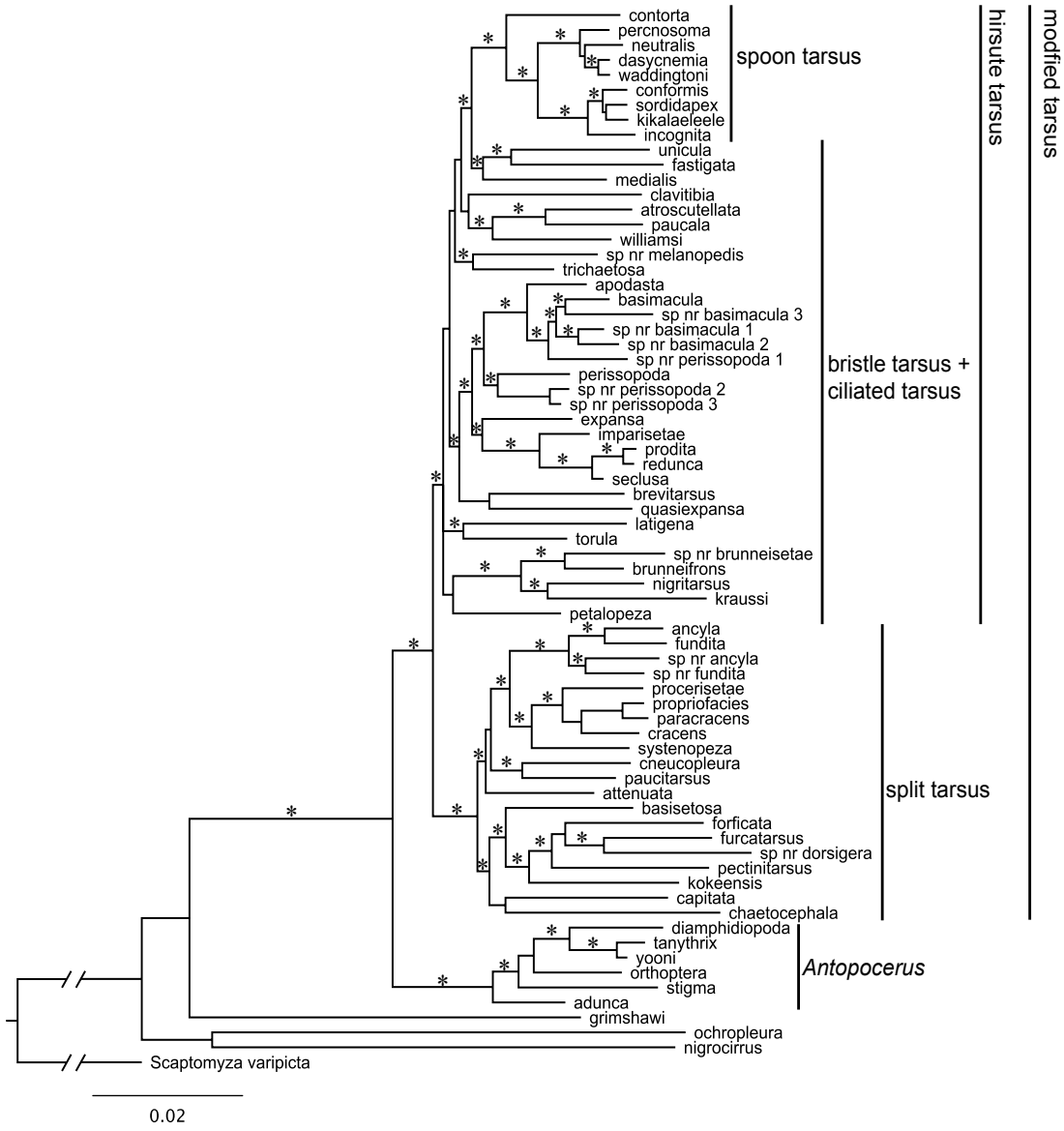


Figure 11. Phylogeny of the species tree inferred using STEM

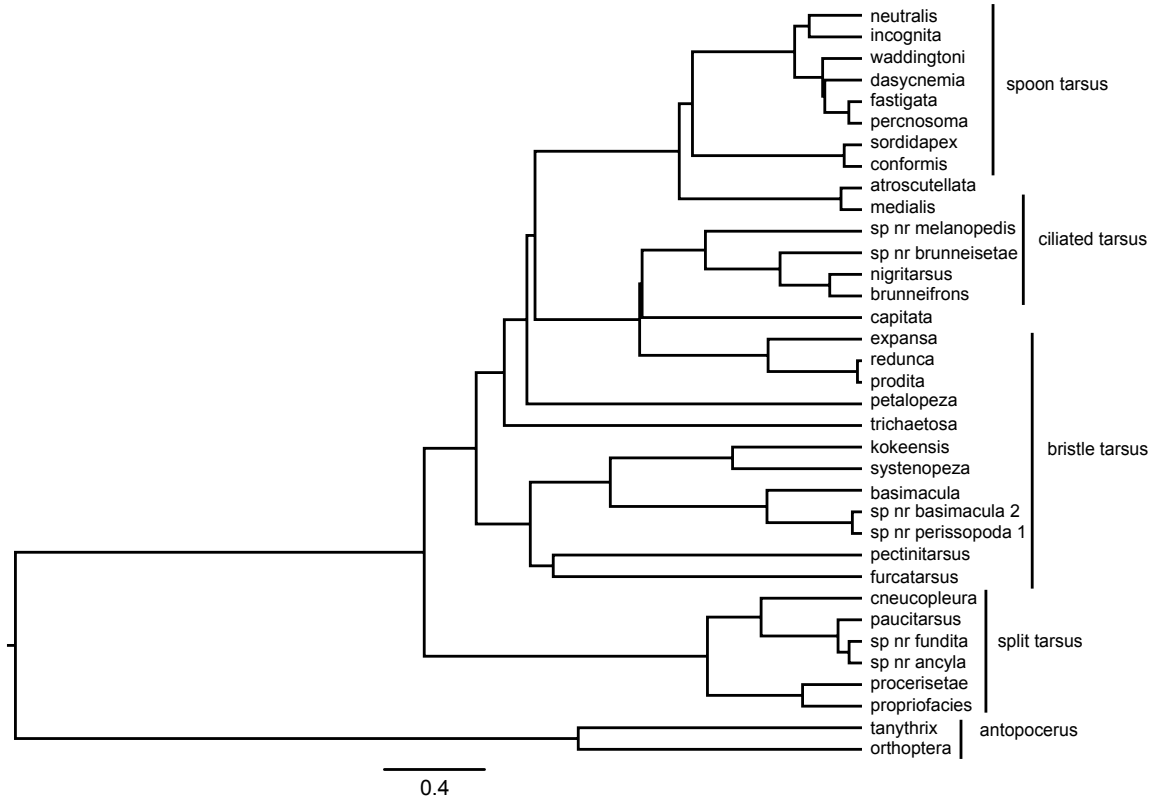


Figure 12. Chronogram of the AMC estimated in BEAST

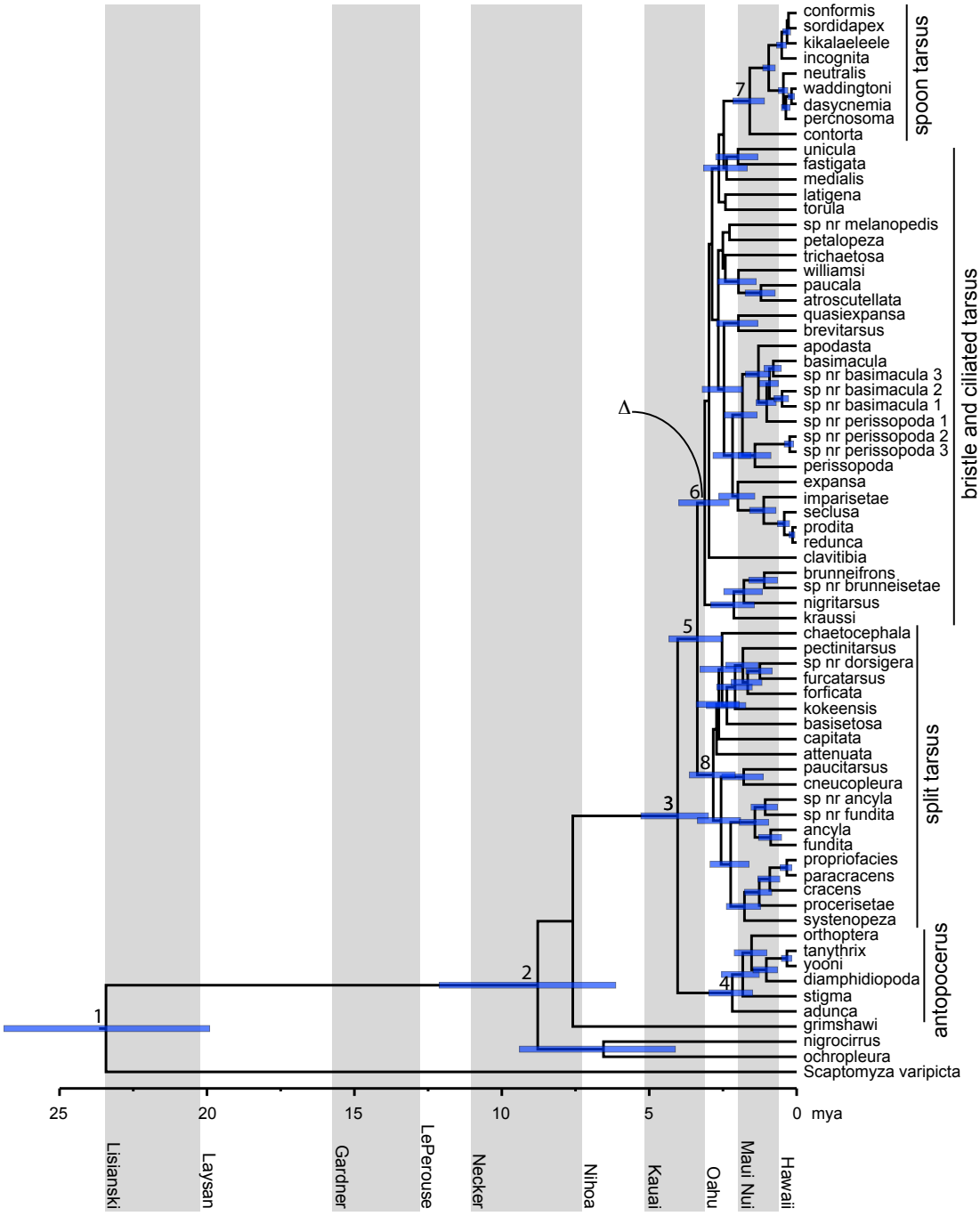


Figure 13. Lineage through time plot

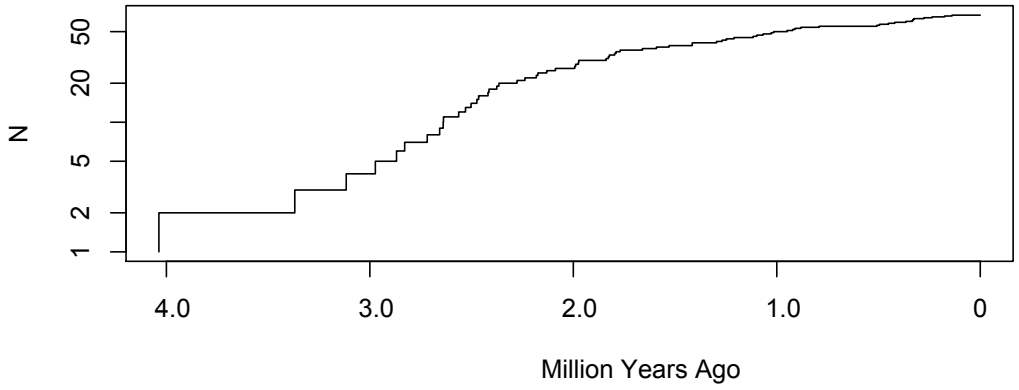


Figure 14. Ancestral range reconstruction estimated under a DEC model

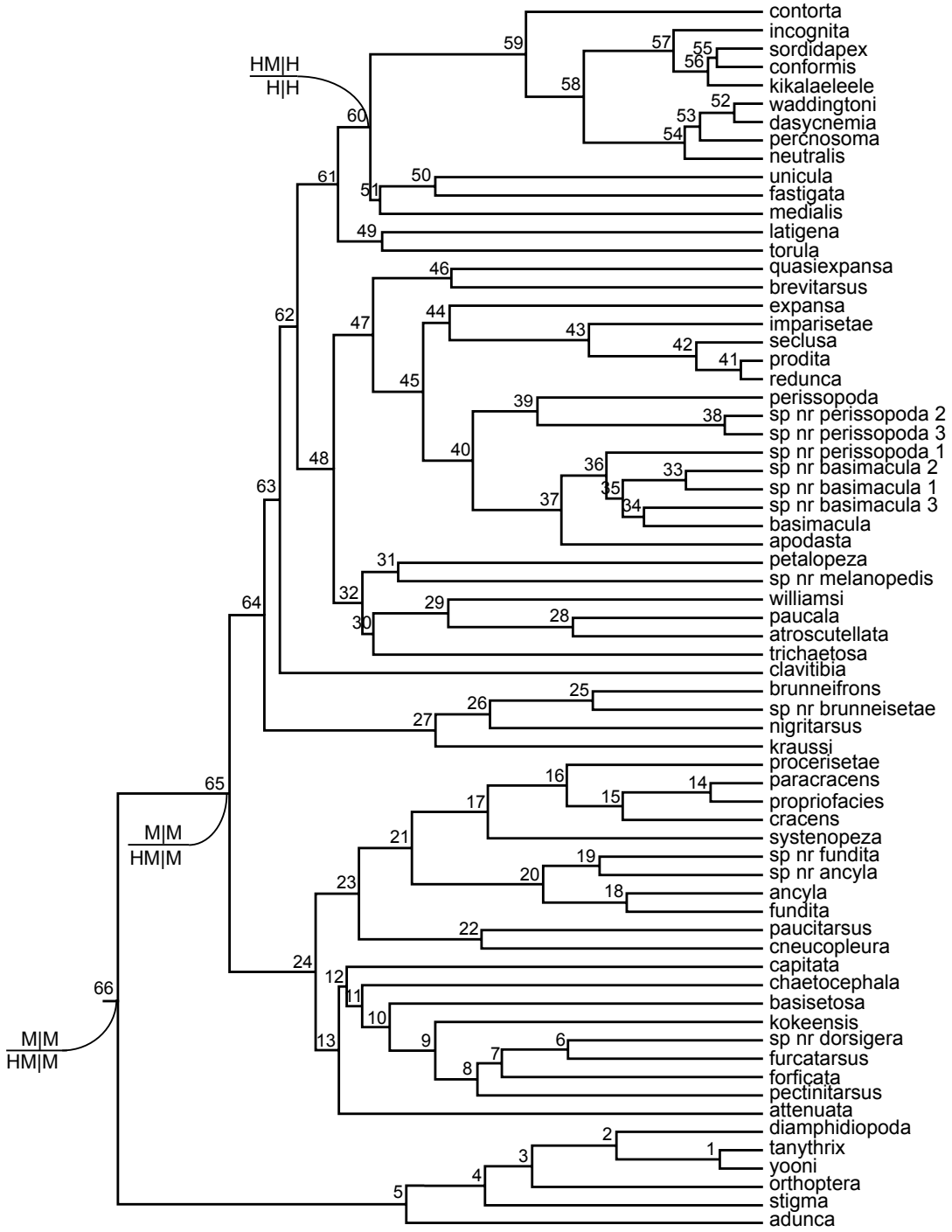
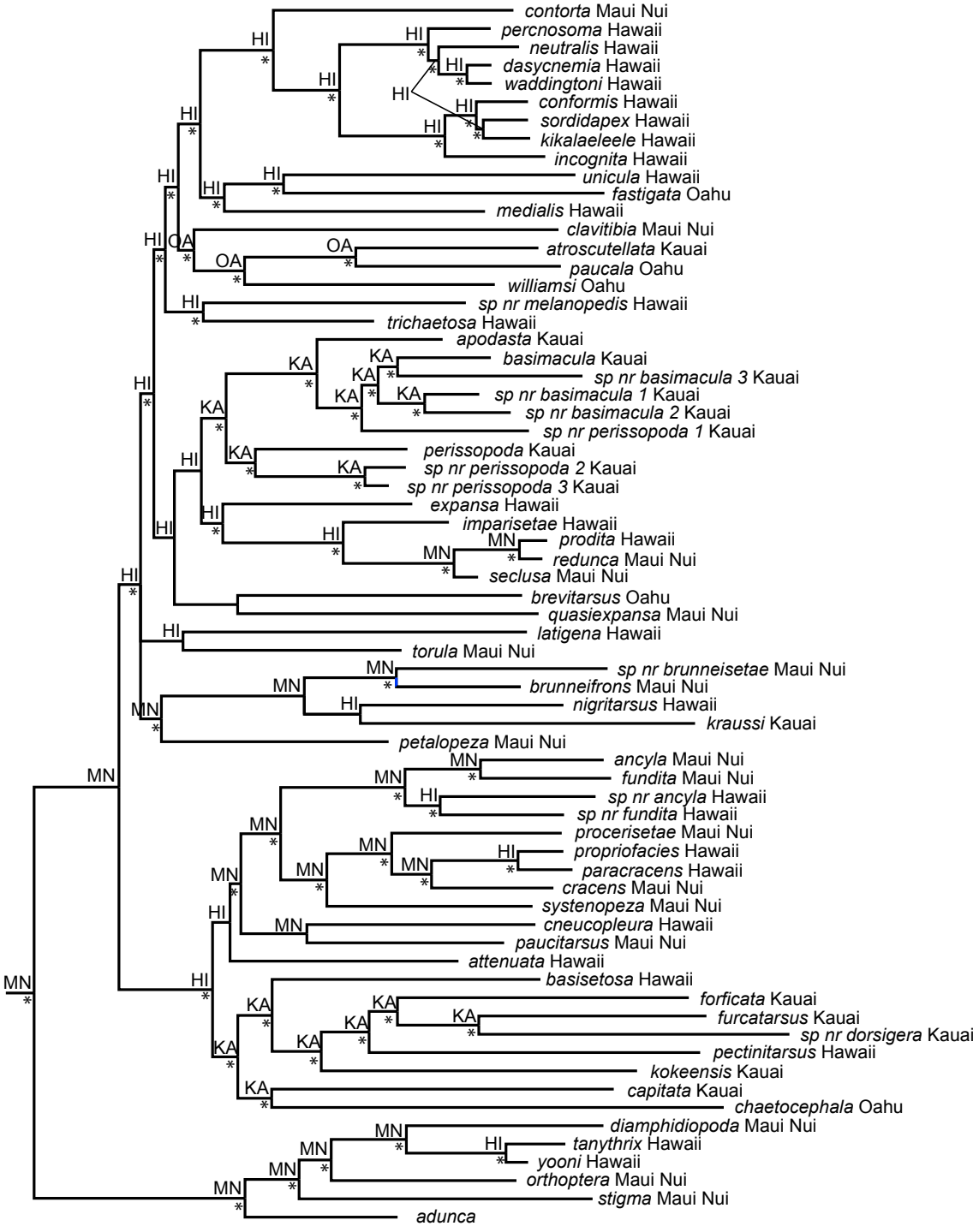


Figure 15. Ancestral range reconstruction estimated under a stochastic mapping model



Chapter 2: Review of the *spoon tarsus* subgroup of Hawaiian *Drosophila* (Drosophilidae: Diptera), with a description of one new species

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Lapoint, R.T., Magnacca, K.N., and O'Grady, P.M., 2009. Review of the *spoon tarsus* subgroup of Hawaiian *Drosophila* (Drosophilidae: Diptera), with a description of one new species. *Zootaxa* 2003: 58-63.

Abstract

The spoon tarsus species subgroup is revised and this clade is placed in the *modified tarsus* group of Hawaiian *Drosophila*. The species boundaries in this group are discussed in light of diagnostic secondary sexual characters of males. *Drosophila septuosa* Hardy is regarded as a junior synonym of *Drosophila percnosoma* Hardy. A new species, *Drosophila kikalaeleele* Lapoint, Magnacca & O'Grady is described. *Drosophila fastigata* Hardy, a species endemic to O'ahu, is added to the species subgroup, bringing the total number of known species to 12. An updated key to species is provided to the spoon tarsus subgroup.

Introduction

The Hawaiian Drosophilidae is an impressive example of an adaptive radiation, with an estimated 1,000 species filling diverse ecological niches (Heed 1968; Kambysellis *et al.* 1995; Magnacca, Foote & O'Grady 2008; Montgomery 1975) exhibiting an impressive array of behaviors (Spieth 1966). Males of many species display extreme sexual dimorphism in wing, foreleg, and mouthpart characters. These characters, in combination with the elaborate mating displays, have led many researchers to propose that sexual selection may play a role in the rapid diversification observed in this group (Kaneshiro 1988; Spieth 1966; Carson 1997).

The Hawaiian Drosophilidae are placed in two genera, *Drosophila* and *Scaptomyza*. The Hawaiian *Drosophila* lineage has been divided into a number of species groups and subgroups based on taxonomic (Hardy 1965), chromosomal (Carson & Stalker 1968), ecological (Heed 1968), and phylogenetic (Throckmorton 1966; Thomas & Hunt 1993; Kambysellis *et al.* 1995; Baker and DeSalle 1997; Remsen & DeSalle 1998; Bonacum 2001; Remsen & O'Grady 2002) analyses (Fig. 1). The *haleakalae* species group (sensu Hardy *et al.* 2001) is basal within the Hawaiian *Drosophila* lineage. Throckmorton suggested that the *ciliated tarsus* species group, characterized by having elongate setulae on the foretarsi of males, is also a more basal member of this group, although more recent molecular phylogenetic analyses have contradicted this placement and consider these species to be a subgroup within the *modified tarsus* species group (Bonacum 2001). A large assemblage of species belonging to the *modified mouthpart* and *picture wing* species groups form a poorly supported clade that is sister to two species groups, *antopocerus* and *modified tarsus*. The latter two groups together form what is referred to as the "leaf breeder clade" as the majority of species in this group utilizes decaying leaves as a larval substrate. The *modified tarsus* species group is divided into four subgroups, *split*, *bristle*, *ciliated* and *spoon tarsus*, based on sexually dimorphic characters on male forelegs (Throckmorton 1966). Members of the *bristle* and *ciliated tarsus* subgroups have strong bristles on the apical portion of the basitarsus. Species placed in the *split tarsus* subgroup have only four tarsal segments (the remainder of species in Drosophilidae have five) and the second tarsal segment inserts medially into the first (rather than apically as in all other drosophilids). The *spoon tarsus* species have the second tarsal segment of the forelegs shortened and anteriorly concave, similar to a spoon. The *ciliated tarsus* subgroup is characterized primarily by lacking the modifications of the other subgroups, possessing only thin, elongate hairs that are commonly found on the tarsi of many Hawaiian *Drosophila*. While the *split* and *spoon tarsus* subgroups are monophyletic (Bonacum 2001), the *bristle tarsus* subgroup, along with the *ciliated tarsus* species subgroup, forms a paraphyletic grade at the base of the modified tarsus group. Two species of each of the latter were included in Bonacum's (2001) phylogenetic analysis, and were not sister to each other.

Grimshaw (1901) described the first *spoon tarsus* species, *Drosophila sordidapex*, from the island of Hawai'i. Later Hardy (1965) described nine new species in this subgroup when he revised the Hawaiian Drosophillidae: *D. conformis* (Hawai'i), *D. contorta* (Maui), *D. dasycnemia* (Hawai'i), *D. incognita* (Hawai'i), *D. mimiconformis* (Maui Nui), *D. neutralis* (Hawai'i), *D. percnosoma* (Hawai'i), *D. septuosa* (Hawai'i), and *D. waddingtoni* (Hawai'i, Maui Nui). *Drosophila atroscutellata* from Kaua'i was added a year later (Hardy 1966). The *spoon tarsus* species are commonly found in association

with the decaying leaves of Araliaceae (especially *Cheirodendron trigynum* and *Tetraplasandra oahuensis*) and Aquifoliaceae (*Ilex anomala*) (Magnacca *et al.* 2008). These taxa can be quite abundant during times of the year when there is a large amount of leaf material on the ground (Mangan 1978), and are readily attracted to fermented banana and mushroom baits. Characters diagnostic for species identification include the presence or absence of dense setae on the posterior surface of the fore tibia of the males, coloration of the face, thorax, legs and abdominal tergites, and position and intensity of infuscation on the apical portion of the wing. Other characters include degree of curving of the basitarsus (*D. contorta*) and the presence of long setae on the basitarsus (*D. incognita*). Here, we describe one new species in this group, *Drosophila kikalaeleele* from Hawai‘i, and place a second, already described taxon, *D. fastigata* Hardy from O‘ahu, in the spoon tarsus subgroup due to its enlarged second tarsal segment and the high morphological similarity of the basitarsus to that of *D. atroscutellata*. In addition, *D. septuosa* is regarded as a junior synonym of *D. percnosoma*. This brings the total number of spoon tarsus species to 12.

Material examined

Holotypes, paratypes, and series of other specimens are housed in the B. P. Bishop Museum (BPBM), the Natural History Museum (London) (BMNH), and University of Hawai‘i Insect Museum (UHIM). Recent collections (O’Grady, Magnacca, Lapoint, Bennett) were also examined for these descriptions. It is not possible to reliably identify spoon tarsus females to species so all descriptions included here refer only to male characters.

Collector abbreviations are: AB = A. Busck; AC = Andrew Christie; CDS = Chelsea D. Specht; CH = Cheryl Hayashi; CPH = C. P. Hoyt; DO = Deodoro Oliviera; DEH = D. Elmo Hardy; DF = David Foote; EHB = E. H. Bryan; EMC = Elysse M. Craddock; FEC = Francis E. Clayton; GMB = Gordon M. Bennett; GS = Gregor Schuurmann; HLC = Hampton L. Carson; HTS = Herman T. Speith; JBS = Julian B. Stark; JEG = John E. Gatesy; JWB = John W. Beardsley; KNM = Karl N. Magnacca; KRG = Kari Roesch Goodman; KTK, Kevin T. Kaneshiro; KYK = Kenneth Y. Kaneshiro; LHT = Lynn H. Throckmorton; MD = M. Dennis; MG = Mark Giannullo; MPK = Michael P. Kambysellis; MRW = Marshall R. Wheeler; MT = M. Tamashiro; OHS = Otto H. Swezey; PMO = Patrick M. O’Grady; RN = R. Namba; RHR = Richard H. Richardson; RTL = Richard T. Lapoint; SH = Sara Hotchkiss, SLM = Stephen L. Montgomery; TL = T. Lyttle; WBH = William B. Heed; WCM = W. C. Mitchell; WMG = W. M. Giffard; YK = Y. Kondoh.

O’Grady field notes are decimal numbers prefaced with an O (e.g., O38.3); alcohol and DNA stocks maintained in the O’Grady Lab are six digit numbers prefaced with an O (e.g., O201595); frozen material maintained in the American Museum of Natural History’s Ambrose Monell Cryo Collection are six digit numbers prefaced by AMCC.

Key to males in the spoon tarsus subgroup

1. Front tibia densely setose on posterior surface with setae equal to or longer than tibial

spur (Fig. 2b) 2
Front tibia not densely setose on posterior surface, setae present less than length of tibial spur (Fig. 2a) 4

2. Thorax entirely yellow, tinged with brown on dorsum; abdomen reddish brown on dorsum, yellow on sides and venter (Maui) ..... *D. contorta*
Thorax brown to black; abdomen mostly dark brown to black 3

3. Fifth and sixth abdominal tergites yellow; wings hyaline; thorax dark brown to black; femora tinged with brown (Hawai‘i, Maui Nui) ..... *D. waddingtoni*
Abdomen entirely dark brown to black; wings subhyaline with brownish infuscation extending through apical third of cell R1, across cell R2+3, and through anterior ~1/2 of cell R4+5 (Fig. 3d); thorax brown; legs entirely yellow, coxae and apical tarsal segments yellow brown (Hawai‘i) ..... *D. dasycnemia*

4. Spoon reduced, segment not concave (Fig. 2a) 5
Spoon well developed, distinctly concave 6

5. Wing with apical spot extending from cell R2+3 to cell M1 (Fig. 3a); thorax pale yellow (Kaua‘i) ..... *D. atroscutellata*
Wings hyaline; thorax brown to dark brown (O‘ahu) ..... *D. fastigata*

6. Front tibia setose on posterior surface without setae longer than tibial spur (Fig. 2e). Wings slightly darkened; thorax dark brown to black; femora, abdomen black (Hawai‘i) ..... *D. percnosoma*
Coloration not as above; other characters variable 7

7. Anteroapical wing spot dark brown, distinct, covering apical 1/2 of cell R2+3 and extending halfway into cells R1 and R4+5 (Fig. 3b: sometimes reduced to stripes along veins R2+3 and R4+5) 8
Wings with spot absent or not as above, broader and more diffuse 9

8. Anal plate black (Hawai‘i) ..... *D. kikalaeleele*
Anal plate yellow (Hawai‘i) ..... *D. sordidapex*

9. Wings lacking distinct brown markings 10
Wings subhyaline with distinct brownish infuscation extending through apical 1/3 of cell R1, across cell R2+3, and through anterior ~1/2 of cell R4+5 (Fig. 3c) 11

10. Wings subhyaline, faintly infuscated with brown, but lacking distinct markings (Hawai‘i) ..... *D.neutralis*
Wings completely hyaline (Maui Nui) ..... *D. mimiconformis*

11. Front basitarsus with ~7 long setae on apical half of anterior margin (Fig. 2d); dark brown to black thorax; sixth and part of fifth tergites yellow (Hawai‘i) ..... *D. incognita*
Front basitarsus with few weak setae; sixth tergum black, nearly as long as fifth tergum;

Anal plate yellow (Hawai‘i) ..... *D. conformis*

***Drosophila atroscutellata* Hardy**
Figures 2a and 3a

Drosophila atroscutellata Hardy, 1966: 200

Diagnosis. The spoon structure of *Drosophila atroscutellata* males is highly reduced relative to the other taxa in this subgroup. The forebasitarsi is slightly extended apically and densely setose with 7 bristles that start at the apex and extend 2/3 of the length of the segment. The second tarsal segment on the forelegs of the male is barely wider than the third tarsal segment, about as long and lacking concavity. Legs mostly yellow, with a dark brown margin at the apical end of the middle tibia (see Fig. 2a). Thorax light yellow except for the dark brown scutellum. The wings are hyaline except for a dark apical spot extending from the anterior portion of vein R2+3 to halfway through cell M1 (see Fig. 3a).

Types. KAUA‘I: Holotype male (BPBM 11258), Halemanu Valley, 1220m, 28.viii.1964, HTS. Allotype female (BPBM 11258a), same collection as holotype. Type locality reported as “Malemanu Valley” by Evenhuis (1982).

Material Examined. KAUA‘I: 8 males have been studied from the BPBM: males, Kōke‘e, 3600 ft, vii.1963, HLC, FEC & MRW; 2 males, Kōke‘e, 3600 ft., vi.1964, HLC, FEC & MRW; 2 males, Halemanu Valley, 4000 ft, vi.1964, FEC, MRW, DEH & HTS; 3 males, Nu‘alolo Trail, 3800 ft., v.2007, KNM. Over 40 males and 25 females in the UHIM were also examined from the following localities: 4 males and 4 females, from Kōke‘e, 3600', iii.1964, MRW; 1 male, Kōke‘e, 3600', iv.1964, DEH; 15 males, Kōke‘e, 3600', vi. 1964, DEH, HTS, HLC; 1 male and 2 females, Kōke‘e, 3600', vi.1966, DEH; 4 males, Halemanu Valley, 4000', iii.1964, MRW; 16 males and 14 females, Halemanu Valley, 4000', vi.1964, DEH, HTS, HLC; 3 males and 5 females, Halemanu Valley, 4000', viii.1964, DEH, HTS.

Distribution. This species is endemic to wet forest habitat on Kaua‘i.

Chromosomes. The metaphase complement of this species is 5 rods and 1 dot (Clayton 1968; Yoon & Richardson 1978).

Illustrations. Foreleg (Hardy 1966: 201, figs. 3c, d); mouthparts (Hardy 1966: 201, fig. 3a); wing (Hardy 1966: 201, fig. 3b); middle tibia (Hardy 1966: 201, fig. 3e); male genitalia, ventral and lateral (Hardy 1966: 201, figs. 3f, g); female genitalia, lateral (Hardy 1966: 201, fig. 3h).

Discussion. *Drosophila atroscutellata* is the only spoon tarsus species recorded from Kaua‘i. It is considered the most basal member of the group because of its highly reduced spoon.

***Drosophila conformis* Hardy**
Figures 2c and 3c

Drosophila conformis Hardy, 1965: 319–320

Diagnosis. Tibia lacking profuse setae equal to, or longer than, the tibial spur. Legs are completely yellow, except for last tarsal segment being brown (see Fig. 2c). The thorax ranges from light to dark brown. The wings have a diffuse brown marking apically, which extends from cell R1 to halfway through cell R4+5 (see Fig. 3c). The abdomen is completely black.

Types. HAWAI'I: Holotype male (BPBM 6330) Upper Ōla'a Forest, vii.1953, DEH. Allotype female (BPBM 6330a) same collection as holotype (Evenhuis 1982).

Material Examined. HAWAI'I: 16 males have been studied from the BPBM: 4 males, Kīlauea, viii.1958, JWB; 1 male, Kīlauea Field Station, 3900 ft., vi.2006, KNM; 1 male, Manukā vii.2006, KNM; 1 male, Stainback Highway, Tom's Trail, 3200', x.2006, KNM, RTL, GMB; 2 males, Pu'u Huluhulu, x.2006, KNM, RTL, GMB; 2 males, Laupāhoehoe, 3800', x.2006, KNM, RTL, GMB; 5 males, Ōla'a, Tr 18, ii.2006, KNM. 22 males deposited in the UHIM have been examined from the following localities: 3 males, Kīlauea, viii.1958, JWB; 9 males, Upper Ōla'a, viii.1952, DEH; 1 male, Nāpau Crater, Kīlauea, vii.1953, DEH; 1 male, Nāpau Crater, Kīlauea, vii.1953, DEH; 6 males, Keanakolu, vi.1966, WBH; 2 males, Hōnaunau Forest Reserve, ii.1966, WBH. The following material is present at AMNH: males, Stainback Highway, 7–8.ii.1999, O49.2, PMO, SLM; 2 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O112.2, 20.x.2000, PMO; 1 male, Volcanoes National Park, Kīpuka Puaulu, O127.5, 29.vii.2001, PMO; 2 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O128.4, 29.vii.2001, PMO, CDS; 18 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O132.5, AMCC105703, 2.viiI.2001, PMO, CDS; 48 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O140.7, AMCC105686, 12.iv.2002, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: 3 males, Volcanoes National Park, Ōla'a Forest, near pole 48, O247.2; O201320; 6–7.vii.2004, PMO, MG, CDS.

Distribution. This species is endemic to the Big Island of Hawai'i. **Behavior.** Shelly (1987; 1990). **Ecology.** This species has been reared from leaves of *Ilex anomala* (Aquifoliaceae) (Heed 1968). **Illustrations.** Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 220, figs. 66a, b); phallus (Kaneshiro 1976: 267, fig. 5d); terminalia, male, lateral (Hardy 1965: 220, fig. 66c).

Molecular Biology. DNA sequences (O'Grady & DeSalle 2008).

Drosophila contorta Hardy

Drosophila contorta Hardy, 1965: 226–227

Diagnosis. Elongate setae present on the posterior surface of fore tibia. The front basitarsus of the males is curved and the face is distinctly concave. The spoon is as long as the third tarsal segments, nearly as wide and distinctly concave. The coloration of the species is very light. The wings are subhyaline (see Fig. 3e for example).

Types. MAUI: Holotype male (BPBM 6333) Waikamoi, 1220 m, vii.1956, DEH. Locality recorded as "Wai*a*kamoi" on type label (Evenhuis 1982).

Material Examined. MAUI: The following material is present at AMNH: males, Makawao Forest Reserve, Pig Hunter's Trail, O153.K, AMCC105817, 23.iv.2002, PMO, DO.

Distribution. This species is endemic to Maui. **Illustrations.** Foreleg (Hardy 1965: 227, fig. 69b); head (Hardy 1965: 227, fig. 69a.

***Drosophila dasycnemia* Hardy**
Figures 2b and 3d

Drosophila dasycnemia Hardy, 1965: 236–238

Diagnosis. Elongate setae present on the posterior surface of fore tibia. The legs are yellow except for brown coxae and terminal portions of the last tarsi (see Fig. 2b). Wings are lightly infuscated with a brown marking apically, which extends from cell R1 to halfway through cell R4+5 (see Fig. 3d). The thorax is brown, and the abdomen is almost entirely dark brown, with the last tergite being yellow.

Types. HAWAI'I: Holotype male (BPBM 6337) Upper Ōlaʻa Forest, viii.1952, DEH (Evenhuis 1982).

Material Examined. HAWAI'I: 61 males have been deposited in the BPBM: males, Kaiwiki, ix.1918, OHS; 2 males, Stainback Highway, 3600', x.2006, KNM, RTL, GMB; 2 males, Stainback Highway, Tom's Trail, 3200', x.2006, KNM, RTL, GMB; 2 males, Kīpuka 9, Kaūmana Trail, x.2006, KNM, RTL, GMB; 5 males, Ōlaʻa Forest, x.2006, KNM, RTL, GMB; 5 males, Ōlaʻa Tr 18, vi.2006, KNM; 10 males, Ōlaʻa Tr 18, ii.2006, KNM; 3 males, Kawaihae Uka, Kohalas, x.2006, KNM, RTL, GMB; 1 male, Kukuiopaʻe, South Kona Forest Reserve, x.2006, KNM, RTL, GMB; 5 males, Laupāhoehoe, 3700', x.2006, KNM, RTL, GMB. 30 males from the UHIM have been examined from the following localities: 5 males, Upper Ōlaʻa, vii.1956, DEH, WCM; 3 males, Upper Ōlaʻa, viii.1958, DEH, WCM; 12 males, Upper Ōlaʻa, viii.1952, DEH, WCM; 7 males, Kīlauea, viii.1958, JWB; 2 males, Volcano, v.1915, AB; 1 male, Mud Lane, vi.1964, DEH, LHT. The following material is present at AMNH: 5 males, Forest behind Volcano Solid Waste Transfer Station, O40.4, 5.vii.1998, PMO, SLM; 14 males, Stainback Highway, 7–8.ii.1999, O49.3, PMO, SLM; 2 males, Forest behind Volcano Solid Waste Transfer Station, O51.5, 12–14.iii.1999, PMO, JBS; 5 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O93.6, 7.ix.2000, PMO; 16 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O132.6, 2.viIi.2001, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: 8 males, Volcanoes National Park, Ōlaʻa Forest, near pole 48, O247.2; O201321; 6–7.vii.2004, PMO, MG, CDS; 5 males, Tree Planting Road, O256.8; O201497; 11.vii.2004, PMO, MG; 4 males, Puu Makaala Trailhead, O257.1, O201318, 11.vii.2004, PMO, MG; 1 male, Kohala Mountains, Top of Waipio Falls, O269.6, O201595, 16.vii.2004, PMO, CDS, GS, SH, MG; 2 males, Volcanoes National Park, Upper Ōlaʻa Forest, end of Wright Road, O272.F, O200625; 20.vii.2004, PMO, MG, GS, AC.

Distribution. This species is endemic to the island of Hawaiʻi.

Behavior. Speith (1966:272).

Ecology. This species has been reared from leaves of *Cheirodendron trigynum*

(Araliaceae) (Heed 1968; Mangan 1978).

Illustrations. Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 237, fig. 74a); phallus (Kaneshiro 1976: 267, fig. 5); terminalia, male, lateral (Hardy 1965: 237, fig. 74c); wing (Hardy 1965: 237, fig. 74b; Edwards et al 2007: fig7).

Molecular Biology. DNA sequences (Baker & DeSalle 1997).

Drosophila fastigata Hardy

Drosophila fastigata Hardy, 1965: 271–273

Diagnosis. Legs entirely yellow. Apex of the basitarsus extends dorsally into a hatchet shaped lobe with two setae. The second tarsal segment is as long as wide and not concave. Wings hyaline, lacking distinct markings (see Fig. 3e for example). Thorax brown tinged with yellow, darker dorsally. Scutellum ranges from dark brown to black. Abdominal tergites are brown, except for last tergite which is yellow.

Types. O‘AHU: Holotype male (BPBM 6529) Pūpūkea, vii.1958, DEH (Evenhuis 1982).

Material Examined. O‘AHU: 9 males have been studied from the BPBM: males, Poamoho Trail, 1700’, v.1953, DEH; 1 male, Mt. Ka‘ala, iv.1929, EHB; 2 males, Manoa Cliff Trail Trail, ii.2007, KNM; 5 males, Mt. Ka‘ala, 3950’, v.2007, KNM.

Distribution. This species is endemic to the island of O‘ahu.

Ecology. This species has been reared from leaves of *Cheirodendron platyphyllum* (Araliaceae) (Heed 1968).

Illustrations. Foreleg (Hardy 1965: 272, fig. 93a); terminalia, male, lateral (Hardy 1965: 272, figs. 93b, c).

Discussion. *D. fastigata* is the only spoon tarsus species endemic to O‘ahu. Due to its morphological similarity to *D. atroscutellata*, this species is included in this group, but is also considered basal due to the reduced spoon.

Drosophila incognita Hardy

Figure 2d

Drosophila incognita Hardy, 1965: 319–320

Diagnosis. The anterioapical portion of the forebasistarus of males have 6 to 8 long setae (see Fig. 2d). The thorax is dark brown. The apical wing spot is similar to that of *D. conformis* but darker (see Fig. 3c for example). The lateral portion of most of the abdomen and the entire final tergite is yellow.

Types. HAWAI‘I: Holotype male (BPBM 6377) Upper Ōla‘a Forest, viii.1952, DEH (Evenhuis 1982).

Material Examined. HAWAI‘I: males has been studied from the BPBM: Ōla‘a Trail 18, vi.2006, KNM. 6 males have been studied from the UHIM: 2 males, Upper Ōla‘a Forest, vii.1956, DEH; 1 male, Kīlauea, viii.1958, JWB; 2 males, Upper Ōla‘a

Forest, viii.1952, DEH; 1 male, Kaiholena, Kohala Mountains, viii.1952, DEH. The following material is present at AMNH: males, Volcanoes National Park, Ōla‘a Forest, near Pole 44, O132.7, AMCC105701, 2.viIi.2001, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: males, Tree Planting Road, O256.8; O201315; 11.vii.2004, PMO, MG.

Illustrations. Morphological structures of this species are depicted in multiple publications including: forleg (Hardy 1965: 320, fig. 118a); phallus (Kaneshiro 1976: 267, fig. 5c); terminalia, male, lateral (Hardy 1965: 320, fig. 118c); wing (Hardy 1965: 320, fig. 118c)

Discussion. This species is morphologically very close to *D. conformis* and the defining features are the long setae on the basitarsus.

Drosophila kikalaeleele, Lapoint, Magnacca & O’Grady new species

Diagnosis: This species is very similar to *D. sordidapex* in overall morphology, notably the discrete marking at the apical portion of the wing, and is inferred to be the sister species. *Drosophila kikalaeleele* is differentiated by the last two tergites being completely yellow on an otherwise completely brown to black abdomen, and by having the anal plate black.

Male Description. Body length: 2.5 to 3 mm. Wing length: 3 mm. **Head**. Lower portion of frons yellow. Ocellar triangle black. Vertex ranges from brown to black. Face ranges from light to dark brown. Gena yellow. Ocellar and vertical setae ~ as long as antennal arista. Anterior reclinate slightly shorter than proclinate. Clypeus, labella and palpi yellow. Several weak black setae on apical portion of palpi. Labellum fringed with weak yellow setae. Mouthparts not ornate. First and second antennal segments yellow. Third antennal segment completely dark brown to black. 6 dorsal rays, 2 ventral rays and apical fork on arista.

Thorax. Thorax dorsally light brown with four rufous stripes running anterior to posterior. Scutellum entirely yellow. The anepisternum dark brown, otherwise lateral portion of thorax yellow tinged with light brown. Posterior dorsocentral setae 1/3 longer than anterior dorsocentral setae. Apical scutellar setae ~2/3 as long as the basal scutellar setae. Haltere completely yellow.

Legs. Basitarsi lack setae apically; second tarsal segment concave and 1/3 longer than third tarsal segment. Fore and mid legs entirely yellow except for slight darkening of fifth tarsal segment and middle tibia. Mid leg yellow except for brown tibia. Short setae 1⁄2 as long as preapical bristle on tibia.

Wings. Discretely edged wing spot darkens apical half of cell R2+3, lower apical margin of cell R1 and upper apical margin of cell R4+5. Remainder of wing hyaline. Costal fringe extends halfway between apical margin of cell R2+3 (see Fig. 3b).

Abdomen. Dorsal tergites dark brown to black with yellow on posterior margins of each tergite, except for yellow to light brown fifth and sixth tergites. Sternites yellow. Anal plate dark black. Genitalia identical to *D. sordidapex*.

Type Material. Holotype male (BPBM 16909), South Kona Forest Reserve, x.2006, RTL, KNM, GMB.

Material Examined. HAWAI‘I: 2 males have been deposited into the BPBM:

males, Ōlaʻa Trail 18, vi.2006, KNM; 1 male South Kona Forest Reserve, x.2006, RTL, KNM, GMB. 30 males have been deposited in the UHIM from the following localities: 19 males, Hualālai, vii.1970, WBH, MD, TL; 3 males, Hualālai, 3400', xii.1969, KYK; 1 male, Kīpuka No 9, Saddle Rd, vi.1969, WBH; 4 males, Kīpuka No 14, 5100', Saddle Road, vii.1969, WBH; 1 male, Puʻu Huluhulu, vii.1969, RHR; 1 male, Greenwell Ranch, Pauahi, vi.1974; 1 male, Upper Ōlaʻa Forest Reserve, x.1988, KYK.

Distribution and Ecology. Reared from Ilex leaves by W. B. Heed. Collected at Hualālai, Puʻu Huluhulu, the Saddle Road Kīpukas and the Ōlaʻa Forest.

Etymology: A combination of the Hawaiian words ʻeleʻele = black and kikala = buttock, posterior.

Relationships. Based on overall morphology, especially wing patterning, this species is close to *D. sordidapex*.

Drosophila mimiconformis **Hardy**

Drosophila mimiconfromis Hardy, 1965: 367–369

Diagnosis. The labella, palpi and frons are all yellow. The fore-tibia of the males lacks numerous vertical setae present on other species (see Fig. 2c). The wings are completely hyaline (see Fig. 3e). The thorax ranges in color from light to dark brown. The legs are entirely yellow. The abdomen is completely black.

Types. MOLOKAʻI: Holotype male (BPBM 6401) Maunawainui Valley, vii.1952, DEH (Evenhuis 1982).

Material Examined. MOLOKAʻI: 2 males has been studied from BPBM: males, Maunawainui Valley, vii.1952, DEH; 1 male, East Kawela Gulch, ii.2007, KNM. 26 males have been deposited in the UHIM from the following localities: males, Hanalilolilo, viii.1953, MT; 15 males, Maunawainui Valley, vii.1952, DEH; 2 males, Puʻu Aliʻi, vii.1953, DEH, MT; 4 males, Puʻu Kolekole, v.1965, DEH; 3 males, Puʻu Kolekole, vi.1964, DEH; 2 males, South of Hanalilolilo, vii.1964, DEH.

Distribution. This species is endemic to Maui Nui (Molokaʻi, Maui, Lanaʻi).

Ecology. This species has been reared from leaves of *Cheirodendron trigynum* (Araliaceae) on Maui and Molokaʻi; leaves of *Tetraplasandra* sp (Araliaceae) on Molokaʻi; leaves of *Ilex anomala* on Maui (Aquifoliaceae) (Heed 1968).

Illustrations. Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 368, fig. 140a); phallus (Kaneshiro 1976: 267, fig. 5a); terminalia, male, lateral (Hardy 1965: 368, fig. 140b).

Discussion. *D. mimiconformis* is the only species endemic to the island of Molokaʻi. It is very similar to *D. conformis* of the island of Hawaiʻi, but can be easily distinguished by location of collection and a lack of pigmentation in the apical portion of the wing.

Drosophila neutralis **Hardy**

Drosophila neutralis Hardy, 1965: 383–385

Diagnosis. The labella, palpi and frons are all brown. Dense setae on the posterior portion of the tibia of the fore legs of the males are prone, not erect, and never longer than the tibial spur (see Fig. 2c for example). Legs are completely yellow, except for the last tarsal segments which are brown. The apical portion of the wings are hyaline (see Fig. 3e for example). Abdomen is mostly dark brown, except for the last two tergites and the sides of first tergite which are yellow.

Types. HAWAI'I: Holotype male (BPBM 6409) Kīlauea, viii.1958, JWB (Evenhuis 1982).

Material Examined. HAWAI'I: 32 males have been studied in the BPBM from the following localities: 3 males, 29 mi. Ōla'a, viii.1925, WMG; 1 male, Stainback Highway, 3600', x.2006, KNM, RTL, GMB; 2 males, Stainback Highway, Tom's Trail, 3200', x.2006, KNM, RTL, GMB; 5 males, Hionamoa Stream, Ka'ū Forest, x.2006, KNM, RTL, GMB; 3 males, Ōla'a Forest, x.2006, KNM, RTL, GMB; 2 males, Ōla'a Forest, Tr 18, ii.2007, KNM; 4 males, Ōla'a Forest, Tr 18 vi.2006, KNM; 4 males, HAVO escape road, 3900', vi.2006, KNM; 1 male, HAVO Kīpuka Puaulu, 4000', vi.2006, KNM; 1 male, Pu'u Huluhulu, x.2006, KNM, RTL, GMB; 1 male, Kukuiopa'e, South Kona, x.2006, KNM, RTL, GMB; 5 males, Laupāhoehoe, 3700', x.2006, KNM, RTL, GMB. Over 50 males have been deposited in the UHIM from the following localities: 20 males, Upper Ōla'a Forest, viii.1952, DEH; 6 males, Upper Ōla'a Forest, vii.1953, DEH; 8 males, Upper Ōla'a Forest, vii.1956, DEH; 1 male, Nāpau Crater, Kīlauea, vii.1952, DEH; 1 male, Pauahi 4300', Kīlauea, vii.1952, DEH; 10 males, Kīlauea, viii.1958, JWB; 6 males, Fern Forest, Upper Ōla'a Forest, iii.1966, KYK; 2 males, Kīlauea, viii.1966, KYK. The following material is present at AMNH: males, Forest behind Volcano Solid Waste Transfer Station, O40.2, 5.vii.1998, PMO, SLM; 2 males, Forest behind Volcano Solid Waste Transfer Station, O51.4, 12–14.iii.1999, PMO, JBS; 1 male, Volcanoes National Park, Ōla'a Forest, near Pole 44, O128.5, 29.vii.2001, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: males, Tree Planting Road, O256.8; O201316; 11.vii.2004, PMO, MG; 1 male, Kohala Mountains, Top of Waipio Falls, O269.6, O200623, 16.vii.2004, PMO, CDS, GS, SH, MG.

Distribution. This species is endemic to the Big Island of Hawai'i.

Ecology. This species has been reared from the bark of *Tetraplasandra oahuensis* (Araliaceae); the leaves and bark of *Cheirodendron trigynum* (Araliaceae) (Heed 1968; Magnacca et al 2008; Mangan 1978).

Illustrations. Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 384, fig. 148b); phallus (Kaneshiro 1976: 267, fig. 5h); terminalia, male, lateral (Hardy 1965: 384, fig. 148c).

Chromosomes. The metaphase complement of this species is 5 rods and 1 dot (Yoon & Richardson 1978).

***Drosophila percnosoma* Hardy**
Figure 2e

Drosophila percnosoma Hardy, 1965: 410–412

Drosophila septuosa Hardy, 1965:410–412, *syn. nov*.

Diagnosis. A large (~ 4 mm) species. The mouthparts and palpi are dark brown to black, along with the other features of the head. Tibia densely setose on posterior surface without setae longer than the tibial spur (see Fig. 2e). Femora are dark brown while the rest of the leg is yellow. The thorax is a dark brown to black. The wings are subhyaline with a faint infuscation of brown at the apical portion of the wing (see Fig. 3e). The abdomen is dark brown to black.

Types. HAWAI'I: Holotype male (BPBM 6419) Upper Ōlaʻa Forest, viii.1958, DEH. Allotype & (BPBM 6419a) same collection as holotype (Evenhuis 1982).

D. septuosa* Type*: Holotype male (BPBM 6443) Kīlauea, viii.1958, JWB (Evenhuis 1982).

Material Examined. HAWAI'I: 27 males have been studied from the BPBM from the following localities: males, Glenwood, iii.1919, OHS; 1 male 29 mi Ōlaʻa, viii.1925, WMG; 3 males, Stainback Highway, Tom's Trail, 3200', x.2006, KNM, RTL, GMB; 5 males, Hionamoa Stream, Kaʻū Forest, x.2006, KNM, RTL, GMB; 1 male, Kīpuka 9, Kaūmana Trail, x.2006, KNM, RTL, GMB; 4 males, Ōlaʻa Forest, x.2006, KNM, RTL, GMB; 4 males, from Puʻu Huluhulu, x.2006, KNM, RTL, GMB; 5 males, Laupāhoehoe, 3700', x.2006, KNM, RTL, GMB; 3 males, Ōlaʻa Forest, Tr 18 ii.2006, KNM. Over 70 males have been deposited in the UHIM from the following localities: 4 males, Upper Ōlaʻa Forest, viii.1952, WCM; 15 males, Kīlauea, viii.1958, JWB; 24 males, Upper Ōlaʻa Forest, viii.1952, DEH, WCM; 3 males, Kahuku Ranch, vii.1953, DEH; 1 male, Upper Ōlaʻa Forest, vii.1953, DEH, WCM; 2 males, Upper Ōlaʻa Forest, viii.1956, DEH; 1 male, Keauhou Ranch, Kīlauea, viii.1953, DEH; 1 male, Pauahi 4300', Kīlauea, vii.1952, DEH; 7 males, Lower Ōlaʻa Forest, vii.1964, LHT; 2 males, Upper Ōlaʻa Forest, vii.1964, LHT; 4 males, Upper Ōlaʻa Forest, ix.1964, HTS; 4 males, Upper Ōlaʻa Forest, vi.1966, WBH; 2 males, Upper Olaʻa Forest, vii.1963, WBH; 3 males, Nāpau Crater, Kīlauea, vii.1956, DEH; 1 male, Upper Ōlaʻa Forest, vii.1956, DEH. The following material is present at AMNH: 8 males, Forest behind Volcano Solid Waste Transfer Station, O40.3, 5.vii.1998, PMO, SLM; 10 males, Stainback Highway, 7–8.ii.1999, O49.1, PMO, SLM; 6 males, Forest behind Volcano Solid Waste Transfer Station, O51.1, 12–14.iii.1999, PMO, JBS; 5 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O93.5, 7.ix.2000, PMO; 2 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O112.3, 20.x.2000, PMO; 3 males, Volcanoes National Park, Kīpuka Puaulu, O127.3, 29.vii.2001, PMO; 2 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O128.3, 29.vii.2001, PMO, CDS; 44 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O132.4, AMCC105700, 2.viIi.2001, PMO, CDS; 49 males, Volcanoes National Park, Ōlaʻa Forest, near Pole 44, O140.8, AMCC105685, 12.iv.2002, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: 12 males, Volcanoes National Park, Ōlaʻa Forest, near pole 48, O247.2; O201444; 6–7.vii.2004, PMO, MG, CDS; 4 males, Tree Planting Road, O256.8; O201317; 11.vii.2004, PMO, MG; 3 males, Puu Makaala Trailhead, O257.1, O201500, 11.vii.2004, PMO, MG; 2 males, Volcanoes National Park, Upper Ōlaʻa Forest, end of Wright Road, O272.F, O200640; 20.vii.2004, PMO, MG, GS, AC; 24 males, Volcanoes National Park, Ōlaʻa Forest, near pole 44, O307.6, O201114; 8.viii.2005; PMO, GMB, CH, JEG.

Maui: 10 males have been studied from UHIM from Waikamoi Forest, v.1966, WBH.

Distribution. This species is endemic to the Big Island of Hawai‘i.

Behavior. Bell and Kipp (1994).

Chromosomes. The metaphase complement of this species is 5 rods and 1 dot (Clayton 1968).

Ecology. This species has been reared from leaves of *Cheirodendron trigynum* and *Tetraplasandra oahuensis* (Araliaceae); leaves of *Clermontia* sp (Campanulaceae) (Heed 1968; Magnacca et al 2008; Mangan 1978).

Illustrations. Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 411, figs. 163a, b); terminalia, male, lateral (Hardy 1965: 411, fig. 163c) ***as septuosa*:** foreleg (Hardy 1965: 462, fig. 187d); phallus (Kaneshiro 1976: 267, fig. 5g); terminalia, male, lateral (Hardy 1965: 462, fig. 187c).

Chromosomes. The metaphase complement of this species is 5 rods and 1 dot (Yoon & Richardson 1978).

Molecular Biology. DNA sequences (O'Grady & DeSalle 2008).

Discussion. Based on our morphological analysis, *Drosophila septuosa* (Hardy 1965) is synonymous with *Drosophila percnosoma* (Hardy 1965). The descriptions of both species by Hardy are very similar, with only minor differences between species, all well within normal variation for the group. We have examined both holotypes in the BPBM as well as the above series and compared both to the description found that all the characters used by Hardy to describe each species describe both holotypes and their respective series. On closer inspection the minor character differences Hardy utilizes in his descriptions are not present in one of the two holotypes. *Drosophila percnosoma* is chosen because of its page priority in the original description and greater usage in the literature: 7 for *D. percnosoma* (Bell & Kipp 1994; Clayton 1968; Hardy 1965; Heed 1968; Magnacca et al 2008; Mangan 1978; O'Grady & DeSalle 2008) and 5 for *D. septuosa* (Hardy 1965; Heed 1968; Kaneshiro 1976; Magnacca et al 2008; Yoon & Richardson 1978). We have identified 10 individuals from UHIM that were reared from *Pittosporum* leaves by W. B. Heed in 1966 from Waikamoi Forest on Maui, but this is the only instance of *D. percnosoma* being found off of the island of Hawai‘i.

***Drosophila sordidapex* Grimshaw**
Figure 3b

Drosophila sordidapex Grimshaw, 1901: 63

Diagnosis. The palpi and mouthparts are light brown. The thorax is yellow to light brown and the legs are completely yellow. The wing posseses a distinct marking that completely darkens the apical half of cell R2+3, the posterior apical margin of cell R1 and anterior apical margin of cell R4+5. Unlike other species, this spot is much darker and is not diffuse, with defined margins (see Fig. 3b), although in some specimens (including the holotype), it may be distinctly following the veins with a small clear area in the middle of cell R2+3. The abdomen is dark brown to black dorsally with yellow sides except for the completely yellow last tergite.

Types. HAWAI‘I: Holotype male (BMNH) Ōla‘a Forest, vii. 1895. [probably collected by R. C. L. Perkins].

Material Examined. HAWAI‘I: 11 males have been deposited in the BPBM from the following localities: males, Stainback Highway, Tom's Trail, 3200', x.2006, KNM, RTL, GMB; 4 males, Kahuku, Pu‘u Akihi Gulch, i.2006, KNM; 1 male, HAVO escape road, 3900’, vi.2006, KNM; 1 male, Hionamoa Stream, Ka‘ū Forest, x.2006, KNM, RTL, GMB; 1 male, Ōla‘a Forest, x.2006, KNM, RTL, GMB; 1 male, Kukuiopa‘e, South Kona Forest Reserve, x.2006, KNM, RTL, GMB; 2 males, Laupāhoehoe, 3700', x.2006, KNM, RTL, GMB. Over 50 males have been deposited in the UHIM from the following localities: 23 males, Keanakolu, x.1952, DEH; 13 males, Upper Ōla‘a Forest, viii.1952, DEH; 5 males, Upper Ōla‘a Forest, viii.1953, DEH; 5 males, Upper Ōla‘a Forest, vii.1963, WBH; 3 males, Upper Ōla‘a Forest, vii.1964, LHT; 7 males, Upper Ōla‘a Forest, vii.1956 DEH; 4 males, Pu‘u Hualalai, vi.1966, WBH; 3 males, Kaiholeua, Kohala Mountains, viii.1952, DEH; 1 male, Forest above Pa‘auilo, vii.1953, DEH; 1 male, Keauhou Ranch, Kīlauea, vii.1953, DEH; 1 male, Honaunau Forest, vii.1966 KYK. The following material is present at AMNH: males, Neuneu Road, Kaloko Mauka, North Kona, O38.6, 3.vii.1998, PMO, SLM; 5 males, Volcanoes National Park, Ōla‘a Forest, near Pole 44, O117.4, 23.ii.2001, PMO; 2 males, Volcanoes National Park, Ōla‘a Forest, transect 16, O120.2, 25.ii.2001, PMO, DF; 1 male, Volcanoes National Park, Ōla‘a Forest, near Pole 44, O132.8, AMCC105696, 2.viIi.2001, PMO, CDS; 5 males, Volcanoes National Park, Ōla‘a Forest, near Pole 44, O140.8, AMCC105684, 12.iv.2002, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: males, Volcanoes National Park, Ōla‘a Forest, near pole 48, O247.H; O201459; 6–7.vii.2004, PMO, MG, CDS.

Distribution. This species is endemic to the Big Island of Hawai‘i.

Behavior: Speith (1966: 274).

Ecology. This species has been reared from leaves of *Cheirodendron trigynum* (Araliaceae); leaves of *Ilex anomala* (Aquifoliaceae) (Heed 1968).

Illustrations. Morphological structures of this species are depicted in multiple publications including: foreleg (Hardy 1965: 470, fig. 190a); phallus (Kaneshiro 1976: 267, fig. 5b); terminalia, male, lateral (Hardy 1965: 470, fig. 190c); wing (Hardy 1965: 470, fig. 190b).

***Drosophila waddingtoni* Hardy**
Figure 3e

Drosophila disticha Hardy, 1965: 249–252
Drosophila waddingtoni Basden, 1976: 185

Diagnosis. Dense setae are present on the posterior surface of the tibia equal to, or longer than, the tibial spur (see Fig. 2b for example). The legs are yellow, except for brown front coxae and femora. The second tarsal segment is the widest of the group, being 1/3 wider than long. The wings are without any noticeable pigmentation (see Fig. 3e). The thorax is dark brown to black, and the abdomen is mostly dark brown with the last tergum yellow,

and the preceding tergites marked with yellow laterally.

Types: MAUI: Holotype male (BPBM 6344) Waikamoi, 1220 m, vii.1956, DEH. Locality recorded as "Wai*a*kamoi" on type label (Evenhuis 1982).

Synonym. *disticha* Hardy 1965: 249 preoccupied

Material Examined. HAWAI'I: 11males have been studied from the BPBM from the following localities: 3 males, Keanakolu, x.1952, DEH, CPH; 3 males, Kīlauea, xi.1919, WMG; 5 males, from Kawaihae Uka, Kohalas, x.2006, KNM, RTL, GMB. 30 males have been deposited in the UHIM from the following localities: 7 males, Kīlauea, viii.1958, JWB; 5 males, Upper Ōla'a Forest, vii.1953, DEH; 3 males, Upper Ōla'a Forest, viii.1952, DEH; 4 males, Upper Ōla'a Forest, vii.1956, DEH; 4 males, Kūlani 5200', vii.1952, WCM; 2 males, Keanakolu, x.1952, DEH, CPH; 5 males, Kīpuka Kī, vii.1966, KYK. The following material is present at AMNH: males, Volcanoes National Park, Kīpuka Puaulu, O127.4, 29.vii.2001, PMO; 65 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O132.3, AMCC105705, 2.viIi.2001, PMO, CDS; 40 males, Volcanoes National Park, Ōla'a Forest, near Pole 44, O140.C, AMCC105687, 12.iv.2002, PMO, CDS. The following material is in the Essig Museum of Entomology at UC Berkeley: 2 males, Puu Makaala Trailhead, O257.1, O201319, 11.vii.2004, PMO, MG; 1 male, Volcanoes National Park, Upper Ōla'a Forest, end of Wright Road, O272.F, O200624; 20.vii.2004, PMO, MG, GS, AC.

LANA'I: Over 60 males have been deposited in the UHIM the following localities: males, Lāna'ihale, vi.1953, DEH; 12 males, Lāna'ihale 3300', iii.1965, KYK; 46 males, Lāna'ihale 3000', vii.1956, dEH; 4 males, Lāna'ihale 3300', viii.1964, HLC.

MAUI: Over 180 males have been deposited in the UHIM from the following localities: 2 males, Pu'u Kukui, vi.1953, DEH; 2 males, Pu'u Kukui, iv.1954, DEH; 1 male, Haelaau, xii.1928, OHS; 1 male, Kula Pipeline, vi.1927, OHS; 16 males, Kula Pipeline, vii.1956, DEH; 1 male, Waikamoi, i.1926, OHS; 59 males, Waikamoi, vii.1956, RN; 13 males, Waikamoi, viii.1958, DEH; 88 males, Waikamoi, vii.1964, HLC; 2 males, Waikamoi, vii.1956, DEH; 1 male, Waikamoi, iii.1966, WBH. The following material is present at AMNH: 9 males, Upper Waikamoi Forest Reserve, 5500 ft., O41.4, 6.vii.1998, PMO, SLM; 10 males, 2 females, Waikamoi Forest Reserve, Heed Trail, O50.A, 8.iii.1999, PMO, EMC, MPK; 3 males, Waikamoi Forest Reserve, Heed Trail, O55.B, 16–18.iii.1999, PMO, JBS; 1 male, Makawao Forest Reserve, Pig Hunter's Trail, O56.1, 18.vii.1999, PMO, JBS; 20 males, Waikamoi Forest Reserve, Heed Trail, O71.A, 2.vi.1999, PMO; 3 males, Hanaula, O72.9; 15–16.vi.1999, PMO, KYK, KTK, YK; 6 males, 2 females, Waikamoi Forest Reserve, Heed Trail, O73.C, 22.vii.1999, PMO, EMC, MPK; 2 males, Waikamoi Forest Reserve, Heed Trail, O74.I, 4.ii.2000, PMO; 10 males, Makawao Forest Reserve, Pig Hunter's Trail, O153.8, AMCC105805, 23.iv.2002, PMO, DO; 4 males, Waikamoi Forest Reserve, Heed Trail, O154.H, 23.iv.2002, PMO, DO. The following material is in the Essig Museum of Entomology at UC Berkeley: males, Waikamoi Forest Reserve, Flume stream, O300.8, O201347, 4.viii.2005, PMO, GMB, CH, JEG; 4 males, Waikamoi Forest Reserve, Heed Trail, O301.A, O201362, 4.viii.2005, PMO, GMB, CH, JEG; 10 males, Waikamoi Forest Reserve, Heed Trail, O303.5, O201407, 4.viii.2005, PMO, GMB, CH, JEG; 6 males, Waikamoi Forest Reserve, Carson Trail, O305.5, 6.viii.2005, PMO, GMB; 12 males, Waikamoi Forest Reserve, Heed Trail, O398.1, O200786, 31.vii.2007; PMO, KNM, RTL, GMB, KRG.

MOLOKA'I: 23 males have been deposited in the BPBM from the following

localities: 3 males, Kamakou Preserve, forest near Hanalilolilo Lookout, O376.C, O201865, 19.ii.2007, PMO, KNM, RTL, GMB; 13 males Kamakou Preserve, Puu Kolekole, O377.6, O201874, 19.ii.2007, PMO, KNM, RTL, GMB, males Kamakou Preserve, makai of Puu Kolekole Cabin, O378.4, 201890,19.ii.2007, PMO, RTL, GMB; 1 male Kamakou Preserve, Tunnel on trail to Puu Kolekole, O379.1, O201894, 19.ii.2007, PMO, KNM, RTL, GMB; 11males, Pepe‘opae, vii.1959, DEH; 4 males, Maunawainui Valley, vii.1952, DEH. Over 50 males have been deposited in the UHIM from the following localities: 16 males, Pu‘u Kolekole, vii.1952, DEH, MT; 16 males, Pu‘u Kolekole, vii.1953, DEH, MT; 4 males, Pu‘u Kolekole, iii.1963, DEH; 1males, Pepe‘opae, vii.1959, DEH; 4 males, Maunawainui Valley, vii.1952, DEH. The following material is present at AMNH: 46 males, Kamakou Preserve, Puu Kolekole, O35.1, 1.vii.1998, PMO, SLM; 83 males, Kamakou Preserve, Puu Kolekole, O58.5, 19–21.iii.1999, PMO, JBS; 37 males, Kamakou Preserve, Puu Kolekole, O101.C, 26–27.vii.1999, PMO, EMC, MPK; 26 males, 55 females, Kamakou Preserve, Puu Kolekole, O146.2, AMCC105707, 15–16.iv.2002, PMO, CDS, DO; 1males, 13 females, Kamakou Preserve, Pepeopae Boardwalk Trail, O150.2, AMCC105755, 16.iv.2002, PMO, CDS, DO; 2 males, 7f, Kamakou Preserve, forest near Hanalilolilo Lookout, O151.C, AMCC105765, 17.iv.2002, PMO, CDS, DO. The following material is in the Essig Museum of Entomology at UC Berkeley: 35 males, Kamakou Preserve, Puu Kolekole, 3854 ft., O283.1, O201705, 28–29.vii.2004, PMO, CDS.

Distribution. This species is endemic to Maui Nui and the Big Island of Hawai‘i.

Behavior. Speith (1966: 273); Grossfield (1968); Kambysellis and Heed (1971).

Chromosomes. The metaphase complement of this species is 5 rods and 1 dot (Clayton 1969; Yoon & Richardson 1978).

Ecology. Nutritional requirements (Robertson et al 1968); This species has been reared from leaves of *Cheirodendron trigynum* (Araliaceae) from Hawai‘i, Maui, Moloka‘i and Lana‘i; leaves of *Tetraplasandra* sp (Araliaceae) from Hawai‘i; leaves and stems of *Clermontia* sp (Campanulaceae) from Hawai‘i and Lana‘i; leaves of *Myrsine lessertiana* (Myrsinaceae) from Moloka‘i; leaves of *Pittosporum* sp (Pittosporaceae) from Moloka‘i (Heed 1968; Magnacca et al 2008; Mangan 1978).

Illustrations. Morphological structures of this species are depicted in multiple publications including: egg (Kambysellis and Heed 1971: 34, fig. 2.3; 36, figs. 4.1, 4.2); foreleg (Hardy 1965: 250, fig. 82a); ovary (Kambysellis and Heed 1971: 35, fig. 3.1); phallus (Kaneshiro 1976: 267, fig. 5e); terminalia, female, lateral (Hardy 1965: 250, fig. 82b); terminalia, male, lateral (Hardy 1965: 250, fig. 82c); Takada 1966: 318, fig. 1.8).

Molecular Biology. DNA sequences (Kambysellis & Craddock 1997; O’Grady & DeSalle 2008; O’Grady & Zilversmit 2004).

Discussion. *Drosophila waddingtoni* has the widest range of any spoon tarsus group species and is found throughout Hawai‘i and Maui Nui. Several individuals from Maui have been collected that grade in coloration of the tibia from yellow (normal) to dark brown.

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Figures

Figure 1. The phylogenetic relationships between the major Hawaiian *Drosophila* groups. a) phylogeny of species groups based on internal morphology (Throckmorton 1966), b) phylogeny of species groups based on molecular data (Bonacum 2001).

Figure 2. Fore-legs of selected males a) *D. atroscutellata*, b) *D. dasycnemia*, c) *D. conformis*, d) *D. incognita*, and e) *D. percnosoma*.

Figure 3. Wings of selected species a) *D. atroscutellata*, b) *D. sordidapex,* c) *D. conformis*, d) *D. dasycnemia*, and e) *D. waddingtoni*.

Figure 1. The phylogenetic relationships between the major Hawaiian *Drosophila* groups

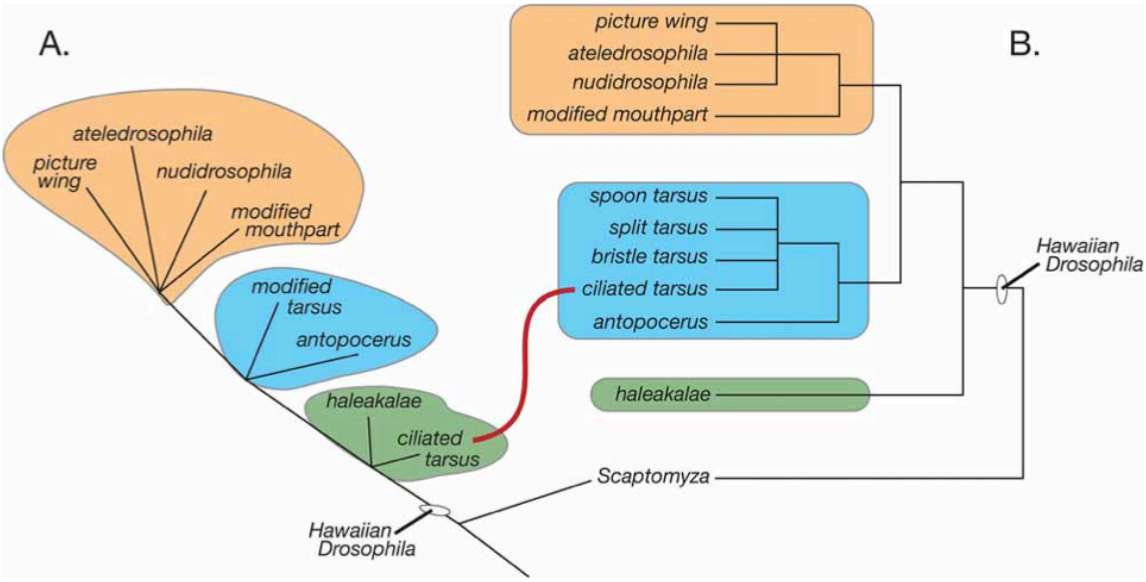


Figure 2. Fore-legs of selected males

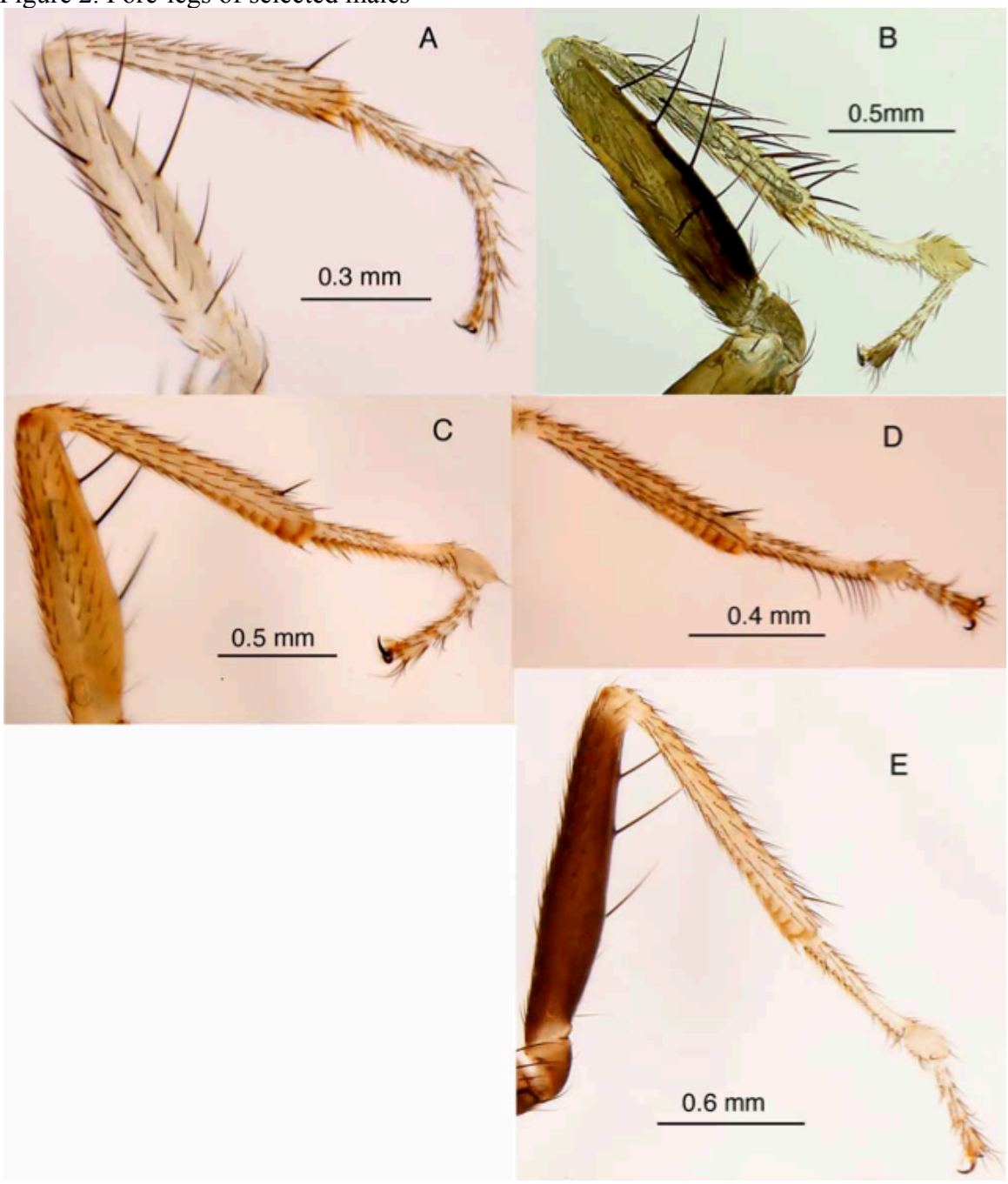
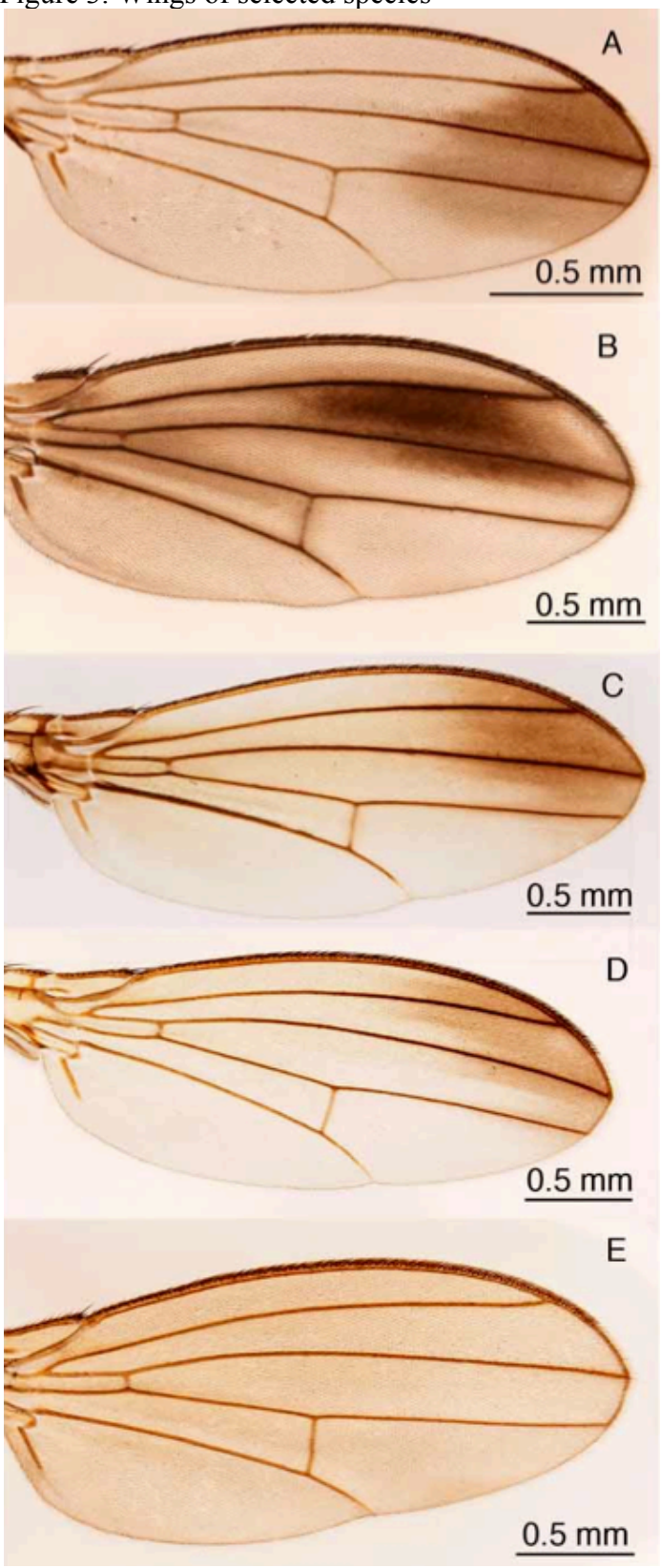


Figure 3. Wings of selected species



Chapter 3: Phylogenetic relationships in the spoon tarsus subgroup of Hawaiian *Drosophila*: Conflict and concordance between gene trees

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Lapoint, R.T., Gidaya, A., and 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.

Abstract

The Hawaiian Drosophilidae contains approximately 1000 species, placed in species groups and subgroups based largely on secondary sexual modifications to wings, forelegs and mouthparts. Members of the spoon tarsus subgroup possess a cup-shaped structure on the foretarsi of males. Eight of the twelve species in this subgroup are found only on the Big Island of Hawaii, suggesting that they have diverged within the past 600,000 years. This rapid diversification has made determining the relationships within this group difficult to infer. We use 13 genes, including nine rapidly evolving nuclear loci, to estimate relationships within the spoon tarsus species, as well as to test the monophyly of this subgroup. A variety of analytical approaches are used, including individual and concatenated analyses, Bayesian estimation of species trees and Bayesian untangling of concordance knots. We find widespread agreement between phylogenetic estimates derived from different methods, although some incongruence is present. Notably, our analyses suggest that the spoon tarsus subgroup, as currently defined, is not monophyletic.

Introduction

The Hawaiian Islands, located approximately 4000 km from the nearest continent, are the most isolated island chain in the world (Carson and Clague, 1995). These volcanic islands rise out of the Pacific where lava seeps through a ‘‘hot spot’’ in the Earth’s crust, and over time becomes large enough to sustain life. As the Pacific Plate moves, islands are carried northwest. When an island moves off the hotspot, it become dormant and gradually erodes back into the sea (Carson and Clague, 1995). This leads to a datable progression with young islands in the southwest and sequentially older islands in the northwest (see Fig. 1). High mountains that gradually slope into the sea characterize the young islands. Older islands are shorter, steeper and deeply carved by the action of wind and water (Price and Clague, 2002).

The Hawaiian chain is currently composed of eight high islands, those that are high enough to catch the moist trade winds and produce rainforest habitats. The high percentage of species found on the Hawaiian Islands has made it home to some of the highest levels of endemism in the United States (Eldredge and Evenhuis, 2003). Price and Clague (2002) have recently reviewed several lineages endemic to Hawaii that have originated from a single or few initial colonizers. In each of the lineages of endemic Hawaiian species that were reviewed it appears that the colonization occurred after the formation of the current high islands (Baldwin and Sanderson, 1998; Fleischer et al., 1998). However a few radiations are estimated to have colonized the island chain well before the formation of the current high islands, and have subsequently progressed down to the younger islands as they form (Givnish et al., 1996; Jordan et al., 2003).

The oldest inhabitants of the island chain are the Hawaiian Drosophilidae, a radiation derived from a single colonization event approximately 25 million years ago (Russo et al., 1995). Since this time they have diversified into a clade of 1000 species (O’Grady et al., 2010), occupying a wide variety of ecological niches (Heed, 1968; Montgomery, 1975; Magnacca et al., 2008) and displaying impressive morphological diversity (Hardy, 1965). They have diversified into many different niches, exploiting nearly 40% of the native plant families and even more bizarre substrates like spider eggs (Wirth, 1952). Most species also display marked sexual dimorphism, with males possessing elaborate secondary characters, such as wing patterning, elongate antennae, tusk-like mouthparts and elaborate processes on the tarsi (e.g., Stark and O’Grady, 2009), that they use in copulation. Mating displays are likewise diverse (Spieth, 1966). Morphological and molecular characters have been useful in defining several groups that have been tested by rigorous phylogenetic analysis (Baker and DeSalle, 1997; Bonacum, 2001; Bonacum et al., 2005; Carson and Stalker, 1969; Kambysellis et al., 1995; O’Grady and Zilversmit, 2004; O’Grady et al., in press).

Males in the spoon tarsus clade have an eponymous cup-like second tarsi on their forelegs used in positioning females during mating (Spieth, 1966; Stark and O’Grady, 2009). All species are ecologically similar, utilizing leaves of the endemic plant group Araliaceae as larval substrate (Magnacca et al., 2008) and are found in a similar range of habitats (Lapoint et al., 2009). Of the 12 species in this group there are eight present on the island of Hawaii (Big Island) seven of which are endemic (Lapoint et al., 2009). Since the Big Island is less than 600,000 years old these species are expected to have diverged recently and personal observations of density in the field suggest they exist in large

population sizes, making them prone to the effects of deep coalescences.

Most recent radiations, where the coalescent is large due to either a recent time common ancestor or large population sizes, are known as difficult subjects for phylogenetic estimation (Belfiore et al., 2008; Degnan and Rosenberg, 2006). Resolving the species tree for such groups is especially important since these radiations can offer insight into many different aspects of biological processes that are better understood by placing the questions in a phylogenetic context. There are four main issues when estimating phylogenies for recent radiations: (1) few variable loci, (2) introgression, (3) cryptic species, (4) incomplete lineage sorting. First, there is a difficulty in finding markers variable enough to be informative at shallow divergences. With the advent of genomic resources (*Drosophila* 12 Genomes Consortium, 2007) the first issue has become less daunting since marker discovery methods have vastly improved (Edwards, 2008; Thomson et al., 2008; Wahlberg and Wheat, 2008). Introgression between species is another factor that can confound phylogenetic reconstruction. In the case of recent divergences, the expectation that all alleles follow a coinherited evolutionary history is not met. Mating barriers, for example, may not be complete and gene flow between incipient species may complicate phylogenetic estimation (Maddison, 1997; Leache and McGuire, 2006). Cryptic species are distinct evolutionary lineages that have not diverged morphologically and when included in phylogenies can make identified ‘‘species’’ paraphyletic. In recent radiations, lineages may become genetically distinct, but may not have had time to diverge morphologically and can warrant a reevaluation of species delimitation.

Finally, incomplete lineage sorting is a common and difficult issue to address in recent radiations (Degnan and Rosenberg, 2006). Incomplete lineage sorting is expected to be widespread in the case of recent radiations, and, by chance alone, genealogies that support the wrong topology can be more common than those that support the true topology (Rokas et al., 2003; Pollard et al., 2006). Clades with large population sizes (h) and short divergence times are especially prone to this issue in simulation studies. In the case of Galapagos finches, silverswords and African cichlids, their rates of diversification and population sizes are within this range and they can be inferred to be prone to lineage sorting issues (McCormack et al., 2009). Empirical evidence shows that the issue of deep coalescences is real, as phylogenetic estimation within these groups has proven to be very difficult (Freeland and Boag, 1999; Lopez-Fernandez et al., 2010).

Several methods have been developed to address this issue of gene tree conflict. Concatenation is a total evidence method that combines all available information and analyzes it together as a single gene, assuming that the independent loci have evolved with a single evolutionary history that is predominantly displayed in the data (Degnan and Rosenberg, 2006). It has proven to be fairly robust in empirical studies in that this method generally finds well-resolved trees with good support (Belfiore et al., 2008; Thomson et al., 2008; Brumfield et al., 2008). A concatenated dataset can be partitioned into classes of data that are assumed to have evolved under the same model, such as gene or codon position (Ronquist and Huelsenbeck, 2003). In datasets composed of sequences evolving under different models, under parameterization can be mediated by partitioning and phylogenetic estimation improved (Brandley et al., 2005). Despite these benefits, drawbacks exist that make the results of this method questionable. Long branches separated by short internal nodes are especially prevalent when large amounts of data are

used and can lead to positively misleading topologies (Gadagkar et al., 2005).

Another way of addressing the issue of lineage sorting is a suite of methods that utilize the coalescent. They attempt to find he best species tree by estimating each gene genealogy independently and assuming that conflict between gene trees is due solely to incomplete lineage sorting. Under these approaches, the best estimate of phylogeny is the one that minimizes the number of deep coalescence events. Several algorithms (Liu et al., 2008; Kubatko, 2009) can perform this estimate in either a Bayesian or maximum likelihood framework. Although these methods are very parameter rich and can be computationally intensive (Knowles, 2009), they provide a good estimate of phylogeny when most of the conflict between gene trees is due to incomplete lineage sorting. Here we reconstruct phylogenetic relationships in the spoon tarsus subgroup analyzing data in individual and partitioned concatenated matrices using both coalescent and concordance frameworks to compare their ability to find a resolved and robust phylogeny. Specifically, we are interested in testing recent taxonomic hypotheses about the composition of this group, to determine evolutionary relationships between closely related taxa, and ascertain whether the Big Island species cluster is comprised of discrete species or an irresolvable species cluster.

Materials and methods

Sampling

Eleven of the 12 spoon tarsus species were obtained for this analysis. *Drosophila mimiconformis*, a rare species endemic to the rainforests of Molokai, was not collected. We included multiple representatives from different volcanic mountains or different sides of the same mountain (Kona or Hilo) for species found on the Big Island in order to rigorously test the monophyly of these species. Multiple populations of *Drosophila waddingtoni* from various localities on Maui and Molokai were also included to test whether: (a) Maui Nui populations were ancestral to Big Island populations and follow the progression rule down the island chain (Wagner and Funk, 1995), or (b) if the Maui Nui populations were recent and resulted from back colonization from the Big Island (Wagner and Funk, 1995). We included *Drosophila grimshawi* (picture wing group), *Drosophila diamphidiopoda* (antopocerus group) and *Drosophila expansa* (bristle tarsus subgroup) as outgroups to test the monophyly of the spoon tarsus subgroup (Table 1). Both the antopocerus group and bristle tarsus subgroup are part of the same species clade as the spoon tarsus subgroup, the AMC (antopocerus, modified tarsus and ciliated tarsus clade). *D. grimshawi* is from a distantly related clade (O'Grady et al., in press) and was used as a known outgroup for all analyses.

Collections were made by sweeping leaf litter and aspirating specimens directly from sponges baited with fermenting banana. Specimens were stored in 95% EtOH for identification and DNA extraction at UC, Berkeley. Species identifications were performed by the authors using the key provided in Lapoint et al. (2009). Genomic DNA was extracted from individual flies using the Qiagen DNeasy DNA extraction kit (Qiagen, Inc). The only departure from this protocol was that individuals were soaked in Proteinase K instead of being macerated and subsequently preserved as point mounted vouchers. All voucher material has been deposited in either the B.P. Bishop Museum or the Essig Museum of Entomology at UC Berkeley. Table 1 lists the collection

information and 6-digit collection code.

DNA amplification and sequencing

All individuals were sequenced for 10 nuclear loci and four mitochondrial loci. Mitochondrial loci were amplified using universal primers (Simon et al., 1994). Primers to amplify nuclear loci were designed by searching Flybase (Tweedie et al., 2009) for genes with exons conserved between *D. grimshawi*, *Drosophila mojavensis* and *Drosophila virilis*, but containing highly divergent introns. Primers spanning these introns were anchored within the conserved exons. We specifically excluded all multicopy genes and members of multigene families. Loci were selected from different chromosome arms or were at least 5 Mbp apart on the same chromosome arm. Gene identity was assessed via BLAST to the annotated *Drosophila melanogaster* and *D. grimshawi* genomes. Details for each locus are listed in Tables 2 and 3.

PCR conditions included an initial denaturing at 95 °C for 5 min, followed by 35 cycles of the following sequence: denaturation at 95 °C for 30 s, annealing between 54 °C and 62 °C (depending on locus) for 30 s, and an extension at 72 °C for 30 s. A final extension step at 72 °C was held for 5 min. PCR products were cleaned using standard ExoSAP-IT (USB) protocols. Cleaned products were sent to the UC Berkeley Sequencing Facility and sequenced in both directions on an ABI 3730 capillary sequencer. Contigs were assembled using Sequencher, ver. 4.7 (GeneCodes, Corp). Because of the recent divergence between the taxa in this study, alignment was trivial and easily performed by eye using MacClade, ver. 4.06 (Maddison and Maddison, 2002). All sequences generated in this study have been deposited in GenBank (Table 1).

Partitioned concatenated analysis

Sequences were concatenated, partitioned and analyzed in Bayesian (mrbayes, ver. 3.1.2; Ronquist and Huelsenbeck, 2003) and maximum likelihood (RAxML, version 7.2.6; Stamatakis, 2006). Maximum likelihood analyses were performed on the Abe Teragrid, accessed through the CIPRES portal (Miller et al., 2009). Likelihood searches were partitioned by locus and the GTRGAMMA model was used for each partition to estimate the tree, since the authors suggest against using proportion of invariant sites and simpler models (Stamatakis, 2006). Two thousand bootstrap replicates were performed to assess support for the inferred relationships. The Akaike information criterion (AIC), implemented in MrModeltest, ver. 2.3 (Nylander, 2004), was used to estimate the best-fit model of substitution for each of the 14 loci in the Bayesian analyses (Tables 2 and 3). The concatenated analysis (with 14 partitions) was run for 10 million generations and was sampled every 100 generations.

Genealogies

Gene trees derived from individual analyses were estimated using MrBayes. An assumption of the coalescent is that there is recombination between loci. We identified which genes to concatenate into one locus based on their chromosomal locations and amount of recombination between them for the gene tree analyses. Loci on the Y chromosome and mitochondria are known to have no recombination so each was analyzed as one non-recombining unit. Loci residing on the same chromosome arm were tested to ensure recombination was occurring between them using the 4-gamete test

(Hudson and Kaplan, 1985) implemented in DnaSP (Librado and Rozas, 2009). All were found to be in linkage equilibrium. Therefore the combined mitochondrial loci, both Y chromosome loci, and every other locus were considered a single non-recombining unit. Each recombining unit was used to estimate a gene tree for a total of 10 gene trees. Gene trees were run for 1 million generations and were sampled every 100 generations. By examining the cumulative split frequencies plot calculated by AWTY (Wilgenbusch et al., 2004) and identifying when the potential scale reduction factor (PSRF) approached 1, convergence in all Bayesian analyses was assessed.

Bayesian estimation of species trees

BEST, ver. 2.3.1 (Liu et al., 2008) was used to estimate the species tree while taking into account incomplete lineage sorting by minimizing the bifurcations based on deep coalescent events. Allelic data were used to build the gene trees used in this analysis. Alleles for heterozygous sequences were identified using PHASE2.1.1 (Stephens and Scheet, 2005). The alleles identified with a probability greater than 0.5 were kept, and any with lower posterior probabilities were cloned and re-sequenced to confirm the identity of each allele at that gene. BEST assumes gene tree conflict is due to incomplete lineage sorting, and not hybridization. To allow the analysis to reach convergence in a reasonable amount of time, only one allele was used per species (Table 1). Exceptions were made for *Drosophila conformis* and *Drosophila incognita* since they were found not be monophyletic in the partitioned concatenated analysis, and populations of *D. waddingtoni* found on different islands. Since BEST is sensitive to missing data and we were unable to amplify some taxa for the Ge-1 and Bin genes, we removed these loci from the dataset. The partitioned concatenated analysis was reanalyzed without these genes to test the impact they had on the topology, and the only difference was a reduction in support in a few nodes, with no topology change. Gene partitions in this analysis were identified as non-recombining blocks. Substitution models for the combined loci on the Y chromosome and mitochondria were estimated using MrModeltest v2.3 (Nylander, 2004).

Two Markov Chain Monte Carlo searches were run for 45 million generations and were sampled every 1000 generations. Four chains were used for each run, with a heating factor set using temp = 0.10 to allow for adequate mixing. Convergence was estimated using AWTY and PSRF values (Wilgenbusch et al., 2004). h was set at 0.0023 ($\alpha = 3$, $\beta = 0.0047$). h was calculated as the average of the average pair wise divergence for each nuclear allele for *Drosophila dasycnemia*, *Drosophila neutralis*, and *Drosophila sordidapex* from sequences used in this paper.

Bayesian untangling of concordance knots

BUCKy, ver. 1.3.2 (Ané et al., 2007) was used to estimate the primary concordance tree and assess agreement between different gene trees. Concordance analyses estimate the overall tree quickly and without assuming that conflict is due to any one issue. BUCKy estimates the overall history of the species tree assuming that the dominant signal from independent gene trees is that of the true evolutionary history. This method allows for uncertainty in gene tree estimation and also estimates a level of support by identifying how much of the genome supports each relationship. Since each nuclear locus did not estimate each species as reciprocally monophyletic, the sampling scheme used for the BEST analysis was also used for the BUCKy analysis to circumvent

conflict. Two analyses were performed, one with the a priori level of discordance (α) set at 1, the other with a set at 0.1. This allowed for a range of expected discordance, with the analyses with $\alpha = 1$ predicting that most of the gene trees support the species tree, and an $\alpha = 0.1$ predicting up to a different tree per genealogy. Each analysis was run twice for 10 million generations with four chains.

Bayesian concordance analysis infers concordance factors (CF), useful in determining how much support there is for a given topology. CF values can indicate how well the sampled genome corroborates a certain tree (sample CF). Alternatively, genome wide CF values assume that the sampled loci are representative of the genome as a whole, given an assumed level of expected discordance (a). Under these assumptions CF values indicate the degree to which the entire genome is expected to produce a certain topology.

Results

Partitioned analyses

Partitioned, concatenated Bayesian and maximum likelihood analyses produce the same topology, with high levels of support (Fig. 2). The first 2000 trees were discarded as burn in for the partitioned concatenated analysis run in MrBayes based on the differences in split posteriors calculated via AWTY and observed in Tracer. In contrast to the partitioned concatenated analyses, individual gene trees displayed a high degree of conflict and poor resolution, possibly due to the fewer characters in each analysis or conflict due to incomplete lineage sorting or hybridization. Analyses for individual gene trees converged quickly, and the first 1000 trees were discarded as burn in based off of convergence statistics. These figures are available as Supplementary material online. The partitioned concatenated analysis suggests that the spoon tarsus subgroup as currently defined (Lapoint et al., 2009) is not monophyletic. Two species considered basal within the spoon tarsus subgroup *Drosophila atroscutellata* and *Drosophila fastigata*, are actually basal to the spoon tarsus subgroup plus bristle tarsus subgroup, represented by *D. expansa*. This result is in agreement with a larger analysis of all Hawaiian *Drosophila* lineages (O'Grady et al., in press).

The species present on the Big Island form a clade, with the Maui Nui populations of *D. waddingtoni* nested within this larger monophyletic group (Fig. 2). There appear to be two well-supported lineages within Big Island, the *dasycnemia* species complex (sensu Hardy, 1965) that includes *D. dasycnemia*, *D. waddingtoni*, *D. neutralis* and *Drosophila percnosoma* and the *sordidapex* species complex (sensu Hardy, 1965), which includes *D. sordidapex*, *Drosophila kikalaeleele*, *D. conformis*, and *D. incognita* (Fig. 2). While these complexes are supported as monophyletic, several of the species included in each are not. For example, *Drosophila waddingtoni* is nested within a paraphyletic *D. dasycnemia*, although support for the relationships within *D. dasycnemia* are not well supported. Furthermore, both *D. incognita* and *D. conformis* are paraphyletic (Fig. 2).

BEST

The BEST analysis produced a similar phylogeny to the partitioned concatenated analyses (Fig. 3a). The monophyly of the Big Island clade and both the *dasycnemia* and *sordidapex* species complexes are well supported. However, posterior probabilities at the species nodes were reduced in comparison to the partitioned analyses. Species

relationships within the *dasycnemia* complex were poorly supported and showed a different order of divergence. Within the *sordidapex* complex species relationships were poorly supported and appear to be recently diverged. The BEST analysis produced a phylogeny that included *D. atroscutellata* and *D. fastigata* within the spoon tarsus subgroup. However, the relationships between *D. atroscutellata*, *D. fastigata* and *D. expansa* were poorly supported (posterior probabilities <0.7) and with very short internodes.

BUCKy

The Bayesian Concordance analyses estimated relationships within the spoon tarsus subgroup (Fig. 3b) to be very similar to those estimated using concatenation or BEST (e.g., Figs. 2 and 3a). The taxa that were identified as paraphyletic in the concatenated analysis are also found to be paraphyletic in this analysis. The primary concordance values are low throughout the tree, indicating a high level of discord between gene genealogies (see Table 4).

Discussion

Relevance to phylogeny

The placement of the bristle tarsus species, *D. expansa*, in all of these analyses renders the monophyly of the spoon tarsus subgroup, as currently described, suspect. The BUCKy analysis found *D. fastigata* sister to *D. expansa*, making its inclusion in the spoon tarsus subgroup questionable. While the BEST analysis still reconstructs the spoon tarsus subgroup as monophyletic, the internal nodes are very short and poorly supported. This lack of resolution is not entirely unexpected. Whereas all other spoon tarsus species have a second tarsomere characterized by being cuplike filled densely with hair, *D. atroscutellata* and *D. fastigata* do not (Stark and O'Grady, 2009). These two leaf breeder species have been included in the spoon tarsus subgroup due to the second tarsal segment being merely slightly enlarged and moderately concave. This enlargement and concavity is not nearly as great as the other spoon tarsus species. Indeed, both *D. atroscutellata* and *D. fastigata* both have slight lobes at the apical end of their basitarsi, with some setae. This could cause them to be better included in the bristle tarsus group, though again, these characters are not as developed as other members of that group (Stark and O'Grady, 2009). A revision of the entire AMC – including the antopocerus and the modified tarsus species groups – is warranted to further resolve the relationships within this species clade. The rest of the spoon tarsus subgroup is well supported as a monophyletic group in all analyses, including *D. contorta* and the Big Island species, which includes the multi-island *D. waddingtoni*. Unlike *D. atroscutellata* and *D. fastigata*, these species share many common characteristics, including the overall morphology of their spoon and genitalic characters. We feel that the available evidence requires the reexamination of the validity of the inclusion of *D. atroscutellata* and *D. fastigata* in the spoon tarsus subgroup.

Relationships between species vary from well supported and found in all analyses to poorly supported and conflicting. *Drosophila contorta* is well supported as being basal to the rest of the Big Island spoon tarsus species group in all analyses. This is expected based on morphology (Lapoint et al., 2009) and the progression rule (Wagner and Funk,

1995), which predicts basal lineages to be found on older islands. A well-supported split appears to have occurred on the island of Hawaii between the *dasycnemia* and *sordidapex* species complexes. This is also expected given the morphology of these eight species. Members of the *dasycnemia* species complex are generally larger, darker and possess more robust setae on their basitarsi. Conversely, the *sordidapex* species complex is comprised of mostly smaller, light colored flies with patterning on their wings.

Within these species complexes relationships were generally plastic. The BEST analyses found *D. neutralis* as basal to *D. percnosoma*, which was basal to a clade comprised of *D. dasycnemia* and *D. waddingtoni*. In the BEST analysis *D. sordidapex* and *D. kikalaeleele* are sister to each other, though poorly supported. The partitioned concatenated phylogeny estimates *D. percnosoma* as basal to *D. neutralis*, which was basal to *D. dasycnemia* and *D. waddingtoni*. *Drosophila conformis* and *D. incognita* render the *sordidapex* complex paraphyletic in the BUCKy and partitioned concatenated analysis, and the BEST analysis finds the species within this complex poorly supported and recently diverged.

The monophyly of the species found on the Big Island was tested using multiple individuals per species from multiple populations using partitioned concatenated analyses and paraphyletic taxon were included in both the BUCKy and BEST analyses. The species in the *dasycnemia* complex are well-supported monophyletic groupings, indicating that there has been enough time since divergence for lineage sorting and subsequently high species identity. *Drosophila percnosoma*, *D. neutralis* and *D. waddingtoni* were all found to be reciprocally monophyletic. Despite being found on multiple islands, *D. waddingtoni* was shown to be a recently derived species nested within *D. dasycnemia*.

The *sordidapex* species complex is much more complicated, exhibiting high levels of gene tree/species tree conflict. *Drosophila sordidapex* is monophyletic, but *D. conformis* and *D. incognita* are found throughout this species group. The rarity of *D. kikalaeleele* prevented the collection of more than one individual, and could not be tested. Despite being morphologically diagnosable, the polyphyly in the *sordidapex* species complex indicates possibly porous barriers to gene flow, recent diversification, and an overall reduced differentiation. This group may represent lineages that have not yet begun independent evolutionary trajectories, but increased
gene and taxon sampling should improve estimation of gene flow and species limits.

Biogeographic implications

Hawaiian *Drosophila* diversification has been characterized as being driven by mating behavior and ecological adaption in addition to geographic isolation, but the most obvious pattern has been seen in the progression rule. Older lineages are generally found on older islands and younger lineages on younger islands. This pattern appears to be recapitulated here since *D. fastigata* and *D. atroscutellata* are found on the oldest islands, Oahu and Kauai respectively, *D. contorta* and *D. expansa* are from Maui, and the youngest lineages are found on the youngest island, Hawaii.

In addition to the progression down the island chain we find evidence for recent back colonization up the island chain. *Drosophila waddingtoni* is unique for being a multi-island endemic (Nitta and O'Grady, 2008) and for originating on the Island of Hawaii and back colonizing the islands of Maui Nui, in apparently a stepping stone

pattern from the youngest volcano on Eastern Maui, then up to the older volcanoes of Maui and Molokai. This pattern is the opposite of the progression rule prevalent in the Hawaiian *Drosophila* (Bonacum, 2001; Bonacum et al., 2005; O'Grady et al., in press) and is not expected since they would have to invade very complex and mature ecosystems (Gillespie et al., 2008). Further exploration of the biogeography of this species is warranted to identify the nature of this interesting back colonization.

Comparison of methods

Large data sets containing many independently evolving loci are becoming more common in phylogenetic inference, especially in recent radiations like Hawaiian *Drosophila*. However, using multiple loci means that the very real issue of genealogical conflict must be addressed. A number of computational methods have recently become available to incorporate incomplete lineage sorting into phylogenetic reconstruction. These methods are improving our understanding of evolutionary relationships within groups that have recently and rapidly diverged and for which morphological or single gene phylogenies were unable to resolve relationships. These are allowing researchers to address important, yet computationally difficult, evolutionary questions within robust phylogenetic context.

This analysis improves on the most recent estimation of this group that uses solely mitochondrial sequences to infer the phylogeny of the entire Hawaiian *Drosophila* (O'Grady et al., in press). While mitochondrial sequences are useful in phylogenetics due to the ease of sequence generation via universal primers (Simon et al., 1994), high variability (Moritz et al., 1987) and rapid lineage sorting as a result of their maternal mode of inheritance (Avise, 2004), the non-recombining nature of mtDNA means that all mitochondrial loci present the same evolutionary history, a history that may not reflect actual relationships among species. Furthermore, incomplete lineage sorting, introgression and selection can obscure the phylogenetic signal present in this one marker and can lead to complications when estimating phylogeny (Maddison, 1997; Leache and McGuire, 2006). The addition of the nuclear loci greatly improves the estimation of the species relationships within the spoon tarsus subgroup since they represent multiple independent genealogies (Edwards, 2009). This should improve the inference of the phylogeny under the assumptions of total evidence (Kluge, 1989), the true signal should swamp out misleading signal caused by demography and selection (Rokas et al., 2003). In addition, by exploring analyses like BEST that use the amount of conflict between genealogies as a source of information, multiple loci should improve phylogenetic estimation (Knowles, 2009).

The partitioned concatenated analyses and BEST analysis differed in several important aspects. First, the concatenated analyses displayed higher resolution and higher levels of support, possibly due to the larger numbers of informative sites. BEST analyzes the species tree as a sum of the individual gene trees, which were poorly resolved due to a lack of informative characters per gene, and this is reflected in the phylogeny. Since one gene is unlikely contain this much information this support and resolution may therefore be artificially inflated in concatenated analyses, particularly if the actual history of the species tree is comprised of very recent and simultaneous divergences that should be poorly supported given the data. BEST infers phylogeny in a more biologically realistic way for young lineages by taking into account the process of incomplete lineage sorting.

The BEST and concordance analyses were similar, but also differ is some respects. BEST requires you to a prior assign alleles to species, difficult in very recent radiations. This is also detrimental when one of your goals is testing for species level monophyly as we are. BUCKy is much less computationally intensive, with gene trees being inferred independently from the species tree, while BEST not only simultaneously estimates gene trees and a species tree but many other parameters as well, which can make the analyses prohibitively long. Concordance analyses also do not assume what is the cause of conflict between species, and just attempts to minimize this (Ané et al., 2007). This can be helpful if conflict is expected to be due to more than just retention of ancestral polymorphism, but conversely is less biologically defensible since it is not modeling any method. BEST improves on BUCKy in this way at taxonomic levels that are not confounded by gene flow, and support on these topologies shows this.

Causes of conflict

There are several reasons species phylogenies are to difficult to directly infer from genealogies: incomplete lineage sorting obscuring species level relationships by drift; introgression of alleles from one lineage to another; and cryptic species can assumptions of monophyly. When multiple loci are analyzed in either a concatenated or coalescent approach, *D. incognita* and *D. conformis* are still found to be paraphyletic or influence the species tree to the point of reducing support for other species relationships. There is a high degree of conflict between the different genealogies in our current analysis, possibly due to any of these issues.

Cryptic species are morphologically indistinguishable species that represent distinct evolutionary lineages (Bickford et al., 2006). The *sordidapex* complex is comprised of nondescript spoon tarsus species with only a few defining characters to discern each and the paraphyly in this group could be attributed to morphologically similar species being described as a single species when in fact they represent distinct lineages. One *D. incognita* specimen is on a relatively distinct branch in the partitioned concatenated analysis, and is the most likely candidate for a cryptic species. The rest of the species in this complex appear to suffer from a shared evolutionary history, instead of representing distinct lineages.

Introgression is a possible cause of the genealogical conflict in the spoon tarsus. Picture wing Hawaiian *Drosophila* species have been shown to be capable of hybridization in wild and laboratory settings (Yang and Wheeler, 1969; Carson et al., 1989), but while *D. dasycnemia* displays to *D. sordidapex* and vice versa, these displays do not lead to copulation (Spieth, 1966). Hybrid phenotypes have not been documented but the partially overlapping mating behaviors of the different species of spoon tarsus (Spieth, 1966) still make hybridization a possibility. The individual genealogies show widespread admixture in both the *sordidapex* and *dasycnemia* complexes, but this could be due to either incomplete lineage sorting or introgression. Differentiating introgression and incomplete lineage sorting can be difficult in recently divergent species since both produce the same pattern of shared polymorphisms between morphologically identifiable species (Holder et al., 2001). A more exhaustive study on the permeability of species boundaries in the spoon tarsus subgroup is warranted.

Despite the possibility of cryptic species and introgression we believe that incomplete lineage sorting in a very young radiation with large population sizes is the

most likely cause of the conflict. We assume the *dasycnemia* and *sordidapex* complexes of the spoon tarsus subgroup are no more than half a million years old, since the Island of Hawaii could not have been colonized before then (Price and Clague, 2002). In such recent radiations it is possible that drift will cause more genealogies to misrepresent the phylogenetic history of the species than those that corroborate it (Knowles and Carstens, 2007). The methods employed in this study address this issue of incomplete lineage sorting and find that there has not been enough time for lineages to coalesce into discrete lineages in the *sordidapex* complex.

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Tables

Table 1. [a] These individuals were also used in the BEST analysis
[b] For more information on specific collection records contact authors with corresponding barcodes.
[c] Taxonomically defined species group that each specimen has been placed in. ST refers to spoon tarsus subgroup, BT refers to bristle tarsus subgroup, Anto refers to the antopocerus subgroup, and PW refers to the picture wing clade.
[d] Island names are in bold.

Table 2. [a] Gene named after orthologous, annotated gene in D. melanogaster.
[b] Number of taxa sampled for this loci. 31 individuals were used in total.
[c] Linkage groups include X and Y sex chromosomes, mitochondrion, or Muller's Elements.
[d] Most likely substitution model for the gene identified by the Akaike Information Criterion (AIC) implemented in MrModeltest v2.3.

Table 3. [a] Gene named after orthologous, annotated gene in D. melanogaster.
[b] Number of taxa sampled for this loci. 31 individuals were used in total.
[c] Linkage groups include X and Y sex chromosomes, mitochondrion, or Muller's Elements.
[d] Most likely substitution model for the gene identified by the Akaike Information Criterion (AIC) implemented in MrModeltest v2.3.

Table 4. [a] Sample wide mean concordance factors with 95% confidence intervals.
[b] Genome wide mean concordance factors with 95% confidence intervals at α=0.1 and α=1.0.

Table 1. Species Sampling

Species Name	Barcode[1]	Group[2]	Location[3]
atroscutellata#	070236a	ST	**Kauai:** Nualolo Trail, 3800'
conformis	201312a	ST	**Hawaii:** HVNP, Olaa Tract
conformis	202493a	ST	**Hawaii:** Laupahoehoe, NARS, 4000'
conformis#	202486a	ST	**Hawaii:** Kau Forest Reserve, Hionamoa Stream
contorta#	200120a	ST	**Maui:** Waikamoi Forest Preserve, Pig Hunter's Trail
dasycnemia	202320a	ST	**Hawaii:** Saddle Road, Kipuka 9, Kaumana Trail
dasycnemia	202326b	ST	**Hawaii:** Kawaihae Uka, Waimea, 5000ft
dasycnemia	202331a	ST	**Hawaii:** Laupahoehoe, NARS, 3700ft
dasycnemia#	200122a	ST	**Hawaii:** HVNP, Olaa Tract
fastigata#	070069a	ST	**Oahu:** Manoa Cliff Trail, 1800'
incognita	202464a	ST	**Hawaii:** Ola'a Forest, pole 44, 3900'
incognita#	202333a	ST	**Hawaii:** Laupahoehoe, NARS, 3700ft
kikalaeleele#	202385a	ST	**Hawaii:** Kukui Opae, South Kona Forest Reserve, 3400ft
neutralis	202311a	ST	**Hawaii:** Stainback Highway, Tom's Trail, 3200'
neutralis	202319b	ST	**Hawaii:** Kau Forest Reserve, Hionamoa Stream
neutralis	202330a	ST	**Hawaii:** Laupahoehoe, NARS, 3700ft
neutralis#	202329b	ST	**Hawaii:** Kukui Opae, South Kona Forest Reserve, 3400ft
percnosoma	202394e	ST	**Hawaii:** Laupahoehoe, NARS, 3700ft
percnosoma	200125a	ST	**Hawaii:** HVNP, Olaa Tract
percnosoma#	202343d	ST	**Hawaii:** Kau Forest Reserve, Hionamoa Stream
sordidapex	202318a	ST	**Hawaii:** Kau Forest Reserve, Hionamoa Stream
sordidapex	202327a	ST	**Hawaii:** Kukui Opae, South Kona Forest Reserve, 3400ft
sordidapex	202332c	ST	**Hawaii:** Laupahoehoe, NARS, 3700ft
sordidapex#	202321a	ST	**Hawaii:** Ola'a Forest, Small tract, Transect 1
waddingtoni#	202431c	ST	**Hawaii:** Kawaihae Uka, Waimea, 5000ft
waddingtoni#	202526a	ST	**Maui:** East Maui Irrigation, Haiku Uka, Heed Trail, 4200'
waddingtoni#	202554b	ST	**Maui:** Puu Kukui Trail
waddingtoni#	202415b	ST	**Molokai:** Puu Kolekole, 3854 ft.
diamphidiopoda#	200785a	Anto	**Maui**
expansa#	201012a	BT	**Maui**
grimshawi	Flybase	PW	**Maui**

Table 2. Nuclear Gene Sampling

Gene[1]	name	Primer Sequence	Number Sampled[2]	Linkage Group[3]	#Chars (PICs)	Substitution Model[4]	Accession Numbers
PRY	PRYL	5'-CCGATGTCCTATGGATAGCTTTA-3'	31	Y	626(24)	HKY	HQ703659 - HQ703688
	PRYR	5'-AAGTGGAACGAATGTGTAAAACG-3'					
Kl-2	Kl2L	5'-TAATACAGAACGGTGGTATGGGTAT-3'	31	Y	587(14)	HKY+I	HQ703629 - HQ703658
	kl2R	5'-GTTGCTTGGCTAATTCGTAAAGAGT-3'					
Fz4	Fz4L	5'-GCGTCTTTCTATTGCGCTACTAT-3'	31	X	1063(39)	SYM+G	HQ703599 - HQ703628
	Fz4R	5'-GCTTGTACGGACTGCTGATTATT-3'					
Smox	SmxL	5'-AATTGAAACCGYTSGAGCA-3'	31	X	855(9)	GTR+G	HQ728839 - HQ728868
	SmxR	5'-CTTAGGCATTCGGCAAAGAC-3'					
Ge-1	Ge1L	5'-ATTGAAATGCAATTGTCCAAACT-3'	28	B	767(33)	HKY+I	HQ728723 - HQ728749
	Ge1R	5'-ATACGCATTAAACTCCACCATGA-3'					
Pds5	Pds5L	5'-GGATACTTTGTGGACAATTCAGAGT-3'	31	C	595(28)	HKY+G	HQ728779 - HQ728808
	Pds5R	5'-AGATATTTCACGAACTCTTCAGCAC-3'					
Dip3	Dip3L	5'-GTTAGAGTGGACATATGGGATCG-3'	31	C	811(25)	HKY+I	HQ703743 - HQ703772
	Dip3R	5'-GCACTGTTTCCATACTGTTGTTG-3'					
Bin	BinL	5'-CGGCTGGYGCATAATCACTT-3'	29	D	542(22)	HKY+I+G	HQ728698 - HQ728722
	BinR	5'-CACAATCTCAGCCTGAACGA-3'					
Osi9	OsiL	5'-AGCAGCGGCATCAGRTACTT-3'	30	E	476(9)	GTR+G	HQ728750 - HQ728778
	OsiR	5'-CCCAAGGACTCCATACAGGA-3'					
Rad23	RadL	5'-GCGGAATATTTGGTGGAAAA-3'	31	F	456(8)	HKY	HQ728809 - HQ728838
	RadR	5'-GAAGCCACTGTGCGATTGTA-3'					

Table 3. Mitochondrial Gene Sampling

Gene[1]	name	Primer Sequence	Number Sampled[2]	Linkage Group[3]	#Chars (PICs)	Substitution Model[4]	Accession Numbers
ND2	192	5'-AGCTATTGGGTTCAGACCCC-3'	31	mt	520(39)	HKY+I+G	HQ703773 - HQ703790
	732	5'-GAAGTTTGGTTTAAACCTCC-3'					HQ170926 - HQ170935, HQ170877
COI	2183	5'-CAACATTTATTTTGATTTTTTGG-3'	31	mt	829(89)	GTR+I+G	HQ703707 - HQ703724
	3041	5'-TYCATTGCACTAATCTGCCATATTAG-3'					HQ170816 - HQ170826, HQ170766
COII	3037	5'-ATGGCAGATTAGTGCAATGG-3'	31	mt	749(62)	GTR+I+G	HQ703725 - HQ703742
	3791	5'-GTTTAAGAGACCAGTACTTG-3'					HQ170712 - HQ170721 HQ170724, HQ170652
16s	16sF	5'-CCGGTTTGAACTCAGATCACGT-3'	31	mt	510(6)	HKY+I	HQ703689 - HQ703706
	16sR	5'-CGCCTGTTTAACAAAAACAT-3'					HQ171019 - HQ171029, HQ170967

Table 4. BUCKy Primary Concordance Factors

Splits	Sample-Wide[1]	Genome-Wide[2] a=0.1	Genome-Wide a=1.0
1	0.799 (0.625,0.875)	0.789 (0.414,0.990)	0.710 (0.353,0.955)
2	0.545 (0.375,0.750)	0.539 (0.189,0.871)	0.488 (0.167,0.816)
3	0.409 (0.250,0.625)	0.404 (0.070,0.812)	0.364 (0.062,0.751)
4	0.349 (0.125,0.500)	0.344 (0.052,0.717)	0.310 (0.046,0.662)
5	0.320 (0.125,0.500)	0.317 (0.038,0.712)	0.288 (0.035,0.659)
6	0.313 (0.125,0.625)	0.310 (0.008,0.737)	0.282 (0.009,0.682)
7	0.306 (0.125,0.500)	0.303 (0.034,0.677)	0.273 (0.030,0.624)
8	0.284 (0.125,0.500)	0.281 (0.008,0.667)	0.252 (0.007,0.614)
9	0.279 (0.125,0.375)	0.276 (0.033,0.642)	0.248 (0.029,0.590)
10	0.267 (0.125,0.500)	0.264 (0.006,0.671)	0.241 (0.006,0.619)
11	0.210 (0.000,0.500)	0.208 (0.000,0.644)	0.190 (0.000,0.593)
12	0.202 (0.000,0.375)	0.200 (0.000,0.602)	0.183 (0.000,0.555)
13	0.199 (0.000,0.375)	0.197 (0.000,0.580)	0.177 (0.000,0.532)
14	0.198 (0.125,0.375)	0.196 (0.007,0.583)	0.179 (0.007,0.537)
15	0.192 (0.000,0.375)	0.189 (0.000,0.579)	0.171 (0.000,0.531)
16	0.164 (0.125,0.250)	0.162 (0.006,0.511)	0.146 (0.005,0.469)

Figures

Figure 1. Map of the Hawaiian Island Chain with the ages of each island based on K–Ar dating. The age of the islands increase from east to west.

Figure 2. Bayesian concatenated, partitioned phylogeny of the spoon tarsus subgroup. Maximum likelihood topology is identical the Bayesian topology. Nodes with posterior probabilities greater than 0.90 and bootstrap supports greater than 70% are indicated by an * above branches. All taxa were collected on the Island of Hawaii except where indicated in bold. KAU = Kau Forest Reserve; OLAA = Olaa Tract, HVNP; LAUP = Laupahoehoe NAR; KONA = Kona Forest Reserve; SADL = Upper Waiakea Forest Reserve; KOHL = Puu O'umi NAR.

Figure 3. (a) BEST phylogeny of the spoon tarsus subgroup. Nodes with posterior probabilities greater than 0.90 are indicated by an *. (b) Bayesian concordance analysis of nine loci. Values above branches are primary concordance values, values below are genome wide mean concordance factors for $\alpha = 0.1$ and $\alpha = 1$.

Supplementary Figures 1-5. Bayesian estimates of gene genealogies, name of each gene used is above phylogeny. See Table 2 for details on evolutionary models used for each gene. Posterior probabilities are numbers above branches.

Figure 1.

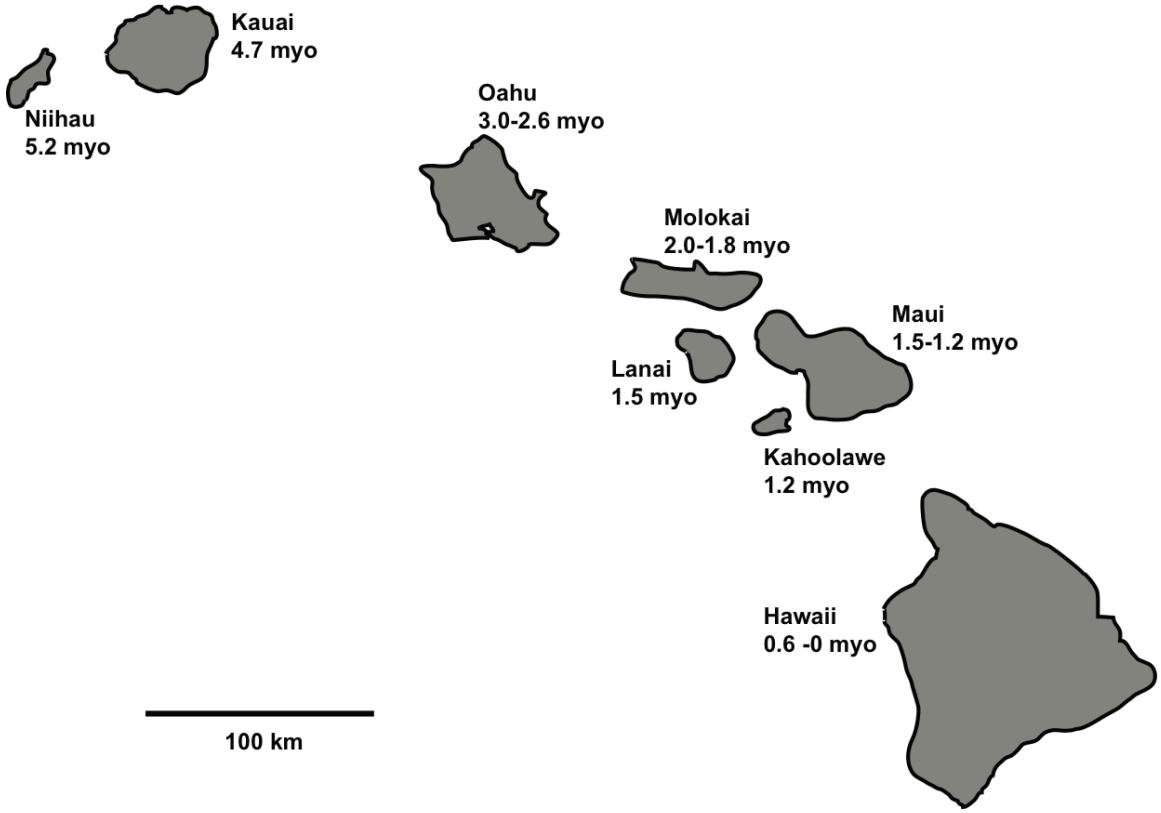

Kauai
4.7 myo
Niihau
5.2 myo
Oahu
3.0-2.6 myo
Molokai
2.0-1.8 myo
Maui
1.5-1.2 myo
Lanai
1.5 myo
Kahoolawe
1.2 myo
Hawaii
0.6 -0 myo
100 km

Figure 2.

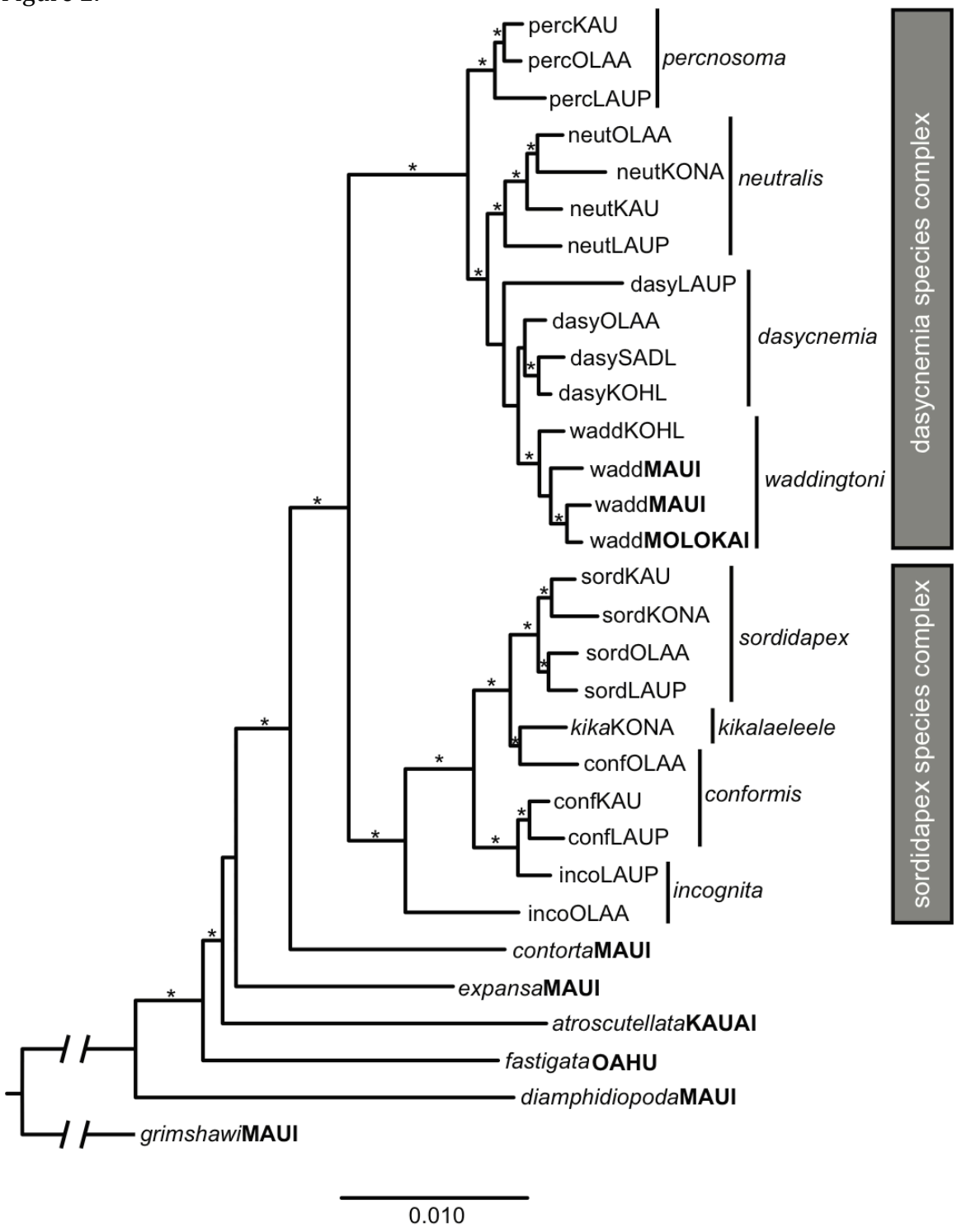
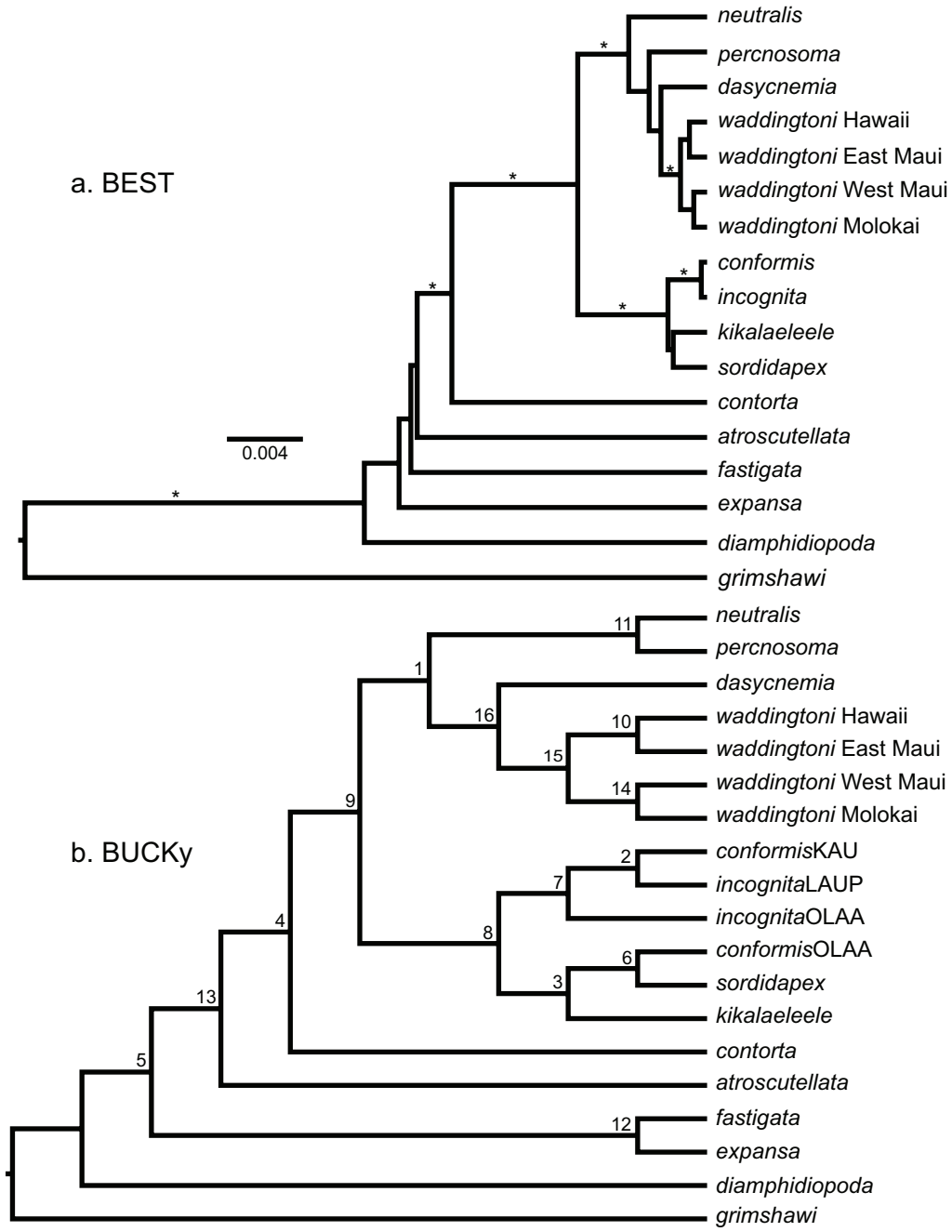
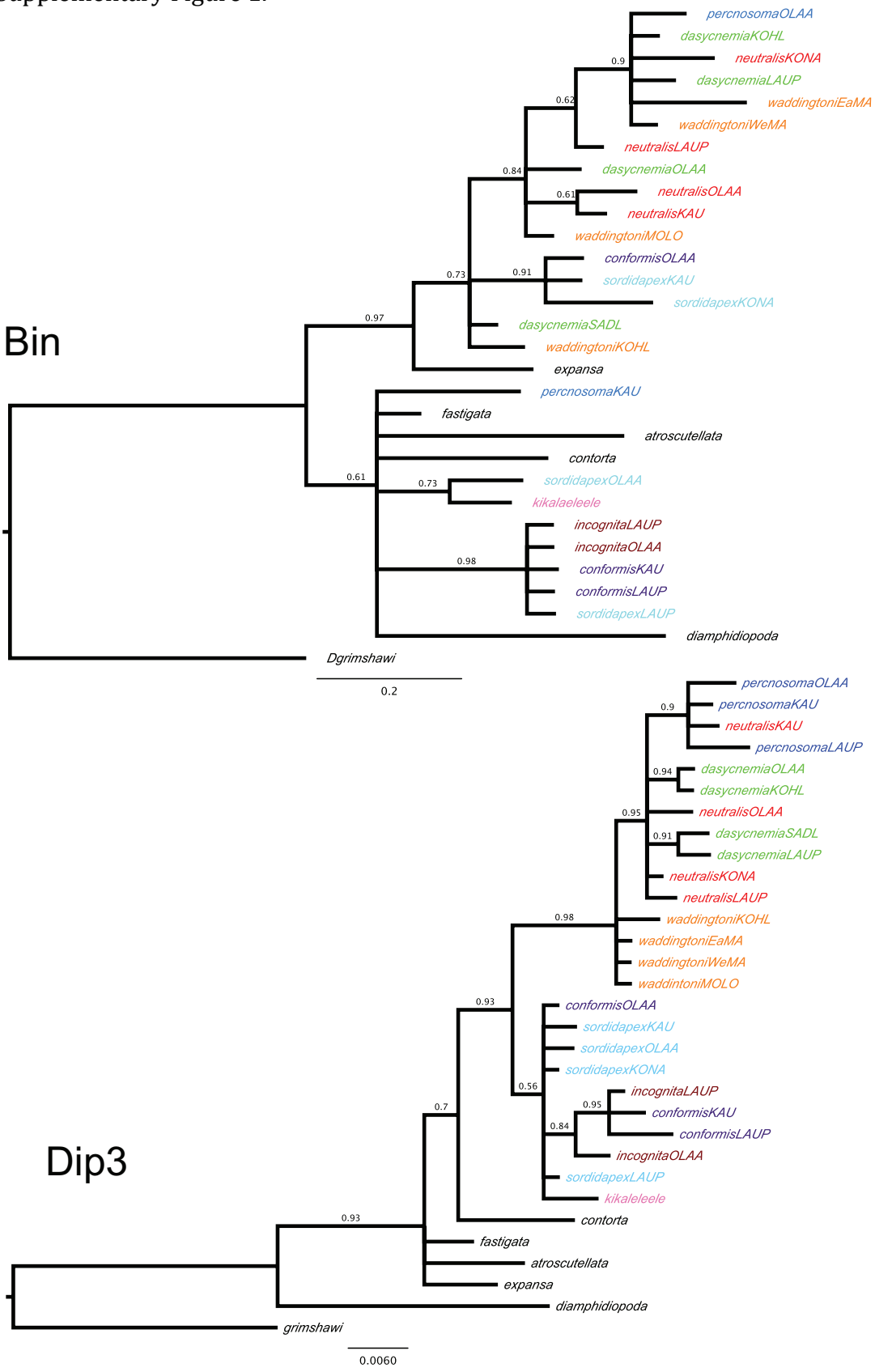

percKAU
percOLAA
percLAUP
percnosoma
neutOLAA
neutKONA
neutKAU
neutLAUP
neutralis
dasyLAUP
dasyOLAA
dasySADL
dasyKOHL
dasycnemia
waddKOHL
waddMAUI
waddMAUI
waddMOLOKAI
waddingtoni
dasycnemia species complex
sordKAU
sordKONA
sordOLAA
sordLAUP
sordidapex
kikaKONA
kikalaeleele
confOLAA
confKAU
confLAUP
conformis
incoLAUP
incoOLAA
incognita
sordidapex species complex
contortaMAUI
expansaMAUI
atroscutellataKAUAI
fastigataOAHU
diamphidiopodaMAUI
grimshawiMAUI
0.010

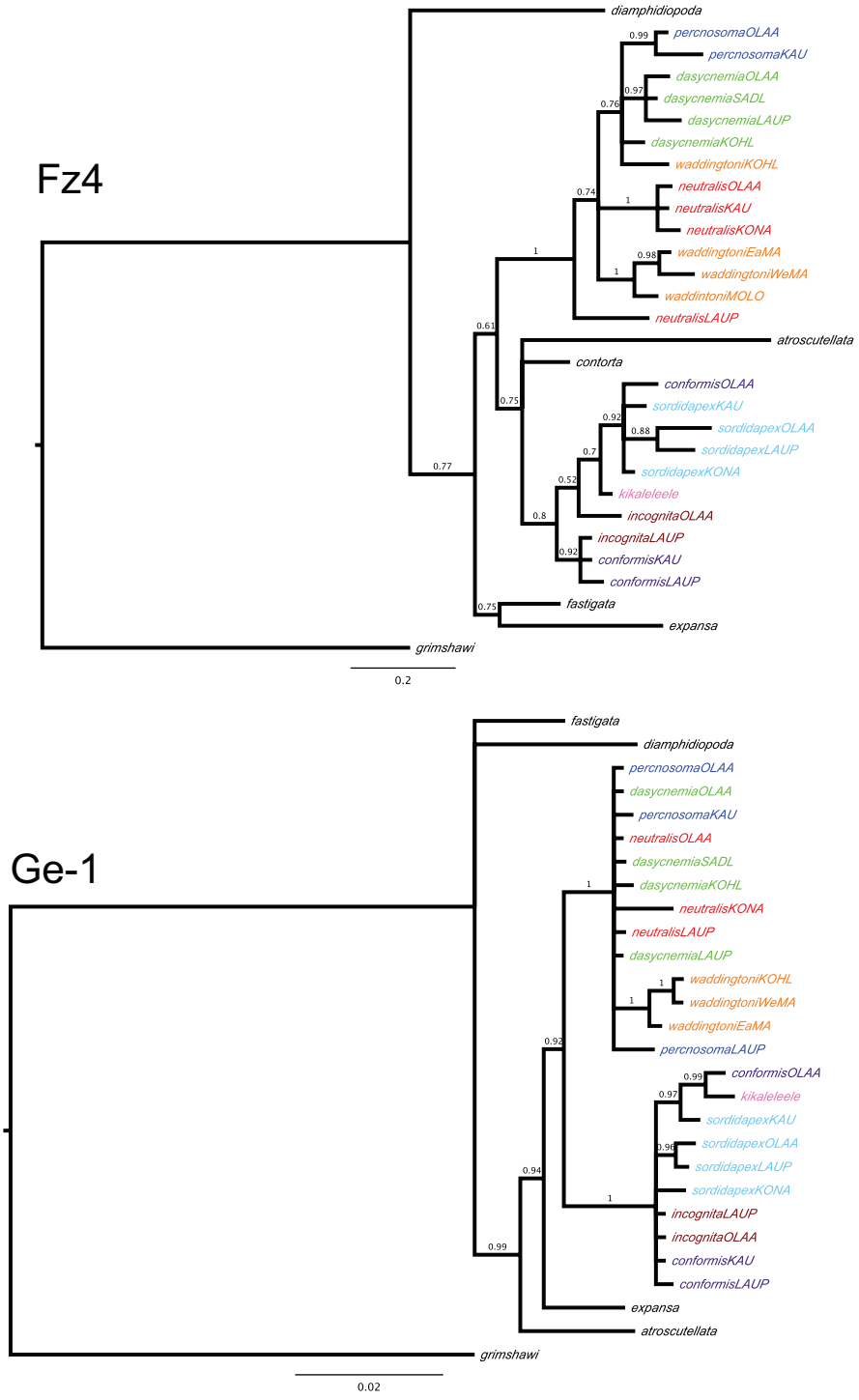
Figure 3.


a. BEST
0.004
neutralis
percnosoma
dasycnemia
waddingtoni Hawaii
waddingtoni East Maui
waddingtoni West Maui
waddingtoni Molokai
conformis
incognita
kikalaeleele
sordidapex
contorta
atroscutellata
fastigata
expansa
diamphidiopoda
grimshawi
b. BUCKy
neutralis
percnosoma
dasycnemia
waddingtoni Hawaii
waddingtoni East Maui
waddingtoni West Maui
waddingtoni Molokai
conformisKAU
incognitaLAUP
incognitaOLAA
conformisOLAA
sordidapex
kikalaeleele
contorta
atroscutellata
fastigata
expansa
diamphidiopoda
grimshawi

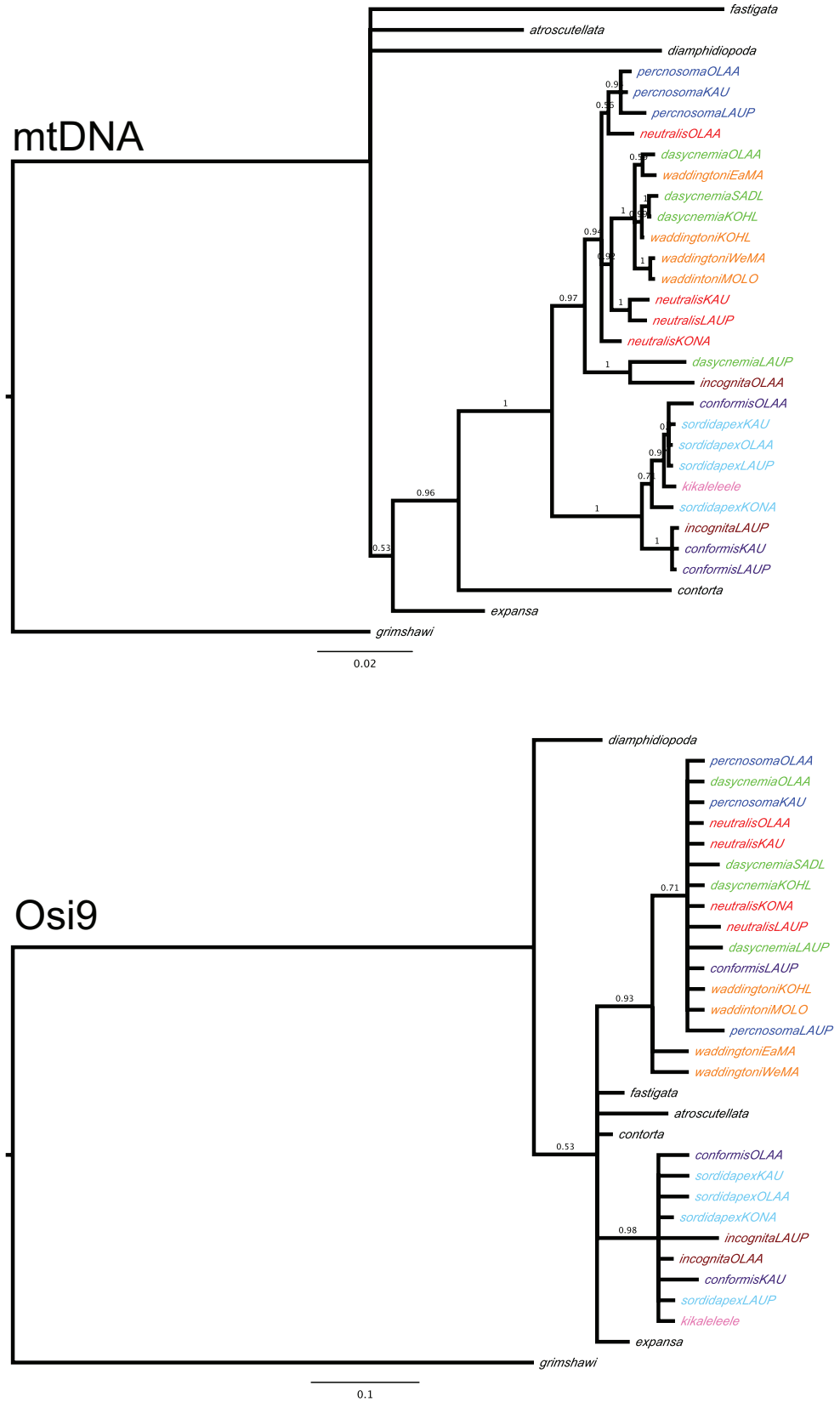
Supplementary Figure 1.



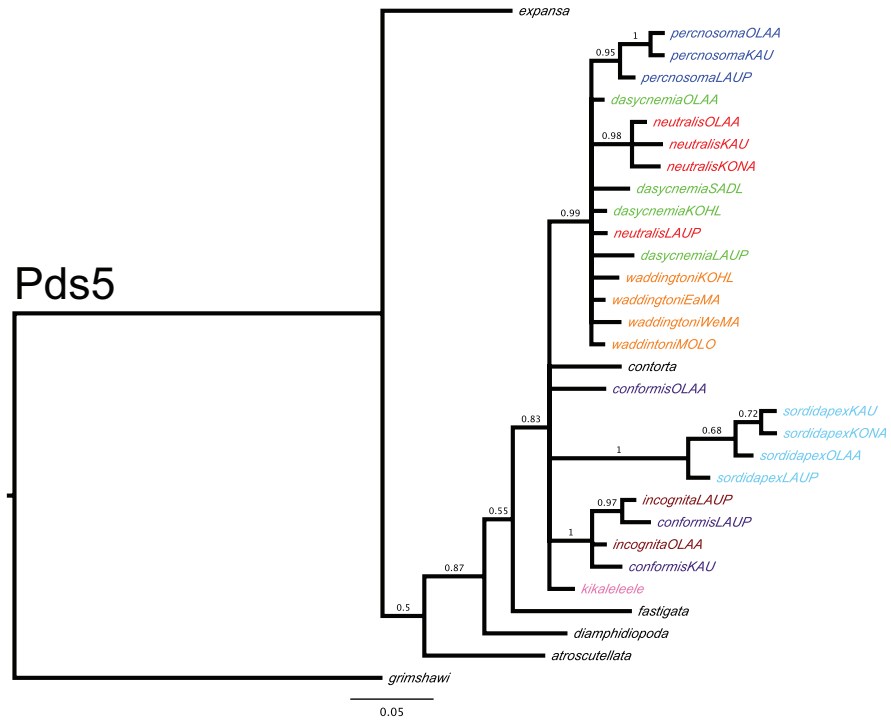
Supplementary Figure 2.

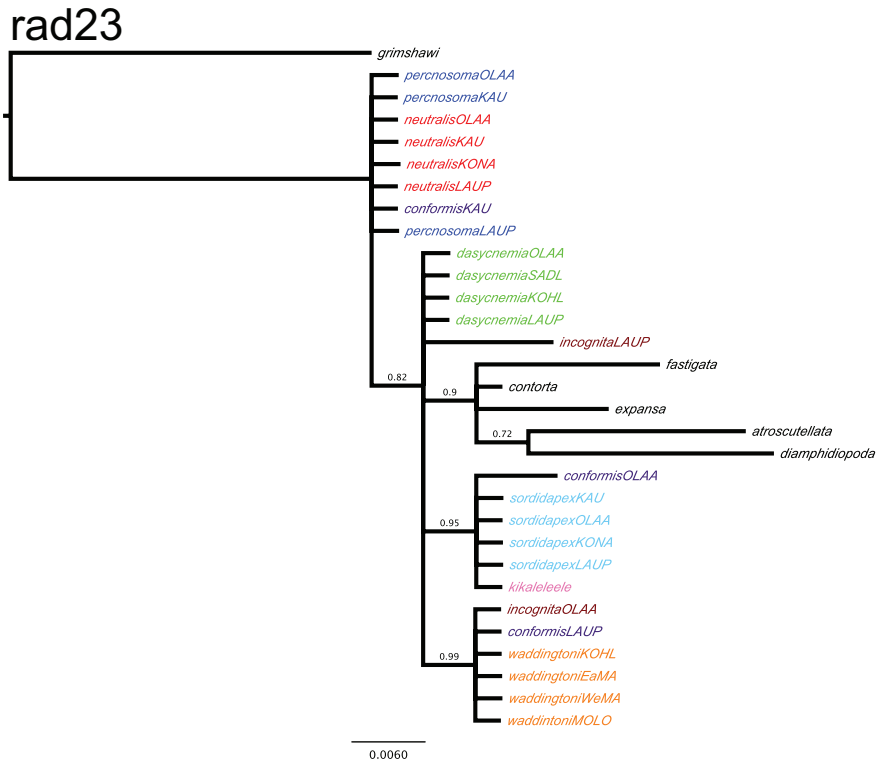


Supplementary Figure 3.

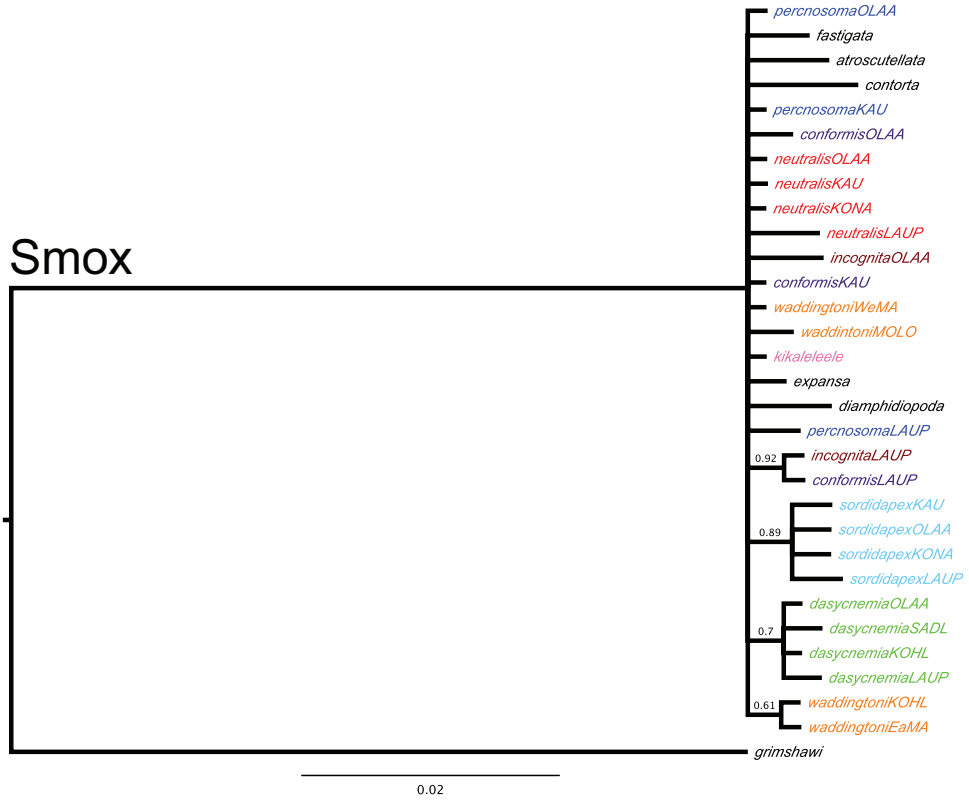


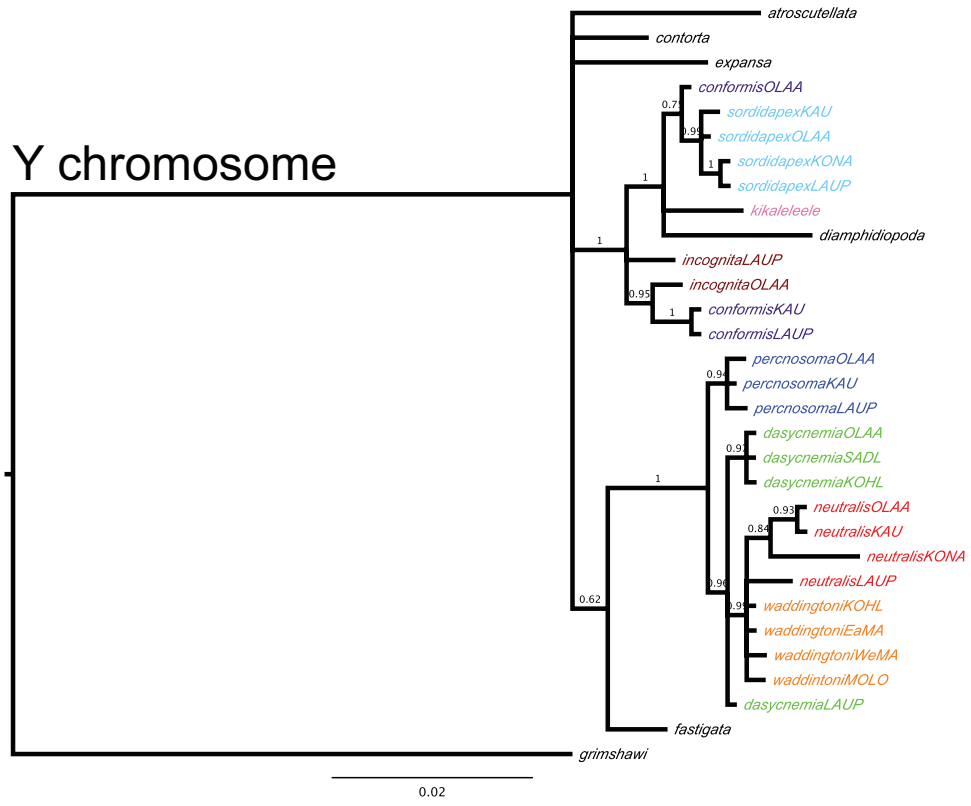
Supplementary Figure 4.





Supplementary Figure 5.





Chapter 4: Patterns of within and between island colonization in the Hawaiian Drosophilidae: *Drosophila waddingtoni*

Abstract

A number of biogeographic patterns have been proposed for taxa within the Hawaiian archipelago. Perhaps the most common is the progression rule, where most basally branching species on a phylogeny are found on older islands and more recently divergent taxa are found on younger islands. Recent work shows that *Drosophila waddingtoni* originated on the youngest island, Hawaii, and subsequently colonized the older islands of the Maui Nui complex, the opposite direction predicted by the progression rule. The recent origin of Hawaii suggests that these founding events have occurred within the past 500,000 years and further examination of this species will provide unique insight into the dynamics of recent island colonization events. Three nuclear and three mitochondrial genes are used to study gene flow and divergence following founder events. Biogeographic patterns, coupled with divergence time estimates suggest that the back colonization to Maui Nui occurred within the past 250,000 years and has since not led to significant population differentiation. These data also demonstrate that while migration between islands is possible and present in this species, it is not frequent enough to homogenize populations. Finally this study finds little evidence for Kaneshiro's asymmetric hybridization theory and suggests that the impact of founder events may not be as significant as previously suggested.

Introduction

The Hawaiian Archipelago is an ideal place to study the biogeography of remote islands (Cowie & Holland, 2008 for review). Separated by 3200 km from the nearest continent the extreme isolation of the Hawaiian Island chain makes their colonization a statistical rarity, and the few lineages that do make it will either go extinct or diverge and diversify (Gillespie et al., 2008). Endemic lineages therefore represent a disharmonic set of diversity adapted to the available niches (Roderick & Gillespie, 1998). The present high level of diversity in the Hawaiian Islands is believed to have evolved in situ and as a consequence of the unique geographic features of remote volcanic archipelagoes (Kambysellis & Craddock 1997; Baldwin & Sanderson 1998; Cryan et al 2001; Jordan et al., 2005; Magnacca & Danforth, 2006; Gillespie, 2005). Erupting through a thin area of the Earth's crust, the Hawaiian Islands rise out of the Pacific Ocean. As the volcano erupts, the lava it produces increases the area of the island. Eventually the island becomes stable enough to support an ecosystem. As the plate moves northwest, the volcanic island does as well. Further away from the center of volcanic activity, the islands become dormant and start to erode and subside back into the sea (Craddock, 2000). Due to this pattern young islands with high elevation wet rainforest are in the southeast and get progressively older and smaller to the northwest (Price & Clague 2002).

Organisms are expected to colonize older islands and subsequently speciate down the island chain to younger islands as they form in a process known as the progression rule (Funk & Wagner, 1995). Many Hawaiian species do display this pattern of radiation, with older lineages on older islands and recent speciation on young islands (e.g. Baldwin & Sanderson, 1998; Jordan et al., 2003; Gillespie, 2004; Mendelson & Shaw, 2005; Bonacum et al., 2005). There are exceptions to this observation, but this is an uncommon pattern (Cowie & Holland, 2008; Funk & Wagner, 1995). This has been seen in the endemic *Hylaeus* (Magnacca & Danforth, 2006), and some *Megalagrion* (Jordan et al., 2005) and *Platynini* species (Cryan et al., 2001). For most terrestrial species native to this archipelago, migrating from one island to another is rare, as evidenced by the high degree endemicity on each island (Nitta & O'Grady, 2008). Appreciable distances separate the current high islands due to historical pauses in volcanic activity and wider distances separated now submerged islands in the chain (Juvik & Juvik, 1995). These older and further distances are expected to have caused lineage wide bottlenecks in the past (Price & Clague, 2002).

Elevation and community-restricted species are subjected to high degrees of intra-island isolation in addition to between island isolation (Carson et al., 1990; Vandergast et al., 2004; Jordan et al., 2005; Muir & Price, 2008). The Hawaiian landscape is constantly changing, allowing for populations to become isolated and eventually reconnect. Active volcanoes will periodically erupt and the resulting lava flows will divide older contiguous habitats into relictual islands of intact forest known as Kipukas (Vandergast et al., 2004). Erosional forces start to wear away at the islands as soon as the volcanoes become dormant, creating valleys and dividing once connected islands, like the islands of the Maui Nui complex (Price & Elliot-Fisk, 2004). Once connected, oceanic barriers exist between the now less active volcanoes. These geologic forces combine to drive much of the species level diversity (Carson et al., 1990; Muir & Price, 2008; Jordan et al., 2005).

The demographic changes associated with the founding of new populations have

been hypothesized to drive Hawaiian *Drosophila* speciation for decades (for review Carson, 1997). Carson expanded on Mayr's theory of founder effect speciation through genetic revolutions and Wright's shifting balance theory with his own founder flush theory (Wright, 1931; Carson, 1968). He inferred that as a small founder population colonizes a new location, it is no longer constrained by the selective pressures the original population dealt with and expands in size and explores less fit gene combinations. As the population becomes large again a build up of less fit gene combinations reasserts selection pressure and the population crashes. Carson used this theory to explain the order and degree of polytene chromosome rearrangements in Hawaiian *Drosophila*. Later, Templeton expanded on this idea with his transilience model, which was also applied to Hawaiian *Drosophila* (Carson & Templeton, 1984). By combining the above theories on founder events with observations on Hawaiian *Drosophila* mating behaviors, an asymmetric hybridization hypothesis, Dr. Kenneth Kaneshiro proposed a model that would encourage pre-mating isolation (Kaneshiro, 1976, 1983, 1988). In this verbal model initial colonists would experience such a reduced population size that normally stringent female sexual selection would have to relax due to a reduced pool of males to choose from. When the founding and source populations come back into contact, the females with reduced preference from the population that underwent a bottleneck will accept the males from their own and the source population. Females from the source population will have not experienced a reduction in choosiness and will not accept males from the founding population. While founder events and low levels of gene flow have been identified in the Hawaiian *Drosophila* and has been shown to be a factor influencing within island diversity (DeSalle & Giddings, 1986; Carson et al., 1990) it is not considered the only factor driving the Hawaiian *Drosophila* radiation (Carson, 1997). Outside of the Hawaiian *Drosophila* there is little evidence that founder events have a large impact on other species that have colonized remote habitats (Clegg et al., 2002; Estoup et al., 2004; Jordan et al., 2005). While the importance of founder events has been downplayed in recent years due to increased evidence of adaptive divergence, the impact of allopatric isolation following a colonization event is still known to generate diversity (Vandergast et al., 2004; Gillespie, 2005; Jordan et al., 2005), and understanding the early stages of these colonization events is important to understanding how these lineages diversify.

While over 90% of all Hawaiian *Drosophila* are single island endemics *Drosophila waddingtoni* is a rare example of a species with a multi-island distribution. *Drosophila waddingtoni* is an ideal species in which to study the effects of recent intra-island colonization. A member of the *spoon tarsus* subgroup of the *antopocerus*, *modified tarsus* and *ciliated tarsus* (AMC) clade of the Hawaiian *Drosophila*, *D. waddingtoni* is found on Hawaii and all islands of the Maui Nui complex. Phylogenetic studies have shown that this species originated on Hawaii and subsequently back colonized the islands of Maui, Molokai and Lanai (Lapoint et al., 2011). The *spoon tarsus* subgroup is comprised of 10 species, with a young clade of eight species that have originated on the Island of Hawaii (Lapoint et al., 2011). Of this young clade only *D. waddingtoni* is found on any island besides Hawaii (Lapoint et al., 2009). Since the Big Island is about 500,000 years old the *spoon tarsus* species endemic to the Island of Hawaii are expected to have diversified within the recent past (Price & Clague, 2002). The young age of the entire clade can be used to infer that the back colonization of the islands of Maui Nui is even

more recent of an event. The barriers between these island populations are expected to be less porous and the bottlenecks more severe than the previous intra-island studies which encompass Hawaiian *Drosophila* and other terrestrial arthropods (Carson et al., 1990; Vandergast et al., 2004; Muir & Price, 2008), and are therefore a more reasonable test of the early stages of divergence for the many Hawaiian *Drosophila* lineages that have speciated betweens islands.

This study will test several hypotheses linked to the development and maintenance of diversity following dispersal events. For example, I will identify when *D. waddingtoni* migrated from Hawaii to Maui Nui, and how much gene flow has occurred since then. This study will also test if there is an effect of topographic disparity within islands in addition to between island isolation that may be driving genetic diversity. In addition to between island structure it is expected that there may be some effect of within island diversity that may scale in relationship to the topographic complexity within each island. This is expected for the populations on Maui and Molokai given the fluctuating availability of territory across the islands of Maui Nui (Price & Elliot-Fisk, 2004). I employ coalescent methods implemented in IMa2 to test when the initial colonization of Maui Nui and the subsequent split between populations occurred and how present day gene flow between islands is influencing the structure of the island populations. Structure will be further explored using classic phylogenetic and population genetic methods to test how it is partitioned throughout the range of *D. waddingtoni*. I will also test the impact of a between island colonization event on the effective population size of *D. waddingtoni*. Since the colonization of Maui Nui is expected to be recent I expect the genetic signal of a bottleneck to be present if it was severe enough to lead to the sort of genetic revolutions anticipated in classic models (Carson, 1968; Kaneshiro, 1976; Carson & Templeton, 1984). Bayesian skyline plots are used to identify the changes in population sizes over time measures, as well as measures of standing diversity that test for changes in population size, such as Fu's F and mismatch distributions. *Drosophila waddingtoni* is used in this study to provide a case study on the effect of geography on how species colonize and diversify across the Hawaiian archipelago.

Materials and Methods

Sampling, DNA amplification and Sequencing

Two to ten specimens of *D. waddingtoni* were collected from localities across the islands of Hawaii, Maui and Molokai (Table 1; Figure 1), for a total of 60 individuals sampled. Hawaiian *Drosophila* are adapted to high elevation habitats, and in addition to oceanic barriers, low elevation is considered a barrier to gene flow. I collected multiple populations from Hawaii and Maui that are separated by areas of low elevation. No samples were found or collected from Lanai, though records of this specimen exist from this island. *Drosophila sordidapex* and *Drosophila percnosoma* belong within the *spoon tarsus* subgroup of the AMC clade, and were used as outgroups, as identified by Lapoint et al. (2011). Specimens were collected by sweeping leaf litter or by aspirating specimens directly from sponges baited with fermenting banana and stored in 95% EtOH for identification and DNA extraction at UC, Berkeley. The author identified the specimens to species with the key provided in Lapoint et al. (2009). Genomic DNA was extracted from individual flies using the Qiagen DNeasy DNA extraction kit (Qiagen, Inc). The

only departure from this protocol was that individuals were soaked in Proteinase K instead of being macerated and subsequently preserved as point mounted vouchers. All voucher material has been deposited in either the B.P. Bishop Museum or the Essig Museum of Entomology at UC Berkeley. For details on collections and current deposition locations contact the author with the 6 digit barcodes listed for each specimen in Table 1.

All individuals were sequenced for 3 nuclear loci and 3 mitochondrial loci. The mitochondrial loci NADH dehydrogenase subunit 2, cytochrome oxidase I and cytochrome oxidase II were amplified using universal mitochondrial primers (Simon et al., 1994). The nuclear loci included were *fz4*, *pds5* and *osi9* (Lapoint et al., 2011). *Fz4* is an X-linked locus, while *pds5* and *osi9* are autosomal loci (Table 2). PCR products were cleaned using standard ExoSAP-IT (USB) protocols. Cleaned products were sent to the UC Berkeley Sequencing Facility and sequenced in both directions on an ABI 3730 capillary sequencer. Contigs were assembled using Sequencher, ver. 4.7 (GeneCodes, Corp). Because of the recent divergence between the taxa in this study, alignment was trivial and easily performed by eye using MacClade, ver. 4.06 (Maddison & Maddison, 2002). Alleles for diploid loci were identified using PHASE2.1.1 (Stephens & Scheet, 2005). The alleles identified with the greatest probability were used in all further analyses. Any with alleles that were phased with exceptionally low posterior probabilities (Posterior Probability < 0.5) were cloned using an Invitrogen TA cloning kit (Invitrogen, Carlsbad, CA USA) and re-sequenced to confirm the identity of each allele at that gene. I generated 58 sequences for COI and COII, and 59 for ND2. I also generated 57 sequences for nuclear gene Fz4 and 116 and 114 sequences respectively for the heterozygous osi9 and pds5 after allelic states were identified via PHASE v2.1.1.

Population Genetic Diversity and Structuring

Standard genetic diversity measures were calculated for each gene. DnaSP v5.10.01 was used to measure number of haplotypes, haplotypic diversity, and θ_S and θ_π per nucleotide (Librado & Rozas, 2009) (Table 2). To identify the amount of genetic structure within and between populations of *D. waddingtoni* I performed a hierarchical analysis of molecular variance, subdividing the dataset into both within island populations and between island populations, using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010). AMOVA identifies the variation due to the difference between groups – in this case islands - (Φ_{CT}), difference between populations within islands (Φ_{SC}), and differences within populations (Φ_{ST}) (Excoffier, et al., 1992). The statistical significance was estimated via 10,000 permutation tests. In addition, Arlequin (Excoffier & Lischer, 2010) was used to calculate pairwise F_{ST} values between island populations and volcano-restricted populations (Wright, 1951).

Phylogenetic Analyses

The Akaike information criterion (AIC), implemented in MrModeltest, ver. 2.3 (Nylander, 2004), was used to estimate the best-fit model of substitution for each of the different genes used in these analyses. Gene trees derived from individual analyses were estimated using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Since recombination is not expected to occur within the mitochondria the genes from this organelle were concatenated into a single locus, and partitioned by gene with a model of substitution

selected for each gene. Each recombining unit was used to estimate a gene tree for a total of 4 gene trees. Gene trees were run for 5 million generations and were sampled every 1000 generations. By examining the cumulative split frequencies plot calculated by AWTY (Wilgenbusch et al., 2004) and identifying when the potential scale reduction factor (PSRF) approached 1, convergence in all Bayesian analyses was assessed

Sequences were also concatenated, partitioned and analyzed in Bayesian (MrBayes, ver. 3.1.2; Ronquist and Huelsenbeck, 2003) and maximum likelihood (RAxML, version 7.2.6; Stamatakis, 2006). Since haploid and diploid sequences were included in this analysis one allele from each diploid sequence was sampled to avoid inflating the impact of the X-linked and mitochondrial alleles by doubling their occurrence to reduce the amount of missing data in this matrix. Maximum likelihood analyses were performed on the Abe Teragrid, accessed through the CIPRES portal (Miller et al., 2009). Likelihood searches were partitioned by locus and the GTRGAMMA model was used for each partition to estimate the tree, since some authors suggest against using proportion of invariant sites in conjunction with a gamma distributed rate matrix to avoid parameter conflict (Stamatakis, 2006). Two thousand bootstrap replicates were performed to assess support for the inferred relationships. The Akaike information criterion (AIC), implemented in MrModeltest, ver. 2.3 (Nylander, 2004), was used to estimate the best-fit model of substitution for each of the loci in the Bayesian analyses (Tables 2 and 3). The concatenated analysis (with 6 partitions) was run for 10 million generations and was sampled every 100 generations. Convergence was assessed via the same methods described above.

Since this species is expected to have originated recently and the populations on Maui Nui to have diverged even more recently, there is a high likelihood that incomplete lineage sorting will obscure the relationships between the populations of *D. waddingtoni*. Incomplete lineage sorting is where drift will cause genealogies to conflict by chance, especially when coalescence times are short and population sizes are large. To deal with this issue I estimated the species tree in a coalescent framework using *BEAST (Heled & Drummond, 2010). *BEAST jointly estimates gene tree topologies, population sizes, divergence times and species tree topologies in a Bayesian framework. I used the same dataset implemented in the concatenated phylogenetic analysis, partitioned it by gene and implemented the substitution model identified by MrModeltest v2.3 (Nylander, 2004) (Table 2). I classified each individual as belonging to the island it was collected from. The initial analysis was run for 50 million generations to identify prior interactions and improve chain mixing. Each gene was initially analyzed under an uncorrelated lognormal relaxed clock in BEASTv1.6.1 and Tracer v1.5 (Drummond & Rambaut, 2007) was used to observe if the *ucld.stdev* parameter deviated from 0 to test whether the loci are evolving in a clock-like manner (Drummond & Rambaut, 2007). All loci were identified as clock-like and this was implemented in the final *BEAST analysis for each loci. The operators were adjusted to improve searching tree space. The final analysis was run twice for 50 million generations and convergence was assessed by observing traces and ESS values for all parameters in Tracer v1.5 (Drummond & Rambaut, 2007). An empty alignment was run simultaneously to identify the influence of priors on the posterior distributions of each parameter.

Population Demographics

To identify recent bottlenecks or founder events following colonization of a different island I inferred mismatch distributions for each island for each locus and compared them to the expectation of an expanding population using Arlequin v3.5.1.2. To test how well the mismatch distribution fit the unimodal (expanding population) model, I estimated the sum of squares deviations and Harpending's raggedness statistic (Harpending, 1994). I also estimated Tajima's D and Fu's F, tests of selection which are also sensitive to population size changes (Tajima, 1989; Fu & Li, 1993), also in Arlequin. Negative values significantly different from zero indicate a population expansion or purifying selection and significantly positive values are expected for While these tests will identify selection in a single gene, if disparate and unlinked loci have significantly negative values for these tests, an expanding population can be inferred.

The above methods can be used to infer the presence or absence of a population size change, but for a detailed examination of population size changes over time I inferred Bayesian skyline plots using the program BEAST v1.6.1 (Drummond et al., 2005; Drummond & Rambaut, 2007). This method uses sequence data from multiple loci to estimate population size changes through time. The same dataset used for the concatenated phylogenetic analysis (excluding the outgroup taxa) was subdivided into three populations based on island of origin: Hawaii, Maui and Molokai. The substitution models were partitioned by gene, genealogies were partitioned by whether they were mitochondrial, autosomal or X-linked. Each gene was found to be evolving in a clock like manner and were estimated under a strict clock model. The substitution rates for the other loci were estimated as relative to the mutation rates estimated below. This analysis was initially run twice to optimize chain mixing and models. Each analysis was run for $5x10^7$ generations until stationarity was attained and ESS values were greater than 200.

Mutation rates are required to calibrate both the Bayesian skyline plot analyses and IMa2 analyses. The substitution rates for the mitochondrial loci and pds5 were estimated using the same dataset from chapter 1 that included representative taxa from the Hawaiian Drosophilidae, with extensive sampling from the AMC clade. The same calibrations were used as in that study including the expected divergence of Hawaiian *Drosophila* and *Scaptomyza* (Russo et al., 1995), as well as the divergence of closely related sister lineages on adjacent islands. The analyses were run in BEAST, partitioned by gene and run for $10x10^8$ generations. The mitochondrial substitution rate estimate of 2.09% (95% highest posterior density (HPD) 1.53%-3.73%) divergence per million years. The mutation rate for Pds5 was estimated at 0.77% (95% HPD 0.59%-0.95%) divergence per million years was very close to the value previously estimated for *Drosophila* nuclear loci - 1.1% (Tamura et al., 2004). The other mutation rates were jointly estimated as relative to these rates.

To address the amount and direction of gene flow, as well of time divergence, the dataset was analyzed under an isolation with migration model as implemented in IMa2 (Hey, 2010). This program estimates six parameters for each pair of populations, including gene flow in each direction between populations, effective population size for each population and their ancestral population, and time of divergence between all populations, all scaled by mutation rate, and can compare multiple populations at one time. This multiple population analysis requires an accurate inference of relationships between the populations involved, and the topology inferred from the *BEAST analysis was used (Hey, 2010). This program first uses a Markov Chain Monte Carlo method to

estimate possible genealogies and divergence times (M mode) and then uses this information to estimate the posterior distributions of all other parameters (L mode). For sake of computational efficiency, ten individuals were randomly selected from each island but include all sampled localities. To test the assumptions of asymmetric hybridization the dataset was divided into and subsequently analyzed as a mitochondrial (maternally inherited) and nuclear (average background) dataset.

Multiple preliminary runs were performed to infer the adequate priors for parameters and burn in (Hey, 2010). The final M mode run consisted of 5 independent runs all started from unique seed values for 330000 generations and sampled every 100 generations, with the first 30000 generations discarded as burn in, for a total of $1.5x10^6$ steps with $1.5x10^5$ steps saved. All runs consisted of 25 chains and employed a geometric heating scheme with an alpha of 0.9 and a beta of 0.8. Mutation rates are required to convert posterior values to demographically meaningful values, and the mutation rates inferred for the Bayesian Skyline plot analyses were used to this end, after converting the rates from substitution per site per million years rate to substitutions per locus per year (Hey, 2010).

Results

This study looks at how the effects of inter island affect populations of a native Hawaiian *Drosophila*, *D. waddingtoni*. To this end we identify how populations are structured across the range of *D. waddingtoni*, detect when the colonization events occurred and then observe how this affects the demographic history of this species. This study looks at the pattern of colonization across the islands using phylogenetic and coalescent methods, and confirms *D. waddingtoni* has indeed back colonized the islands of Maui Nui. The impact of oceanic barriers on the genetic structure of these populations is further tested using methods that display how diversity is divided among populations (AMOVA and F_{ST}) as well as using coalescent based methods that take into genetic similarity between populations due to incomplete lineage sorting and gene flow (IMa2). Finally I use multiple tests to identify how severe of a founder event occurred as this species colonized the islands of Maui Nui and Molokai using both measures of current diversity (Tajima's D, Fu's F and mismatch distributions) as well as methods with a time component to test how population size has changed over time (Bayesian Skyline Plots). The results of these analyses are described below.

Phylogeography of waddingtoni

The concatenated mitochondrial genes display a high degree of resolution, but do not define monophyletic groups that can be used to identify individuals as belonging to discrete populations (Figure 2). This is likely due to a combination of incomplete coalescence and some degree of gene flow between the different populations. In contrast with the mitochondrial genealogy, the nuclear loci are not well resolved and show a much higher incidence of shared haplotypes (Figures 3-5). The difference in the amount of structure may be related to the inheritance scalar of each locus, as the mitochondrion is well resolved and has the smallest effective population size, followed by the X-linked locus, and the autosomal loci are most poorly resolved, both with increasingly larger effective population sizes. The decrease in resolution proportional to the increase in

inheritance scalar is expected given the assumptions of the coalescent (Kingman, 1982). Concatenating both the nuclear and mitochondrial loci and partitioning substitution models by gene better delimits phylogenetic relationships within *D. waddingtoni* (Figure 6). The population from Hawaii is basal, as expected from all previous analyses (Lapoint et al., 2011; O'Grady et al., 2011). The Maui Nui populations form a clade nested within Hawaii samples and is divided into a mostly East Maui clade and a West Maui/Molokai clade, indicating a gene flow between Maui and Molokai. The *BEAST analysis recovers a similar topology with high support (Figure 7). While hampered by the a priori designation of alleles to populations, this method accounts for incomplete coalescence, which is expected to have a strong influence in cases of recent divergence such as this. This topology inferred using *BEAST is similar to that estimated under the concatenated dataset, but because it takes into account incomplete coalescence is the preferred topology for future analyses.

Time to Most Recent Common Ancestor

To identify that these species have recently colonized the islands of Maui and Molokai and originated on the island of Hawaii, the ages of these divergences were estimated using IMa2. A species group level analysis has estimated the divergence of *D. waddingtoni* and *Drosophila dasycnemia* at about 175,500 (95% HPD 81700/296500) years ago (see chapter 3, this dissertation). Using a molecular clock inferred from that data, the times of divergence were estimated between the populations of *D. waddingtoni* in IMa2. A slightly older date for the split between the Hawaiian and Maui Nui *waddingtoni* populations – 245,251 (95% HPD 117560/374472) years ago – which overlaps the previous estimate. This date also indicates that the divergence occurred soon after the species originated. The split between Maui and Molokai is most likely to have occurred soon after, about 186,376 (95% HPD 18542/330124) years ago.

Population Structure and Gene Flow

Several measures were utilized to identify how genetic diversity is partitioned across the populations of *D. waddingtoni*. The F_{ST} analyses indicate structure between the different islands, with significant differences between islands at most loci (Table 3). This pattern indicates that overwater distances do present a significant barrier to gene flow, with some gene flow between Maui and Hawaii. The within island F_{ST} analyses also indicate that populations found on different volcanoes represent very distinct populations and dispersal across low elevations is also restricted. The hierarchical AMOVA describes a similar situation: most of the variation observed in the sequences is due to variance within populations of *D. waddingtoni* (Table 4). Fz4 is the exception, and its negative Φ_{CT} indicates significant out breeding between other islands, most likely between Maui and Hawaii. The significant, or nearly significant, Φ_{SC} values indicate that there is large amount of diversity present between populations within the same island, and this species is highly structured across its entire range.

A lack of genetic divergence between populations can be due to 2 factors: 1) incomplete lineage sorting, where not enough time has passed for sequences to sort by identity into separate populations, or 2) gene flow homogenizes the gene pool of poorly isolated populations. The directionality and magnitude of migration were estimated with the program IMa2. The isolation with migration model implemented in IMa2 infers a

coalescent process to identify what degree of similarity is due to gene flow. IMa2 allows for multiple populations and will infer direction and magnitude of migration between the current islands of Hawaii, Maui and Molokai, as well as between Hawaii and the ancestral lineage that led to the now present Maui and Molokai populations (Figure 8). The greatest degree of gene flow is between the islands of Hawaii and Maui, with similar levels of gene flow in both directions, corroborating the F_{ST} results. While the posterior distribution of migration from Hawaii to Maui includes an initial peak at zero, the second peak is more likely given the observed structure between these islands. There appears to be no migration between Hawaii and Molokai, and low levels of migration from Maui to Hawaii, but not vice versa. The IMa2 analysis also confirms the initial pattern of back colonization from the Island of Hawaii to the islands of Maui Nui seen in previous phylogenetic analyses. The analysis was partitioned to compare the amounts of migration between islands by whether the loci are nuclear or mitochondrial to test if there is a sex bias in dispersal direction. There is no difference in the directionality of greater gene flow between these islands based on either female or male movement (Table 6).

Population Size Changes

To explore the impact of founder events on populations that have colonized Maui and Molokai tests of diversity were employed to observe how diversity was partitioned in these populations. The analyses indicate that all island populations of *D. waddingtoni* are expanding. Tajima's D and Fu's F_S statistic are almost all negative for each gene, though not all are significantly so (Table 5). This pattern is indicative of a weak population expansion. The mismatch distributions also exhibit this pattern of population growth (Figures 9-11). I was not able to reject a model of population growth for any locus or population, except in the case of pds5, which was significantly different in all populations. This departure may be due to the extremely low level of variation observed for this locus. The Bayesian Skyline plots show this trend as well. Skyline plots show the effective population size (Ne) of a population through time (Drummond et al., 2005). The population on Hawaii is the largest, followed by Maui, and the smallest is Molokai. Around 20,000 years ago all populations start to expand (Figure 12). The smallest population, Molokai is estimated to have a Ne of 4.03×10^5 (95% HPD $4.95 \times 10^2 / 1.251 \times 10^6$), while Maui's Ne is estimated at 9.031×10^6 (95% HPD $4.7 \times 10^4 / 30.542 \times 10^6$). Hawaii maintains the oldest population of *D. waddingtoni*, and it has expectedly the largest population, with a Ne of 2.5372×10^7 (95% HPD $1.83 \times 10^5 / 8.1200 \times 10^7$). The estimates of Ne from the IMa2 analysis had difficulty converging and are not reported (Figure 8).

Discussion

This study looks at how inter and intra-island dispersal affects populations of a native Hawaiian *Drosophila*, *D. waddingtoni*. To this end we identify how populations are structured across the range of *D. waddingtoni*, detect when the colonization events occurred and then observe how this or subsequent events affects the demographic history of this species. This study looks at the pattern of colonization across the islands using phylogenetic and coalescent methods, and confirms *D. waddingtoni* has indeed back colonized the islands of Maui Nui (Lapoint et al., 2011). The impact of oceanic barriers

on the genetic structure of these populations is further tested using methods that display how diversity is divided among populations (Excoffier & Lischer, 2010) as well as using coalescent based methods that take into account genetic similarity between populations due to incomplete lineage sorting and gene flow (IMa2) (Hey, 2010). Finally, I use multiple methods to test whether a founder event occurred as this species colonized the islands of Maui Nui and Molokai using measures that consider current sequence diversity (Tajima's D, Fu's F and mismatch distributions) as well as those which examine how population size has changed over time (Bayesian Skyline Plots) (Drummond & Rambaut, 2007).

Back Colonization of Maui Nui

The back colonization of *D. waddingtoni* from Hawaii to Maui Nui is interesting for several reasons. First, the order of colonization that is predominantly observed across the Hawaiian Islands is from the oldest to youngest islands. This is not the only example of this pattern in Hawaiian terrestrial arthropods; *Hylaeus* (Magnacca & Danforth, 2006), *Megalagrion* (Jordan et al., 2003) and even other Hawaiian *Drosophila* species groups (Carson, 1983) are known to have back colonized older islands in the archipelago. However, the vast majority of lineages examined show this pattern of older lineages on older islands and younger lineages on younger islands (Funk & Wagner, 1995; Bonacum et al., 2005; Cowie & Holland, 2008). Examples of within-species back colonization are even more rare as Hawaiian arthropod species are rarely endemic to multiple islands and it is often difficult to interpret the origin of these species (Piano, et al., 1997; Holland & Cowie, 2007; Jordan et al., 2005). The only other multi-island Hawaiian *Drosophila* species that has been studied, *D. grimshawi*, has been similarly cryptic in its origin but is likely to have followed the progression rule pattern of diversification (Piano et al., 1997). Second, given the expectations of community assembly terrestrial arthropods are expected to colonize from less to more ecologically open islands (Roderick & Gillespie, 1998). This is not always the case, and many examples exist where species are able to exploit open niche space on older islands (Gillespie et al., 2008). However, species from the AMC clade, which *D. waddingtoni* belongs to, inhabits superficially similar ecological niches (Magnacca et al., 2008), ovipositing on decaying leaves of plants from mostly the Aquifoliaceae and Araliaceae family. To put it into perspective, *D. waddingtoni* is competing against 40 other species in the AMC clade endemic to the islands of Maui Nui, almost all of which exploit nearly identical niches. Finally, when considering the *spoon tarsus* subgroup of the AMC, *D. waddingtoni* appears to be especially unique. The Hawaiian clade of the *spoon tarsus* subgroup includes eight species that are morphologically and genetically closely allied (Lapoint et al, 2009; Lapoint et al., 2011). They are found in sympatry in locations across the Island of Hawaii (Lapoint et al., 2009) and have been reared from nearly identical substrates (Magnacca et al., 2008) and exploit similar mating sites and utilize similar mating behaviors (Spieth, 1966; Bell & Kipp, 1990; Shelly, 1987, 1988, 1990). Despite all of these similarities, *D. waddingtoni* is the only species in this clade to have colonized other islands.

Dating estimates indicate that the colonization of Maui Nui and subsequently the split between Maui and Molokai occurred during periods of low sea level when the islands of the Maui Nui complex were connected (Price & Elliot-Fisk, 2004). Oxygen isotopes have been used to estimate when glacial maxima have occurred identify periods

of low sea level as recently as 20,000 years ago, as well as further in the past approximately 160,000 to 190,000 years ago and 260,000 to 290,000 years ago. During these periods Maui Nui would be overall a larger target for migrant individuals and home to a greater amount of suitable habitat (Price & Elliot-Fisk, 2004). This research indicates that dispersal between islands is rare and facilitated by changes in sea level. This pattern of gene flow between islands within the Maui Nui complex during periods of low sea is found in other species and explains the patterns of diversity seen in these groups (Piano et al., 1997; Jordan et al., 2005). These short but predictable periods of increased area of these islands may be what facilitates colonization of Maui Nui, and future research may lead to identifying more examples of recent back colonization to these islands because of this process.

The stochastic processes that lead to some lineages being better island colonizers than others has been documented (Gillespie et al., 2008), and chance alone may have allowed *D. waddingtoni* to back colonize Maui Nui and not other Hawaiian *Drosophila* species. However, there are two possible factors that may have predisposed this species to back colonize Maui and Molokai. Hawaii has the largest number Drosophilidae species (Hardy, 1965), many of which share similar ecologies (Magnacca et al., 2008) on the island with similar ecologies. The Hawaiian Drosophilidae community on Hawaii is therefore assumed to be highly competitive. This could give the species that have evolved on Hawaii an advantage when colonizing other islands in the archipelago. There are nine other AMC species that are multi-island endemics, excluding those found only on multiple islands of the Maui Nui complex, and seven of these species co-occur on Hawaii and the islands of Maui Nui (Hardy, 1965). The direction of colonization in these cases is unknown but worth investigating. The second possible factor that may facilitate the colonization of Maui Nui by *D. waddingtoni* is the slightly wider breadth of host use this species exhibits. In addition to the common host plant families listed above, *D. waddingtoni* has also been reared from decaying leaves from the plant families Myrsinaceae and Pittosporaceae (Magnacca et al., 2008). While possibly of only incidental use, this larger diversity in host use may allow for this species to more easily colonize less than ideal habitats. Generalist species have a wider range than their more specialized sister species, as has been shown in the context of other Hawaiian arthropods (Kambysellis & Craddock, 1997; Vandergast et al., 2004; Arnedo et al., 2007).

Founder Events

Hypotheses on how the Hawaiian *Drosophila* have radiated so spectacularly have historically revolved around the effects of repeated bottlenecks. Founder flush (Carson, 1968) and transilience (Carson & Templeton, 1984) are both verbal models that describe how founder events, in combination with epistatic and environmental adaptations, could cause the rapid development of post mating barriers and species formation. While bottlenecks are expected to be common occurrences for most terrestrial Hawaiian taxa, this result is not surprising since previous tests of these founder speciation hypotheses have been inconclusive (Barton & Charlesworth, 1984). Founder events are currently considered to be unnecessary for speciation to occur, even in the case of the Hawaiian *Drosophila*, due to apparent ecological adaptation, runaway sexual selection and abundant examples of local allopatry (Carson et al., 1990; Kambysellis & Craddock, 1997; Boake, 2005; Muir & Price, 2008; O'Grady et al., 2011). Both the founder flush

and transilience models expect that the populations will undergo a founder event, followed by a population expansion followed by the population reaching an equilibrium size. *Drosophila waddingtoni* does not appear to follow this pattern, which could explain why the isolated island populations are not more divergent. While all analyses indicate that each island's population is rapidly expanding, there does not appear to be any evidence of an actual bottleneck, and the population size does not yet appear to have reached equilibrium. This may be because either the duration of the bottleneck was very short or a large number of migrants founded the initial population. Given that the expansion occurred on the oldest and largest population over time, another possible explanation for this pattern is that migration during the last glacial maxima between islands was at a high enough level to mitigate the effects of a reduced gene pool and increased overall diversity. Current migration indicates the latter hypothesis may be more defensible.

Drosophila waddingtoni is potentially a good model to test the hypotheses of Kaneshiro's asymmetric hybridization theory (Kaneshiro, 1976) and is the first multi-island distributed species to test this hypothesis: In populations at equilibrium, females are very choosy in selecting males with which to mate. However, small, colonizing populations are initially subject to founder effects, in which the scarcity of males forces females to be less selective when choosing a mate: If they are too choosy, they will not find a mate. Additionally, males from this small population display highly variable mating behaviors due to reduced intraspecific competition and relaxed female selection. Choosy females from the larger source population will not mate with the males of the founder population, although females of this founder population will accept males from the larger source population (Kaneshiro, 1976, 1983, 1988). Kaneshiro's hypothesis was tested by exploiting one of the assumptions of this model: founder population females are able to breed with males from both the source and founder population, but females from the source population can only breed with males from the source population. Because of this I assume that nuclear loci will move freely between the two populations, but mitochondrial haplotypes will only migrate from the founder to source population. This data does indicate that each island has recently undergone a population expansion, which for the Maui Nui populations is likely to have followed a bottleneck. When the estimates of directional gene flow are compared there is no pattern of preferential mitochondrial gene flow from small to large populations, or from Maui Nui to Hawaii. In fact, there is more mitochondrial gene flow from the source population of Hawaii to the present day islands of Maui Nui, than the reverse. It is possible that these populations have been stable long enough post isolation, that any signature of founder-flush or asymmetric hybridization has been lost, but these analyses do not indicate this. Though this pattern has been used to be a major component of sexual selection in Hawaiian *Drosophila* it is not thought to be necessary to drive divergence between populations given the topological and environmental gradients commonly found on the Hawaiian Islands (Boake, 2005). While this test of Kaneshiro's hypothesis does not appear to identify this model of founder event speciation as the cause of increased divergence between these populations there is still genetic structure developing between these populations that may lead to eventual speciation. Future work to compare the mating behaviors of each islands population to more thoroughly test if a lack of divergence in behaviors corroborates my findings.

Topographic Effects on Genetic Diversity

Recent molecular evidence indicates that genetic diversity on the Hawaiian Islands is driven by within island allopatry as much as between island isolation (Vandergast et al., 2004; Jordan et al., 2005). The Hawaiian landscape is constantly changing allowing for populations to become isolated and eventually reconnect, driving higher levels of genetic diversity. The AMOVA analyses and diversity estimates confirm that a major factor in genetic diversity is due to population structure between populations on the same island. Indeed, there is greater diversity at all measures for the island of Hawaii than for either the islands of Maui or Molokai. This could be due to either the age of each population – older populations have accumulated more genetic diversity – or because of the increased topographic diversity seen on the island of Hawaii. The age of the populations are unlikely to be a factor since the Maui Nui and Hawaii populations diverged very close in time with one another. Unlike Maui and Molokai which are each made up of two volcanoes, Hawaii is comprised of five shield volcanoes, and *D. waddingtoni* are found on four of these. Since most Hawaiian *Drosophila* species, including *D. waddingtoni*, are found from 1000 to 2000 meters in elevation these high elevation wet forests act as islands themselves, driving divergence through isolation.

Changing sea level has also connected and bisected the islands of Maui Nui. These islands are formed from a total of 6 shield volcanoes that have intermittently been connected to form a single land mass as sea levels subside, and at its maximum size may have been larger than the island of Hawaii. Currently divided into the islands of Maui, Molokai, Lanai and Kahoolawe, Maui Nui was connected as recently as the last glacial maximum, about 20,000 years ago. Overland connections between the islands is not expected to have an effect on high elevation restricted species, but would allow greater mobility between populations and possible homogeneity for low elevation species. Despite this expectation, this study identifies within island structuring as a major component of diversity within the high elevation wet forest adapted *D. waddingtoni* (Table 4), but increased genetic connectedness between island populations during periods of low sea levels (Figure 8). There is an abundance of information showing that over water barriers are powerful restrictors to gene flow (Piano et al., 1997; Jordan et al., 2005; Holland & Cowie, 2007) and over water dispersal is generally expected to reduce gene flow between the islands of Maui Nui when sea levels rise (Table 3). Despite this, vicariance is expected to be a prevalent pattern for Maui Nui lineages due to the high degree of contact the islands experience in the past. *Drosophila waddingtoni* appears to be significantly structured between Maui and Molokai, with migration only going from Maui to Molokai and colonization only occurring during periods of low sea level. Conversely there may be an effect from the gene flow between the two populations that is observable from the change in population size over time. Both the Maui and Molokai populations start to expand at about the same time, when the islands were last connected (Figure 12). The current distribution of the genetic diversity of *D. waddingtoni* is the result of vicariance and dispersal across the islands of Maui Nui.

Conclusions

Drosophila waddingtoni is a useful species to explore the impact of recent colonization events on an endemic taxon. After originating on Hawaii, it has colonized

the older islands of Maui and Molokai, the opposite of what is expected given the progression rule. This analysis finds that colonization of new islands is most likely during periods of low sea level, when more suitable habitat is available and founding populations are large enough populations that the effects of bottlenecks are not presently apparent. While gene flow is apparently more easily accomplished during glacial maxima, it is still possible during periods of high sea level. Mating trials and behavioral studies between the island populations of *D. waddingtoni* will identify if the observed geographic isolation is leading to the development of intrinsic reproductive barriers. In addition to between island structure, within island population structure is an important component of diversity within *D. waddingtoni*. Future work on the biogeography of the Hawaiian *Drosophila* should place this work into a comparative framework and investigate the difference in population structure between *D. waddingtoni* and related species endemic to single islands to test the degree that within and between islands geography drives diversity.

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Tables

Table 1. Individuals sampled and gene coverage. # refers to where the individual was collected on the map in Figure 1. Barcode refers to O'Grady Lab bar-coding conventions and can be referred to identify full collection details. An X indicates that gene was sequenced for that individual. For Osi9 and Pds5, two copies were sequenced per individual.

Table 2. Gene details and diversity. [1] number of different haplotypes. [2] haplotype diversity.

Table 3. Pairwise F_{ST} within and between island populations. Values indicated by an asterisk are significant ($P < 0.05$).

Table 4. Analysis of Molecular Variance results partitioned by gene. Significant values indicated in bold.

Table 5. Measures of effective population size and change in island populations. All significant values indicated by an asterisk. For Tajima's D and Fu's F, negative values indicate population expansion, positive values indicate a bottleneck. For SSD and Raggedness scores, non-significant values indicate that an expansion model cannot be rejected.

Table 6. Directional migration rates between islands partitioned by nuclear and mitochondrial genes. Migration rates estimated for both mitochondrial (mt = COI, COII, ND2) and nuclear (nu = osi9, pds5, fz4) data. Arrow points towards directionality of gene flow. HI = Hawaii, MI = Maui, MO = Molokai, MN = Maui Nui.

Table 1. Individuals sampled and gene coverage

#	Barcode	Population	Locality	ND2	COII	COI	Fz4	Pds5	Osi9
1	202431a	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431b	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431c	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431d	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431e	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431f	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431g	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	
1	202431h	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431i	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
1	202431j	No. Hawaii	Puu Oumi NARS, 5000ft	x	x	x	x	x	x
2	201314a	So. Hawaii	HAVO, Olaa Tract, Pole 44	x	x	x	x	x	x
2	201314b	So. Hawaii	HAVO, Olaa Tract, Pole 45	x	x	x	x	x	x
2	201314c	So. Hawaii	HAVO, Olaa Tract, Pole 46	x	x	x	x	x	x
3	201318a	So. Hawaii	Puu Makaala Trailhead, off Stainback Highway	x	x	x	x	x	x
3	201318b	So. Hawaii	Puu Makaala Trailhead, off Stainback Highway	x	x	x	x	x	x
3	201318c	So. Hawaii	Puu Makaala Trailhead, off Stainback Highway	x	x	x	x	x	x
4	202523a	So. Hawaii	Kau Forest Reserve Hionomoa Strm	x	x	x	x	x	x
4	202523b	So. Hawaii	Kau Forest Reserve Hionomoa Strm	x	x	x		x	x
4	202527a	So. Hawaii	Kau Forest Reserve Hionomoa Strm	x	x	x	x	x	x
4	202517a	So. Hawaii	Kau Forest Reserve N19°10.269, W155°35.804	x	x	x		x	x
4	202517b	So. Hawaii	Kau Forest Reserve N19°10.269, W155°35.805	x	x	x	x	x	x
5	202528a	So. Hawaii	South Kona Forest Reserve, Kukuiopae	x	x	x	x	x	x
6	m015106a	So. Hawaii	Saddle Rd, Kipuka 4900', N19.681°, W155.331°	x	x	x	x	x	x
6	m015106b	So. Hawaii	Saddle Rd, Kipuka 4900', N19.681°, W155.331°	x	x		x		x
6	m015106c	So. Hawaii	Saddle Rd, Kipuka 4900', N19.681°, W155.331°	x	x	x	x	x	x
6	m012303	So. Hawaii	Kaumana Trail 4900'	x	x	x	x	x	x
7	202526a	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526b	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526c	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526d	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526e	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526f	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526g	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526h	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526i	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
7	202526j	Ea. Maui	East Maui Irrigation, Haiku Uka, Heed Trail	x	x	x	x	x	x
8	202555a	Ea. Maui	Makawao Forest Reserve	x	x	x	x	x	x
8	202555b	Ea. Maui	Makawao Forest Reserve	x		x	x	x	x
9	202554a	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554b	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x

Table 1. Individuals sampled and gene coverage (continued)

#	Barcode	Population	Locality	ND2	COII	COI	Fz4	Pds5	Osi9
9	202554c	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554d	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554e	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554f	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554g	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554h	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554i	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
9	202554j	We. Maui	Puu Kukui, Puu Kukui Trail, 2900'-3700'	x	x	x	x	x	x
10	202424a	Molokai	makai of Kolekole Cabin, on 4WD road	x	x	x	x	x	x
10	202424b	Molokai	makai of Kolekole Cabin, on 4WD road	x	x	x	x	x	x
10	202424c	Molokai	makai of Kolekole Cabin, on 4WD road	x	x	x	x	x	x
11	202413a	Molokai	Kamakou Forest, stream before tunnel	x	x	x	x	x	x
10	202415a	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x		x
10	202415b	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x	x	x
10	202415c	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x	x	x
10	202415d	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x	x	x
11	201894a	Molokai	Kamakou Forest, stream before tunnel	x	x	x	x	x	x
10	201874a	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x	x	x
10	201874b	Molokai	Puu Kolekole, 3854 ft.	x	x	x	x	x	x
Outgroups			**Species**						
	202321a	Hawaii	*sordidapex*	x	x	x	x	x	x
	202343d	Hawaii	*percnosoma*	x	x	x	x	x	x

Table 2. Locus details and diversity

Gene	locus	Primers	N	BP	Model	H[1]	Hd[2]	θs	θπ
COI	mt	5'-CAACATTTATTTTGATTTTTTGG-3' 5'-TYCATTGCACTAATCTGCCATATTAG-3'	58	829	HKY+I+Γ	34	0.93	11.8816	8.1222
COII	mt	5'-ATGGCAGATTAGTGCAATGG-3' 5'-GTTTAAGAGACCAGTACTTG-3'	58	750	GTR+I	30	0.9	9.50527	4.05808
ND2	mt	5'-AGCTATTGGGTTCAGACCCC-3' 5'-GAAGTTTGGTTTAAACCTCC-3'	59	520	HKY+I	22	0.859	5.81113	2.86382
fz4	X	5'-GCGTCTTTCTATTGCGCTACTAT-3' 5'-GCTTGTACGGACTGCTGATTATT-3'	57	649	HKY	15	0.829	3.25276	4.16416
osi9	auto	5'-AGCAGCGGCATCAGRTACTT-3' 5'-CCCAAGGACTCCATACAGGA-3'	116	379	HKY+I	23	0.772	3.75482	1.32729
pds5	auto	5'-GGATACTTTGTGGACAATTCAGAGT-3' 5'-AGATATTTCACGAACTCTTCAGCAC-3'	114	473	GTR+I	17	0.748	2.2603	1.06862

Table 3. Pairwise F_{ST} within and between island populations

	Hawaii-Maui	Maui-Molokai	Hawaii-Molokai	North Hawaii-South Hawaii	East Maui-West Maui
mtdna	0.237*	0.317*	0.333*	0.215*	0.672*
fz4	0.004	0.129*	0.183*	0.616*	0.437*
osi9	0.060*	0.109*	0.028*	0.024	0.03
pds5	0.043*	0.049*	0.095*	0.061*	0.111*

Table 4. Analysis of Molecular Variance results partitioned by gene

Locus	Source of Variation	d.f.	Sum of Squares	Variance Components	% of variation	Fixation Indices	*P*
mtDNA	Total	54	385.073	7.837			
	Among Islands	2	90.482	0.313	3.990	Φ_{CT} = 0.040	0.253
	Among populations within Islands	2	78.545	3.204	40.880	Φ_{SC} = 0.426	**<0.001**
	Within populations	50	216.045	4.321	55.130	Φ_{ST} = 0.449	**<0.001**
fz4	Total	56	116.596	2.105			
	Among Islands	2	10.331	-0.805	-38.240	Φ_{CT} = -0.382	0.789
	Among populations within Islands	2	39.127	1.619	76.900	Φ_{SC} = 0.556	**<0.001**
	Within populations	52	67.138	1.291	61.340	Φ_{ST} = 0.387	**<0.001**
osi9	Total	115	76.319	0.680			
	Among Islands	2	4.644	0.031	4.550	Φ_{CT} = 0.046	0.188
	Among populations within Islands	2	2.318	0.024	3.510	Φ_{SC} = 0.038	0.067
	Within populations	111	69.356	0.625	91.940	Φ_{ST} = 0.081	**<0.001**
pds5	Total	113	60.337	0.544			
	Among Islands	2	3.199	-0.002	-0.440	Φ_{CT} = -0.004	0.599
	Among populations within Islands	2	3.428	0.053	9.800	Φ_{SC} = 0.098	**<0.001**
	Within populations	109	53.750	0.493	90.640	Φ_{ST} = 0.094	**<0.001**

Table 5. Measures of effective population size and change in island populations

	Tajima's D	Fu's F_S	SSD	Raggedness	$\theta\pi$
mtDNA					
Hawaii	-0.968	-6.652*	0.013	0.005	18.74892
Maui	-1.018	-4.003	0.020	0.019	7.57576
Molokai	-1.947*	-10.035*	0.027	0.113	2.87273
fz4					
Hawaii	-0.781	-0.361	0.009	0.021	4.83333
Maui	-0.059	0.157	0.028	0.041	3.82251
Molokai	0.117	-2.707*	0.011	0.011	2.10909
osi9					
Hawaii	-1.709*	-8.983*	0.003	0.072	1.13143
Maui	-0.704	-6.125*	0.006	0.082	1.7463
Molokai	-1.650*	-3.720*	0.000	0.099	0.61039
pds5					
Hawaii	-1.440	-5.018*	0.018	0.150*	0.84327
Maui	-0.414	-6.536*	0.017	0.128*	1.36364
Molokai	-1.947*	-1.837	0.039	0.247*	0.75789

Table 6. Directional migration rates between islands partitioned by nuclear and mitochondrial loci

	$M_{HI>MI}$	$M_{MI>HI}$	$M_{HI>MO}$	$M_{MO>HI}$	$M_{MI>MO}$	$M_{MO>MI}$	$M_{HI>MN}$	$M_{MN>HI}$
mt	11.87	5.405	5.685	1.515	29.98	9.995	0.045	0.005
nu	17.98	9.995	1.325	0.355	8.085	0.065	6.905	0.055

Figures

Figure 1. Map of *D. waddingtoni* collection sites. Refer to Table 1 for more details.

Figure 2. Combined mitochondrial genealogy for populations of *D. waddingtoni*. Asterisks indicate posterior probability $\geq$ 0.90 and likelihood bootstraps $\geq$ 70.

Figure 3. Fz4 genealogy for populations of *D. waddingtoni*. Asterisks indicate posterior probability $\geq$ 0.90 and likelihood bootstraps $\geq$ 70.

Figure 4. Osi9 genealogy for populations of *D. waddingtoni*. Asterisks indicate posterior probability $\geq$ 0.90 and likelihood bootstraps $\geq$ 70.

Figure 5. Pds5 genealogy for populations of *D. waddingtoni*. Asterisks indicate posterior probability $\geq$ 0.90 and likelihood bootstraps $\geq$ 70.

Figure 6. Concatenated partitioned phylogeny for populations of *D. waddingtoni*. Asterisks indicate posterior probability $\geq$ 0.90 and likelihood bootstraps $\geq$ 70.

Figure 7. *BEAST phylogeny of *D. waddingtoni*. Values at nodes indicate posterior probability.

Figure 8. IMa2 demographic estimates for island populations of *D. waddingtoni*. Distributions of posterior probabilities of time (scaled to time in millions of years), migration (2Mm) and q (4Nem). Arrow points towards directionality of gene flow. HI = Hawaii, MI = Maui, MO = Molokai, MN = Maui Nui.

Figure 9. Mismatch distributions for Hawaii. Bars indicate number of observed differences, Line is expected distribution under a model of expanding population size.

Figure 10. Mismatch distributions for Maui. Bars indicate number of observed differences, Line is expected distribution under a model of expanding population size.

Figure 11. Mismatch distributions for Molokai. Bars indicate number of observed differences, Line is expected distribution under a model of expanding population size.

Figure 12. Bayesian Skyline Plot detailing population size changes for each island. 95% posterior density of population size estimates indicated by dashed line.

Figure 1. Map of *D. waddingtoni* collection sites

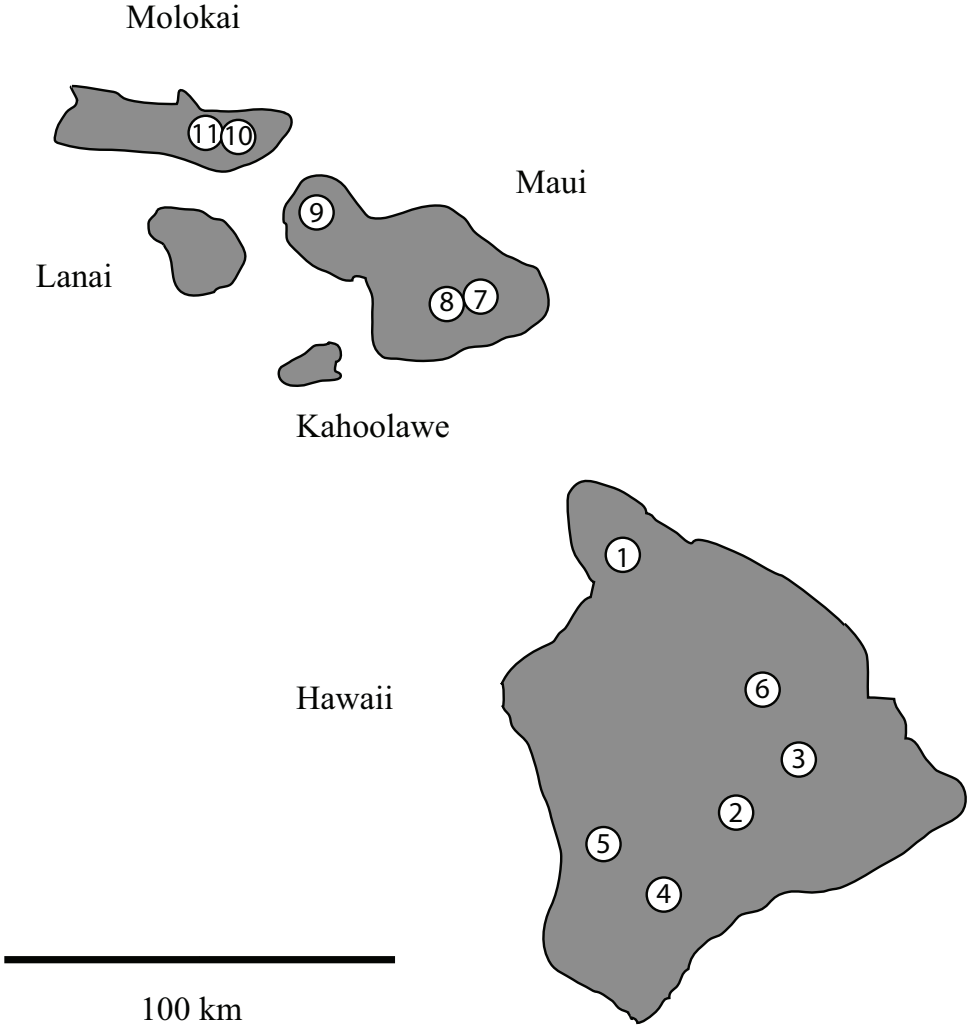


Figure 2. Combined mitochondrial genealogy for populations of *D. waddingtoni*

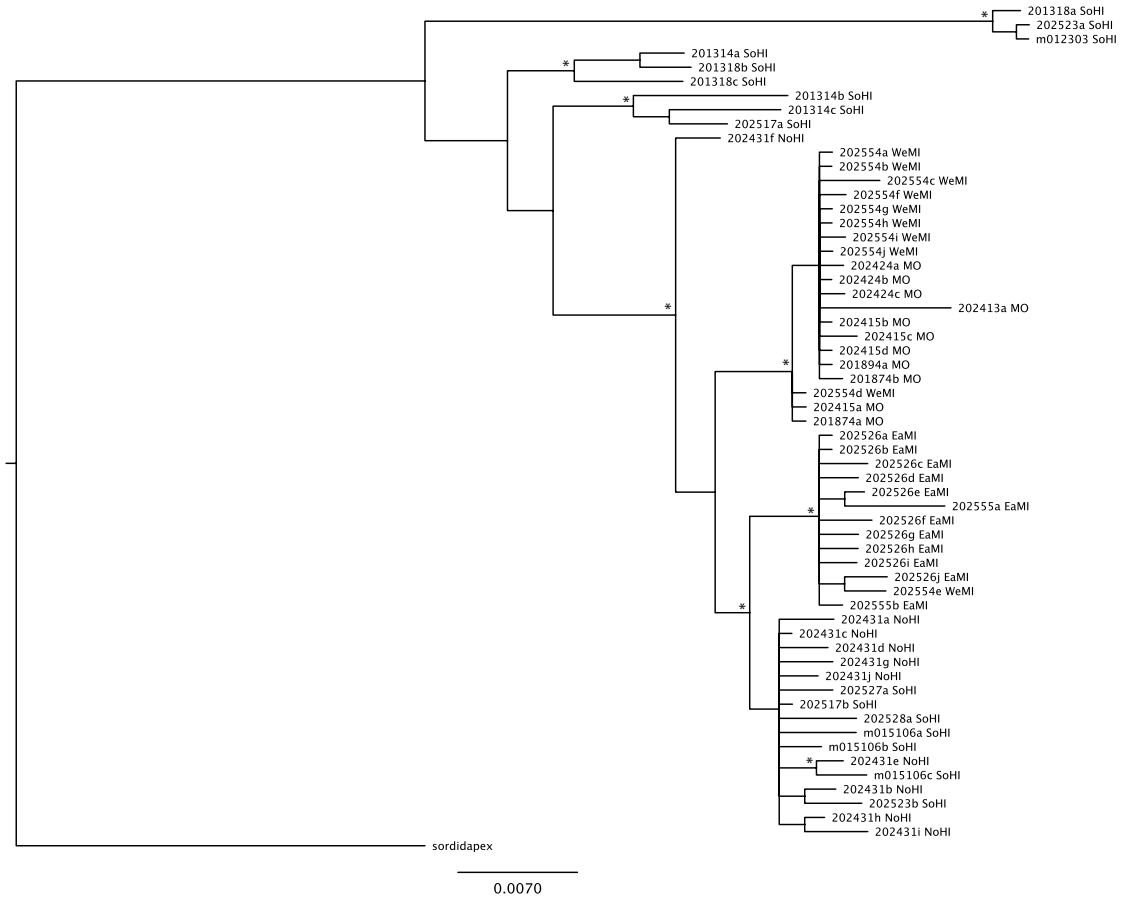


Figure 3. Fz4 genealogy for populations of *D. waddingtoni*

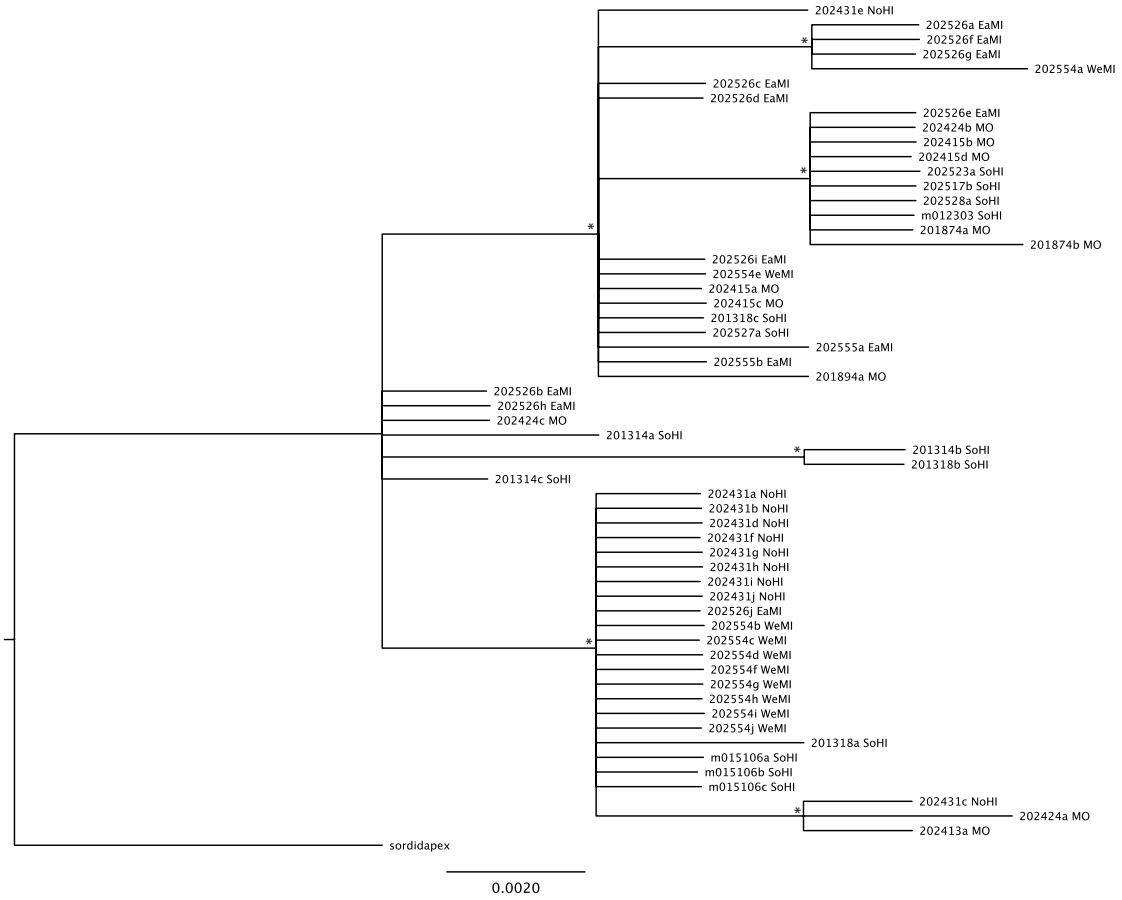


Figure 4. Osi9 genealogy for populations of *D. waddingtoni*

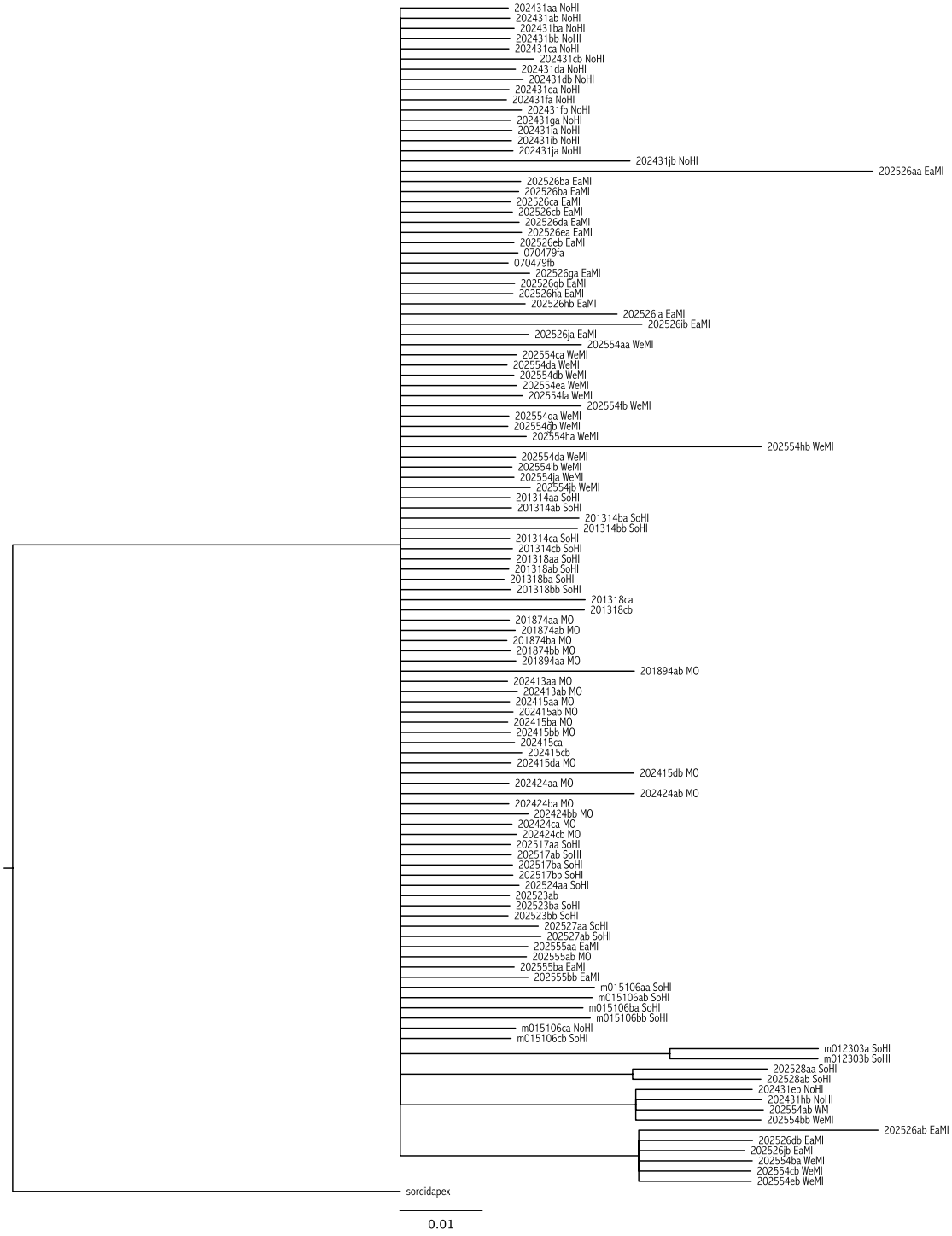


Figure 5. Pds5 genealogy for populations of *D. waddingtoni*

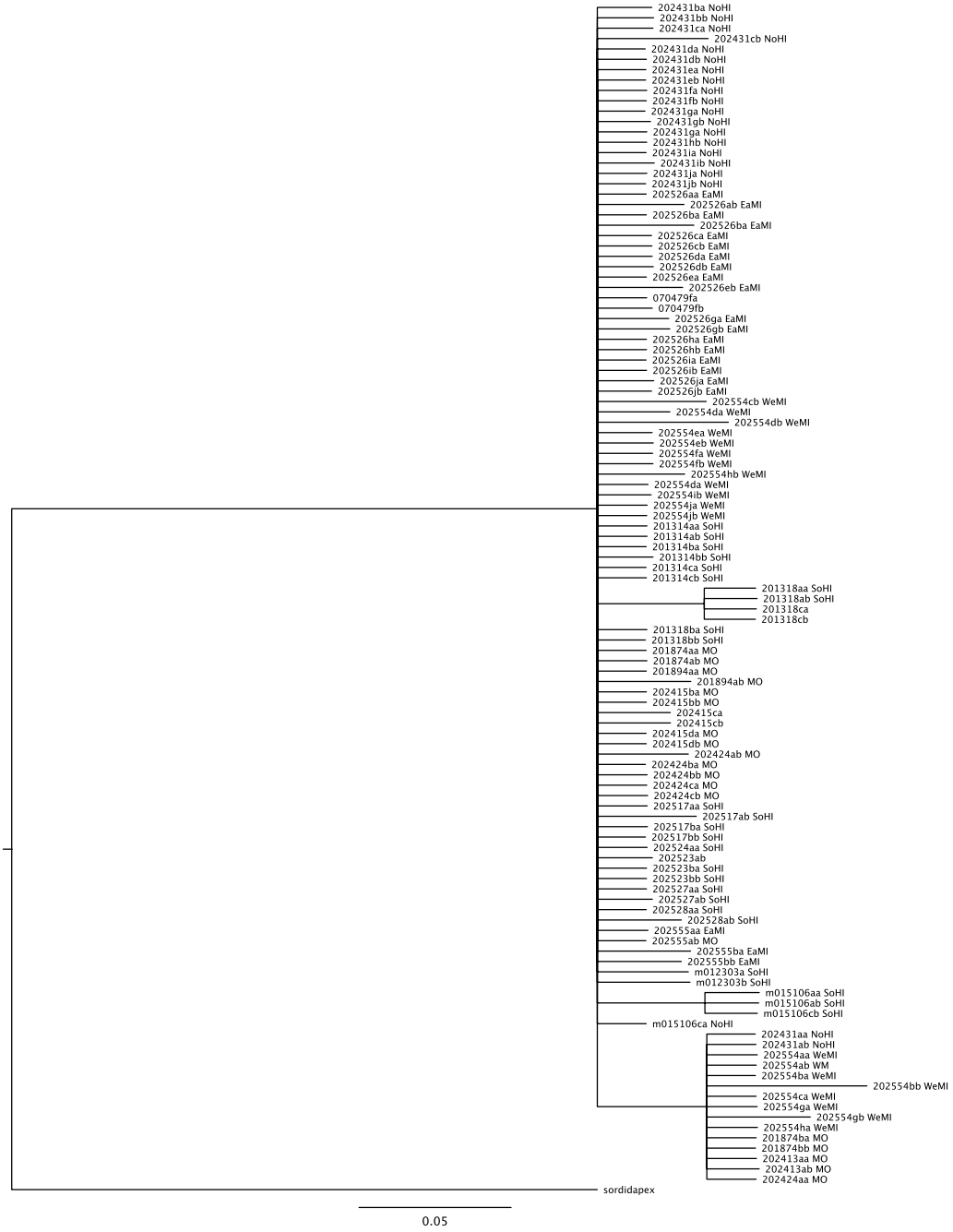


Figure 6. Concatenated partitioned phylogeny for populations of *D. waddingtoni*

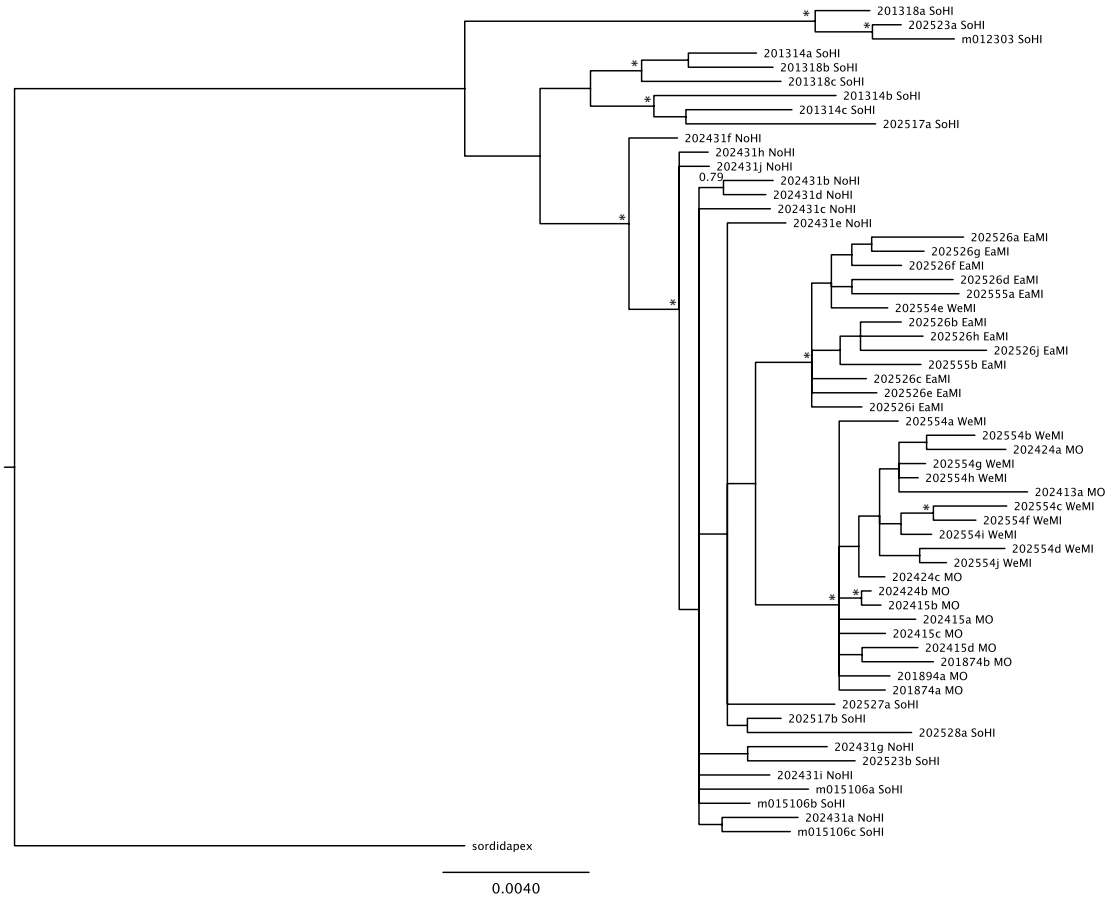


Figure 7. *BEAST phylogeny of *D. waddingtoni*

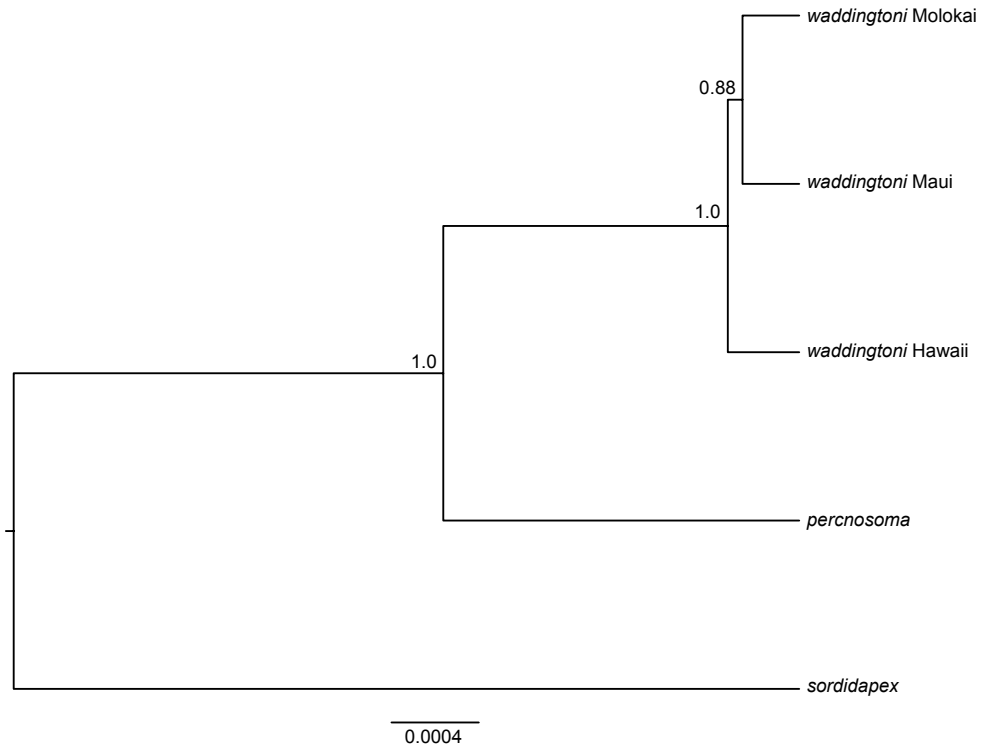


Figure 8. IMa2 demographic estimates for island populations of *D. waddingtoni*

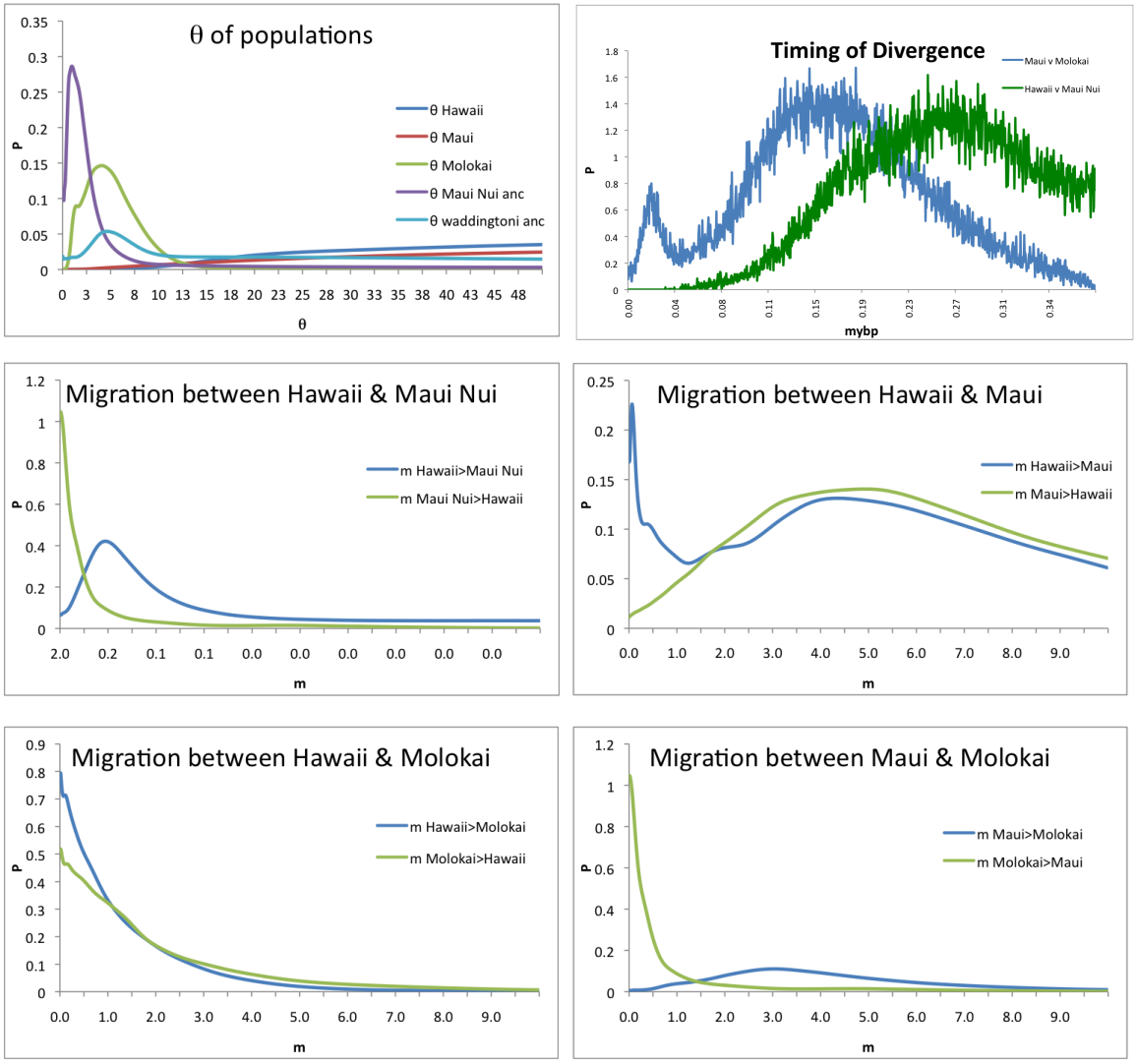


Figure 9. Mismatch distributions for Hawaii

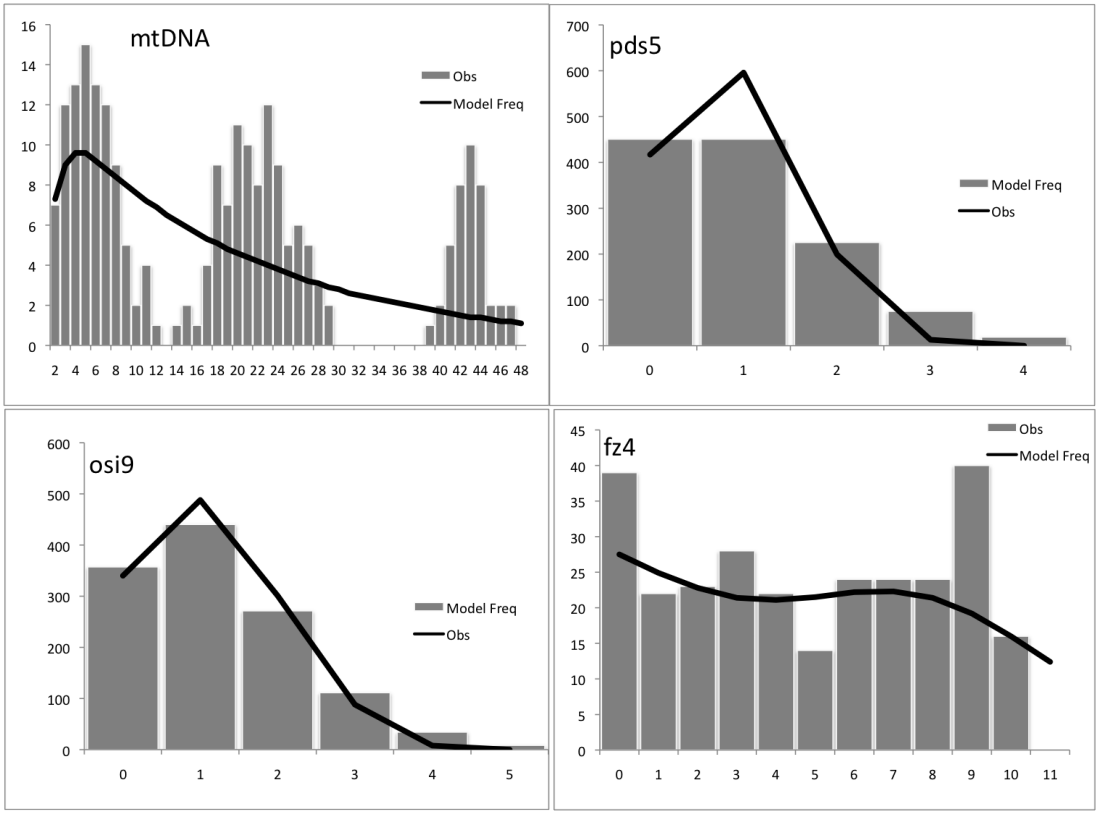


Figure 10. Mismatch distributions for Maui

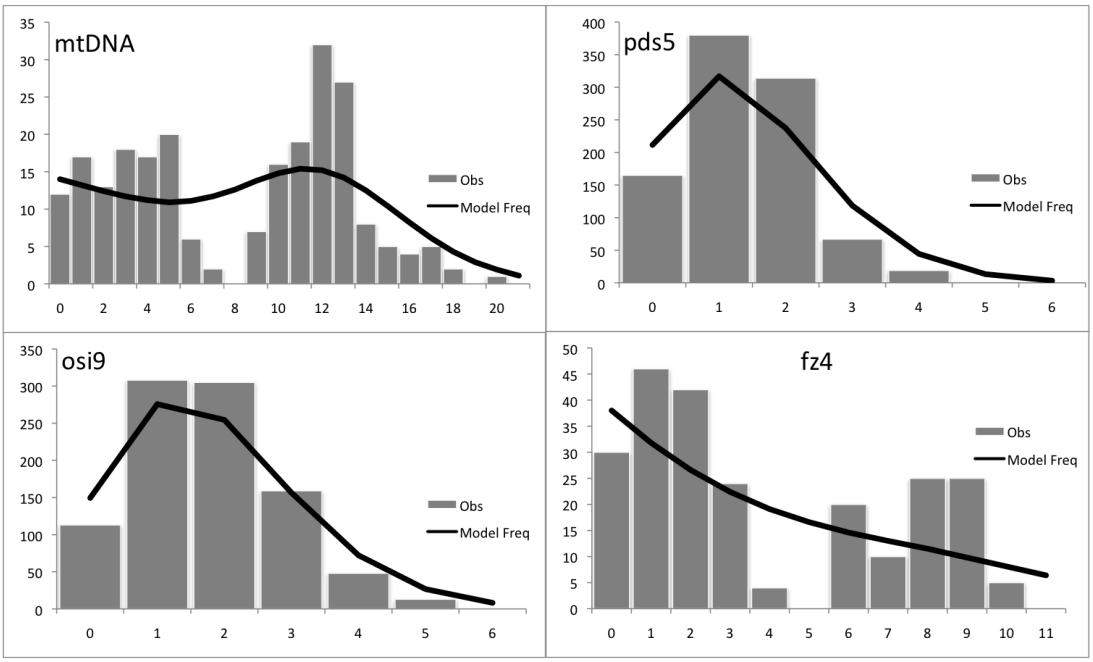
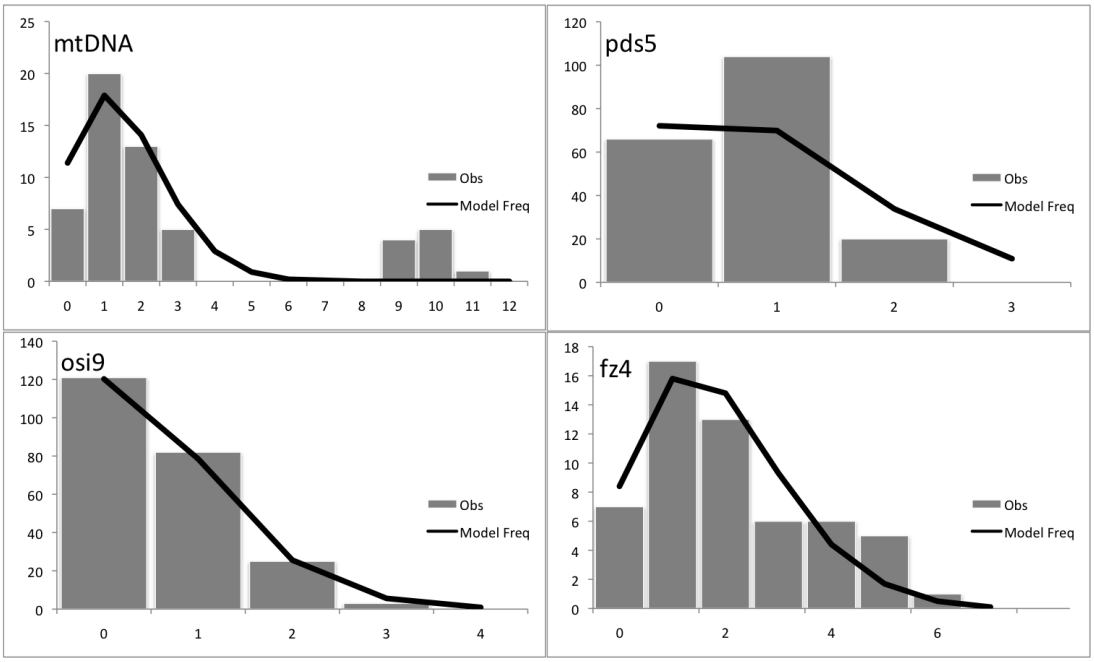


Figure 11. Mismatch distributions for Molokai



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