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Journal
Molecular Phylogenetics and Evolution, 69(1)

ISSN
1055-7903

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Publication Date
2013-10-01

DOI
10.1016/j.ympev.2013.04.032

Peer reviewed

Molecular Phylogenetics and Evolution 69 (2013) 95–108

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Diversification and dispersal of the Hawaiian Drosophilidae: The evolution of *Scaptomyza*

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ARTICLE INFO

Article history:
Received 8 October 2012
Revised 21 March 2013
Accepted 19 April 2013
Available online 10 May 2013

Keywords:
Scaptomyza
Hawaiian Drosophilidae
Dispersal
Hawaii
Leaf-mining

ABSTRACT

The genus *Scaptomyza* is emerging as a model lineage in which to study biogeography and ecological adaptation. To place future research on these species into an evolutionary framework we present the most comprehensive phylogeny of *Scaptomyza* to date, based on 5042 bp of DNA sequence data and representatives from 13 of 21 subgenera. We infer strong support for the monophyly of almost all subgenera with exceptions corroborating hypotheses of conflict inferred from previous taxonomic studies. We find evidence that the lineage originated in the Hawaiian Islands and subsequently dispersed to the mainland and other remote oceanic islands. We also identify that many of the unique ecological niches exploited by this lineage (e.g., herbivory, spider predation) arose singly and independently.

1. Introduction

1.1. Dispersal in island-adapted species

Colonists that become established on oceanic islands can adapt to a wide variety of ecological niches, evolve a range of phenotypes, and diversify into radiations comprising hundreds of species (Baldwin and Sanderson, 1998; Grant, 1999; Shaw, 2002; Jordan et al., 2003; Lerner et al., 2011; O'Grady et al., 2011). However, oceanic islands have historically been considered a "dead end" for diversity (Wilson, 1961; Mayr and Diamond, 2001). Island-adapted taxa are poor dispersers (Darwin, 1859) and as an island erodes and is submerged many resident lineages go extinct. Furthermore, island endemics are considered too naïve to compete with continental species (Cox, 1999), and in many lineages energetically expensive defensive structures (e.g. Hawaiian thornless raspberries) and compounds (e.g. Hawaiian "mintless" mints) are abandoned in the absence of predators (Carlquist, 1974). However, new evidence suggests that island endemics are capable of escaping islands, colonizing continents and other remote islands, and diversifying (Heaney, 2007; Bellemain and Ricklefs, 2008).

The Hawaiian Archipelago is one of the most remote oceanic island chains in the world. Its isolation, located 3200 km from the nearest landmass or archipelago, has led to its characteristically disharmonic and diverse set of biota (Simberloff and Wilson, 1969; Gillespie and Roderick, 2002; Price and Clague, 2002; Cowie and Holland, 2008). Examples of emigrations of lineages that arose in the Hawaiian Islands are emerging from species groups as diverse as sandalwoods, birds and snails (Filardi and Moyle, 2005; Harbaugh and Baldwin, 2007; Rundell and Price, 2009). Another example of this dispersal out of Hawaii is found within the hyper-diverse Hawaiian Drosophilidae (Diptera), a clade that includes two monophyletic groups, the endemic Hawaiian *Drosophila* and its cosmopolitan sister genus *Scaptomyza*. The common ancestor of both the Hawaiian *Drosophila* and *Scaptomyza* lineages colonized the archipelago ca. 25 million years ago and has subsequently diversified into a lineage of an estimated 1000 species (Throckmorton, 1966; Grimaldi, 1990; Russo et al., 1995; Remsen and DeSalle, 1998; Da Lage et al., 2007; O'Grady and DeSalle, 2008; Van der Linde et al., 2010). The present cosmopolitan distribution of *Scaptomyza* is hypothesized to be the result of an ancient dispersal event out of Hawaii (O'Grady and DeSalle, 2008). The majority of the 272 (80%) described *Scaptomyza* species occur on remote oceanic islands including the Hawaiian Islands, the Marquesas, Tristan da Cunha, Ogasarawa Islands, St. Helena Islands, and Juan Fernandez Islands. The remaining 55 species are found on all continents except Antarctica (Evenhuis and Samuelson, 2007; O'Grady et al., 2010). Most drosophilid lineages of similar age are more restricted in their distributions (Russo et al., 1995; Tamura et al., 2004; Morales-Hojas and Vieira, 2012) and lineages like the *immigrans* species group that have dispersed to a similar degree are less speciose (Markow and O'Grady, 2006). The relatively recent origin of the Hawaiian Drosophilidae clade

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http://dx.doi.org/10.1016/j.ympev.2013.04.032

(Hawiian *Drosophila* + *Scaptomyza*), ca. 23–35 million years ago (mya) (Russo et al., 1995; Tamura et al., 2004; Morales-Hojas and Vieira, 2012) provides a temporal interval during which *Scaptomyza* arose and dispersed around the world. The ability of *Scaptomyza* species to disperse large distances may be explained by their derived physiological characteristics. O'Grady and DeSalle (2008) analyzed potential characters that could have predisposed *Scaptomyza* species to be dispersers, allowing them to exist in marginal habitats and to traverse long geographic distances. They found that *Scaptomyza* species are generally smaller and develop more rapidly than other species of Hawaiian Drosophilidae, facilitating the use of ephemeral breeding and feeding substrates.

Dispersal between the Hawaiian Islands may also be a factor in diversification in the Hawaiian *Scaptomyza*, as is seen in other Hawaiian Drosophilidae lineages (Bonacum et al., 2005; Carson, 1997). The Hawaiian Emperor Seamount chain is a volcanic archipelago that has existed for over 80 million years and extends approximately 5500 km across the Pacific Ocean. Islands form in the southeast over a volcanic hotspot near the current position of the island of Hawaii, then migrate northwest on the Pacific plate, erosive forces cause the islands to eventually subside. Many Hawaiian lineages follow the progression rule (Hennig, 1966) where basally branching lineages are found on older islands and more recently derived species are present on younger islands. As new islands form, taxa from older neighboring islands can colonize them, leading to clades that have diversified "down" the chain (e.g. Jordan et al., 2003; Pons and Gillespie, 2004; Rubinoff, 2008). Here, we test the hypothesis that the progression rule can explain the biogeographic history of the Hawaiian endemic *Scaptomyza*.

1.2. Scaptomyza larval ecology

The majority of drosophilid species, including many members of the genus *Scaptomyza*, have saprophagous larvae that feed on yeasts, other fungi, and bacteria living on decomposing plant material (Markow and O'Grady, 2008). In addition to this general saprophagous habit, larvae of some *Scaptomyza* species utilize a variety of substrates not found in other *Drosophila* clades (Markow and O'Grady, 2008). For example larvae of some *Scaptomyza* species are animal predators. Larvae of species in the subgenus *Titanochaeta* were reared from thomisid spider egg sacs (Hardy, 1965), and some species in the subgenus *Elmomyza* feed on insects (Magnacca et al., 2008). The subgenus *Scaptomyza* includes species whose larvae are leaf-miners of mustards (Brassicaceae) and other plant families (Hackman, 1959; Wheeler and Takada, 1966; Maca, 1972; Brncic, 1983; Whiteman et al., 2011), including the genetic reference plant, *Arabidopsis thaliana* (Chittenden, 1902). Species in the subgenera *Hemiscaptomyza* and *Dentiscaptomyza* are also known to associate with mustards, but whether they actively mine the leaf or not is unclear (Wheeler and Takada, 1966; Brncic, 1983). Larvae of species in the subgenus *Exalloscaptomyza* specialize on the microflora living on the corollas of Hawaiian *Ipomoea* spp. flowers (Montague, 1984). These larval ecologies have never been examined in a phylogenetic context.

1.3. Scaptomyza taxonomy and systematics

Hardy erected the genus *Scaptomyza* in 1849 for the type species *S. graminum* Fallen. *Scaptomyza* has a complex taxonomic history, characterized by various shifts in status from genus to subgenus and species transfers from one subgenus to another. Species delimitation in *Scaptomyza* has also been difficult, because of the wide distributions and similar morphology of many taxa. For example, the type series of *S. graminum* contains both *S. pallida* and *S. graminum* specimens (Hackman, 1959). Dissections of male genitalia are required to reliably identify many closely related species in this genus. *Scaptomyza* is currently divided into 21 subgenera, several of which (e.g., *Bunostoma*, *Celidosoma*, *Grimshawomyia*, *Titanochaeta*) were originally described as distinct genera (Malloch, 1932; Throckmorton, 1966), but have since been synonymized with *Scaptomyza* due to other morphological characters and molecular evidence (Hackman, 1959; O'Grady et al., 2003).

Researchers have previously examined the phylogenetic relationships within *Scaptomyza* to place these species into a taxonomic framework. Okada (Okada, 1973) used a phenetic algorithm to propose relationships within the genus *Scaptomyza*. His analysis suggested that all Hawaiian subgenera, with the exception of *Exalloscaptomyza*, were monophyletic and divided into three major lineages (Fig. 1A). This analysis agreed with previously posited taxonomic hypotheses (Throckmorton, 1966). However, neither analysis employed an outgroup and therefore the

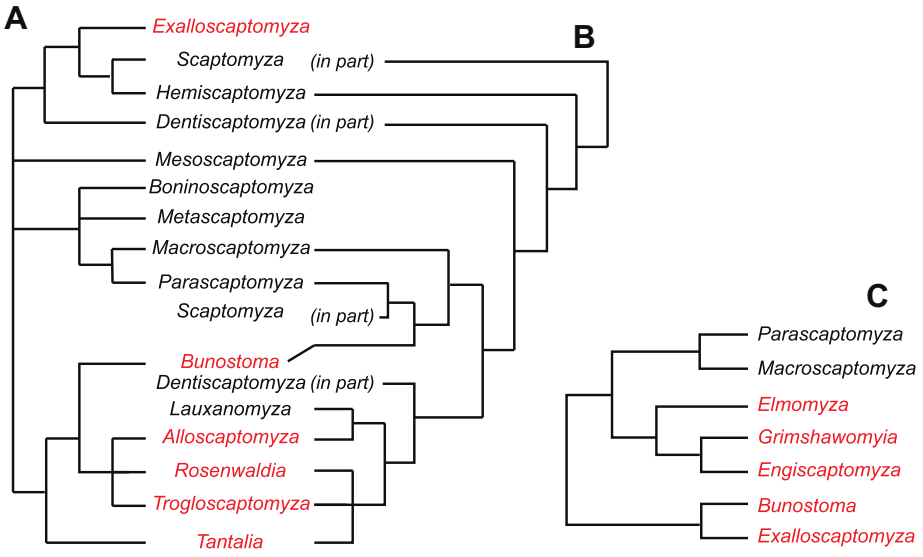

Fig. 1. Previous phylogenetic hypotheses. Subgenera in red include species endemic to the Hawaiian Islands. Relationships within the genus *Scaptomyza* based on A. Okada's phenetic analysis (Okada, 1973), B. Grimaldi's cladistic analysis (Grimaldi, 1990) C. O'Grady and DeSalle's (2008) molecular phylogenetic analysis.

Table 1
Taxon and character sampling. "Spnr" refers to taxa that are not identified to a described species, but morphologically similar to the described species following spnr. Accession numbers in italics are for sequences new to this paper.

Species	Barcode[a]	*co1*	*co2*	*16s*	*nd2*	*cad-r*	*marf*	*n(l)tid*	*gstd1*
S. abrupta	O202704	*KC609723*	*KC609678*	*KC609618*		*KC609590*	*KC609521*		
S. akalae	O201528	HQ170854	HQ170736				*KC609487*	*KC609541*	
S. albovittata	O202712	*KC609725*	*KC609680*			*KC609592*	*KC609523*		
S. ampliloba	O202690	*KC609721*	*KC609676*	*KC609616*	*KC609640*	*KC609588*	*KC609519*	*KC609557*	
S. apicata	L10003	JX160024	JX160028	*KC609623*	*KC609646*	*JX160020*	*JX160038*	*KC609561*	*KC609534*
S. apiciguttula	O202579	*KC609693*	*KC609650*	*KC609601*	*KC609631*		*KC609492*		
S. bryani	O202725	*KC609726*	*KC609681*	*KC609620*			*KC609524*	*KC609558*	*KC609531*
S. buccata	O202560	*KC609690*		*KC609600*	*KC609630*		*KC609488*		
S. caliginosa	O202698	EU493676	EU493805			*KC609589*	*KC609520*		*KC609530*
S. chauliodon	JB	*KC609595*	*KC609684*						
S. connata	O202618	*KC609701*	*KC609656*				*KC609497*	*KC609545*	
S. crassifemur	O202688	EU493677	U94211	*KC609614*	EU493547		*KC609517*	*KC609556*	
S. ctenophora	O202658	*KC609715*	*KC609670*			*KC609581*	*KC609511*		
S. cuspidata	O202656	*KC609714*	*KC609669*	*KC609610*		*KC609580*	*KC609510*		
S. cyrtandrae	O202583	*KC609694*	HQ170737		HQ170936		*KC609493*	*KC609542*	*KC609529*
S. decepta	O202648	*KC609712*	*KC609667*		*KC609638*	*KC609578*	*KC609508*	*KC609551*	
S. dentata	O202625	*KC609703*	*KC609658*	*KC609607*			*KC609499*		
S. devexa	O202663	*KC609716*	*KC609671*			*KC609582*	*KC609512*		
S. elmoi	hdk22A107	HQ170853	HQ170735						
S. exigua	O202592	*KC609697*	*KC609652*	*KC609603*	*KC609634*		*KC609495*	*KC609543*	
S. fastigata	O202631	*KC609706*	*KC609661*			*KC609573*	*KC609502*		
S. flava NZ	O201322	HQ170855	HQ170738	*KC609599*			*KC609486*		*KC609527*
S. flava NA	L10001	JX160022	JX160026	*KC609621*	*KC609644*	JX160018	JX160036	*KC609559*	*KC609532*
S. frustulifera	O108518	EU493679	EU493808						
S. hackmani	O202635	*KC609708*	*KC609663*			*KC609575*	*KC609504*		
S. hamata	JB	*KC609596*	*KC609685*						
S. hsui	L10053	*KC609729*	*KC609687*	*KC609626*		*KC609594*	*KC609480*	*KC609565*	
S. intricata	O202638	*KC609709*	*KC609664*		*KC609637*	*KC609576*	*KC609505*	*KC609548*	
S. latitergum	O202640	*KC609710*	*KC609665*				*KC609506*	*KC609549*	
S. lobifera	O202596		*KC609654*	*KC609604*				*KC609544*	
S. longisetosa	O201057	HQ170856	HQ170739			*KC609567*	*KC609482*	*KC609538*	
S. magnipalpa	O201056	HQ170857	HQ170740			*KC609566*	*KC609481*	*KC609537*	
S. nasalis	O202692	*KC609722*	*KC609677*	*KC609617*	*KC609641*				
S. neocyrtandrae	O201368	*KC609689*	HQ170741					*KC609540*	
S. neosilvicola	O201061	HQ170858	HQ170742		*KC609629*	*KC609569*	*KC609485*		
S. nigrita	L10004	JX160025	JX160029	*KC609624*	*KC609647*	JX160021	JX160039	*KC609562*	*KC609535*
S. palata	JB		*KC609686*						
S. pallida	L10002	JX160023	JX160027	*KC609622*	*KC609645*	JX160019	JX160037	*KC609560*	*KC609533*
S. pallifrons	O202651	*KC609597*	*KC609668*			*KC609579*	*KC609509*	*KC609552*	
S. palmae	O202573	EU493680	EU493809			*KC609571*	*KC609490*		*KC609528*
S. protensa	O202622	*KC609702*	*KC609657*	*KC609606*			*KC609498*		
S. pusilla	O202601	*KC609699*	*KC609655*	*KC609605*			*KC609496*		
S. recava	O202589	*KC609696*			*KC609633*				
S. recta	O202667	*KC609717*	*KC609672*	*KC609611*	*KC609639*	*KC609583*	*KC609513*	*KC609553*	
S. reducta	O202686	*KC609719*	*KC609674*	*KC609613*		*KC609586*	*KC609516*	*KC609555*	
S. remota	O108519		EU493804						
S. scoloplichas	O202593	*KC609698*	*KC609653*						
S. setosiscutellum	O202726	*KC609727*	*KC609682*		*KC609643*	*KC609593*	*KC609525*		
S. spnr anomala	O202575	*KC609692*	*KC609649*			*KC609572*	*KC609491*		
S. spnr cuspidata	O202671	*KC609718*	*KC609673*			*KC609584*	*KC609514*		
S. spnr decepta	O202634	*KC609707*	*KC609662*			*KC609574*	*KC609503*		
S. spnr inflatus	O202689	*KC609720*	*KC609675*	*KC609615*		*KC609587*	*KC609518*		
S. spnr longipecten	O202642	*KC609711*	*KC609666*	*KC609609*		*KC609577*	*KC609507*	*KC609550*	
S. spnr longisetosa	O202571	*KC609691*	*KC609648*			*KC609570*	*KC609489*		
S. spnr mitchelli	O202705	*KC609724*	*KC609679*	*KC609619*	*KC609642*	*KC609591*	*KC609522*		
S. trivittata	O202629	*KC609704*	*KC609659*		*KC609635*		*KC609500*	*KC609546*	
S. tumidula	O201059	KC609688	HQ170743	HQ171050			*KC609483*		
S. umbrosa	O202584	*KC609695*	*KC609651*	*KC609602*	*KC609632*		*KC609494*		
S. undulata	O202684	EU493681	EU493810	EU494407		*KC609585*	*KC609515*	*KC609554*	
S. vagabunda	O202630	*KC609705*	*KC609660*	*KC609608*	*KC609636*		*KC609501*	*KC609547*	
S. varia	O202607	*KC609700*							
S. varifrons	O201058		HQ170744	*KC609598*	*KC609627*				
S. varipicta	O201060	HQ170860	HQ170745	HQ171051	*KC609628*	*KC609568*	*KC609484*	*KC609539*	*KC609526*
D. ancyla	O201019	HQ170749	HQ170632	HQ170952	HQ170861				
D. dissita	O201323	HQ170763	HQ170649	HQ170964	HQ170876				
D. grimshawi	FLYBASE	FLYBASE	FLYBASE	FLYBASE	FLYBASE	FLYBASE	FLYBASE	FLYBASE	FLYBASE
D. malele	O201047	HQ170776	HQ170668	HQ170978	HQ170891				
D. melanocephala	O201799	HQ170778	HQ170670	HQ170980					
D. melanoloma	O105708	EU493662	EU493791	EU494391	EU493536				
D. mimica	O205066	HQ170780	HQ170672	HQ170982	HQ170950				

(*continued on next page*)

Table 1 (*continued*)

Species	Barcode[a]	*co1*	*co2*	*16s*	*nd2*	*cad-r*	*marf*	*n(l)tid*	*gstd1*
D. mitchelli	O202312	HQ170781	HQ170673	HQ170983	HQ170894				
D. nigra	O105821	EU494394	EU493796	EU494394	EU493540				
D. ochropleura	O202558	*KC609728*	*KC609683*	*KC609625*				*KC609564*	*KC609536*
D. percnosoma	O200125	HQ170819	HQ170715	HQ171022	HQ170929				
D. tanythrix	O202520	HQ170828	HQ170726	HQ171031				*KC609563*	

[a] Detailed information on specimens can be found by contacting the authors with the reference barcodes. Hd: Hawaiian *Drosophila* project. O: P.M. O'Grady. L: R.T. Lapoint.

monophyly of the genus *Scaptomyza* was not tested. Grimaldi's (Grimaldi, 1990) morphological study was an improvement over the previous phenetic work because it tested the monophyly of the genus *Scaptomyza* and it's subgenera through more extensive sampling of outgroups and multiple members of subgenera (Fig. 1B). There are two areas of concordance between these morphological studies: both suggest (a) close relationships among the Hawaiian subgenera *Alloscaptomyza*, *Tantalia*, *Rosenwaldia*, and *Elmomyza* and (b) that *Macroscaptomyza* and *Parascaptomyza* are members of the same larger clade. The most recent treatment of this group, based on phylogenetic analyses of molecular characters (O'Grady and DeSalle, 2008), provides evidence that this lineage arose in the Hawaiian Islands, but the paucity of mainland taxa sampled in that study indicates that a more complete sampling of *Scaptomyza* diversity would better test monophyly and relationships among the subgenera (Fig. 1C).

1.4. Objectives

The genus *Scaptomyza* is a diverse lineage that is becoming a useful model system for studies of biogeography and ecological diversification. Here, we present the most comprehensive phylogeny of the genus *Scaptomyza* to date, employing more than twice as many subgenera and molecular characters than any previous molecular analysis to test the relationships within and between the subgenera of *Scaptomyza*. We use this phylogeny to test hypotheses of how and when *Scaptomyza* dispersed globally, and explore when and how often various larval ecologies evolved.

2. Materials and methods

2.1. Specimen collection and vouchers

We included 63 *Scaptomyza* taxa in this analysis, including representatives of 13 of the 21 subgenera. These species span a large portion of the ecological variation and known distribution of the genus. Twelve Hawaiian *Drosophila* species, including multiple taxa from the four major lineages (O'Grady et al., 2011), were used as outgroups. Species were collected via sweeping or reared from plants and immediately placed into 100% EtOH. Voucher specimens are stored at −80 °C in 100% EtOH at the University of Arizona and the University of California, Berkeley in the collections of NKW and PMO, respectively (Table 1).

2.2. DNA amplification and sequencing

DNA was extracted following standard Qiagen DNeasy blood and tissue kit protocol. We aligned the *Scaptomyza flava* transcriptome (Whiteman et al., 2012) with orthologous sequences from the completely sequenced genomes of the closely related *D. mojavensis*, *D. virilis* and *D. grimshawi* from Flybase (Tweedie et al., 2009). We then used the program primer3 (Rozen and Skaletsky, 2000), as implemented in Geneious 5.5.5, to design or modify PCR primers that would amplify partial fragments of four nuclear genes: *gstd1, cad-r, marf* and *n(l)tid*. These loci were included based on their use in other studies (O'Grady and Zilversmit, 2004; Gloss et al., in preparation). Four additional mitochondrial genes (*16s*, *co1*, *co2* and

Table 2
Details of loci used in this study. Tm is the annealing temperature. More than one temperature is listed for primers that were used in touchdown PCRs. #Ind refers to the number of individuals sequenced for that locus for this study. #Sbgn refers to the number of subgenera that had representatives sequenced for that locus in this study.

Gene	Primer	Tm	# Ind	# Sbgn	Chars(PIC)[d]
Nuclear					
n(l)tid[a]	ntidL-GGGYCGCATCTTTGAGCACAAATGG ntidR-TGCTGGGATAGGTGTTCCARCARTA	60C	26	9	618(71)
cad-r[a]	Cad787F-GGSAATACGACNGCCTGYTTTGARCC Cad1098R-TTNGGCAGCTGRCCNCCCAT	62C,58C,54C	36	12	849(208)
Marf[a]	MarfF1-ATGGCGGCCTAYTTGAAYCGCA MarfR1-AAGAAGGCGACCTTCATGTGRTC	62C,58C,54C	51	12	371(84)
gstd-1[b]	gstd1FB-TGTGCTYTTCTAATTATAG gstd1RA-GAATACWCTTTTATTWTAAG	38C	12	7	630(76)
Mitochondrial					
16s[c]	16sF-CCGGTTTGAACTCAGATCACGT 16sR-CGCCTGTTTAACAAAAACAT	56C	43	12	550(25)
co1[c]	2183-CAACATTTATTTTGATTTTTTGG 3041-TYCATTGCACTAATCTGCCATATTAG	56C	71	12	765(246)
co2[c]	3037-ATGGCAGATTAGTGCAATGG 3791-GTTTAAGAGACCAGTACTTG	56C	72	13	700(214)
nd2[c]	192-AGCTATTGGGTTCAGACCCC 732-GAAGTTTGGTTTAAACCTCC	56C	35	12	559 (102)

[a] Bonacum et al., 2001.
[b] Gloss et al., in preparation.
[c] Simon et al., 1994.
[d] Maximum sequence length. Number of parsimony informative characters for in group taxa are in parentheses.

nd2) were also PCR-amplified using universal mitochondrial primers (Simon et al., 1994). See Table 2 for relevant details for each gene.

PCR conditions included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of the following amplification sequence: (1) denature at 95 °C for 30 s, (2) anneal (between 52 and 64 °C depending on gene) for 30 s, and (3) extension at 72 °C for 1 min. A final round of extension at 72 °C was performed for 5 min. A touch down PCR was performed to obtain enough specific PCR product for *cad-r* and *marf*. In these cases, three rounds of amplification sequences were performed: (1) An initial high specificity sequence with a high annealing temperature round for 5 cycles, (2) a second sequence at the calculated annealing temperature for 10 cycles, and (3) a final low specificity sequence at a low annealing temperature at 20 cycles. PCR products were visualized on 1% agarose TAE gels and cleaned using Fermentas ExoSAP-it following manufacturers instructions. Cleaned PCR products were Sanger sequenced in both directions at the University of Arizona Genetics Core Sequencing Facility or the University of California, Berkeley Sequencing Facility using the same primers for PCR amplification and ABI dye terminator chemistry. Raw sequences were assembled into contigs and trimmed in Geneious 5.5.5 (Biomatters). All sequences have been deposited on GenBank (Table 1).

2.3. Phylogenetic inference

Additional drosophilid DNA sequences were downloaded from GenBank and Flybase (Table 1), added to the newly sequenced genes, and aligned in MUSCLE (Edgar, 2004) using the default parameters. The large concatenated DNA sequence matrix was comprised of 5042 bp. Alignment of each gene was trivial, though the intronic region in *marf* was found to be highly variable between species and was removed due to a high degree of ambiguity in that portion of the alignment. The coding regions of *marf* were retained. Most loci contained a high proportion of parsimony informative characters (Table 2). The loci were analyzed both as individual genes and in a concatenated, partitioned phylogenetic analysis under Bayesian (MrBayes v3.1.2) (Ronquist and Huelsenbeck, 2003) and Maximum Likelihood (RAxML) (Stamatakis, 2006) frameworks. To explore the effects of missing data we analyzed a reduced dataset without *gstd1* (which had the lowest level of coverage) of 32 taxa that were sequenced for at least five of seven genes. We will refer to this smaller, higher coverage matrix (~85% complete data matrix) as the "small dataset" and the larger matrix with more gaps (~60% complete data matrix) as the "large dataset." Phylogenies were rooted with Hawaiian *Drosophila* species. Phylogenetic analyses were performed on the CIPRES Science Gateway workbench (Miller et al., 2010).

Partitioned genealogies were estimated for each gene. The genes were partitioned by codon position, RNA sequences were given their own partition, and models of sequence evolution were selected for each partition using MrModeltest (Nylander, 2004). The substitution matrix, base frequencies, and gamma shape parameter were unlinked for each data partition, and the rate prior was set to variable. MrBayes analyses were run twice for 5×10^6 generations, and sampled every 500 generations. Convergence was assessed via a low (<0.01) average standard deviation in split frequencies and by assessing the cumulative split frequency in AWTY (Nylander et al., 2008). Appropriate levels of burn-in were discarded – generally the first 10–20% of the sampled data. RAxML was used to simultaneously infer the optimal topology and to assess bootstrap support. The individual gene datasets were partitioned in the same manner for the maximum likelihood analysis as described for the Bayesian analyses, but the GTRGAMMA model was applied to all partitions. A total of 1000 bootstrap replicates were generated to assess node confidence. Each dataset was analyzed five times with different random starting seeds in RAxML to identify if significant changes in topology and support occurred between runs.

All loci were combined into a partitioned, concatenated dataset. Both the large and small datasets were partitioned by gene, codon position, and RNA – *16s* and part of tRNA-Lys which is sequenced at the end of *co2* – resulting in a total of 23 partitions for the large dataset and 20 partitions for the small dataset. The concatenated phylogenetic analyses were run as described above except that the MrBayes analysis was run for 5×10^7 generations and sampled every 5000 generations and the chain temperature was reduced to 0.15 to improve chain mixing. Convergence was assessed in the same way and the first 30% was discarded as burn-in for the large dataset and 15% was discarded as burn-in for the small dataset.

2.4. Dating

Divergence date estimates were inferred using BEAST v1.6.2 (Drummond and Rambaut, 2007). The concatenated, partitioned dataset was used and the models of substitution identified by MrModeltest were implemented as above. A relaxed clock with an uncorrelated lognormal model of rate variation was implemented. Both a birth–death and Yule speciation process for branching rates were tested, and after comparing Bayes Factors (non-significant for rejecting either model) a birth death model was implemented in all subsequent analyses. The analysis was run for 5×10^7 generations and sampled every 1000 generations. The analysis was run twice independently, 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 (Drummond and Rambaut, 2007). Tracer v1.5 (Rambaut and Drummond, 2004) was used to visually assess convergence and stationarity, and to observe if the effective sample size (ESS) for all parameters was sufficiently high.

To estimate the timing of divergence events we analyzed the large dataset employing two calibration points – a fossil and biogeographic information. Biogeographic calibrations points have been previously used for dating most drosophilid lineages (Russo et al., 1995; Tamura et al., 2004; Bonacum et al., 2005; Morales-Hojas and Vieira, 2012), but there is evidence that this may introduce a large amount of error (Obbard et al., 2012). We used only the fossil calibration to date the small dataset due to a lack of appropriate taxa on which to place the biogeographic calibration. Given the limitations of our dataset we caution against too strict an interpretation of our inferred dates (Parham et al., 2012).

Unlike in many drosophilid lineages, a well-described *Scaptomyza* fossil (*Scaptomyza dominicana*) from Dominican amber is available (Grimaldi, 1987). Placement of fossil calibration points can strongly influence the divergence time estimates, and while this specimen shares many synapomorphies with modern *Scaptomyza* species, including four rows of acrostichal setae, long legs and longitudinal thoracic color patterning, the placement of *S. dominicana* within any *Scaptomyza* subgenus is uncertain. To identify whether the fossil belongs to either the crown or stem group of *Scaptomyza* we performed a partitioned analysis using a mixed data matrix, including our molecular dataset and Grimaldi's (1990) morphological character matrix. We used the morphological matrix of 218 morphological characters compiled by Grimaldi (1990) for *Scaptomyza* subgenera and Hawaiian *Drosophila* (Table S1) including species for which we were not able to get DNA. *Scaptomyza dominicana* was coded based on characters and states described for the fossil (Table S2) (Grimaldi, 1987). The same partitioning scheme was used for this analysis as was used for the concatenated, partitioned molecular phylogenetic analysis, with the addition of another partition for morphology and applying the standard discrete model to the

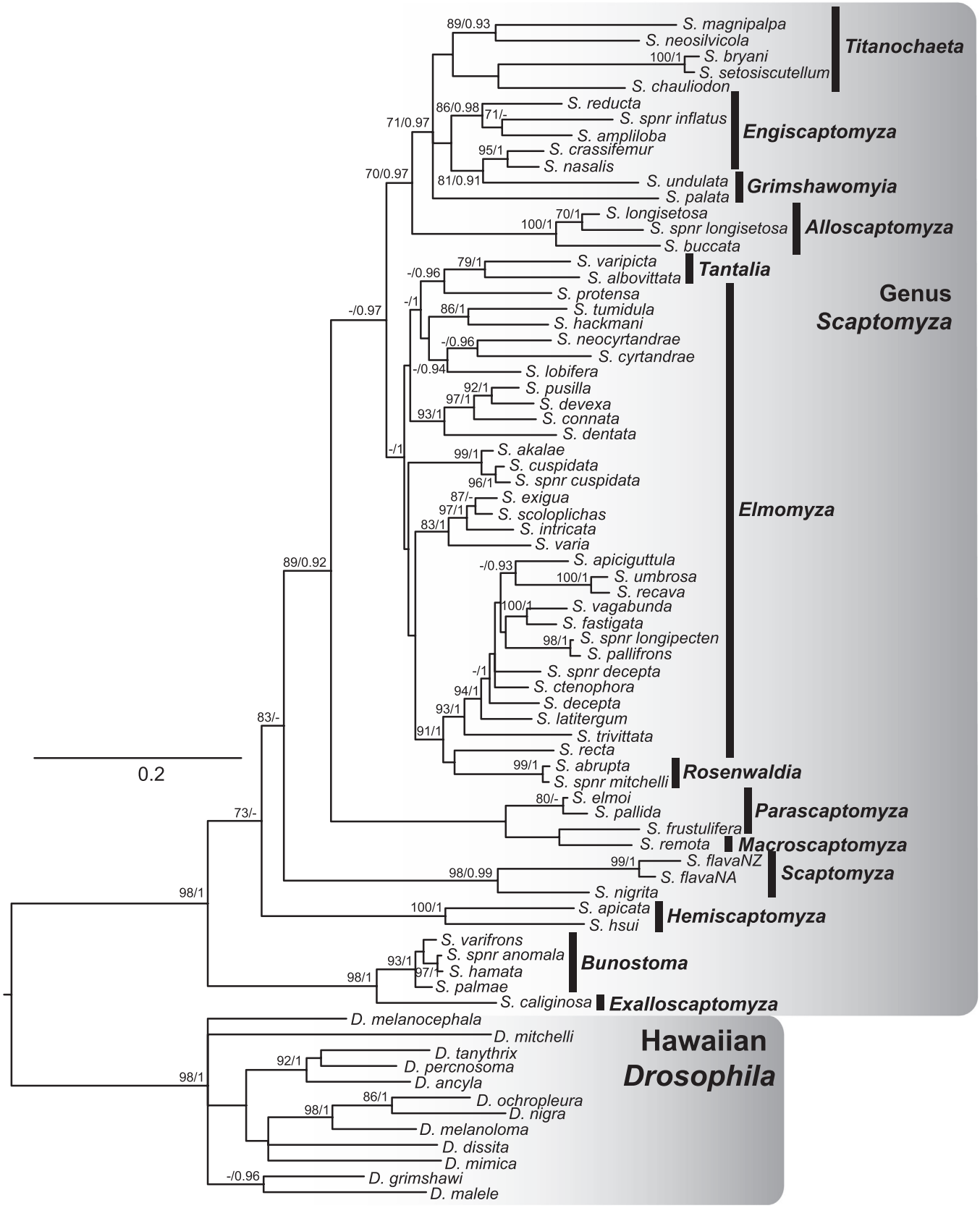

Fig. 2. Bayesian analysis of phylogenetic relationships in the genus *Scaptomyza* based on combined mitochondrial and nuclear loci. Support is indicated at nodes. Bayesian posterior probabilities are to the right, maximum likelihood bootstrap support is to the left. A lack of either posterior probability or bootstrap support is indicated by a –. Nodes with no numbers are unsupported.

Table 3
Dates of nodes of interest based on alternate fossil placements. Dates are in millions of years. The numbers in parentheses are 95% HPD values.

Node	Large dataset fossil calibration on stem of *Scaptomyza*	Large dataset fossil calibration on crown of *Scaptomyza*	Small dataset fossil calibration on stem of *Scaptomyza*	Small dataset fossil calibration on crown of *Scaptomyza*
Hawaiian Drosophilidae	23.9815 (18.4171,30.8063)	27.5188 (19.7108,36.4494)	22.094 (16.9855,30.4300)	26.7188 (18.5686,38.9109)
Hawaiian *Drosophila*	13.5124 (8.5068,18.991)	16.2589 (10.5708,22.9984)	7.3783 (3.7371,12.3473)	8.8566 (4.2898,15.3252)
Genus *Scaptomyza*	17.7695 (12.0609,23.8934)	23.3117 (18.2039,29.3288)	18.0608 (12.0566,26.6593)	22.0651 (17.0523,30.5186)
Subgenus *Bunostoma/ Exalloscaptomyza*	5.0245 (2.6018,8.0539)	6.2836 (3.2876,9.9992)		
Subgenus *Hemiscaptomyza*	6.3179 (3.1055,9.8527)	7.9487 (3.9896,12.3632)	7.3128 (3.2918,12.6882)	8.9594 (4.3753,15.3327)
Subgenus *Scaptomyza*	6.2578 (3.5528,9.3058)	7.8196 (4.2868,11.6125)	6.0421 (2.8436,10.2698)	7.3262 (3.8031,12.5439)
Subgenus *Parascaptomyza/ Macroscaptomyza*	5.0038 (2.3353,8.2453)	6.2199 (2.8278,10.1529)		
Major Hawaiian *Scaptomyza* clade and *Parascaptomyza*	12.4499 (8.6459,16.8388)	15.6811 (11.3817,20.5619)	13.2164 (8.5173,19.3361)	16.1853 (11.5786,23.1262)
Major Hawaiian *Scaptomyza* clade	10.1232 (7.0774,13.5957)	12.5643 (9.0474,16.5584)	10.7234 (6.992,15.8132)	13.1557 (9.1929,18.7188)
Subgenus *Titanochaeta*	7.3799 (5.0047,10.2295)	9.1050 (6.2861,12.2000)	7.2832 (4.9255,10.9465)	8.9665 (6.0078,13.3242)
Subgenus *Alloscaptomyza*	3.6752 (1.9896,5.6053)	4.5275 (2.4184,6.8349)		
Subgenus *Engiscaptomyza/ Grimshawomyia*	7.3432 (4.8311,10.0851)	9.0934 (6.1614,12.2354)	7.0578 (4.359,10.8391)	8.6677 (5.7311,12.9715)
Subgenus *Tantalia*	3.9689 (2.1777,5.8193)	4.8385 (2.6327,7.1144)		
Subgenus *Rosenwaldia*	0.5273 (0.1952,0.9471)	0.6553 (0.2310,1.1546)	0.5342 (0.1785,0.9632)	0.6526 (0.2304,1.2757)
Subgenus *Elmomyza* sensu lato	8.4941 (5.9130,11.4224)	10.5345 (7.4580,13.9344)	8.2522 (5.1581,12.2088)	10.0645 (6.9321,14.7174)

morphological partition. The analyses were run in MrBayes using the same settings as for the concatenated molecular dataset, and convergence was similarly assessed. The first 20% of the dataset was excluded as burn-in. The phylogeny inferred via this method was poorly resolved, especially at deeper nodes, and the fossil's placement as either a stem or crown group species could not be ascertained due to a lack of resolution (Fig. S1).

To explore how the placement of this fossil calibration as either a stem or crown group species effects the dates of divergence we ran the BEAST analysis twice for both the large and small datasets. We placed the fossil calibration as belonging to the crown group of *Scaptomyza* in one analysis and the stem in another analysis. Given the uncertainty in the dating of Dominican amber (Iturralde-Vinent and MacPhee, 1996) we calibrated these nodes with a lognormal distribution prior that allows for a range of divergence dates from 15 to 45 mya with a the median at 24.5 mya, minimum at 15 mya and a 95% HPD greater than 45 mya. To obtain this distribution the log (mean) was set to 2.251, Log (std dev) was set to 0.75, and the offset was 15.

We applied two calibration points to the large dataset dating analyses: (1) the fossil (described above) and (2) the biogeography of the Hawaiian Islands. Clades endemic to the island of Hawaii are not expected to be older than that island (Fleischer et al., 1998), making the most probable time of divergence between Hawaiian endemic lineages and their sister species on the next nearest island, Maui, about 0.5 mya (Price and Clague, 2002). Since the Hawaiian lineage could have diverged before the formation of Hawaii and sister species on Maui Nui went extinct, or the island of Hawaii could have been colonized later than the island's initial formation, we calibrated the time to most recent common ancestor of these groups with a normal distribution prior with a mean of 0.5 and a standard deviation of 0.15 mya. This creates a distribution where the most probable time of divergence is 0.5 mya, but allows for divergence from almost the present and up to 0.9 mya. The ancestral node of *Scaptomyza scoloplichas* and *S. exigua* was calibrated using this prior since both species are found on the island of Hawaii and are sister to a Maui Nui species. We were not able to use this calibration in the small dataset due to the exclusion of *S. scoloplichas*.

2.5. Ancestral state reconstructions

We used two methods to infer the biogeographic history of *Scaptomyza*. For Hawaiian endemic taxa we used the program Lagrange v2.0.1 (Ree and Smith, 2008). Lagrange models dispersal, extinction and cladogenesis (DEC) in a likelihood framework and allows for modeling multiple biogeographic scenarios. This method has proven to be powerful in reconstructing ancestral ranges in simulation and empirical studies (Buerki et al., 2011) and is appropriate for Hawaiian taxa, where dispersal to adjacent islands is associated with diversification (Bonacum et al., 2005; Ree and Smith, 2008; Holland and Cowie, 2009), and is possible within island groups like Maui Nui (which includes the islands of Maui, Molokai and Lanai) that are historically connected or isolated during periods of glacial maxima and minima respectively (Price and Clague, 2002).

The chronogram for the large dataset calibrated with Hawaiian biogeography and the fossil placed as part of the crown *Scaptomyza* was entered into the Lagrange configurator and pruned to include only Hawaiian *Scaptomyza* species (reelab.net/lagrange/configurator). Species' present day ranges were coded based on known ranges (Hardy, 1965; Wheeler and Takada, 1966) and collection localities (Table S1). Each species was coded as being from Hawaii, Maui Nui, Oahu, or Kauai. Species from either Maui, Molokai, Lanai or any combination thereof were treated as being from one island since these islands were connected together into the larger island known as Maui Nui in the past, facilitating dispersal between islands (Price and Elliot-Fisk, 2004). A stepping stone model of evolution was applied in Lagrange: possible node ranges were restricted to single islands or two adjacent islands.

Since the above assumptions are not realistic for lineages separated by large distances (e.g. Hawaiian Island species and North American species), Lagrange was not used to reconstruct biogeographic history of the more distant dispersal events. We instead used SIMMAP v1.5 (Bollback, 2006) to stochastically map the ancestral ranges in a Bayesian framework. Ancestral state reconstructions used 1000 post burn-in BEAST trees and their branch lengths. Species areas were coded as Hawaiian Islands, North America, New Zealand and Tristan da Cunha. The overall substitution rate of each morphological character was modeled using a gamma distribution whose priors α and β were estimated using the two-step procedure suggested in SIMMAP 1.5. An MCMC analysis was used to sample overall rate parameter values. Next, the results of this analysis were analyzed with the R Statistical Package and the sumprmcmc.r script provided with SIMMAP 1.5 to find the best fitting gamma and beta distributions. Based on these analyses, we obtained an $\alpha = 0.668$ and a $\beta = 0.005$ which were used to parameterize further analyses.

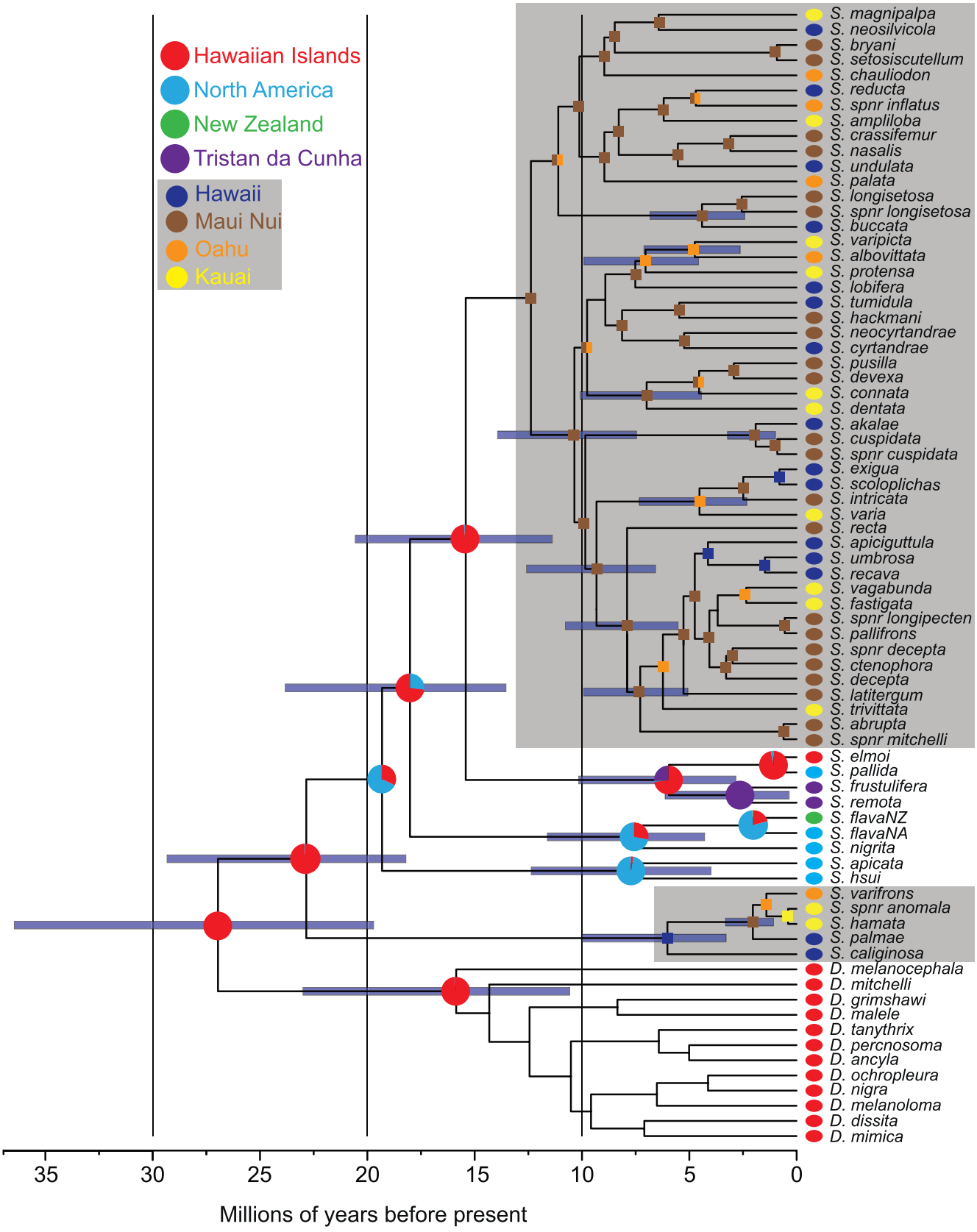

Fig. 3. Chronogram with biogeographic reconstructions. Calibrations include the age of Hawaii for range restricted species of *Elmomyza*, and a Dominican amber calibration placed at the crown of the genus *Scaptomyza*. 95% HPD age estimate distributions are drawn on nodes of interest with a BEAST BI PP > 0.90. Pie charts on nodes indicate biogeographic reconstructions. Ancestral ranges inferred using Lagrange (nodes in gray boxes) or SIMMAP (nodes not in gray boxes). States inferred via SIMMAP indicate posterior probability of each range. Ranges inferred by Lagrange are the most likely state given likelihood reconstructions – divided circles indicate a range of multiple islands.

To identify how often unique larval ecologies have evolved in *Scaptomyza* we reconstructed the ancestral states for lineages using SIMMAP v1.5. Ecological status was coded based on published sources (Stalker, 1945; Maca, 1972; Collinge and Louda, 1989; Martin, 2004; Magnacca et al., 2008) (Table S1). Species were coded as being saprophagous, spider predators, flower-specialists or leaf-miners. Species with no information were coded as unknown. We used the same method as described above to identify substitution model parameters. We obtained an $\alpha = 3.361$ and a $\beta = 0.482$ which were used to parameterize further analyses.

3. Results

3.1. Phylogenetics

The concatenated phylogeny for the large dataset is supported throughout most of the topology and the maximum likelihood estimate ($-\ln L = -36674.8637$) is similar to the Bayesian topology (Figs. 2 and S2). The small dataset corroborates the results of the larger dataset, though with fewer taxa it is not as rigorous a test of monophyly for most groups (Fig. S3). Wiens and colleagues (Wiens 2003, 2006; Wiens and Moen, 2008) have shown that modest amounts of missing data have little impact on phylogenetic inference but a lack of overlapping characters can influence branch lengths. We find little difference between the phylogenies inferred from the large and small datasets in terms of topology and nodal support. Individual gene genealogies broadly agree with one another (Fig. S4a–e) and the concatenated dataset. The conflict observed between individual loci is poorly supported and may be due to stochasticity, incomplete lineage sorting or hybridization.

Almost all subgenera are monophyletic, except: (1) *Elmomyza* is rendered paraphyletic by *Rosenwaldia* and *Tantalia*, (2) *Grimshawomyia* and *Engiscaptomyza* are paraphyletic with respect to one another; and (3) *Macroscaptomyza* is nested within *Parascaptomyza*. This analysis recovered two well-supported clades comprising Hawaiian *Scaptomyza* (Fig. 2), one including the majority of Hawaiian subgenera, and another including only *Bunostoma* and *Exalloscaptomyza*. The non-Hawaiian subgenera are separated by long branches, and relationships between these subgenera are poorly supported in this phylogeny (posterior probability < 0.90).

3.2. Divergence dating

Ages of divergence varied depending on the placement of the fossil calibration, and the completeness of the dataset, though there was overlap between estimated age ranges of all analyses (Table 3, Figs. 3, S5, and S6). The dates inferred with the small dataset had similar 95% highest posterior density (HPD) ranges as the full dataset, even with the exclusion of the biogeographic calibration from the small dataset. The placement of the fossil has the strongest effect on the dating inference. Placing the *S. dominicana* fossil as part of the stem lineage of *Scaptomyza* reduces the timing of divergences of extant *Scaptomyza* species, and if placed as part of the crown group, increases the age of the *Scaptomyza* species. Given the uncertainty of the taxonomic placement of *S. dominicana*, dates should be treated with caution.

Dates of divergence between *Scaptomyza* and Hawaiian *Drosophila* were estimated in all analyses within the range of those estimated from previous analyses using biogeographic or external calibrations (Russo et al., 1995; Tamura et al., 2004; Obbard et al., 2012; Morales-Hojas and Vieira, 2012). The genus *Scaptomyza* is estimated to have arisen between 20 and 30 mya with the majority of diversification in the Hawaiian lineages occurring 7–16 mya, around the time that the islands of Gardner and Necker were high enough to support rainforests (Price and Clague, 2002). Divergence times within the non-Hawaiian subgenera were variable, and ranged from ca 3 to 12 mya, although sparse sampling in some of these lineages reduces confidence in these estimates.

3.3. Biogeography

Stochastically mapped reconstructions of range suggest a Hawaiian origin for the genus *Scaptomyza* (Fig. 3). Due to the lack of support at nodes leading to the mainland subgenera *Hemiscaptomyza* and *Scaptomyza* it is difficult to quantify the number of dispersal events from Hawaii. Stochastic mapping reconstructions indicate that at least one dispersal from the island chain occurred, followed by subsequent worldwide diversification of mainland subgenera. The split between *Hemiscaptomyza* and the remaining *Scaptomyza* is reconstructed as most likely to have occurred on North America. The node at the base of all non-Hawaiian subgenera is weakly supported in all analyses, and improved sampling of mainland subgenera may change the relationships inferred for this node.

The greatest diversity of Hawaiian *Scaptomyza* species belongs to the clade including *Elmomyza*, *Tantalia*, *Rosenwaldia*, *Titanochaeta*, *Engiscaptomyza*, *Alloscaptomyza*, and *Grimshawomyia*. Most species in these subgenera are inferred as originating on Maui Nui (Fig. 2). However, other analyses do not support a Maui Nui ancestral range for these subgenera, because the Maui Nui islands are younger than these subgenera.

3.4. Evolution of larval ecologies

Fig. 4 shows the reconstruction of larval ecology (leaf-mining, spider predation, flower-specialization, etc.) using stochastic character mapping. We defined character states for ~40% of the *Scaptomyza* species included in this study using the available rearing records (Stalker, 1945; Maca, 1972; Collinge and Louda, 1989; Martin, 2004; Magnacca et al., 2008; Table S1). The majority (~60%) of these species are saprophagous and use only one or few rotting host substrates. We only differentiated between flower and non-flower based saprophagy in this analysis. The ancestral state of the *Scaptomyza* larval substrate is saprophagy, the few clades with unique larval ecologies are monophyletic, and the ecologies are reconstructed to their most basal node with high support. Switches between larval ecologies occurred only rarely.

4. Discussion

4.1. Subgeneric relationships

Our results suggest that the majority of subgenera included in our analysis are monophyletic, and the relationships within each group are congruent with morphological and ecological affinities (see Fig. 2). However, there are subgenera that were not recovered as monophyletic. *Parascaptomyza* is rendered paraphyletic by *Macroscaptomyza*. This is not entirely surprising: the close relationship between these two subgenera has been proposed by multiple prior morphological phylogenetic analyses (Okada, 1973; Grimaldi, 1990). Also, *Rosenwaldia* and *Tantalia* are reconstructed as nested within *Elmomyza*. Again, this is not unexpected, given that at least one previous analysis allied these subgenera (Grimaldi, 1990). *Grimshawomyia* is also not monophyletic, but there is low support at the base of the clade including *Grimshawomyia* and part of *Engiscaptomyza*. Increased tax on and character sampling could improve the resolution of these relationships. We found statistical

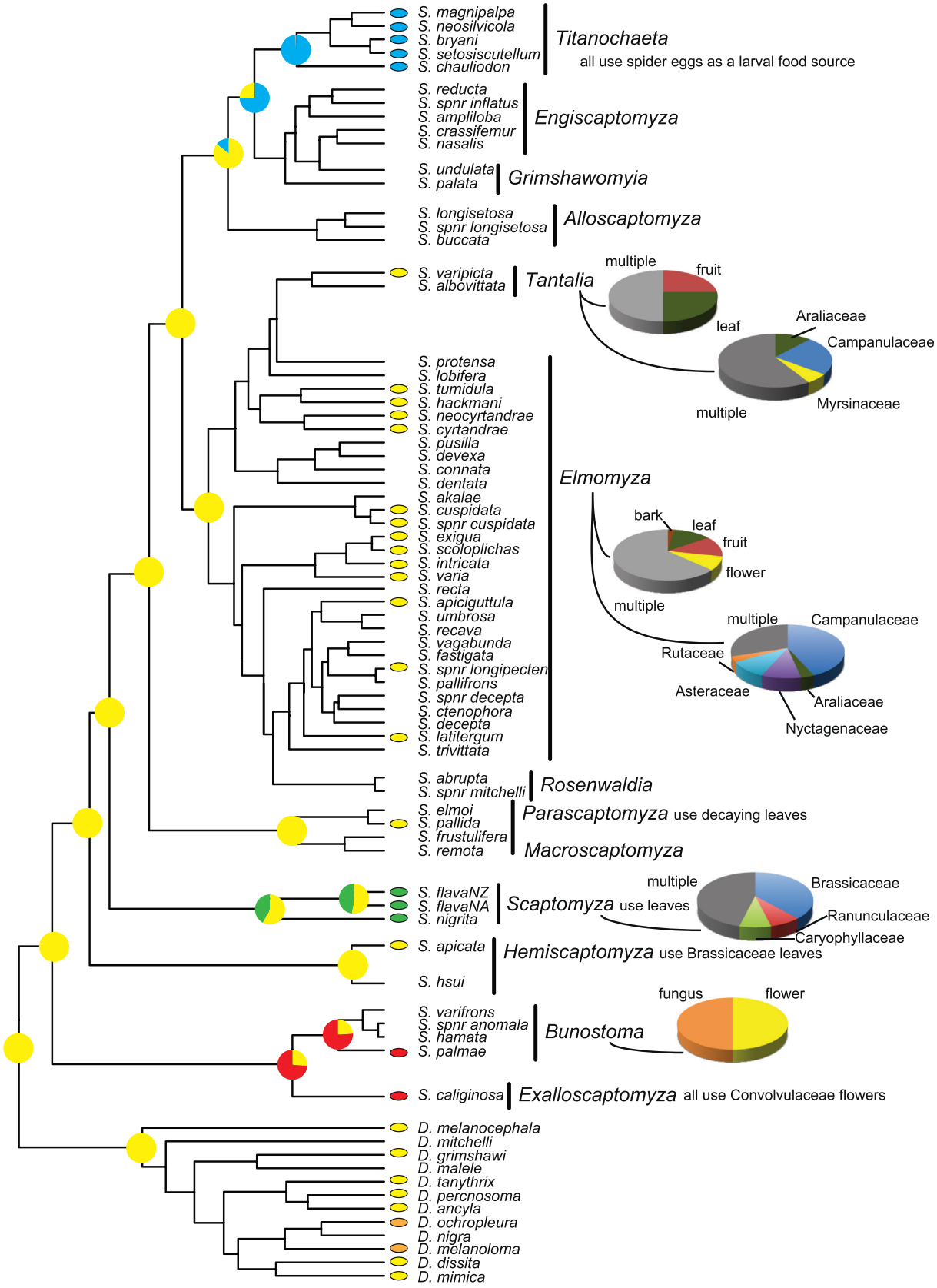

Fig. 4. Ancestral state reconstruction of larval host ecologies. Pie charts at nodes indicate posterior probability of stochastically mapped ancestral states. Yellow = saprophagy, green = leaf-mining, blue = spider predation, red = flower specialist, orange = fungus. Charts to the right of subgenera indicate subgenus wide rearing records parsed by either part of plant that *Scaptomyza* were reared from, or family of plant from which *Scaptomyza* were reared . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

support for the hypothesis that *Parascaptomyza* and *Scaptomyza* are distinct clades, which had been unclear in previous studies.

We identified three clades of Hawaiian *Scaptomyza* separated by well supported nodes: (1) *Bunostoma* and *Exalloscaptomyza*, (2) *Elmomyza*, *Rosenwaldia* and *Tantalia*, and (3) *Grimshawomyia*, *Alloscaptomyza*, *Titanochaeta* and *Engiscaptomyza*. The first clade includes species of flower specialists, though they have been reared from divergent plant families (Magnacca et al., 2008). The second clade of *Elmomyza*, *Rosenwaldia* and *Tantalia*has been combined based on morphological cladistics and genitalic similarity (Hackman, 1959; Grimaldi, 1990). The final clade has been identified as monophyletic in a previous molecular analysis, and while gross morphology had originally placed them as distantly related, scanning electron microscopy of genitalia and polytene chromosome morphology revealed synapomorphies between subgenera (O'Grady et al., 2003).

The non-Hawaiian endemic subgenera included in this study – *Parascaptomyza*, *Macroscaptomyza*, *Scaptomyza*, and *Hemiscaptomyza* – belong to distinct clades, despite a wide overlap in range and morphology. This finding agrees well with previous phylogenetic hypotheses (Okada, 1973; Grimaldi, 1990). The exact relationships between these subgenera are suspect, however, given the low support at a node intermediate between them and relatively sparse taxon sampling. Future studies that include more non-Hawaiian taxa are required to more thoroughly explore these taxonomic relationships.

4.2. Biogeography of Scaptomyza

Throckmorton (1975) suggested that the ancestors of the Hawaiian Drosophilidae arrived in either one or two colonization events. The single colonist theory is supported by the sister relationship between the Hawaiian *Drosophila* and *Scaptomyza* lineages (Russo et al., 1995; Remsen and DeSalle, 1998; Da Lage et al., 2007; O'Grady and DeSalle, 2008; Van der Linde et al., 2010). The two-event colonization hypothesis is supported if Hawaiian *Drosophila* and *Scaptomyza* were not sister taxa, a result that is only seen in a single study (Grimaldi, 1990). O'Grady and DeSalle (2008) further explored the biogeographic history in *Scaptomyza* by proposing a single escape from Hawaii by the ancestor of a subset of mainland subgenera. After emigrating from the Hawaiian Islands this lineage dispersed to remote Atlantic and Pacific Islands, and all continents except Antarctica. Our phylogenetic analyses are an improvement over previous studies in both taxonomic and character sampling. We inferred a more complex pattern of dispersal, but one that cannot reject the one-event colonization hypothesis.

4.2.1. Dispersal from and to Hawaii

The Hawaiian Drosophilidae is recognized as the oldest terrestrial Hawaiian lineage to have persisted to the present day (Price and Clague, 2002), though little dating has been done to identify when subgenera within *Scaptomyza* diverged. Our analyses confirm that the divergence of *Scaptomyza* and the Hawaiian *Drosophila* predates the emergence of the present day high islands and occurred when the atolls of Midway and Laysan were high islands (peak heights 28.7 and 20.7 mya, respectively (Price and Clague, 2002)). The ancestral range of the genus *Scaptomyza* was inferred to be the Hawaiian Islands, and the divergence between the Hawaiian *Drosophila* and *Scaptomyza* fits within the previously estimated range of dates of 23–35 mya (Russo et al., 1995; Tamura et al., 2004).

Our phylogeny recovered a Hawaiian origin of *Scaptomyza* (pp = 0.99), and we inferred an emigration of *Scaptomyza* from the Hawaiian Islands around 20 mya (pp = 0.68). Given the locality of the fossil species in Dominican amber, the dispersal of *Scaptomyza* was rapid. The pattern of movement to and from the Hawaiian Islands is more complicated than expected. One possible conclusion from these analyses is that there is one dispersal event from Hawaii to the mainland by the ancestor of the subgenera *Scaptomyza*, *Hemiscaptomyza*, *Parascaptomyza* and *Macroscaptomyza*. The split between the ancestor of *Bunostoma* and *Exalloscaptomyza* and the ancestor of the remaining genus *Scaptomyza* represents the oldest divergence event in our topology. Following this split a lineage emigrated from Hawaii and another lineage remained in Hawaii. Given the low support at important nodes of our topology we cannot entirely reject this hypothesis (Fig. 2). Alternatively, a second colonization of Hawaii by the ancestor of the large Hawaiian *Scaptomyza* clade may have occurred ca. 12 mya. This Hawaiian clade is found to be sister to the globally distributed subgenera *Macroscaptomyza* and *Parascaptomyza* in our analyses. However, this interpretation requires another long distance colonization event to Hawaii – one colonization event is already an unlikely event (but see Rundell et al., 2004; Arnedo et al., 2007) – and a subsequent radiation.

Other instances of long distance dispersal have occurred. The subgenus *Bunostoma* includes non-Hawaiian endemic species, and future collections will identify if dispersal from or to Hawaii explains this range (Hackman, 1982). Similarly the Hawaiian subgenus *Rosenwaldia* includes species found throughout the Pacific, and represents another independent dispersal event. Further sampling is required to test these hypotheses.

4.2.2. Hawaiian biogeography

Movement of *Scaptomyza* within the Hawaiian Islands is also reconstructed as more complex than hypothesized. Most Hawaiian *Scaptomyza* species are single island endemics, although they display a higher incidence of multi-island endemism than species in their sister lineage, the Hawaiian *Drosophila*. Approximately 25% of described Hawaiian *Scaptomyza* species are multi-island endemics, as compared to 10% of Hawaiian *Drosophila* species (Hardy, 1965). The progression rule is a pattern found in the Hawaiian *Drosophila* (Bonacum et al., 2005) and is observed in our study in one clade of *Elmomyza* including *S. exigua*, *S. intricate, S. scoloplichas*, and *S. varia* which is corroborated by our dating and biogeographic inferences. The remainders of the Hawaiian *Scaptomyza* species do not conform to this pattern. The high vagility of *Scaptomyza* species is one potential explanation for why there is almost no signal of a progression rule for many Hawaiian *Scaptomyza* clades. In contrast, the subgenus *Bunostoma* appears to follow a reverse progression rule, with earlier divergences occurring on younger islands. It is possible that this lineage originated on the island of Hawaii and back-colonized up the island chain. This movement is surprising since this back colonization pattern is not normally observed among Hawaiian taxa (but see Magnacca and Danforth, 2006), and given the young age of the island of Hawaii. This pattern may be a symptom of the high vagility displayed by *Scaptomyza*.

Our analyses indicate that the larger clade of Hawaiian *Scaptomyza* – which includes *Elmomyza, Rosenwaldia, Tantalia*, *Grimshawomyia, Alloscaptomyza, Titanochaeta* and *Engiscaptomyza* – started to diversify across the Hawaiian Islands approximately 10–12 mya. This was the last time multiple islands in the Hawaiian Island chain were as high in elevation as they are today – the islands of Gardner, LaPerouse and Necker were above sea-level and large (Price and Clague, 2002). Given the mobility of this lineage we propose that this increased topographic diversity created more habitats and allowed for increased ecological diversification, rather than an increased incidence of vicariance. This lineage presently comprises the majority of described Hawaiian *Scaptomyza* species (90%).

Maui Nui is reconstructed as the area of origin for many of the Hawaiian *Scaptomyza* subgenera, which is probably an artifact of the present high species diversity within subgenera on Maui Nui. Maui Nui has experienced a high degree of geological heterogeneity as the sea level falls during ice ages and rises during interglacial periods. The islands of Maui, Molokai, Lanai and Kahoolawe were intermittently connected over the past 1.2 million years, and the opportunity for communities to intermingle and then diverge in allopatry via vicariant events is credited with facilitating speciation in many different taxa (Jordan et al., 2003; Gillespie, 2005; Holland and Cowie, 2007). However, this scenario is unlikely given the age of the Hawaiian *Scaptomyza* subgenera and the dispersal capabilities of these species. The subgenera of Hawaiian *Scaptomyza* are not derived from Maui Nui ancestors – we reconstruct these lineages as older than the formation of the current main islands. We hypothesize instead that the Hawaiian subgenera had diversified during periods when older Hawaiian Islands were high enough in elevation to support cloud forests, and these subgenera persisted onto the current high islands. The present diversity seen on Maui Nui may be due to a combination of its size (second largest island in the archipelago in total area today) and age (1.2 myo to present).

4.3. Evolution of larval ecologies

Saprophagy is hypothesized to be the ancestral larval ecology in the family Drosophilidae (Throckmorton, 1975) and our analysis supports the hypothesis that saprophagy was the ancestral state for the genus *Scaptomyza* with high confidence (pp = 0.99). Our analyses conceal a high level of specialization within *Scaptomyza*: many of the saprophagous species utilize decaying plant matter of specific plant families and are restricted to specific parts of the plant (e.g. rotting leaf or stem), similar to their sister lineage, the Hawaiian *Drosophila* (O'Grady et al., 2011; Ort et al., 2012).

Several specialized larval ecologies evolved in *Scaptomyza* that are found only rarely, if at all, in other drosophilid lineages. Our analysis provides basic information about the evolution of these *Scaptomyza* ecologies that have previously been untested. We provide the first phylogenetic evidence for the conservation of ecologies within subgenera of *Scaptomyza*. In almost all cases novel ecologies arise once and relatively recently. This pattern is not surprising given the large number of morphological and physiological changes required of these new lifestyles. These adaptations do not appear to be solely associated with dispersal events, and may be due to other factors such as exploiting newly available resources, or as a potential escape from predation or parasitism (Conner and Taverner, 1997).

Larvae of species in the subgenus *Titanochaeta* are spider egg predators and the group reconstructs strongly as such (pp = 0.98). The subgenus *Titanochaeta* and its spider predation ranges from ca. 7 to 9 mya. This is interesting given that the Hawaiian Thomisidae radiation, the group of spiderson which *Titanochaeta* larvae feed, are inferred to have colonized the Hawaiian Islands during this same time period, between 7 and 9 mya and subsequently radiated across the Hawaiian Archipelago (Garb and Gillespie, 2009). Given the coincident date of diversification of Thomisidae and the origination of spider predation it is possible that *Titanochaeta* radiated alongside the Thomisidae. *Titanochaeta* is phylogenetically nested within a lineage of Hawaiian *Scaptomyza* with completely unknown ecologies – including *Engiscaptomyza*, *Grimshawomyia* and *Alloscaptomyza*. While none of the species from these other subgenera have been reared from spider egg sacs, the ancestor of these subgenera is reconstructed as possibly being predatory (pp = 0.76). Understanding the ecologies of their relatives may illuminate the evolution of this larval ecology unique to *Drosophila*.

The subgenus *Scaptomyza* includes leaf-mining species, and is also monophyletic, although sampling within this group is currently very limited, and its phylogenetic placement is poorly supported. Leaf-mining originated at least 6 mya in *Scaptomyza* (pp = 0.47). Adapting to an herbivorous lifestyle requires overcoming multiple hurdles, especially for leaf-mining species in the subgenus *Scaptomyza*, some of which specialize on mustard oil-rich plant leaves in the Brassicaceae. Extant herbivorous insects are among the most evolutionarily successful radiations, and comprise nearly 25% of all eukaryote species (Bernays and Graham, 1988; Bernays, 1998). The transition to herbivory occurred at least 50 times in the Insecta (Labandeira and Sepkoski, 1993; Labandeira, 2005) and was accompanied by major changes that allowed nascent herbivores to feed on nutritionally different hosts with potent phytotoxins. Moreover, the evolution of herbivory in insects is associated with increases in the rate of lineage diversification (Mitter et al., 1988; Farrell, 1998; Farrell and Sequeira, 2004). However, in many cases, these evolutionary transitions occurred so long ago that understanding the biogeographic, morphological and physiological underpinnings of these transitions is difficult. The relatively recent origin of herbivory in *Scaptomyza* indicates that it might be a good system in which to study these transitions (Whiteman et al., 2012).

Identifying where and when obligate flower specialization arose is not yet possible given that we have only sampled one species of *Exalloscaptomyza*, but we did find that this lineage is sister to *Bunostoma*. *Bunostoma* includes species that have also been reared from flowers but are not specialists. We reconstruct the most probable life history for the *Bunostoma*, *Exalloscaptomyza* clade as flower breeding (pp = 0.76). Placing the mainland lineage *Dentiscaptomyza*, another *Scaptomyza* subgenus that includes flower specialist (Brncic, 1983), in a phylogenetic framework would allow further insight into how this life history evolved. Specialization on morning glory flowers (*Ipomoea* spp.) has occurred at least twice in the Drosophilidae (Remsen and O'Grady, 2002; O'Grady and DeSalle, 2008); once in *Scaptomyza* (subgenus *Exalloscaptomyza*) and independently in the genus *Drosophila* (subgenus *Phloridosa*). These morning glory-adapted taxa converged on similar phenotypes, including dark body color with lightening at the tip of the abdomen and a general shortening and reduction in chaetotaxy. Both species have low fecundity, larvae that feed and adults that breed on yeast on the surface of corollas, and rapid metamorphosis. These species compete for the same resources in the Hawaiian Islands, where *D. floricola* is introduced and breed in *Ipomoea* spp. (Montague, 1984). Whether there are convergent molecular evolutionary underpinnings of these convergent phenotypes is unknown.

4.4. Conclusions

The genus *Scaptomyza* is a model for future studies of biogeography, the evolution of ecology and the associated morphological and physiological changes accompanying these adaptations. To begin exploring these questions and placing the associated research into an evolutionary context we have produced the most complete phylogenetic analysis of this group to date. This study also allows us to identify where future taxonomic sampling will improve our inference of the phylogeny of this genus. Our analyses are an important step in the evaluation of this group, but will be improved by further research. We find support for the Hawaiian origin of *Scaptomyza*, and its subsequent emigration back to the mainland, in accordance with previous studies, but we also find a more complex biogeography than previously predicted. The vagility of this group has been attributed to desiccation tolerance and ecology of *Scaptomyza* (O'Grady and DeSalle, 2008), however a better understanding of the biology of this clade is required to fully

explain how these species are able to disperse across such vast distances and if *Scaptomyza* is pre-adapted to long range dispersal. By including specimens of other ecologically important lineages – such as the flower specializing and possibly leaf-mining *Dentiscaptomyza* and the predacious *Elmomyza* species – we will be able to explore how these ecologies have evolved in relation to those included in this study. Species from more remote areas of the broad range of this genus – such as other subgenera from Tristan da Cunha in the South Atlantic and species of the subgenus *Bunostoma* from around the Pacific Basin – will further refine our understanding of how *Scaptomyza* have dispersed throughout the world.

Acknowledgments

The authors would like to thank the insightful and helpful advice of two anonymous reviewers. We thank the Hawaii Natural Area Reserves and Department of Fish and Wildlife for their help with permitting. Thanks to A. Gloss, K. Magnacca, B. Ort, and A. Gidaya for indispensible help with specimen collecting and sequencing. Thanks to both the Whiteman and O'Grady labs for advice and editing on a previous version of the manuscript. This work was supported by NIH K12 GM000708 PERT funds from the University of Arizona to RTL, Walker funds from the University of California, Berkeley, to RTL, NSF DEB0842348 awarded to PMO, University of Arizona Faculty Seed Grant to NKW, University of Arizona Center for Insect Science Seed Grant to NKW, and a Faculty Seed Grant to NKW, University of Arizona laboratory setup funds to NKW and NSF DEB 1256758 awarded to NKW.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.04.032.

References

Arnedo, M.A., Agnarsson, I., Gillespie, R.G., 2007. Molecular insights into the phylogenetic structure of the spider genus *Theridion* (Araneae, Theridiidae) and the origin of the Hawaiian *Theridion*-like fauna. Zool. Scr. 36, 337–352.

Baldwin, B.G., Sanderson, M.J., 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. USA, USA 95, 9402–9406.

Bellemain, E., Ricklefs, R.E., 2008. Are islands the end of the colonization road. Trends Ecol. Evol. 23, 461–468.

Bernays, E., 1998. Evolution of feeding behavior in insect herbivores. Bioscience 48, 35–44.

Bernays, E., Graham, M., 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69, 886–892.

Bollback, J.P., 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinform. 7, 88.

Bonacum, J., DeSalle, R., O'Grady, P.M., Olivera, D., Wintermute, J., Zilversmit, M., 2001. New nuclear and mitochondrial primers for systematics and comparative genomics in Drosophilidae. Drosophila Inform. Serv. 84, 201–204.

Bonacum, J., O'Grady, P.M., Kambysellis, M., DeSalle, R., 2005. Phylogeny and age of diversification of the planitibia species group of the Hawaiian Drosophila. Mol. Phylogenet. Evol. 37, 73–82.

Brncic, D., 1983. A review of the genus *Scaptomyza* Hardy (Diptera, Drosophilidae) in Chile with description of a new species. Revista Chilena de Historia Natural 56, 71–76.

Buerki, S., Forest, F., Alvarez, N., Nylander, J.A.A., Arrigo, N., Sanmartín, I., 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. J. Biogeogr. 38, 531–550.

Carlquist, S., 1974. Island Biology. Columbia University Press, New York and London.

Carson, H.L., 1997. Sexual selection: a driver of genetic change in Hawaiian Drosophila. J. Hered. 88, 343–352.

Chittenden, F.H., 1902. Some insects injurious to vegetable crops. Bulletin of the US Department of Agriculture, Division of Entomology.

Collinge, S.K., Louda, S.M., 1989. *Scaptomyza nigrita* Wheeler (Diptera: Drosophilidae), a Leaf Miner of the Native Crucifer, *Cardamine cordifolia* A. Gray (Bittercress). J. Kans. Entomol. Soc. 62, 1–10.

Conner, E.F., Taverner, M.P., 1997. The evolution and adaptive significance of the leaf-mining habit. Oikos 79, 6–25.

Cowie, R.H., Holland, B.S., 2008. Molecular biogeography and diversification of the endemic terrestrial fauna of the Hawaiian Islands. Philos. Trans. Royal Soc. Lond. B Biol. Sci. 363, 3363–3376.

Cox, G.W., 1999. Alien Species in North America and Hawaii. Island Press, Washington, DC.

Da Lage, J.L., Kergoat, G.J., Maczkowiak, F., Silvain, J.F., Cariou, M.L., Lachaise, D., 2007. A phylogeny of Drosophilidae using the Amyrel gene: questioning the *Drosophila melanogaster* species group boundaries. J. Zool. Syst. Evol. Res. 45, 47–63.

Darwin, C., 1859. On the Origin of Species by Means of Natural Selection, Or the Preservation of Favoured Races in the Struggle for Life. John Murray, London.

Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.

Evenhuis, N.L., Samuelson, A., 2007. The Insect and Spider Collections of the World Website of the Bishop Museum. Honolulu, Hawaii.

Farrell, B.D., 1998. "Inordinate Fondness" explained: why are there So many beetles? Science 281, 555–559.

Farrell, B.D., Sequeira, A.S., 2004. Evolutionary rates in the adaptive radiation of beetles on plants. Evolution 58, 1984–2001.

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

Fleischer, R.C., McIntosh, C.E., Tarr, C.L., 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K–Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Mol. Ecol., 533–545.

Garb, J.E., Gillespie, R.G., 2009. Diversity despite dispersal: colonization history and phylogeography of Hawaiian crab spiders inferred from multilocus genetic data. Mol. Ecol. 18, 1746–1764.

Gillespie, R.G., 2005. Geographical context of speciation in a radiation of Hawaiian Tetragnatha spiders (Araneae, Tetragnathidae). J. Arachnol. 33, 313–322.

Gillespie, R.G., Roderick, G.K., 2002. Arthropods on islands: colonization, speciation, and conservation. Ann. Rev. Entomol. 47, 595–632.

Gloss, A.D., Vassaõ, D.G., Schramm, K., Reichelt, M.,Hailey, A.L., Rast, T.J., Weichsel, A., Gershenszon, J., Montfort, W.R., Whiteman, N.K. (In preparation). Evolution of herbivory by co-option of an ancient detoxification pathway.

Grant, P.R., 1999. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton, New Jersey.

Grimaldi, D.A., 1990. A phylogenetic, revised classification of genera in the Drosophilidae (Diptera). Bull. Am. Mus. Nat. Hist. 197, 123–128.

Grimaldi, D., 1987. Amber fossil Drosophilidae (Diptera), with particular reference to the Hispaniolan taxa. Am. Mus. Novitates 1.

Hackman, W., 1982. The relation between the genera *Scaptomyza* and *Drosophila* (Diptera, Drosophilidae). Ann. Entomol. Fennicae 48, 97–104.

Hackman, W., 1959. On the genus *Scaptomyza* Hardy (Diptera, Drosophildae). Acta Zool. Fenn. 97, 3–73.

Harbaugh, D.T., Baldwin, B.G., 2007. Phylogeny and biogeography of the sandalwoods (Santalum, Santalaceae): repeated dispersals throughout the Pacific. Am. J. Bot. 94, 1028–1040.

Hardy, D.E., 1965. Diptera: Cyclorrhapha II, Series Schizophora, Section Acalypterae I, Family Drosophilidae. Insect. Hawaii 12, 1–814.

Hardy, J., 1849. Notes on the remedies of the turnip-fly amongst the ancients, a new genus (*Scaptomyza*) and species (*S. graminum* and *S. apicalis*) of Diptera. Proc. Berwicksh. Nat. Club. 2, 359–362.

Heaney, L.R., 2007. Is a new paradigm emerging for oceanic island biogeography? J. Biogeogr. 34, 753–757.

Hennig, W., 1966. Phylogenetic Systematics. Univ. Illinois Press, Urbana.

Holland, B.S., Cowie, R.H., 2007. A geographic mosaic of passive dispersal: population structure in the endemic Hawaiian amber snail *Succinea caduca* (Mighels, 1845). Mol. Ecol. 16, 2422–2435.

Holland, B.S., Cowie, R.H., 2009. Land snail models in island biogeography: a tale of two snails. Am. Malac. Bull. 27 (1–2), 59–68.

Iturralde-Vinent, M.A., MacPhee, R.D.E., 1996. Age and paleographical origin of Dominican amber. Science, 1850–1852.

Jordan, S., Simon, C., Polhemus, D., 2003. Molecular Systematics and Adaptive Radiation of Hawaii's Endemic Damselfly Genus *Megalagrion* (Odonata: Coenagrionidae). Syst. Biol. 52, 89–109.

Labandeira, C.C., 2005. Invasion of the continents: cyanobacterial crusts to tree-inhabiting arthropods. Trends Ecol. Evol. 20, 253–262.

Labandeira, C.C., Sepkoski, J.J., 1993. Insect diversity in the fossil record. Science 261, 310–315.

Lerner, H.L., Meyer, M., James, H., Hofreiter, M., Fleischer, R., 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Curr. Biol. 21, 1838–1844.

Maca, J., 1972. Czechoslovak species of the genus *Scaptomyza* Hardy (Diptera, Drosophilidae) and their bionomics. Acta Entomologica Bohemoslovaca 69, 119–132.

Magnacca, K.N., Danforth, B.N., 2006. Evolution and biogeography of the native Hawaiian Hylaeus bees (Hymenoptera: Colletidae). Cladistics 22 (5), 393–411.

Magnacca, K.N., Foote, D., O'Grady, P.M., 2008. A review of the endemic Hawaiian drosophilidae and their host plants. Zootaxa 1728, 1–58.

Malloch, J.R., 1932. New species and other records of Otitidae (Ortalidae), Piophilidae, clusiidae, chloropidae, and drosophilidae from the Marquesas. Bull. Bernice P. Bishop Mus. 98, 205–223.

Markow, T.A., O'Grady, P.M., 2006. *Drosophila:* A Guide to Species Identification and Use. Academic Press, London.

Markow, T.A., O'Grady, P., 2008. Reproductive ecology of *Drosophila*. Funct. Ecol. 22, 747–759.
Martin, N.A., 2004. History of an invader, *Scaptomyza flava* (Fallen, 1823) (Diptera: Drosophilidae). N.Z. J. Zool. 31, 27–32.
Mayr, E., Diamond, J., 2001. The Birds of Northern Melanesia. Oxford University Press, New York.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE) 2010, 1–8.
Mitter, C., Farrell, B.D., Wiegmann, B., 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? Am. Nat. 132, 107–128.
Montague, J.R., 1984. The ecology of Hawaiian flower-breeding drosophilids. I. Selection in the larval habitat. Am. Nat. 124, 712–722.
Morales-Hojas, R., Vieira, J., 2012. Phylogenetic patterns of geographical and ecological diversification in the subgenus *Drosophila*. PLoS One 7 (11), e49552.
Nylander, J.A.A., 2004. MrModeltest v2.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.
Obbard, D.J., Maclennan, J., Kim, K.W., Rambaut, A., O'Grady, P.M., Jiggins, F.M., 2012. Estimating the divergence dates and substitution rates in the *Drosophila* phylogeny. Mol. Biol. Evol. 29, 3459–3473.
O'Grady, P.M., Bonacum, J., Val, F.C., 2003. The placement of the *Engiscaptomyza, Grimshawomyia,* and *Titanochaeta*, three clades of endemic Hawaiian Drosophilidae. Zootaxa 159, 1–16.
O'Grady, P.M., Magnacca, K.N., Lapoint, R.T., 2010. Taxonomic relationships within the endemic Hawaiian Drosophilidae. Records of the Hawaii Biological Survey 108, 3–35.
O'Grady, P., DeSalle, R., 2008. Out of Hawaii: the biogeographic history of the genus *Scaptomyza* (Diptera: Drosophilidae). Biol. Lett. 4, 195–199.
O'Grady, P.M., Lapoint, R.T., Bonacum, J., Lasola, J., Owen, E., Wu, Y., DeSalle, R., 2011. Phylogenetic and ecological relationships of the Hawaiian *Drosophila* inferred by mitochondrial DNA analysis. Mol. Phylogenet. Evol. 58, 244–256.
O'Grady, P.M., Zilversmit, M., 2004. Phylogenetic relationships within the *haleakalae* species group inferred by molecular and morphological characters (Diptera: Drosophilidae). Bishop Mus. Bull. Entomol. 10, 117–134.
Okada, T., 1973. Descriptions of four new species of drosophilidae of the Bonins, with taxometrical analyses of the *Scaptomyza* species (Diptera). Kontyu 41, 83–90.
Ort, B.S., Bantay, R.M., Pantoja, N.A., O'Grady, P.M., 2012. Fungal diversity associated with Hawaiian *Drosophila* host plants. PLoS One 7, e40550.
Parham, J.F., Donoghue, P.C.J., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J.G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., et al., 2012. Best practices for justifying fossil calibrations. Syst. Biol. 61, 346–359.
Pons, J., Gillespie, R.G., 2004. Evolution of satellite DNAs in a radiation of endemic Hawaiian spiders: does concerted evolution of highly repetitive sequences reflect evolutionary history? J. Mol. Evol. 59, 632–641.
Price, J.P., Elliot-Fisk, D., 2004. Topographic history of the Maui Nui complex, Hawaii, and it's implications for biogeography. Pac. Sci. 58, 27–45.
Price, J.P., Clague, D.A., 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Philos. Trans. Royal Soc. Lond. B Biol. Sci. 269, 2429–2435.
Rambaut, A., Drummond, A.J., 2004. Tracer v1.4..
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Remsen, J., O'Grady, P.M., 2002. Phylogeny of Drosophilidae (Diptera), with comments on combined analysis and character support. Mol. Phylogenet. Evol. 24, 248–263.
Remsen, J., DeSalle, R., 1998. Character congruence of multiple data partitions and the origin of the Hawaiian Drosophilidae. Mol. Phylogenet. Evol. 9, 225–235.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rozen, S., Skaletsky, H., 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132, 365.
Rubinoff, D., 2008. Phylogeography and ecology of an endemic radiation of Hawaiian aquatic case-bearing moths (Hyposmocoma: Cosmopterigidae). Philos. Trans. Royal Soc. B 363, 3459–3465.
Rundell, R.J., Holland, B.S., Cowie, R.H., 2004. Molecular phylogeny and biogeography of the endemic Hawaiian Succineidae (Gastropoda: Pulmonata). Mol. Phylogenet. Evol. 31, 246–255.
Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. Trends Ecol. Evol. 24, 394–399.
Russo, C.A.M., Takezaki, N., Nei, M., 1995. Molecular phylogeny and divergence times of drosophilid species. Mol. Biol. Evol. 12, 391–404.
Shaw, K.L., 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA, 16122–16127.
Simberloff, D.S., Wilson, E.O., 1969. Experimental zoogeography of islands: the colonization of empty islands. Ecology 50, 278–296.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651.
Stalker, H.D., 1945. On the biology and genetics of *Scaptomyza graminum* Fallen (Diptera, Drosophilidae). Genetics 30, 266–279.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Tamura, K., Subramanian, S., Kumar, S., 2004. Temporal patterns of fruit fly (*Drosophila*) *evolution revealed by mutation clocks*. Mol. Biol. Evol. 21, 36–44.
Throckmorton, L.H., 1975. The phylogeny, ecology, and geography of *Drosophila*. In: King, R.C. (Ed.), Handbook of Genetics, vol. 3. Plenum, New York, pp. 421–469.
Throckmorton, L.H., 1966. The relationships of the endemic Hawaiian Drosophilidae. Univ. Tex. Publ. 6615, 335–396.
Tweedie, S., Ashburner, M., Falls, K., Leyland, P., McQuilton, P., Marygold, S., Millburn, G., Osumi-Sutherland, D., Schroeder, A., Seal, R., et al., 2009. FlyBase: enhancing *Drosophila* Gene Ontology annotations. Nucl. Acids Res. 37, D555–D559.
Van der Linde, K., Houle, D., Spicer, G.S., Steppan, J., 2010. A supermatrix-based molecular phylogeny of the family Drosophilidae. Genet. Res. 92, 25–38.
Wheeler, M.R., Takada, H., 1966. The Nearctic and Neotropical species of *Scaptomyza* Hardy (Diptera: Drosophilidae). Univ. Tex. Publ. 6615, 35–78.
Whiteman, N.K., Groen, S.C., Chevasco, D., Bear, A., Beckwith, N., Gregpry, T.R., Denoux, C., Mammarella, N., Ausubel, F.M., Pierce, N.E., 2011. Mining the plant-herbivore interface with a leafmining *Drosophila* of Arabidopsis. Mol. Ecol. 20, 995–1014.
Whiteman, N.K., Gloss, A.D., Sackton, T.B., Groen, S.C., Humphrey, P.T., Lapoint, R.T., Sønderby, I.E., Halkier, B.A., Kocks, C., Ausubel, F.M., et al., 2012. Genes involved in the evolution of herbivory by a leaf-mining, drosophilid fly. Genome Biol. Evol. 4, 788–804.
Wiens, J.J., 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52, 528–538.
Wiens, J.J., 2006. Missing data and the design of phylogenetic analyses. J. Biomed. Inform. 39, 34–42.
Wiens, J.J., Moen, D.S., 2008. Missing data and the accuracy of Bayesian phylogenetics. J. Syst. Evol. 46, 307–314.
Wilson, E.O., 1961. The nature of the taxon cycle in the Melanesian ant fauna. Am. Nat. 95, 169–193.