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Emerging Biodiversity: Diversification of the Hawaiian Nesosydne planthoppers

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Emerging Biodiversity: Diversification of the Hawaiian *Nesosydne* planthoppers

By

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Professor Stephen C. Welter, co-Chair
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Abstract

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Large species radiations provide exceptional opportunities for understanding the processes involved in the formation of new species. This research is focused on a species-rich lineage of ecologically diversified planthoppers in the Hawaiian islands, *Nesosydne* (Hemiptera: Delphacidae). In order to examine the factors promoting diversification in this lineage at multiple slices through evolutionary time, I used an integrative approach combining three classes of methods. First, I used molecular phylogenetics and comparative methods to characterize the features of the *Nesosydne* adaptive radiation. I found that the Hawaiian *Nesosydne* are ecologically divergent and have undergone substantial within-island diversification. Second, I used population genetics and phylogeographic approaches in order to characterize within-species genetic structure. I found that the species *Nesosydne chambersi* comprises a very recently diverged, highly structured set of populations that I hypothesize to be in the early stages of diversification. Geographic isolation due to natural fragmentation from volcanic activity, not ecological adaptation to different host plant species, appears to be associated with population structure in *N. chambersi*. In addition, I documented a stable zone of secondary contact, which suggests that partial reproductive isolation develops rapidly between populations. Finally, using methods from animal communication, I established that: a) vibratory sexual signals in *N. chambersi* vary among populations on small temporal and spatial scales, b) sexual signal divergence occurs both in the absence and presence of ecological shifts, and c) signal divergence is evident in the zone of secondary contact in a pattern consistent with reproductive character displacement.

Based on my findings, I propose that the Hawaiian *Nesosydne* radiation represents an unusually clear case study in which the initial divergence within species is decoupled from the ecological diversification observed in the phylogeny. Species within the lineage first fracture into multiple genetic pools under the influence of geographic isolation. Sexual signals then shift among populations and they become reproductively isolated. This leads to the formation of populations that are set on independent evolutionary trajectories where

they are free to either differentiate through adaptive means, differentiate through non-adaptive means, or go extinct. In other words, the formation of multiple reproductively isolated genetic pools results in a set of incipient species that are primed to radiate, given the appropriate conditions – but ecological forces are not responsible for the initial divergence. In a dynamic landscape such as the Hawaiian islands, plant and arthropod communities are both assembling and diversifying in concert. Consequently, the diversity of host plants used by the Hawaiian *Nesosydne* may best be explained by speciation first followed by a process of ecological fitting during a period of ecological opportunity as plant communities establish in novel terrain. Despite repeated observations of ecological diversification from classic adaptive radiations and plant-insect systems representing a wide variety of taxonomic groups, it is difficult to pinpoint with certainty the initial causes of divergence in any of these groups. The Hawaiian *Nesosydne* offer a rare vantage point into this enigmatic phase of diversification.

For my family

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Introduction

Large species radiations provide exceptional opportunities for understanding the formation of species. The study of adaptive radiation has focused primarily on the phylogenetic scale examining differences among species (Schluter, 2000), and this approach has contributed enormously to our understanding of macroevolutionary patterns, providing insight into the processes accompanying diversification and extinction (e.g., Baldwin and Sanderson, 1998; Gillespie, 2004; Losos, 2009; Mahler et al., 2010; Rabosky, 2009; Schluter, 2000). However, focusing on differences among species necessarily obscures the details of dynamics occurring within species at the earliest stages of divergence – within the tips of phylogenetic trees. What promotes the initial stages of adaptive radiation is very poorly understood (Schluter, 2000).

There are a variety of ways that speciation may relate to ecological diversification (Rundell and Price, 2009). The classic conception of adaptive radiation (Rundle and Nosil, 2005; Schluter, 2000, 2009) assumes that both speciation and subsequent diversification is driven by divergent ecological selection. Under this model, gene flow would be disrupted by divergent natural selection or some forms of sexual selection in alternate selective environments (Rundle and Nosil, 2005) – or ecological selection followed by adaptive differentiation. However, an alternative, and one that would produce the same signature of ecological diversification at the phylogenetic level, is that the disruption of gene flow and subsequent speciation may occur prior to diversification into divergent selective environments as a result of geographic isolation or some other non-adaptive force (Rundell and Price, 2009) – or non-ecological speciation followed by adaptive differentiation. Distinguishing these alternatives is difficult in practice, and requires either (1) a study system in which speciation and diversification were decoupled for a sufficiently long period of time that the effects may be discriminated using dated molecular phylogenies (Rundell and Price, 2009), (2) an experimental evolution system (Kassen, 2009), or (3) a system that offers an ecologically diversified lineage in which species are presently in a variety of stages of diversification and thus may be observed directly – the option I pursue in work presented here.

Despite the known importance of geographic isolation, natural selection and/or sexual selection (Dobzhansky, 1937; Mayr, 1963) in promoting diversification among populations, their relative roles in the speciation process are still not well understood (Coyne and Orr, 2004; Ramsey et al., 2003). For example, while empirical and theoretical work have demonstrated that divergent natural selection can drive the development of reproductive isolation (Berlocher and Feder, 2002; Kirkpatrick and Ravigne, 2002; Via, 2001), debate continues as to whether (or how much) geographic isolation is necessary in the early stages of speciation, as well as its role relative to natural selection in driving divergence (Coyne and Orr, 2004; Grant and Grant, 2008; Mayr, 1959). In addition to natural selection, factors associated with sexual selection (i.e., female choice and male-male competition) can accelerate or refine the process of speciation (West-Eberhard, 1983).

Effects of geographic isolation are hypothesized to be a prevalent feature in diversification (Kirkpatrick and Ravigne, 2002; Lack, 1947; Mayr, 1959, 1963) and as a

result, geographic isolation and associated genetic drift serves as a null expectation for what drives initial divergence between populations. In some groups, diversification can be explained well by simple geographic isolation. For example, diversification in Hawaiian *Orsonwelles* spiders appears to be entirely a result of limited dispersal to new islands and subsequent allopatric separation (Hormiga et al., 2003). However, natural selection with accompanying ecological specialization appears to be the most important driver in many species radiations (Schluter, 2000), with classic examples resulting in tight phenotype-environment correlations: e.g., Caribbean *Anolis* lizard limb length and perch size (Losos, 2009); Hawaiian *Tetragnatha* spiders body color and habitat background color (Gillespie, 2004); and Darwin's Galapagos finches beak size and seed size (Grant and Grant, 2008). The importance of divergence promoted by ecological factors is also supported by comparative studies that associate ecological shifts with increased species richness among lineages: e.g., enhanced diversification among phytophagous insects compared with their non-phytophagous sister groups (Farrell and Mitter, 1993; Farrell, 1998; Mitter et al., 1988). Similarly, sexual selection, particularly selection acting directly on courtship signals, may also be an important force in driving diversification (discussed in: West-Eberhard, 1983). Indeed, sexual selection appears to act in the apparent absence of natural selection, for example, in Hawaiian *Laupala* crickets (Mendelson and Shaw, 2005), *Habronattus* jumping spiders of the Sky Islands of the western US (Masta and Maddison, 2002), and sections of the Hawaiian *Drosophila* (Carson, 1997). Other evidence for the importance of sexual selection in diversification comes from comparative studies that show an association between indicators of the intensity of sexual selection and species richness: for example, groups of birds that have promiscuous mating systems are more speciose than their sister groups (Mitra et al., 1996).

While theory predicts that geographic isolation, natural selection, and sexual selection can each separately drive the evolution of reproductive isolation (review, Coyne and Orr, 2004), and studies are accumulating that convincingly demonstrate each process in nature, it is not well understood how these mechanisms interact with one another along the diversification pathway. Some authors have hypothesized, based on a survey of diversification in vertebrate groups, that there may be a predictable sequence to what promotes diversification at various stages of radiation, in which taxa diversify 1st in habitat, 2nd in trophic morphology and 3rd in communication (Salzburger, 2009; Streelman and Danley, 2003). Based on a summary of existing theoretical work on speciation, Gavrilets (2004) has suggested that that divergence in adaptive radiations proceeds through predictable stages, whereby taxa diversify: 1st between *macro*habitats, 2nd between *micro*habitats, 3rd between magic traits (traits that control both local adaptation and nonrandom mating), and 4th between traits that control survival and reproduction. In other words, both empirical and theoretical work suggest that holding all else constant, forces associated with ecology should be relatively more important early in radiations with those associated with sexual behavior following (Gavrilets and Losos, 2009). However, applying phylogenetic tools alone may not provide the resolution necessary to examine the forces acting early in diversification because of uncertainty inherent in estimating ancestral states for rapidly evolving traits (Losos, 2010; Oakley and Cunningham, 2000; Schluter et al., 1997).

Phytophagous insects in diversification studies

Plant-feeding insects are useful models for studies of resource-based divergence and speciation (Funk et al., 2002) because of their relative abundance in the wild, the ease with which they can be manipulated, and because their host associations represent an important and easily measured ecological variable. Plant diversity is clearly associated with the great diversity of phytophagous insects, which may include approximately 25% of all multi-cellular species (Strong et al., 1984). Analyses of the diversity of herbivorous clades versus their non-herbivorous sister clades (Mitter et al., 1988) demonstrate a significant association between phytophagy and species richness. Numerous plant-related factors have been shown in different systems to affect how herbivorous insects interact with their hosts, including adaptation to novel host plant chemistry leading to coevolutionary arms races between plants and insects (e.g. Erlich and Raven, 1964); community ecological effects such as enemy or competitor free space (e.g. Murphy, 2004; Singer and Stireman III, 2005); neural constraints in the ability to locate new hosts (e.g. Bernays, 2001); host plant assortative mating (e.g. Feder et al., 1994); and genetic variation in the ability to adapt to new hosts (e.g. Jaenike and Holt, 1991; Via, 1990).

Detailed work in understanding the role of host variation in insect speciation has concentrated on a few systems, including the apple maggot fly (e.g. Feder, 1998), the pea aphid (e.g. Via, 1999), *Enchenopa* treehoppers (e.g. Cocroft et al., 2010) and *Timema* walking sticks (e.g. Nosil, 2007). For many species with large geographic ranges such as these, it can be difficult to assess how diversification proceeds. Indeed, the near-ubiquitous presence of population substructure within species and the variation in ecological factors across a species' range guarantees that dynamics of diversification will vary throughout a single species (Thompson, 2005). For this reason, radiations of ecologically specialized insects on oceanic islands are well-suited to address issues associated with ecological divergence – they contain populations and species in various stages of evolutionary divergence within the same phylogenetic context in an explicit and time-calibrated geographic framework (Gillespie and Roderick, 2002; Roderick and Gillespie, 1998; Roderick and Percy, 2008).

Phytophagous insects and sexual signaling

Sexual behavior is one mechanism that may underlie rapid divergence in animals (Mendelson and Shaw, 2005; West-Eberhard, 1983), and many groups of plant-feeding insects rely on sexual signals to locate and choose mates. The role of sexual signaling has been shown to be important at different times in the divergence of populations and species of a variety of phytophagous insect groups. Rodriguez et al (2007) experimentally demonstrated that a herbivore's shift to a novel host plant can promote changes in the dynamics of sexual selection and suggested that this can serve to initiate divergence among populations in the early stages of the colonization of a novel environment. This is consistent with Endler's (1992) "sensory drive" hypothesis—as substrate-borne environments strongly constrain signal propagation, host plant shifts would lead to signal divergence (as novel environments distort male signals) before any preference divergence. Alternatively, Mendelson and Shaw (2005) suggest that shifts in sexual signals in Hawaiian *Laupala* crickets take place in the absence of ecological shifts, and that sexual

selection alone is the primary driving force behind the rapid speciation of this group. In addition, founder events and subsequent genetic drift are also thought to be able to interact with sexual selection to lead to rapid shifts in sexual signal and preference divergence between populations. This process may be particularly relevant to taxa in island systems where populations can be quite small (Coyne and Orr, 2004; Kaneshiro, 1980; Uyeda et al., 2009; Wagner and Funk, 1995).

Hawaiian Nesosydne planthoppers

Here I study a speciose lineage of ecologically diverse and specialized planthoppers in the largest genus in the Delphacidae (Hemiptera) family. Known only from islands in the eastern Pacific Ocean, a radiation of 82 species has occurred in the Hawaiian Islands where the remarkable species richness is associated with an extraordinarily diverse host range (Asche, 1997; Denno and Perfect, 1994; Roderick, 1997; Wilson et al., 1994; Zimmerman, 1948). Like other members of their family (Claridge, 1985a, b), they utilize acoustic signals transmitted through their host plants to locate and select mates (O'Connell, 1991).

The islands in the Hawaiian Archipelago are arrayed in an age progression from youngest in the southeast to oldest in the northwest, providing temporal evolutionary snapshots, and detailed work by the USGS on the dating of island ages (Price and Clague, 2002), and even soils and lava flows on the island of Hawaii (Trusdell et al., 1996), has provided an explicit temporal context both within and between islands, allowing me to relate divergence time estimates between populations based on molecular data to the local geological dynamics of the islands, providing much greater inferential power than is possible in ordinary landscapes.

In order to understand the relationship between speciation and ecological diversification, over the course of the following chapters I aim to describe the evolutionary context of the Hawaiian *Nesosydne* and evaluate the forces acting on populations early in the divergence process. These are ideas that are difficult to approach with only phylogenetic tools, and in the following chapters I present evidence by integrating methods from molecular phylogenetics and comparative methods (Chapter 2), population genetics, phylogeography (Chapter 3 & 4) and animal communication (Chapter 5).

Overall conclusions

Based on my findings, I propose that the Hawaiian *Nesosydne* radiation represents an unusually clear case study where speciation is decoupled from ecological diversification. Species within the lineage first fracture into multiple genetic pools under the influence of geographic isolation. Sexual signals then shift among populations and they become reproductively isolated – which forms populations that are set on independent evolutionary trajectories where they are free to either: differentiate through adaptive means, differentiate through non-adaptive means, or go extinct. In other words, the formation of multiple reproductively isolated genetic pools results in a set of incipient species that are primed to radiate, given the appropriate conditions – but ecological forces

are not responsible for the initial divergence. In a dynamic landscape such as the Hawaiian islands, plant and arthropod communities are both assembling and diversifying in concert. Consequently, the diversity of host plants used by the Hawaiian *Nesosydne* may best be explained by speciation first followed by a process of ecological fitting (Janzen, 1980) during a period of ecological opportunity as plant communities establish in novel terrain. Despite repeated observations of ecological diversification from classic adaptive radiations and plant-insect systems representing a wide variety of taxonomic groups, it is difficult to pinpoint with certainty the initial causes of divergence in any of them. The Hawaiian *Nesosydne* offer a rare vantage point into this enigmatic phase of diversification.

Literature cited

Asche, M., 1997, A Review of the Systematics of Hawaiian Planthoppers (Hemiptera: Fulgoroidea): Pacific Science, v. 51, p. 366-376.

Baldwin, B.G., and Sanderson, M.J., 1998, Age and rate of diversification of the Hawaiian silversword alliance (Compositae): Proceedings of the National Academy of Sciences of the United States of America, v. 95.

Berlocher, S.H., and Feder, J.L., 2002, Sympatric speciation in phytophagous insects: Moving beyond controversy?: Annual Review of Entomology, v. 47.

Bernays, E.A., 2001, Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation: Annual Review of Entomology, v. 46, p. 703-727.

Carson, H.L., 1997, Sexual Selection: A Driver of Genetic Change in Hawaiian *Drosophila*: Journal of Heredity, v. 88, p. 343-352.

Claridge, M.F., 1985a, Acoustic behavior of leafhoppers and planthoppers: Species problems and speciation, *in* Nault, L.R., and Rodriguez, J.G., eds., The Leafhoppers and Planthoppers: New York, John Wiley and Sons, p. 103-125.

—, 1985b, Acoustic Signals in the Homoptera: Behavior, Taxonomy, and Evolution: Annual Review of Entomology, v. 30, p. 297-317.

Cocroft, R.B., Rodriguez, R.L., and Hunt, R.E., 2010, Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects: Biological Journal of the Linnean Society, v. 99, p. 60-72.

Coyne, J.A., and Orr, H.A., 2004, Speciation: Massachusetts, Sinauer Associates, Inc., 545 p.

Denno, R.F., and Perfect, T.J., 1994, Planthoppers: Their ecology and management: New York, Chapman & Hall, 799 p.

Dobzhansky, T.G., 1937, Genetics and the origin of species: New York, Columbia Univerisy Press.

Endler, J.A., 1992, Signals, signal conditions, and the direction of evolution: The American Naturalist, v. 139, p. s125-s153.

Erlich, P.R., and Raven, P.H., 1964, Butterflies and plants: a study in coevolution: Evolution, v. 18, p. 586-608.

Farrell, B., and Mitter, C., 1993, Phylogenetic determinants of insect/plant community diversity, *in* Ricklefs, R.E., and Schluter, D., eds., Species Diversity in Ecological Communities: Chicago, University of Chicago Press, p. 263-266.

Farrell, B.D., 1998, "Inordinate Fondness" Explained: Why are there so many beetles?: Science, v. 281, p. 555-559.

Feder, J.L., 1998, The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation?, *in* Howard, D., and Berlocher, S.H., eds., Endless Forms: Species and Speciation: Oxford, Oxford University Press.

Feder, J.L., Opp, S., Wazlo, B., Reynolds, K., Go, W., and Spizak, S., 1994, Host fidelity as an effective premating barrier between sympatric raxes of the apple maggot fly: Proceedings of the National Academy of Sciences, v. 91, p. 7990-7994.

Funk, D.J., Filchak, K.E., and Feder, J.L., 2002, Herbivorous insects: model systems for the comparative study of speciation ecology: Genetica, v. 116, p. 251-267.
Gavrilets, S., 2004, Fitness Landscapes and the Origin of Species: Princeton, N.J., Princeton University Press.
Gavrilets, S., and Losos, J.B., 2009, Adaptive Radiation: Contrasting Theory with Data: Science, v. 323 p. 732-737.
Gillespie, R.G., 2004, Community Assembly Through Adaptive Radiation in Hawaiian Spiders: Science, v. 303, p. 356-359.
Gillespie, R.G., and Roderick, G.K., 2002, Arthropods on islands: colonization, speciation, and conservation: Annual Review of Entomology, v. 47, p. 595-632.
Grant, P.R., and Grant, B.R., 2008, How and Why Species Multiply: The Radiation of Darwin's Finches: Princeton, NJ, Princeton University Press.
Hormiga, G., Arnedo, M.A., and Gillespie, R.G., 2003, Speciation on a conveyor belt: sequential colonization of the Hawaiian islands by Orsonwelles spiders (Araneae: Linyphiidae): Systematic Biology, v. 52, p. 70-88.
Jaenike, J., and Holt, R.D., 1991, Genetic variation for habitat preference: evidence and explanations: American Naturalist, v. 137, supplement.
Janzen, D.H., 1980, On ecological fitting: Oikos, v. 45, p. 308-310.
Kaneshiro, K.Y., 1980, Sexual selection, speciation and the direction of evolution: Evolution, v. 34, p. 437-444.
Kassen, R., 2009, Toward a General Theory of Adaptive Radiation: Insights from Microbial Experimental Evolution: The Year in Evolutionary Biology 2009: Annals of the New York Academy of Sciences, v. 1168, p. 3-22.
Kirkpatrick, M., and Ravigne, V., 2002, Speciation by Natural and Sexual Selection: Models and Experiments: The American Naturalist, v. 159, p. S22-S35.
Lack, D., 1947, Darwin's Finches: Cambridge, Cambridge University Press.
Losos, J.B., 2009, Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles Berkeley, CA, University of California Press.
—, 2010, Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism: American Society of Naturalists E.O. Wilson Award Address: The American Naturalist, v. 175, p. 623-639.
Mahler, D.L., Revell, L.J., Glor, R.E., and Losos, J.B., 2010, Ecological opportunity and the rate of morphological evolution in the diversification of greater Antillean anoles: Evolution, v. Accepted Article.
Masta, S.E., and Maddison, W.P., 2002, Sexual selection driving diversification in jumping spiders: Proceedings of the National Academy of Sciences, v. 99, p. 4442-4447.
Mayr, E., 1959, Isolation as an evolutionary factor: Proceedings of the American Philosophical Society, v. 103, p. 221-230.
—, 1963, Animal species and evolution: Cambridge, MA, Harvard University Press.
Mendelson, T.C., and Shaw, K.L., 2005, Rapid speciation in an arthropod: the likely force behind an explosion of new Hawaiian cricket species is revealed: Nature, v. 433, p. 375-376.

Mitra, S., Landel, H., and Pruett-Jones, S., 1996, Species richness covaries with mating system in birds: Auk, v. 113, p. 544-551.
Mitter, C., Farrell, B., and Wiegmann, B., 1988, The phylogenetic study of adaptive zones: has herbivory promoted insect diversification? : American Naturalist, v. 132, p. 107-128.
Murphy, S., 2004, Enemy-free space maintains swallowtail butterfly host shift: Proceedings of the National Academy of Sciences, v. 101, p. 18048-18052.
Nosil, P., 2007, Synthesis: Divergent Host Plant Adaptation and Reproductive Isolation between Ecotypes of *Timema cristinae* Walking Sticks: The American Naturalist, v. 169, p. 151-162.
O'Connell, C., 1991, Species Barriers and Breakdowns in Hawaiian Planthoppers: Manoa, University of Hawaii.
Oakley, T.H., and Cunningham, C.W., 2000, Independent contrasts succeed where ancestor reconstruction fails in a known bacteriophage phylogeny: Evolution, v. 54, p. 397-405.
Price, J.P., and Clague, D.A., 2002, How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence: Proceedings of the Royal Society of London, B v. 269, p. 2429-2435.
Rabosky, D.L., 2009, Heritability of extinction rates links diversification patterns in molecular phylogenies and fossils: Systematic Biology, v. 58, p. 629-640.
Ramsey, J., Bradshaw Jr, H.D., and Schemske, D.W., 2003, Components of Reproductive Isolation Between the Monkeyflowers *Mimmulus lewisii* and *N. cardinalis* (Phrymaceae): Evolution, v. 57, p. 1520-1534.
Roderick, G.K., 1997, Herbivorous Insects and the Hawaiian Silversword Alliance: Coevolution or Cospeciation?: Pacific Science, v. 51, p. 440-449.
Roderick, G.K., and Gillespie, R.G., 1998, Speciation and phylogeography of Hawaiian terestrial arthropods: Molecular Ecology, v. 7, p. 519-531.
Roderick, G.K., and Percy, D.M., 2008, Host plant use, diversification, and coevolution: Insights from remote oceanic islands, *in* Tilmon, K.J., ed., Evolutionary Biology of Plant and Insect Relationships: Berkeley, University of California Press.
Rodriguez, R.L., Sullivan, L.M., Snyder, R.L., and Cocroft, R.B., 2007, Host shifts and the beginning of signal divergence: Evolution, v. 62, p. 12-20.
Rundell, R.J., and Price, T.D., 2009, Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation: Trends In Ecology & Evolution, v. 24, p. 394-399.
Rundle, H.D., and Nosil, P., 2005, Ecological speciation: Ecology Letters, v. 8, p. 336-352.
Salzburger, W., 2009, The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes: Molecular Ecology, v. 18, p. 169-185.
Schluter, D., 2000, The Ecology of Adaptive Radiation: Oxford, Oxford University Press, 288 p.
—, 2009, Evidence for Ecological Speciation and Its Alternative: Science, v. 323, p. 737-741.
Schluter, D., Price, J.P., Moores, A.O., and Ludwig, D., 1997, Likelihood of Ancestor States in Adaptive Radiation: Evolution, v. 51, p. 1699-1711.

Singer, M.S., and Stireman III, J.O., 2005, The tri-trophic niche concept and adaptive radiation of phytophagous insects: Ecology Letters, v. 8, p. 1247-1255.
Streelman, J.T., and Danley, P.D., 2003, The stages of vertebrate evolutionary radiation: Trends In Ecology & Evolution, v. 18, p. 126-131.
Strong, D.R., Lawton, J.H., and Southwood, R., 1984, Insects on Plants: Cambridge, Harvard University Press.
Thompson, J.N., 2005, The Geographic Mosaic of Coevolution: Chicago, The University of Chicago Press, 400 p.
Trusdell, F.A., Wolfe, E.W., and Morris, J., 1996, Digital Database of the Geologic Map of the Island of Hawaii: Data Series 144: USGS Series IMAP, Report Number 2524-A.
Uyeda, J.C., Arnold, S.J., Hohenlohe, P.A., and Mead, L.S., 2009, Drift promotes speciation by sexual selection: Evolution, v. 63, p. 583-594.
Via, S., 1990, Ecological genetics of herbivorous insects: the experimental study of evolution in natural and agricultural systems: Annual Review of Entomology, v. 35, p. 421-446.
—, 1999, Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice.: Evolution, v. 53, p. 1446-1457.
—, 2001, Sympatric speciation in animals: the ugly duckling grows up: Trends In Ecology & Evolution, v. 16, p. 381-390.
Wagner, W., and Funk, V., 1995, Hawaiian Biogeography: Evolution on a Hot Spot Archipelago: Washington DC, Smithsonian Institution Press, 467 p.
West-Eberhard, M.J., 1983, Sexual selection, social competition, and speciation: Quarterly Review of Biology, v. 58, p. 155-183.
Wilson, W.W., Mitter, C., Denno, R.F., and Wilson, M.R., 1994, Evolutionary patterns of host plant use by delphacid planthoppers and their relatives, *in* Denno, R.F., and Perfect, T.J., eds., Planthoppers: Their Ecology and Management: New York, Chapman & Hall, p. 7-113.
Zimmerman, E.C., 1948, Homoptera: Auchenorhyncha: Honolulu, HI, University of Hawaii Press.

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Chapter 1. Molecular Phylogeny of the Hawaiian *Nesosydne* (Hemiptera: Delphacidae): Ecological and Geographic Modes of Diversification

Introduction

Plant-feeding insects are excellent models for studies of adaptive radiation and ecological speciation (1998; Funk et al., 2002; Nyman et al., 2006; Rundle and Nosil, 2005) because plant diversity is clearly associated with the great diversity of phytophagous insects (Mitter et al., 1988). Island systems around the world have produced some fantastic examples of radiations of phytophagous insects whose diversity of host use appears to be associated with species proliferation (Roderick and Percy, 2008). Hypotheses as to what promotes host range expansion among phytophagous insects on islands include ecological opportunity and ecological release (Hutchinson, 1978; Wilson et al., 1994) and multiple colonizations (Asche, 1997; Roderick and Percy, 2008). However, although these factors may be involved in providing the appropriate starting conditions, they don't provide a satisfactory explanation for what drives the evolution of novel host associations.

Islands are notorious for promoting geographic isolation (Wagner and Funk, 1995), and it can be difficult to distinguish the effects of isolation and host switches on diversification in these systems. In two island plant-insect systems where these associations have been rigorously examined – psyllids in the Macronesian Islands (Percy, 2003a, b) and bark beetles in the Canary Islands (Jordal et al., 2004) – both groups of researchers concluded that geographic isolation was associated with diversification events in many more cases than host switches. This is an interesting result, and suggests that in the explicit geographic context that islands provide, it is possible to disentangle multiple forces acting together in the diversification of ecologically specialized lineages. It also suggests that the rapid proliferation of phytophagous insect species on oceanic islands begs a more detailed explanation than simply multiple colonizations or ecological release and opportunity. The *Nesosydne* planthoppers in the Hawaiian Islands utilize a remarkably wide diversity of host plants, making them a prime candidate for a group that has undergone adaptive radiation. In this study, I test for the association between diversification events, geographic isolation and host shifts in the *Nesosydne* phylogeny.

The Hawaiian Islands provide an ideal backdrop for the study of adaptive radiation because of their recent geologic history, extreme isolation and topographic complexity (Gillespie and Roderick, 2002; Roderick and Gillespie, 1998; Wagner and Funk, 1995). The islands have been forming in a linear temporal sequence, and plant communities in the islands are zoned by elevation and precipitation into predictable assemblages across the island chain (Wagner et al., 1999), providing replicated habitats on each island. The formation of each island has provided a period of ecological opportunity for the flora and fauna as communities assemble, which is predicted by the ecological theory of adaptive radiation to promote diversification (Gavrilets and Losos, 2009; Lack, 1947; Schluter, 2000). Indeed, a myriad of taxa have undergone radiation in these islands, e.g.:

Tetragnatha (Gillespie, 2004), silverswords (Baldwin and Sanderson, 1998), honeycreepers (Pratt, 2005), *Drosophila* (O'Grady and DeSalle, 2008; Wagner and Funk, 1995), demonstrating their efficacy as natural speciation and diversification factories.

Nesosydne is the largest genus (126 described species) in the planthopper family Delphacidae (Hemiptera). Its distribution is restricted entirely to the eastern Pacific islands: Juan Fernandez (7 species), Galapagos (7), Austral Islands (6), Society Islands (2), Marquesas (22) and the Hawaiian Islands (82) (Fennah, 1955; Fennah, 1958; Fennah, 1967; Zimmerman, 1948). The majority of the diversity within this genus is endemic to the Hawaiian islands, and its origin – continental or otherwise – is unknown. It has been suggested, based on morphology (Asche, 1997), that diversity in this group may actually be the result of several distinct colonization events. Although some notable adaptive radiations appear to include multiple colonizations in their histories (e.g.: Losos, 2009), one criteria for diagnosing an adaptive radiation is that the ecologically diversified forms have arisen from a common ancestor (Schluter, 2000). Across the high Hawaiian islands, this genus is distributed primarily as single island endemics (68 species – 83%: Zimmerman, 1948), suggesting that allopatry and limited gene flow among islands plays an important role in diversification.

Like other members of the Delphacidae family, species within the Hawaiian *Nesosydne* are highly host specific – feeding on phloem, and mating and ovipositing on only one or a couple of closely related host plants species (Asche, 1997; Denno and Perfect, 1994; Roderick, 1997; Wilson et al., 1994; Zimmerman, 1948). Seventy-one species (87%) are host specific, documented from a single host plant species (Zimmerman, 1948). However, *Nesosydne* are distinct from other members of their family in their exceptionally wide host range. Despite the host specificity of individual species, the set of species within *Nesosydne* from across the Hawaiian archipelago have been documented on a wide diversity of host plants from 28 different families, primarily dicots (Asche, 1997; Fennah, 1958; Hasty, 2005; Roderick and Metz, 1997; Wilson et al., 1994; Zimmerman, 1948). This pattern contrasts with host feeding patterns of the majority of delphacid planthoppers, which are also highly host specific but feed predominantly on grasses (Poaceae) and sedges (Cyperaceae) (Wilson et al., 1994). Thus, the pattern of host use in the Hawaiian *Nesosydne* suggests that shifts among divergent hosts could be associated with diversification in this group.

However, while host range within this genus is remarkably diverse, certain plant families serve as hosts to a disproportionate number of planthopper species (Asteraceae = 17, Gesneriaceae = 11, Campanulaceae = 10, Fabaceae = 4), indicating niche conservatism at some scale among members of this genus. Two hypotheses have proposed subclades associated with different host plant groups. Asche (1997) proposed that species associated with *Acacia koa* (Fabaceae), a dominant overstory tree throughout the Hawaiian islands, form a monophyletic clade (Asche's "koae" group). O'Connell (1991) suggested that diversification in this clade, which maintains a similar ecology across the islands, is dominated by shifts in both geography and sexual signals. Among the Asteraceae-feeding *Nesosydne,* a hypothesis of cospeciation has been proposed between this clade and plants in the Hawaiian silversword alliance (Roderick, 1997), which assumes that Asteraceae-

feeding is a conserved trait among species with this feeding mode. Neither idea has been tested using a multilocus phylogeny with broad taxonomic sampling.

In order to characterize the features of the *Nesosydne* adaptive radiation in the Hawaiian islands, I present the first molecular phylogenetic hypothesis for the genus within Hawaii and use it to determine: (a) the history of colonization to the islands, (b) whether there is a significant amount of within-island diversification, and (c) whether host shifts are a prominent feature associated with diversification. My results reveal a genus that is prone to diversification under the influence of both geographic (primarily within-island) and ecological forces (shifts and conservatism of host plant use).

Materials and Methods

Taxon Sampling

Planthopper specimens were collected into 95% ethanol in the field from Kauai, Oahu, Lanai, Molokai, Maui and Hawaii between 2000 and 2008 and were stored at -20°C at the University of California at Berkeley. Most areas with historical records of Hawaiian *Nesosydne* were visited during the course of the survey. An additional two *Nesosydne* species from the Society Islands were included in the analysis. Specimens were identified using Zimmerman (1948) as the primary reference, Asche (1998). Trees were rooted using *Leialoha* from the Hawaiian Islands as outgroups (Table 1).

DNA extraction, PCR amplification and Sequencing

DNA nucleotide sequence data were generated from three loci: cytochrome oxidase I (COI), 12S rDNA (12S), and wingless (Wg) (Table 2). I extracted genomic DNA using a QIAGEN DNeasy DNA extraction kit, following the manufacturer's protocol and performing a double elution step of 50μL each into a final volume of 100μL.

The CO1 and 12S PCR reactions were performed in 25 μL volumes with 2 μL DNA, 2.5μL of 10X PCR Buffer (Applied Biosystems), 5 μL Betaine (Sigma), 2 μL of 10 mM dNTPs (Promega), 1.25 μL of each primer (1:9 dilution), 2 μL of 25 mM $MgCl_2$ (Applied Biosystems), 0.2 μL of 5U/μL AmpliTaq® (Applied Biosystems) and 8.8 μL ddH_2O (Wg PCR recipes had the following minor alterations: 3 μL DNA, 1.5 μL of 25 mM $MgCl_2$ (Applied Biosystems), and 8.3 μL ddH_2O).

Thermal cycling for COI and 12S was performed using a touchdown protocol with an initial activation cycle at 96°C for 2.5 min. This was followed by 25 cycles of 30s denaturing at 96°C, 30s annealing through a touchdown series starting from 55°C (or 60°C) and stepping down 0.4°C per cycle, with 45s extension at 72°C. This was followed by 15 cycles of 30s denaturing at 96°C, 30s annealing at 45°C and 45s extension at 72°C. Thermal cycling was completed by a final extension for 7 min at 72°C. For Wg, thermal cycling was performed with an initial activation cycle at 96°C for 2.5 min. This was followed by 40 cycles of 30s denaturing at 95°C, 30s annealing at 56.3°C, and extension at 72°C. Thermal cycling was completed by a final extension for 7 min at 72°C.

Sequencing products were purified using *ExoSAP-IT* (USB Corporation, Cleveland, OH) and cycle sequenced using the primers described above. PCR products were cycle sequenced in both directions in 10 μL volume reactions: 2 μL of the cleaned PCR product, 1 μL BigDye v3.1 (Applied Biosystems) 1.5 μL 5X sequencing buffer, 0.4 μL primer (1:9 dilution), and 5.1 μL ddH20, purified using Sephadex (GE Healthcare, Piscataway, NJ) and visualized on an ABI 3730. Finally, raw sequences were edited and forward/reverse aligned using Sequencher 4.0 (Gene Codes Corporation, Ann Arbor, MI).

Sequence alignment

Sequences of the protein-coding loci, COI and Wg, were aligned in Sequencher 4.0 (Gene Codes Corporation, Ann Arbor, MI) and checked for coding consistency in MacClade (Maddison and Maddison, 2000). Codon positions were determined in MacClade (Maddison and Maddison, 2000) using the *Drosophila* mitochondrial (COI) and universal (Wg) genetic codes by choosing to minimize the number of stop codons in each locus. 12S was aligned using MUSCLE (Edgar, 2004). Gaps were treated as missing data in the phylogenetic analysis, and all the alignments were unambiguous.

Phylogenetic analysis

Phylogenetic analyses were conducted using two different optimality criteria: (1) maximum likelihood (ML) using the Randomized Axelerated Maximum Likelihood v.7.0.4 (RAxML) algorithm (Stamatakis, 2006) and (2) Bayesian Inference (BI) using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The ML analysis used a general time-reversible (GTR) model (Tavare, 1986) with gamma – distributed rate variation (Yang, 1994) across sites estimated for each data partition and1000 non-parametric bootstrap replicates. The gene sequences from the three loci were concatenated into one alignment and partitioned (1) by gene and by codon position (seven partitions), (2) by gene (three partitions), and (3) by whole data set (no partitions).

For the BI analysis, the best-fit model of sequence evolution for each data partition was selected using the Akaike Information Criterion (AIC; Akaike, 1973) calculated in Mr.Modeltest2.2 (Nylander, 2004). Models selected for each partition are listed in Table 3. There is persistent concern in the statistical phylogenetic community regarding the simultaneous inference of Γ and proportion of invariable sites (see discussion on pg. 20 of the RAxML manual, v. 7.0.4; Yang, 2006, pp. 113-114), so when the I+Γ model was chosen for a given partition using AIC, I compared the parameter distributions from the resulting analysis with the parameter distributions from a twin analysis that used the next best-scoring model selected by AIC but had only one type of among site rate variation (Table 4). I found no difference in the resulting topologies or parameter distributions, so for the final analyses the AIC-selected models were implemented.

In order to select the partitioning scheme that most accurately represented the sequence evolution in the data, I partitioned the data a priori and then selected the best strategy. I did this in two phases. First, I partitioned the data based on gene identity (COI, 12S and Wg) and within each of these gene classes, I created more partitions based on

constraints appropriate to that gene. For each of the two protein-coding genes, COI and Wg, three partitioning strategies were tested: (1) whole gene, (2) codon positions one and two together and position three modeled separately, and (3) three separate partitions within each gene. The structural gene 12S was treated as one partition. Second, I concatenated the data set and tested two alternative partitioning strategies: (1) the three genes, each with the best partitioning strategy from the first set of comparisons, and (2) the whole data set, unpartitioned.

A Bayesian analysis of each gene given each data partition was run for 15,000,000 generations with 4 independent runs each. Initial runs were performed using default parameters, but inspection of these runs revealed two issues: (1) bimodality of alpha and m parameter distributions, and (2) a large discrepancy between the total length (TL) parameter for the fully partitioned analyses (single gene – COI and Wg; concatenated data set) relative to analyses with fewer partitions (Table 4). I also compared these results with the TL parameter resulting from the ML analyses (Table 3) and concluded that the branches are growing too long in the fully partitioned Bayesian analyses. In order to compensate, I reduced the mean of the branch length prior (Brown et al., 2010; Marshall, 2010). This resulted in unimodal m parameter distributions and TL parameters that were on a similar scale between partitioning schemes as well as between tree-building methodologies. I unlinked model parameters and estimated them independently across partitions, and finally, in order to improve MCMC performance by achieving optimal levels of chain mixing (swap rates between .1 and .7), I also reduced the temperature prior from the default setting.

I used a Bayes Factor analysis (Brandley et al., 2005; Brown and Lemmon, 2007) in order to select the best partitioning scheme (Table 5). I calculated the harmonic means of the (log) tree likelihoods for each scheme tested in Tracer (Rambaut and Drummond, 2009; Suchard et al., 2001) and compared them using the cutoffs of: 2ln BF>=10 (good evidence against the competing hypothesis), 10<2ln BF>-10 (ambiguous, select least complex strategy), and 2ln BF <=-10 (good evidence for the competing hypothesis) (Brandley et al., 2005; Kass and Raftery, 1995). Based on these results, for my final analysis I created a concatenated alignment of the three genes with seven partitions (COI – 3 partitions; 12S – 1 partition; Wg – 3 partitions) (Table 6 – final Bayes block).

MCMC convergence diagnostics: For each of the BI analyses, I assessed stationarity within and convergence among each of the four runs using several complimentary approaches: (1) I checked the convergence metrics provided by Mr. Bayes (Huelsenbeck and Ronquist, 2001) – that the maximum standard deviation of split frequencies of any of the runs was under 0.05 and that the potential scale reduction factor for all parameters approached 1.0, (2) I compared the posterior probabilities of all splits for pairwise comparisons of each MCMC run using AWTY (are we there yet?) (Nylander et al., 2008), and (3) I plotted the log-likelihood values for each run, checked the effective sample sizes to ensure I had an adequate number of independent samples and examined the posterior distributions of all parameters using Tracer v.1.5 (Rambaut and Drummond, 2009) to ensure the distributions were smooth and not bimodal and that the traces were uniformly fuzzy and not jagged. I also used Tracer v.1.5 to determine the burn-in phase by assessing each run's plot of log-likelihood values over generations – I assumed stationarity

had been reached when the log likelihood values reached a stable plateau. Finally, I created a 50% majority rule consensus tree from the resulting 54,000 post burn-in trees (Bayesian tree).

Comparative Analyses: Is diversification associated with conservatism or shifts in islands & host use?

I performed all comparative analyses on the Bayesian tree pruned to include only a single representative from each taxa (comparative tree), except in a few cases where taxa have wide geographic distributions and the Bayesian tree indicated a large amount of genetic structure – thus maintaining them is informative as to evolutionary history. The comparative analyses were rooted using *Leialoha* from the Hawaiian Islands with outgroup character states coded as missing as there is no phylogenetic hypothesis for the ancestral state of this group. Ingroup terminal taxa were assigned five multistate traits representing (1) islands (six ingroup states), (2a) plant family (21 ingroup states), (2b) Asteraceae (2 ingroup states), (2c) Fabaceae (2 ingroup states), and (2d) Campanulaceae (2 ingroup states). Gesneriaceae was not included in a separate character analysis because there was limited sampling from this plant. I recorded characters in the field, with verification by botanists when necessary. It must be noted that treating Asteraceae as one character state is an extremely conservative way to classify these plants, because all Asteraceae feeding within the *Nesosydne* occurs across plants in the Hawaiian silversword alliance, which, with its extreme physiological and morphological diversity at the species level, is well known as one of the classic examples of adaptive radiation in plants (Schluter, 2000).

In order to infer the evolutionary history of geographic and host plant associations, first I reconstructed the history of character states over my comparative tree using maximum parsimony and maximum likelihood in Mesquite v.2.72 (Maddison and Maddison, 2006; Maddison and Maddison, 2009). Next, I tested for phylogenetic conservatism in each of the characters using randomizations (Maddison and Slatkin, 1991); I compared the number of observed steps under parsimony to the distribution of values for steps I obtained by reshuffling the character states on the tips of my comparative phylogeny 10,000 times. I expected that if there were conservatism in any of the characters examined, the observed number of steps to reconstruct its character states on the comparative tree would be fewer than would be expected if the character tested were not associated with the phylogeny.

In order to quantify the effect host plant shifts vs. tracking and within versus between island shifts on diversification, I evaluated each terminal node and tallied whether the descendents were on the same or different host plants and island. I then performed a 2-tailed Fisher's exact test. Three nodes were found to descend to a bifurcation that resulted in tips with either character state. These were analyzed both ways and then excluded from the analysis after finding that it does not change the outcome of the results.

Results

Species Sampling

Twenty-five species of *Nesosydne* were identified and included as ingroup specimens in this analysis, along with five *Aloha* and one *Nesothoe* species. The remaining 33 ingroup specimens are unidentified and require further taxonomic work (Table 1). Based on the structure of the phylogeny, I estimate that these unidentified specimens represent an additional 25 species. Although this only provides sampling of approximately 56% of the species that are described, it represents a significant sampling effort to most of the known historical locations of these species. Certainly some species are extant and have been missed in our sampling. However, the results indicate that many species are quite rare and some are possibly extinct (see discussion in Hasty, 2005). While collections from each of the Hawaiian islands are included, Maui and Hawaii are best represented in this sample, and thus my conclusions about geographic modes of diversification are derived from analysis of the fauna of these two islands.

Phylogenetic Results

Three gene fragments: COI (698 bp), 12S (333 bp) and Wg (327 bp) were amplified, yielding a total data set of 1358 bp.

Maximum Likelihood Analysis: The ML analysis of the total, 7 partition data set resulted in a tree with a -ln likelihood score of 10514.201 (Fig. 1). Outgroup branch lengths are long relative to the ingroup taxa, so Figure 1 shows relationships among the Hawaiian *Nesosydne* as the primary figure, with the inset depicting the shape of the total tree. Bootstrap values are shown next to nodes. Overall, support values were higher towards the tips of the tree and indicated less support toward the backbone.

Bayesian Analysis: The harmonic mean of the log likelihood for the Bayesian tree was -ln likelihood score of -10605.535±0.237 and very similar total length (TL: Table 3, Table 4) and topology to the ML tree – all groups identified in the ML analysis were recovered in the Bayesian analysis (Figure 2; it is important to note that the -ln likelihood scores reported for each analysis are calculated differently and are thus not directly comparable). Posterior probabilities are shown next to the nodes and indicate good support overall for the majority of the nodes, although there are some unresolved relationships within the tree.

My results demonstrate some issues with the taxonomy of the Hawaiian *Nesosydne,* suggested by the *Nesothoe* (H266) and *Aloha* (L001, H010, H012, H113, H114, H145) specimens nested within the tree (Figures 1 & 2). The phylogeny presented here is consistent with a single colonization of this lineage to Hawaii, based on the distance of the ingroup to the outgroup.

Comparative Results: Is diversification associated with conservatism or shifts in islands & host use?

Of the nodes on the comparative tree involving terminal diversification events, 13 involved host shifts, 14 involved host tracking (4 involved both), 21 involved within-island diversification and nine involved between-island diversification (1 involved both). Thus, both within and between host plant and island diversification events appear to contribute to diversification in this group. However, the results of the test for phylogenetic signal in geographic distribution and host plant association (Table 7) reveal that islands, host plant family (all plant families included), Asteraceae and Fabaceae all show significant phylogenetic signal, indicating that diversification has occurred more often within each of these characters than would be expected by chance, given the topology of the tree and the distribution of sampling. Although incomplete taxon sampling may have interfered with my inference of diversification modes, it is unlikely that the sampling, at least on Hawaii and Maui, is biased with respect to taxa resulting from within versus between island or host plant colonization.

Islands: The significant phylogenetic signal observed in this character (Table 7) indicates that diversification within islands is the dominant mode in the data set. However, it also appears that insects shifting to new islands accounts for a significant portion of the biodiversity of this group as well. Mapping the characters of island onto the comparative tree and performing maximum likelihood estimation of ancestral states revealed that most of the diversity on Hawaii is nested within the clade on Maui, but also indicates several back colonizations at the tips. Specimens from "Maui Nui" (Molokai, Lanai and Maui) all group together, consistent with the biogeographic history of this group of islands – and may either represent shifts among these islands or "within-island" diversification driven by fluctuating ocean levels (Figure 3).

Host plant: Overall, the character "plant family" is significantly associated with the comparative tree (Table 7), indicating some level of conservatism in this character, but each of the three plant families examined shows a different trend. Host plant conservatism is evident in the case of the Fabaceae-feeding species, whose feeding association is significantly associated with the phylogeny (Table 7) and appear to have colonized Fabaceae only once or twice (Figures 2 & 3). In contrast, the Campanulaceae-feeding species show no phylogenetic signal and appear to have independently colonized the Campanulaceae multiple times (Figures 2 & 3). Finally, the Asteraceae-feeding *Nesosydne* do show significant phylogenetic signal, but this character is distributed across the tree. The very large number of character states and high number of host shifts make inferring the ancestral host plant family of this group with any level of certainty unrecoverable (Schluter et al., 1997).

Relationship between host use and islands: Of the terminal nodes, 11 involved host tracking within islands, three involved host tracking between islands, eight involved host shifts within islands and five involved host shifts between islands (three involved both host shifts and tracking within islands and one involved host shifts and tracking between islands). Fisher's exact test of association between the two diversification modes revealed that there is no tendency towards one combination over the others ($p=0.4197$).

Discussion

Nesosydne has diversified substantially in the Hawaiian Islands, and here I developed a molecular phylogeny to examine what generates diversity in this group. My results reveal a lineage that is prone to diversification under the influence of both geographic and ecological forces, primarily occurring within islands and associated with both shifts in host use and conservatism.

Biogeography within the Hawaiian Islands

Island systems are excellent places to study diversification (Mayr, 1954), and whether it occurs as a result of within or between island colonization events is an important question to address when studying island radiations (Parent and Crespi, 2006). In this group, it appears that while both are evident, within-island diversification events dominate the pattern – providing the geographic context necessary for adaptive radiation.

Several additional patterns emerge from these data. First, it is clear that the bulk of Hawaiian (Big Island) species diversified from within a Maui clade, which is consistent with a progression rule wherein colonization trends from oldest to youngest islands (Wagner and Funk, 1995). However, back colonizations from younger to older islands are also evident, indicating that even if the overall pathway of colonization is from older to younger islands, it is far from a strict pattern. These organisms, even with their relatively low vagility, are able to disperse among islands and then diversify with some regularity. Finally, based on the distance of the ingroup to the outgroups, the structure of the Hawaiian *Nesosynde* (and the additional taxa found to be paraphyletic) phylogeny is consistent with a hypothesis of a single colonizing ancestor. However, this conclusion must be treated with caution as it has the potential to change in the future with additional intra and extra-Hawaiian sampling.

Open questions in Nesosydne biogeography

Data presented here cannot address two very compelling questions regarding the *Nesosydne,* but suggest them as fruitful future research directions. First, what are its continental origins and pan-Pacific pathways of colonization? Beevis and Gillespie (*in prep*) have generated a hypothesis of colonization syndromes for the Hawaiian biota (air transport, bird phoresy and rafting by ocean currents), with associated predictions for each syndrome. According to their predictions, the patterns observed in the Hawaiian *Nesosydne* are consistent with a syndrome of aerial colonization from the Americas, and their presence in the Galapagos and Juan Fernandez islands (Fennah, 1955, 1967) suggests a South American origin. Research in other genera of delphacid planthoppers has demonstrated that wing length and the corresponding ability to disperse, including long-distance migration, is a somewhat labile trait (Denno and Perfect, 1994; Denno and Roderick, 1990; Denno et al., 1991; Denno et al., 1996), and within the Hawaiian *Nesosydne*, there exists a spectrum from complete brachyptery to fully macropterous forms (Zimmerman, 1948). Thus, it is plausible that the islands were colonized by a migratory

ancestor that experienced the strong selection against dispersal ability typical for island biota (Carlquist, 1965). Second, the extreme diversity of host plant use in this genus on the Hawaiian islands is in stark contrast to the very limited host range observed in most continental genera of Delphacidae (Wilson et al., 1994), which suggests that this genus may be an excellent example of ecological release (Hutchinson, 1978). However, both questions demand a family-level phylogenetic hypothesis for the Delphacidae, which is under development but is not currently available (Cryan and Bartlett, *in prep*).

Host shifts and conservatism of Fabaceae and Asteraceae feeding

Although the tests of phylogenetic signal in host plant use by plant family indicated conservatism within the Fabaceae and the Asteraceae, Fabaceae-feeding appears to be the only truly conserved host plant association within the *Nesosydne* (discussed below). Furthermore, the number of diversification events characterized by a host shift versus host plant conservatism is almost exactly equal. Thus, in addition to some conservatism of host use, the evolution of the ability to use new hosts – with an associated loss of the use of the old host – must also be a characteristic of this group.

***Acacia koa* (Fabaceae):** These data provide support of Asche's (1997) hypothesis that there is a monophyletic "koae" group within this genus that feeds on *Acacia koa* (Fabaceae). Inspection of the phylogeny indicates that it is in a relatively derived position within the radiation, and that it includes specimens from Kauai, Maui and Hawaii – demonstrating that following initial colonization of this plant, dispersal and diversification occurred across the island chain within *A. koa*. Why is there such strong host plant conservatism in this clade relative to the other host plants used by the Hawaiian *Nesosydne*? *A. koa* is one of the two dominant overstory forest tree species in the Hawaiian islands, providing suitable habitat across wide elevation and precipitation gradients. Most other host plant species used by this group are either geographically limited, patchy in distribution, or both (Wagner et al., 1999). Thus, it may simply be a question of numbers and varied structure within this single host plant species (Denno, 1994) – *A. koa* provides the insects more opportunity. Expanded geographic sampling within this clade will help to elucidate patterns of diversification within this group.

The Hawaiian Silversword Alliance (Asteraceae): Treating Asteraceae as one character state is an extremely conservative way to classify these plants, because all Asteraceae feeding within the *Nesosydne* occurs across plants in the Hawaiian silversword alliance, which, with its extreme physiological and morphological diversity at the species level, is well known as one of the classic examples of adaptive radiation in plants (Schluter, 2000). As such, although I do observe significant host conservatism within this plant family, I believe the relationship of diversification to host use to be quite a bit more complicated.

Observations of the pattern of Asteraceae feeding within this group indicate that it is both significantly conserved (at the plant family level) and distributed in multiple places across the phylogeny. One of two models may explain this pattern: (1) it was produced by

multiple independent colonizations of the Asteraceae followed by diversification at low taxonomic levels, or (2) Asteraceae-feeding is a much more ancestral state and shifts occurred from Asteraceae plants to all the other plant families. As I stated above, the diversity of host plants used across this radiation and large number of host shifts among host plant families make inferring the ancestral host plant species with any degree of confidence, particularly at deep nodes, extremely difficult (Schluter et al., 1997). Consequently, it may never be possible to determine what the ancestor of this group fed on. However, either possibility does not change the overall conclusion much – shifts to new hosts occur with enough frequency that tight *Nesosydne*-Asteraceae associations do not exist in this group, except at low taxonomic levels. Accordingly, as suggested by Hasty (2005), host shifting must be included in an overall model of what drives diversification in this group. Cospeciation (Roderick, 1997) between plants in the silversword alliance and *Nesosydne* does not appear to be a major force explaining its diversity.

The relationship of ecological factors to speciation and diversification?

These data reveal that diversification in this group may arise by any combination of host or island shift or conservatism, but that host shifts and within-island speciation dominate the pattern. Thus, many of the expectations for adaptive radiation appear to be met in this lineage – diversification of a variety of ecologically specialized forms from a single colonizing ancestor in the face of ecological opportunity. However, other criteria for diagnosing adaptive radiation: the phenotype-environment correlation, trait utility and quantifying the speed of diversification have yet to be addressed in this group (Schluter, 2000).

Although it is clear that much of this lineage is ecologically differentiated, it is worth considering how involved the hosts themselves may have been in providing the divergent selection necessary to drive the initial diversification of the group. Does the pattern of ecological diversification apparent in the phylogeny truly divulge a history initiated by ecological speciation? If shifts among host plants were an easy proposition for *Nesosydne*, one would expect to see only one to a few generalist species. Instead, the lineage is comprised of numerous highly specialized forms – strongly indicating that gene flow between insect populations using the ancestral and novel host plants must somehow become disrupted easily upon colonization of new host plants.

Under an ecological speciation model, gene flow would be disrupted by divergent natural or some forms of sexual selection on alternate hosts (Rundle and Nosil, 2005). However, an alternative is that the disruption of gene flow and subsequent speciation may have occurred preceding diversification onto new hosts (non-ecological speciation followed by adaptive differentiation) as a result of geographic isolation or some other non-adaptive force (Rundell and Price, 2009). In this scenario, the diversity of host plant associations in the Hawaiian *Nesosydne* would best be explained by speciation first followed by a process of ecological fitting (Janzen, 1980) during a period of ecological opportunity as plant communities establish, providing open niche space. Data presented here cannot distinguish these alternatives. Elucidating the relationship between speciation

and adaptive diversification in this radiation will require an integrative research program aimed at understanding the dynamics of speciation at its earliest stages.

Literature Cited

Akaike, H., 1973, Information theory as an extension of the maximum likelihood principle, *in* Petrov, B.N., and Csaki, F., eds., Second International Symposium on Information Theory: Akademiai Kiado, Budapest.

Asche, M., 1997, A Review of the Systematics of Hawaiian Planthoppers (Hemiptera: Fulgoroidea): Pacific Science, v. 51, p. 366-376.

—, 1998, A review of the planthopper genus *Nesodryas kirkaldy* and related taxa (Homoptera: Fulgoroidea: Delphacidae). Proceedings of the Hawaiian Entomological Society, v. 33, p. 89-124.

Baldwin, B.G., and Sanderson, M.J., 1998, Age and rate of diversification of the Hawaiian silversword alliance (Compositae): Proceedings of the National Academy of Sciences of the United States of America, v. 95.

Brandley, M.C., Schmitz, A., and Reeder, T.W., 2005, Partitioned Bayesian Analyses, Partition Choice, and the Phylogenetic Relationships of Scincid Lizards: Systematic Biology, v. 54, p. 373-390.

Brown, J.M., Hedtke, S.M., Lemmon, A.R., and Lemmon, E.M., 2010, When Trees Grow Too Long: Investigating the Causes of Highly Inaccurate Bayesian Branch-Length Estimates: Systematic Biology, v. 59, p. 145-161.

Brown, J.M., and Lemmon, A.R., 2007, The Importance of Data Partitioning and the Utility of Bayes Factors in Bayesian Phylogenetics: Systematic Biology, v. 56, p. 643-655.

Carlquist, S., 1965, Island Life: Garden City, New York, The Natural History Press, 451 p.

Denno, R.F., 1994, Life history variation in planthoppers, *in* Denno, R.F., and Perfect, J.T., eds., Planthoppers: Their Ecology and Management: New York, Chapman & Hall, p. 163-215.

Denno, R.F., and Perfect, T.J., 1994, Planthoppers: Their ecology and management: New York, Chapman & Hall, 799 p.

Denno, R.F., and Roderick, G.K., 1990, Population Biology of Planthoppers: Annual Review of Entomology, v. 35, p. 489-520.

Denno, R.F., Roderick, G.K., Olmstead, K., and Dobel, H.G., 1991, Density-related migration in planthoppers: the role of habitat persistence: American Naturalist, v. 138, p. 1513-1541.

Denno, R.F., Roderick, G.K., Peterson, M.A., Huberty, A.F., Dobel, H.G., Eubanks, M.D., Losey, J.E., and Langellotto, G.A., 1996, Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers: Ecological Monographs, v. 66, p. 389-408.

Edgar, R.C., 2004, MUSCLE: multiple sequence alignment with high accuracy and high throughput: Nucleic Acids Research, v. 32, p. 1792-1797.

Farrell, B.D., 1998, "Inordinate Fondness" Explained: Why are there so many beetles?: Science, v. 281, p. 555-559.

Fennah, R.G., 1955, Delphacidae from Juan Fernandez (Homoptera: Fulgoroidea): Proc. R. ent. Soc. London (B), v. 24, p. 129-138.

—, 1958, Fulgoroidea of South-Eastern Polynesia: Transactions of the Royal Entomological Society of London, v. 110, p. 117-220.
—, 1967, Fulgoroidea from the Galapagos Archipelago: Proc Calif Acad Sci, v. 35, p. 53-102.
Funk, D.J., Filchak, K.E., and Feder, J.L., 2002, Herbivorous insects: model systems for the comparative study of speciation ecology: Genetica, v. 116, p. 251-267.
Gavrilets, S., and Losos, J.B., 2009, Adaptive Radiation: Contrasting Theory with Data: Science, v. 323, p. 732-736.
Gillespie, R.G., 2004, Community assembly through adaptive radiation in Hawaiian spiders: Science, v. 303, p. 356-359.
Gillespie, R.G., and Roderick, G.K., 2002, Arthropods on islands: colonization, speciation, and conservation: Annual Review of Entomology, v. 47, p. 595-632.
Hasty, G.L., 2005, Host-plant specialization affects species diversity and vulnerability to endangerment in a group of island-endemic phytophagous insects (Delphacidae, Hawaiian Islands): Berkeley, University of California at Berkeley.
Huelsenbeck, J.P., and Ronquist, F., 2001, MrBayes: Bayesian inference of phylogenetic trees, v. 17, p. 754-5.
Hutchinson, G.E., 1978, An Introduction to Population Ecology: New Haven, Connecticut, USA, Yale University Press.
Janzen, D.H., 1980, On ecological fitting: Oikos, v. 45, p. 308-310.
Jordal, B.H., Kirdendall, L.R., and Harkestad, K., 2004, Phylogeny of a Macronesian radiation: host-plant use and possible cryptic speciation in *Liparthrum* bark beetles: Molecular Phylogenetics and Evolution, v. 31, p. 554-571.
Kass, R.E., and Raftery, A.E., 1995, Bayes Factors: Journal of the American Statistical Association, v. 90, p. 773-795.
Lack, D., 1947, Darwin's Finches: Cambridge, Cambridge University Press.
Losos, J.B., 2009, Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles Berkeley, CA, University of California Press.
Maddison, W.P., and Maddison, D., 2006, StochChar: A package of Mesquite modules for stochastic models of character evolution.
Maddison, W.P., and Maddison, D.R., 2000, MacClade version 4: Analysis of phylogeny and character evolution: Sunderland, Massachusetts, Sinauer Associates.
—, 2009, Mesquite: a modular system for evolutionary analysis.
Maddison, W.P., and Slatkin, M., 1991, Null Models for the Number of Evolutionary Steps in a Character on a Phylogenetic Tree: Evolution, v. 45, p. 1184-1197.
Marshall, D.C., 2010, Cryptic Failure of Partitioned Bayesian Phylogenetic Analyses: Lost in the Land of Long Trees: Systematic Biology, v. 59, p. 108-117.
Mayr, E., 1954, Change of genetic environment and evolution, *in* Huxley, J., Hardy, A., and Ford, E., eds., Evolution as a process: London, U.K., Allen & Unwin, p. 157-180.
Mitter, C., Farrell, B., and Wiegmann, B., 1988, The phylogenetic study of adaptive zones: has herbivory promoted insect diversification? : American Naturalist, v. 132, p. 107-128.

Nylander, J.A.A., 2004, MrModeltest v2. Program distributed by the author: Evolutionary Biology Centre, Uppsala University.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., and Swofford, D.L., 2008, AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics: Bioinformatics, v. 24, p. 581-583.
Nyman, T., Farrell, B.D., Zinovjev, A.G., and Vikberg, V., 2006, Larval Habits, Host-Plant Associations, and Speciation in Nematine Sawflies (Hymenoptera: Tenthredinidae): Evolution, v. 60, p. 1622-1637.
O'Connell, C., 1991, Species Barriers and Breakdowns in Hawaiian Planthoppers: Manoa, University of Hawaii.
O'Grady, P., and DeSalle, R., 2008, Out of Hawaii: the origin and biogeography of the genus *Scaptomyza* (Diptera: Drosophilidae): Biology Letters, v. 4, p. 195-199.
Parent, C.E., and Crespi, B., 2006, Sequential Colonization and Diversification of Galapagos Endemic Land Snail Genus *Bulimulus* (Gastropoda, Stylommatophora): Evolution, v. 60, p. 2311-2328.
Percy, D.M., 2003a, Legume-feeding psyllids (Hemiptera, Psylloidea) of the Canary Islands and Madeira: Journal of Natural History, v. 37, p. 397-461.
—, 2003b, Radiation, diversity, and host-plant interactions among island and continental legume-feeding psyllids: Evolution, v. 57, p. 2540-2556.
Pratt, H.D., 2005, The Hawaiian Honeycreepers: New York, Oxford University Press.
Rambaut, A., and Drummond, A.J., 2009, Tracer v1.5, *in* http://beast.bio.ed.ac.uk/Tracer, A.f., ed.
Roderick, G.K., 1997, Herbivorous Insects and the Hawaiian Silversword Alliance: Coevolution or Cospeciation?: Pacific Science, v. 51, p. 440-449.
Roderick, G.K., and Gillespie, R.G., 1998, Speciation and phylogeography of Hawaiian terestrial arthropods: Molecular Ecology, v. 7, p. 519-531.
Roderick, G.K., and Metz, E.C., 1997, Biodiversity of Planthoppers (Hemiptera: Delphacidae) on the Hawaiian Silversword Alliance: Effects of Host Plant Phylogeny and Hybridisation: Memoirs of the Museum of Victoria, v. 56, p. 393-399.
Roderick, G.K., and Percy, D.M., 2008, Host plant use, diversification, and coevolution: Insights from remote oceanic islands, *in* Tilmon, K.J., ed., Evolutionary Biology of Plant and Insect Relationships: Berkeley, University of California Press.
Rundell, R.J., and Price, T.D., 2009, Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation: Trends In Ecology & Evolution, v. 24, p. 394-399.
Rundle, H.D., and Nosil, P., 2005, Ecological speciation: Ecology Letters, v. 8, p. 336-352.
Schluter, D., 2000, The Ecology of Adaptive Radiation: Oxford, Oxford University Press, 288 p.
Schluter, D., Price, J.P., Moores, A.O., and Ludwig, D., 1997, Likelihood of Ancestor States in Adaptive Radiation: Evolution, v. 51, p. 1699-1711.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P., 1994, Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a

compilation of conserved polymerase chain reaction primers: Annals of the Entomological Society of America, v. 86, p. 651-701.
Stamatakis, A., 2006, RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models: Bioinformatics, v. 22, p. 2688-2690.
Suchard, M.A., Weiss, R.E., and Sinsheimer, J.S., 2001, Bayesian selection of continuous-time Markov chain evolutionary models: Molecular Biology and Evolution, v. 18, p. 1001-1013.
Tavare, S., 1986, Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences, *in* Miura, R.M., ed., Annual Meeting of the American Association for the Advancement of Science, Symposium on Some Mathematical Questions in Biology: DNA Sequence Analysis, Volume 17, Lectures on Mathematics in the Life Sciences: New York, New York, The American Mathematical Society, p. 57-86.
Wagner, W., and Funk, V., 1995, Hawaiian Biogeography: Evolution on a Hot Spot Archipelago: Washington DC, Smithsonian Institution Press, 467 p.
Wagner, W.L., Herbst, D.R., and Sohmer, S.H., 1999, Manual of the flowering plants of Hawaii, revised edition: Honolulu, HI, Universiy of Hawaii Press, 1919 in 2 volumes p.
Wilson, W.W., Mitter, C., Denno, R.F., and Wilson, M.R., 1994, Evolutionary patterns of host plant use by delphacid planthoppers and their relatives, *in* Denno, R.F., and Perfect, T.J., eds., Planthoppers: Their Ecology and Management: New York, Chapman & Hall, p. 7-113.
Yang, Z., 1994, Maximum likelihood phylogenetic estimation from dna sequences with variable rates over sites: Journal of Molecular Evolution, v. 39, p. 306-314.
—, 2006, Computational Molecular Evolution: New York, Oxford University Press.
Zimmerman, E.C., 1948, Homoptera: Auchenorhyncha: Honolulu, HI, University of Hawaii Press.

Tables

Table 1 – Taxon and gene sampling included in this study. Samples used as outgroups indicated in gray. Collectors (Col.): GLH=G. Luke Hasty, KRG=Kari Roesch Goodman, PTO=Peter T. Oboyski, GKR=George K. Roderick. Genes used in this study: COI (Cytochrome Oxidase I), 12S (12S rDNA), Wg (Wingless)

ID	*Insect Genus species*	Island	Plant Family	*Plant Genus species*	Collection location	Latitude	Longitude	Col.	CO1	12S	Wg
H278	*Nesosydne naenae*	Kauai	Asteraceae	*Dubautia sp.*	Mohihi-Waialae Trail	22.112	-159.561	GLH	yes	yes	yes
H258	*Nesosydne koae*	Kauai	Fabaceae	*Acacia koa*	Kohua Trail	22.104	-159.624	GLH	yes	no	yes
H266	*Nesothoe terryi*	Kauai	Fabaceae	*Acacia koa*	Kohua Trail	22.104	-159.624	GLH	yes	yes	yes
H308	*Nesosydne ? (female)*	Kauai	Fabaceae	*Acacia koa*	Pihea Trail	22.153	-159.617	GLH	yes	yes	yes
H317	*Nesosydne koae*	Kauai	Fabaceae	*Acacia koa*	Kauhao Ridge Road	22.094	-159.624	GLH	yes	yes	no
H329	*?*	Kauai	Fabaceae	*Acacia koa*	Kolo Ridge Road	22.079	-159.677	GLH	yes	no	no
H346	?	Kauai	Fabaceae	*Acacia koa*	Kolo Ridge Road	22.079	-159.677	GLH	yes	no	yes
H318	*Nesosydne ?*	Oahu	Pandanaceae	*Freycinetia arborea*	Aiea Ridge Trail	21.407	-157.879	GLH	yes	no	no
H322	*Nesosydne pipturi*	Oahu	Urticaceae	*Pipturus albidus*	Manoa Cliffs Trail	21.337	-157.813	GLH	yes	yes	no
2306IM	*Nesosydne ?*	Maui	Aquifoliaceae	*Ilex anomala*	Pu'u Kukui	20.911	-156.592	KRG	yes	yes	yes
106DD	*Nesosydne bridwelli*	Maui	Asteraceae	*Dubautia menzeizii*	Haleakala	20.720	-156.187	KRG	yes	yes	yes
1106BP	*Nesosydne raillardicola*	Maui	Asteraceae	*Dubautia menzeizii*	Haleakala, gulch	20.758	-156.240	KRG	yes	no	yes
1506EA	*Nesosydne argyroxiphium*	Maui	Asteraceae	*Argyroxiphium sandwicensis*	Haleakala	20.709	-156.231	KRG	yes	yes	yes
18PTO132	*Nesosydne ?*	Maui	Asteraceae	*Dubautia sp.*	Paliku Ridge	20.719	-156.138	PTO	yes	no	no

2606IF	*Nesosydne ?*	Maui	Asteraceae	*Dubautia laxa*	Pu'u Kukui	20.911	-156.592	KRG	yes	yes	yes
306AD	*Nesosydne bridwelli/ osbornii*	Maui	Asteraceae	*Dubautia menzeizii*	Haleakala	20.754	-156.225	KRG	yes	yes	yes
3506HL	*Nesosydne eeke*	Maui	Asteraceae	*Argyroxiphium calignis*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
3606HO	*?*	Maui	Asteraceae	*Argyroxiphium grayanum*	Pu'u Kukui	20.890	-156.586	KRG	yes	no	yes
806BB	*Nesosydne raillardicola*	Maui	Asteraceae	*Dubautia menzeizii*	Haleakala, gulch	20.758	-156.240	KRG	yes	no	yes
2106IB	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia micrantha*	Pu'u Kukui	20.911	-156.592	KRG	yes	yes	yes
2406HY	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia grandiflora Munroi*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
2706HZ	*?*	Maui	Campanulaceae	*Lobelia glorimantus*	Pu'u Kukui	20.890	-156.586	KRG	yes	no	yes
3006HS	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia grandiflora grandiflora*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
3106IO	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia arborescens*	Pu'u Kukui	20.916	-156.593	KRG	yes	no	yes
3306IL	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia kakeana*	Pu'u Kukui	20.911	-156.592	KRG	yes	yes	yes
47KRG0708	*Nesosydne ?*	Maui	Campanulaceae	*Clermontia*	Waikaloa	19.900	-155.432	KRG	yes	no	yes
1406CE	*Nesosydne nigrinervis*	Maui	Epacridaceae	*Styphelia tameiameiae*	Haleakala crater	20.717	-156.160	KRG	yes	yes	yes
1006DR	*Nesosydne geranii*	Maui	Geraniaceae	*Geranium ?*	Haleakala	20.755	-156.223	KRG	yes	yes	yes
3406IC	*Nesosydne stenogynicola*	Maui	Lamiaceae	*Stenogyne kamehamehae*	Pu'u Kukui	20.909	-156.592	KRG	no	yes	yes
2006HT	*Nesosydne painu*	Maui	Liliaceae	*Astelia menziesiana*	Pu'u Kukui	20.890	-156.586	KRG	no	yes	yes

2906HP	*Nesosydne ?*	Maui	Myrtaceae	*Metrosideros polymorpha*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
L022	*Nesosydne ? (female)*	Maui	n.d.	*n.d.*	Waikamoi Preserve	20.784	-156.228	GLH	yes	yes	yes
L023	*Nesosydne amaumau*	Maui	n.d.	*n.d.*	Waikamoi Preserve	20.784	-156.228	GLH	yes	no	yes
2806IE	*?*	Maui	Plantaginaceae	*Plantago pachyphylla*	Pu'u Kukui	20.911	-156.592	KRG	yes	no	yes
3206HX	*Nesosydne ?*	Maui	Rubiaceae	*Coprosma (ochracea or pubens)*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
2206HV	*Nesosydne ?*	Maui	Smilacaceae	*Smilax melastomifolia*	Pu'u Kukui	20.890	-156.586	KRG	yes	yes	yes
L003	*Nesosydne ? (female)*	Molokai	Dennstedtiaceae	*Microlepia strigosa*	near Puuau	21.106	-156.868	GLH	yes	yes	yes
H203	*Nesosydne ? (female)*	Lanai	Dicksoniaceae	*Cibotium glaucum*	Munro Trail	20.818	-156.887	GLH	yes	yes	yes
H204	*Nesosydne ? (female)*	Lanai	Dicksoniaceae	*Cibotium glaucum*	Munro Trail	20.818	-156.887	GLH	yes	yes	yes
H205	*Nesosydne lanaiensis*	Lanai	Epacridaceae	*Styphelia tameiameiae*	Munro Trail	20.818	-156.887	GLH	yes	yes	yes
H206	*Nesosydne lanaiensis*	Lanai	Epacridaceae	*Styphelia tameiameiae*	Munro Trail	20.818	-156.887	GLH	yes	yes	yes
1206GH	*Nesosydne raillardiae*	Hawaii	Asteraceae	*Dubautia ciliolata*	Hilina Pali Rd, HIVO	19.369	-155.244	KRG	yes	yes	yes
13DPlantag	*Nesosydne ?*	Hawaii	Asteraceae	*Dubautia plantaginea*	Kohala Mountains	20.063	-155.728	KRG	yes	yes	yes
406GW	*Nesosydne chambersi*	Hawaii	Asteraceae	*Dubautia scabra*	Saddle Road	19.689	-155.455	KRG	yes	yes	yes
41DLIN	*Nesosydne raillardiae*	Hawaii	Asteraceae	*Dubautia linearis*	Pu'u Laau	19.753	-155.815	KRG	yes	no	no
43KCR45	*Nesosydne chambersi*	Hawaii	Asteraceae	*Dubautia ciliolata*	Pu'u Kawiiwi	19.817	-155.383	KRG	yes	yes	yes

48L2JUNE	*N. ?*	Hawaii	Asteraceae	*Dubautia arborea*	Pu'u Laau	19.840	-155.580	KRG	yes	no	yes
606GP	*Nesosydne raillardiae*	Hawaii	Asteraceae	*Dubautia scabra*	Manuka	19.180	-155.765	KRG	yes	no	no
706FM	*Nesosydne chambersi*	Hawaii	Asteraceae	*Dubautia ciliolata*	Mauna Loa Low	19.508	-155.396	KRG	yes	yes	yes
H00WH0961	*Nesosydne ?*	Hawaii	Asteraceae	*Dubautia ciliolata/ arborea*	Waipahoehoe	19.810	-155.399	KRG	yes	no	no
5Clermon	*Nesosydne ?*	Hawaii	Campanulaceae	*Clermontia?*	Ola'a	19.481	-155.280	KRG	no	yes	yes
L021	*Nesosydne ?*	Hawaii	Campanulaceae	*Clermontia sp.*	Puu Huluhulu	19.688	-155.465	GLH	yes	no	yes
206IS	*Nesosydne ipomoeicola*	Hawaii	Convolvulaceae	*Ipomoecola*	Kipuka Puaulu (Bird Park)	19.440	-155.304	KRG	yes	yes	yes
H015	*Nesosydne ipomoeicola*	Hawaii	Convolvulaceae	*Ipomoema sp.*	Manuka State Forest Reserve	19.121	-155.821	GLH	yes	yes	yes
H074	*Nesosydne ? (female)*	Hawaii	Convolvulaceae	*Ipomoema sp.*	Manuka State Forest Reserve	19.121	-155.821	GLH	yes	yes	yes
L001	*Aloha swezyi*	Hawaii	Cucurbitaceae	*Sicyos macrophyllus*	unnamed kipuka	19.687	-155.445	GLH	yes	no	yes
906HK	*Nesosydne koae*	Hawaii	Fabaceae	*Acacia koa*	Mauna Loa Road, HIVO	19.439	-155.309	KRG	yes	no	yes
H001	*Nesodydne pseudorubescens*	Hawaii	Fabaceae	*Acacia koa*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	yes
H002	*Nesosydne rubescens pele*	Hawaii	Fabaceae	*Acacia koa*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	yes
H095	*Nesosydne koae-phyllodii*	Hawaii	Fabaceae	*Acacia koa*	Puu Huluhulu	19.688	-155.465	GLH	yes	yes	yes
H101	*Nesosydne koae*	Hawaii	Fabaceae	*Acacia koa*	Puu Huluhulu	19.688	-155.465	GLH	yes	yes	yes
17PTOGer	*Nesosydne ?*	Hawaii	Geraniaceae	*Geranium sp.*	?	?	?	PTO	yes	yes	yes

42GER	*Nesosydne ?*	Hawaii	Geraniaceae	*Geranium sp.*	Waipahoehoe Hybrids	19.811	-155.399	KRG	yes	yes	no
H010	*cf.Aloha myoporicola*	Hawaii	Myoporaceae	*Myoporum sandwicense*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	yes
H012	*Aloha myoporicola*	Hawaii	Myoporaceae	*Myoporum sandwicense*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	yes
H013	*Aloha myoporicola*	Hawaii	Myoporaceae	*Myoporum sandwicense*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	yes
H073	*?*	Hawaii	Myoporaceae	*Myoporum sandwicense*	Puu Huluhulu	19.688	-155.465	GLH	yes	yes	yes
H114	*Aloha myoporicola*	Hawaii	Myoporaceae	*Myoporum sandwicense*	Puu Huluhulu	19.688	-155.465	GLH	yes	yes	yes
H145	*Aloha ipomoeae*	Hawaii	n.d.	*n.d.*	Humuula Saddle	19.684	-155.452	GLH	yes	yes	no
39PISONIA	*Nesosydne ?*	Hawaii	Nyctaginaceae	*Pisonia (brunoniana or sandwicensis)*	Kipuka Puaulu (Bird Park)	19.440	-155.304	KRG	yes	yes	yes
H079	*Nesosydne anceps*	Hawaii	Pandanaceae	*Freycinetia arborea*	Olaa State Forest	19.499	-155.224	GLH	yes	yes	yes
H082	*Nesosydne nigriceps*	Hawaii	Pandanaceae	*Freycinetia arborea*	Olaa State Forest	19.499	-155.224	GLH	yes	yes	yes
H084	*Nesosydne anceps*	Hawaii	Pandanaceae	*Freycinetia arborea*	Olaa State Forest	19.499	-155.224	GLH	yes	yes	yes
H005	*Nesosydne coprosmicola/ phyllostegiae*	Hawaii	Rubiaceae	*cf. Coprosma waimeae*	Hamakua Ditch	20.067	-155.670	GLH	yes	yes	yes
H008	*Nesosydne umbratica*	Hawaii	Urticaceae	*Pipturus albidus*	Hamakua Ditch	20.067	-155.670	GLH	yes	yes	yes
H044	*Nesosydne umbratica*	Hawaii	Urticaceae	*Pipturus albidus*	Hamakua Ditch	20.067	-155.670	GLH	yes	yes	yes
GJ008	*Nesosydne scorpinaca*	Tahiti, Society Islands	n.d.	*n.d.*	Mt. Marau	-17.600	-149.533	GKR	yes	no	yes

GJ009	*Nesosydne ?*	Moorea, Society Islands	nd	*nd*	Mt. Rotui	-17.500	-149.833	GKR	yes	no	no
H255	*Leialoha ohiae*	Kauai	Myrtaceae	*Metrosideros polymorpha*	Kohua Trail	22.104	-159.624	GLH	yes	yes	yes
H296	*Leialoha lehuae*	Kauai	Myrtaceae	*Metrosideros polymorpha*	Pihea Trail	22.153	-159.617	GLH	yes	yes	yes
H076	*Leialoha hawaiiensis*	Hawaii	Myrtaceae	*Metrosideros polymorpha*	Hamakua Ditch	20.067	-155.670	GLH	yes	no	yes

Table 2. Primers used in this study

Locus	Primer name	Primer sequence (5'-3')	Reference
Cytochrome Oxidase I	HCO-2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	(Simon et al., 1994)
	LCO-1490	GGT CAA CAA ATC ATA AAG ATA TTG G	(Simon et al., 1994)
12s rDNA	SR-N-14588 (12Sai)	AAA CTA GGA TTA GAT ACC CTA TTA T	(Simon et al., 1994)
	SR-J-14233 (12Sbi)	AAG AGC GAC GGG CGA TGT GT	(Simon et al., 1994)
Wingless	Wg-3	GGC TCG TGC ACG GTG AAG AC	(Hasty, 2005)
	Wg-4	GTG CAG TGACAG CGG TCG GTG	(Hasty, 2005)

Table 3. Results from RAxML runs: Partitioning strategies, Likelihoods, Total Tree Length (TL). All partitions used the GTR+G model.

Name	Partitioning strategy	Likelihood	TL
Cytochrome Oxidase I – COI			
LC1	Whole gene	-7173.509	1.91
LC3	Codon positions 1, 2, 3	-6857.785	2.11
Wingless - Wg			
LW1	Whole gene	-1324.14	0.35
LW3	Codon positions 1, 2, 3	-1209.81	0.67
All genes concatenated			
LA1	No partitions	-11207.33	1.69
LA7	7 partitions	-10514.20	1.82

Table 4. Results from Mr. Bayes runs: Partitioning strategies, Models used, Likelihoods, Total Tree Length (TL). C1-C3: Cytochrome Oxidase I (COI), W1-W3: Wingless (Wg), A1, A7a, A7b: concatenated CO1, 12S, Wg. Unless noted otherwise, all analyses below were performed with short branch length priors. The white side of the table presents the models selected by AIC and results from analyses of the different partitions. The grey side shows the results from twin analyses I performed using the model with the next-highest AIC score but only one type of among site rate variation (I or Γ). All parameter distributions were inspected in Tracer following each run, and no differences were observed, so I implemented the final analysis using the AIC-selected models. Partitioning strategy A7b with AIC-selected models was used in the final analysis.

Name	**Partitioning strategy**	**Partitioning strategies using AIC-selected models**			**Partitioning strategies using models with only one type of among site rate variation**		
		Models used	**Harmonic mean: (log) tree Likelihood**	**TL**	**Models used**	**Harmonic mean: (log) tree Likelihood**	**TL**
C1	Whole gene	GTR+I+Γ	-7255.888± 0.42	2.339	GTR+Γ	-7303.109± 0.203	2.58
C2	Codon positions 1+2, 3	HKY+I+Γ GTR+I+Γ	-6975.605± 0.218	2.588	HKY+I GTR+Γ	-6998.602± 0.227	2.581
C3	Codon positions 1, 2, 3	GTR+I+Γ HKY+I GTR+I+Γ	-6956.131± 0.222	2.577	GTR+Γ HKY+I GTR+Γ	-6960.907± 0.226	2.64
W1	Whole gene	GTR+I	-1445.703± 0.153	0.948	na	na	na
W2	Codon positions 1+2, 3	HKY+I F81	-1431.59± 0.171	0.935	na	na	na
W3	Codon positions 1, 2, 3	K80+I GTR+Γ F81	-1381.367± 0.156	1.132	na	na	na
A1	No partitions	GTR+I+Γ	-11260.959± 0.224	2.213	GTR+Γ	-11332.938± 0.26	2.55
A7a	7 partitions, *default branch length priors*	GTR+I+Γ HKY+Γ GTR+I+Γ GTR+Γ F81 K80+1 GTR+Γ	-10684.425 0.328±	12.899	GTR+Γ HKY+Γ GTR+Γ GTR+Γ F81 K80+1 GTR+Γ	-10709.152± 0.995	12.333
A7b	7 partitions, *short branch length priors*	GTR+I+Γ HKY+Γ GTR+I+Γ GTR+Γ F81 K80+1 GTR+Γ	-10605.535± 0.237	2.37	GTR+Γ HKY+Γ GTR+Γ GTR+Γ F81 K80+1 GTR+Γ	-10617.194± 0.257	2.408

Table 5. Bayes Factors comparisons (of partitioning strategies using AIC selected models from Table 4): table is arranged with most complex partitioning strategy first, comparisons are made between more complex vs. less complex strategies.

	C3	**C2**
C2	19.474	-
C1	299.757	280.283
	W3	**W2**
W2	50.223	-
W1	64.336	14.113
	A7b	**A7a**
A7a	78.89	-
A1	655.424	576.534

Table 6. Final Bayes block, partitioning strategy A7b.

```
BEGIN mrbayes;
log start filename=7parts.log replace;
CHARSET beginning=1-205;
CHARSET endCO1=659-698;
CHARSET CO11stpos  =  2-698\3;
CHARSET CO12ndpos  =  3-696\3;
CHARSET CO13rdpos  =  1-697\3;
CHARSET 12S  =  699-1031;
CHARSET wg1stpos=1033-1357\3;
CHARSET wg2ndpos=1034-1358\3;
CHARSET wg3rdpos=1032-1356\3;
TAXSET  outgroup  =  40-43 56 68 72;
TAXSET  ingroup  =  1-39 44-55 57-67 69-71 73-83;
partition parts=7: CO11stpos, CO12ndpos, CO13rdpos, 12S, wg1stpos, wg2ndpos, wg3rdpos;
set partition=parts;
lset applyto=(1) nst=6  rates=invgamma;
lset applyto=(2) nst=2  rates=propinv;
lset applyto=(3) nst=6  rates=invgamma;
lset applyto=(4) nst=6  rates=gamma;
lset applyto=(5) nst=1  rates=equal;
lset applyto=(6) nst=2  rates=propinv;
lset applyto=(7) nst=6  rates=gamma;
prset applyto=(all) ratepr=variable;
prset applyto=(1,2,3,4,6,7) statefreqpr=dirichlet(1,1,1,1);
prset applyto=(5) statefreqpr=fixed(equal);
prset applyto=(all) brlenspr=Unconstrained:Exponential(100);
unlink revmat=(all) shape=(all) pinvar=(all) statefreq=(all) tratio=(all);
mcmc ngen=15000000 printfreq=1000 samplefreq=1000 nchains=4 nruns=4 temp=0.04 savebrlens=yes;
log stop;
END;
```

Table 7. Results of phylogenetic constraint analysis. Significant results are marked with an asterisk and indicate that the characters are non-randomly distributed on the tree, considering the topology of the tree and number of observations of each character state in the data set. Analyses are based on 10,000 replicates.

Character	**# Ingroup States**	**Observed # of steps (parsimony)**	**Distribution of # of steps under character reshuffling** *median (range)*	**p-value**
Island	6	18	26 (22-29)	p<0.0004*
Plant Family	16	26	36 (33-38)	p<0.0001*
Asteraceae	2	9	14 (10-15)	p=0.0022*
Fabaceae	2	3	9 (6-9)	p<0.0001*
Campanulaceae	2	5	6 (4-6)	p=0.1812

Figures

Figure 1. Maximum Likelihood Tree showing ingroup taxa. Inset shows *Nesosydne* relative to outgroup (*Leialoha*).

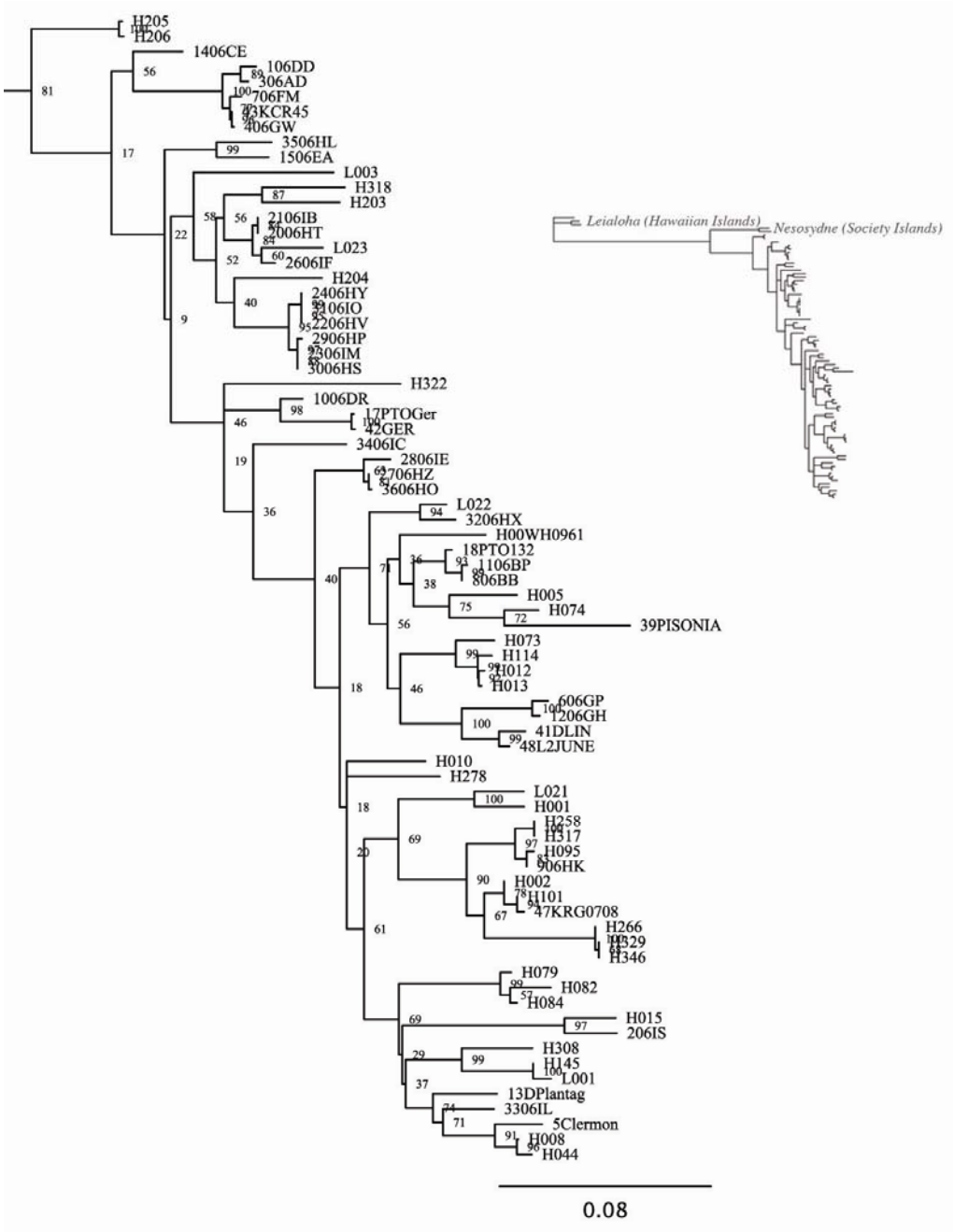


Figure 2. Consensus 50% majority rule Bayesian phylogram of the Hawaiian *Nesosydne* with islands denoted in colors and host plant families denoted with shapes (see inset legends). Posterior probabilities are near the nodes, and outgroup taxa are shown on the inset.

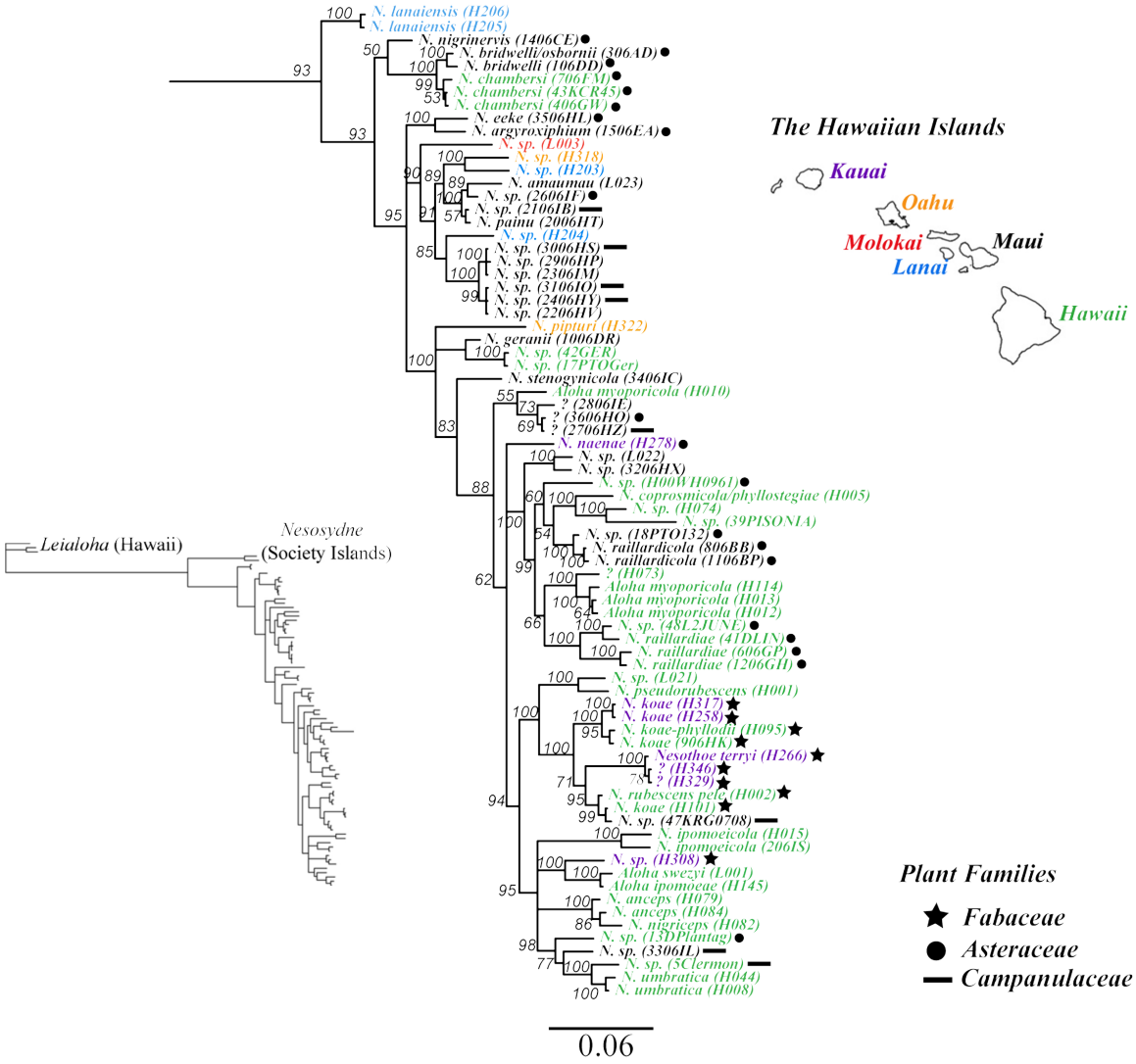
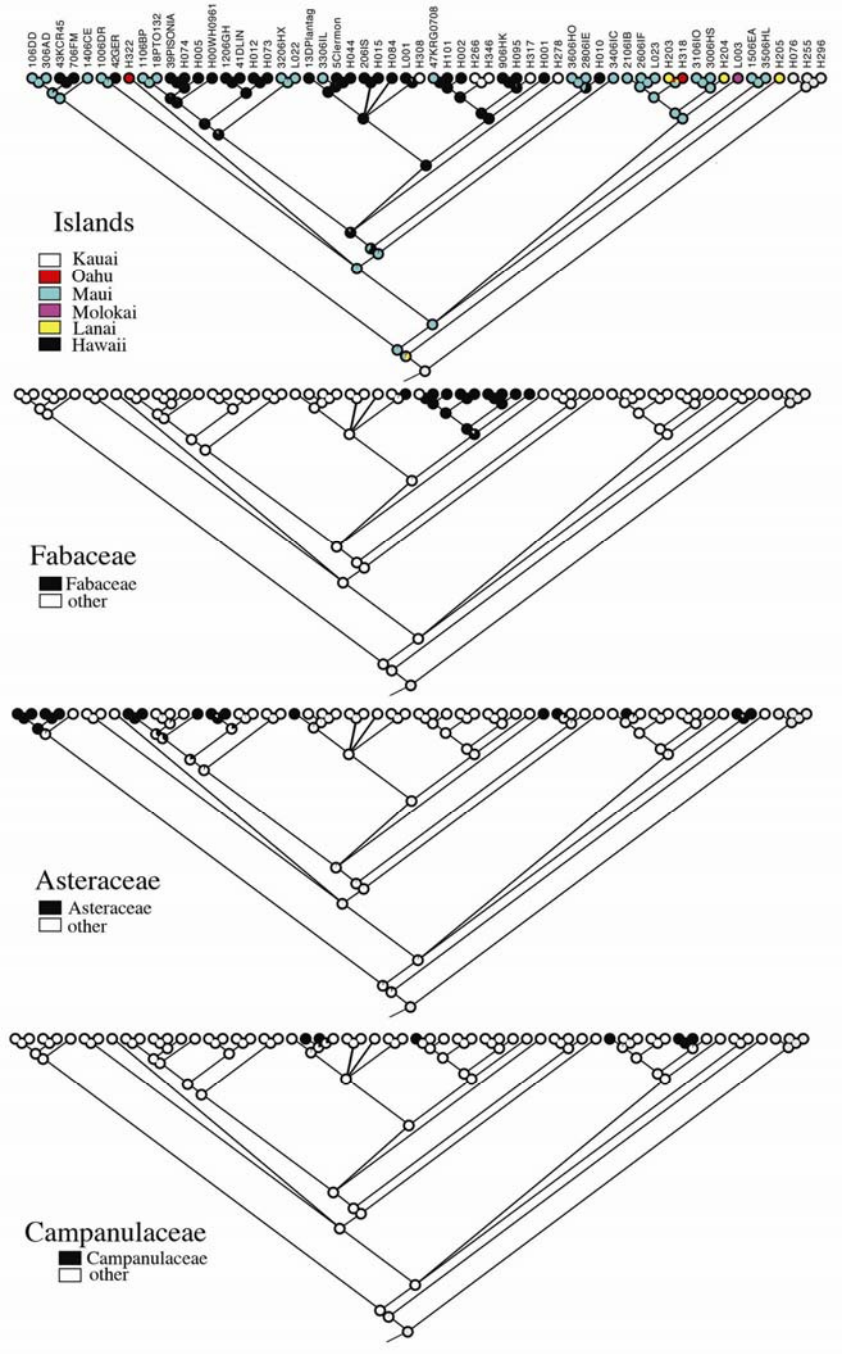


Figure 3. The 50% majority rule Bayesian phylogeny for the Hawaiian *Nesosydne* pruned for comparative analyses. Maximum likelihood estimates of ancestral states reconstructions were performed in Mesquite. Outgroup characters were coded as missing and are depicted here in gray on all trees.



Chapter 2. Isolation and characterization of microsatellite markers in an endemic Hawaiian planthopper (*Nesosydne chambersi:* Delphacidae)

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Abstract

We have isolated and characterized 17 microsatellite loci for the endemic Hawaiian planthopper *Nesosydne chambersi* (Delphacidae), a member of a large Hawaiian *Nesosydne* radiation. Thirty individuals from one population and 10 individuals from 2 populations across its range were tested to investigate polymorphism. The observed loci contained 2 - 9 alleles per locus. Expected heterozygosity within this species ranged from 0.2 to 0.85. These markers will be used to assess intraspecific differentiation and population structure within *N. chambersi*.

Introduction

The Hawaiian Islands have served as laboratories for evolutionary studies, in part because of rapid rates of diversification observed in many groups of species as a result of adaptive radiation (Gillespie and Roderick 2002; Wagner and Funk 1995). In many groups, this process has resulted in large, closely related lineages within the Hawaiian island chain: examples include the Hawaiian honeycreepers, plants in the silversword alliance, *Drosophila* flies, *Laupala* crickets, *Tetragnatha* spiders, and *Nesosydne* planthoppers. Population level studies using multiple nuclear markers, such as microsatellites, have the potential to provide detailed information about the demographic and ecological factors that lead to divergence and ultimately speciation within individual species. *Nesosydne chambersi* is a planthopper (Delphacidae) that specializes on members of another Hawaiian radiation: plants in the silversword alliance (Zimmerman 1948; Swezey 1954; Roderick 1997). This planthopper species has a relatively widespread distribution across both major volcanoes (Mauna Kea and Mauna Loa) on the island of Hawaii. Here, we report the isolation of microsatellite markers, which will be used to assess intraspecific differentiation and population structure within *N. chambersi*.

Nesosydne chambersi specimens were collected from *Dubautia ciliolata* ssp. *glutinosa,* in the Waipahoehoe Exclosure in the Mauna Kea Forest Reserve at approximately 9200 ft on the island of Hawaii (HI DOFAW Permit FHM07-143). Genomic DNA was extracted for the development of these primers using a QIAGEN DNeasy DNA extraction kit, following the manufacturer's protocol. DNA from one individual was used to construct a library enriched for microsatellite repeats, following the methods presented in Glen and Schable (2005) and briefly described below. Four hundred and fifty-two ng of extracted DNA was digested by RSAI (New England BioLabs), then ligated to superSNX linkers. This restriction-ligation mixture was hybridized to the following biotinylated oligonucleotides to probe for repeats: $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$; $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACAG)_6$ $(ACCT)_6$ $(ACTC)_6$ $(ACTG)_6$; $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ $(AGAT)_8$. These hybridized repeat fragments were captured using Dynabeads (Dynal) and recovered using polymerase chain reaction (PCR). A second enrichment was performed on the PCR product, and this amplified, doubly enriched DNA was cloned using a TOPO TA kit (Invitrogen), following the manufacturer's protocol. Successful transformations were selected using ampicillin and screened for inserts using b-galactosidase and 768 clones with inserts were isolated and grown up in deep well plates. Of these, 192 plasmids were sequenced with M13 forward and reverse primers using Big Dye version 3.1 (Applied Biosystems) on an ABI 3730 capillary sequencer.

Sequences were edited in Sequencher 4.0 (GeneCodes Corp., Ann Arbor, MI, USA). Microsatellite repeats were identified both visually and with the aid of Microsatellite Repeats Finder (Bikandi 2006). Primers were designed using PRIMER3 (Rozen & Skaletsky 2000). Finally, IDT's OligoAnalyzer 3.0 (www.idtdna.com/analyzer/Applications/OligoAnalyzer) was used to check for secondary structure and primer-dimer formation. A total of 60 unlabeled primers were ordered. These were tested for consistency in amplification and polymorphism on 2 to 3 individuals from 4 populations each by PCR amplification and visualization on 1.8% agarose gels stained with ethidium bromide, yielding 19 suitable primers.

Flourescent-labeled forward primers were ordered for each of these 19 and were tested using 30 individuals from a population on Mauna Kea (Pu'u Kanakaleonui), as well as on five individuals from two populations across its range (Saddle Road and Mauna Loa Trail). PCR solutions contained 2.5 μL of extracted DNA (10 ng/μL), 2.5 μL 10x PCR gold buffer (Applied Biosystems), 4mM MgCl2, 25μg/mL BSA, 0.52 μM each primer, 150 μM dNTPs, 10.325 μL ddH2O and 1U Taq polymerase (Invitrogen). Thermal cycling conditions consisted of an initial activation cycle at 95° C for 10 min. This was followed by two cycles of 1 min denaturing at 94° C, 1 min annealing at 60° C, and 35s extension at 70° C. Next were 18 cycles of 45 s denaturing at 93° C, 45 s annealing through a touchdown series starting from 59° C and stepping down 0.5° C per cycle, with 45 s extension at 70° C. This was followed by 20 cycles of 30 s denaturing at 92° C, 30 s annealing at 50° C and 1 min extension at 70° C. Thermal cycling was completed by a final extension for 5 min at 72° C. All loci amplify well using the same PCR conditions.

This PCR product was mixed in a solution of 0.5 μL PCR product, 9.215 μL of HiDi formamide and 0.285 μL of Liz500 size standard (Applied Biosystems), then run on

an ABI 3730 automated capillary sequencer. The genotypes were analyzed using GENEMAPPER version 3.0™ (Applied Biosystems)**.**

Testing of the 19 primers on 30 individuals of *Nesosydne chambersi* from Pu'u Kanakaleonui on Mauna Kea yielded 17 primer pairs that were polymorphic within the populations examined here and produced at most two alleles per individual per locus (Table 1). Numbers of alleles, allele size ranges and observed and expected heterozygosities (H_O and H_E) were calculated using Genepop (Raymond & Rousset 1995). The number of alleles per locus ranged from 2 to 9 and the expected heterozygosities ranged from 0.20 to 0.85 (Table 1). Genepop was also used to test for deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). Two loci (NC1 and NC12) were found to be significantly out of HWE after a correction for multiple comparisons (sequential Bonferroni correction: Rice 1989). Departure from HWE in locus NC1 may be due to the presence of null alleles. No locus pairs were in significant LD after applying the sequential Bonferroni correction (Rice 1989). Analysis of the five individuals each from the Saddle Road and Mauna Loa populations indicate that these loci amplify reliably and are polymorphic among populations.

In summary, the 17 loci reported here will be useful for investigating the population genetic structure among populations of *Nesosydne chambersi*, allowing a detailed assessment of the intraspecific differentiation within a diversifying lineage.

Acknowledgements

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References

Bikandi, J (2006) Microsatellite repeats finder. http://biophp.org/minitools/microsatellite_repeats_finder/demo.php. Accessed March 2007.

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

Glen, TC & Schable, NA (2005) Isolating microsatellite DNA loci. Methods in Enzymology, **395**, 202-222.

IDTDNA IDT's Oligo Analyzer version 3.0. http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/. Accessed April 2007.

Raymond, M & Rousset, F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, **86**, 248-249.

Rice, WR (1989) Analyzing Tables of Statistical Tests. Evolution, **43**, 223-225.

Roderick, GK (1997) Herbivorous Insects and the Hawaiian Silversword Alliance: Coevolution or Cospeciation? Pacific Science, **51**, 440-449.

Rozen, S & Skaletsky, H (2000) Primer 3 on the www for general users and biologist programmers. IN Krawetz, S. & Misener, S. (Eds.) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, N.J., Human Press.

Swezey OH (1954) Forest Entomology in Hawaii, Honolulu, HI, Bishop Museum Press.

Wagner, W & Funk, V (1995) Hawaiian Biogeography: Evolution on a Hot Spot Archipelago, Washington DC, Smithsonian Institution Press.

Zimmerman, EC (1948) Homoptera: Auchenorhyncha, Insects of Hawaii: volume 4. Honolulu, HI, University of Hawaii Press.

Tables

Table 1. Characteristics of seventeen microsatellite loci isolated from *Nesosydne chambersi*. Repeat motifs are those characterized from the cloned allele.

GenBank Accession no.	Primer	Sequence 5'- 3'	Repeat Sequence	Fwd primer label	Allele range (bp)	No. of Alleles	H_O	H_E
EU622228	Nc1	F: AACGCCTTCAGCCGTAATC	$(ACAG)_6$	NED	227-239	4	.13*	.59
		R: GGCGACAGGGCTATTTTCTA						
EU622229	Nc2	F: GACCGAGCTAAGTGAGGTCAT	$(GCTC)_4$	PET	201-259	9	.73	.75
		R: TGAAGGGTATTCTTTAATTTTCCTTT						
EU622230	Nc3	F: CATGAAACCAGCACTAGAGCAA	$(GTCT)_5$	VIC	157-165	3	.47	.42
		R: TCTTCATGCCAAATCTCACG						
EU622231	Nc4	F: TCGCGCAGTTCAGAAAGTAA	$(GACA)_4$	VIC	223-231	3	.30	.33
		R: CGCCTCGAACTGGAATAGAA						
EU622232	Nc5	F: CGTTGGGAACAGTCAGACAA	$(CTGT)_4$	VIC	191-207	5	.40	.50
		R: TCGAGCAGTCCAAAAAGGAT						
EU622233	Nc6	F: TCGACGCACAGTTCAAAAAG	$(GACA)_7$	6FAM	168-192	6	.75	.64
		R: TCCAAAAATTTTCCGAACAAAC						
EU622234	Nc7	F: TTCGAAAGTTATCGTCGAACA	$(AACA)_5$	NED	161-165	3	.48	.40
		R: CCTTTGAATCCGGTGTGAAC						
EU622235	Nc8	F: AGCCGTTCGAAAGTTATCGT	$(CAAA)_4(CAGA)(CGGA)(CAAC)$	VIC	146-172	6	.77	.71
		R: CACTTGAGGGTCCGCTGAT						

EU622236	Nc9	F: TACTGGCGTGTCTTGTGGTC R: GCGCAGTTCAAAAAGGAATC	$(GTCT)(GTTT)_3(GTCT)_4$	PET	196-216	5	.38	.38
EU622237	Nc10	F: TGCTTTCCTCCTCAATTCATC R: AACAAACAAGTGCCAATCACA	$(TTA)_5$	6FAM	96-99	2	.15	.20
EU622238	Nc11	F: ATGTGAACAGATCGGCCTTC R: GCCATTATCTCATATTCTGTTATTTGG	$(TGAG)_3$sequence$(GA)_3$sequence$(GA)_3$	NED	195-207	5	.27	.30
EU622239	Nc12	F: AAGGTATAGCCGTCCCATGA R: TCCCAACAACACGACAACAT	$(CTCA)_3(CGCT)(CA)_3(CTCA)(CT)_5$ sequence$(CA)_3$	PET	184-191	3	.77*	.49
EU622240	Nc13	F: GCGATGTATCCACATGAAACTC R: TCCCAATCTGGGATCTAAGC	$(AGATT)_4$	VIC	171-181	3	.20	.21
EU622241	Nc14	F: TATCTACGCGTTTGGCCGTA R: TCGACAGAAGAAATCAGGCTAA	$(ACAG)_5(ACAA)_3$	PET	217-253	6	.63	.72
EU622242	Nc15	F: ATTTGCGATTCGAGGTGACT R: CATTCCGCCAAGAAGTTGAT	$(GT)_4(TGAGT)_3$	NED	147-157	4	.55	.64
EU622243	Nc16	F: TGGAGCTTTCGAGCTAGACC R: CGCGAATATTCAGGAACGAA	$(TTC)_6$sequence$(TTC)_{15}$sequence$(TTC)_{16}$	PET	122-298	9	.68	.85
EU622244	Nc17	F: CGGGAGCTTTCCTTCTTATTTT R: TATTCCCTATGGTGCCAAGC	$(CTT)_{13}$	6FAM	136-183	9	.67	.68

N_A, number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity; * Significant deviation from Hardy-Weinberg Equilibrium with $\propto$=0.05 corrected for multiple comparisons using the sequential Bonferroni correction.

Chapter 3: Genetic divergence is decoupled from ecological diversification in the *Hawaiian* Nesosydne planthoppers

Introduction

Adaptive radiations provide some of the most compelling examples of adaptive divergence leading to speciation, but what promotes the initial stages is very poorly understood (Schluter, 2000, Losos, 2009). There are a variety of ways that speciation may relate to ecological diversification (Rundell and Price, 2009). The classic conception of adaptive radiation (Rundle and Nosil, 2005, Schluter, 2000, Schluter, 2009) assumes that both speciation and subsequent diversification is driven by divergent ecological selection. Under this model, gene flow would be disrupted by divergent natural or some forms of sexual selection in alternate selective environments (Rundle and Nosil, 2005) – or ecological selection followed by adaptive differentiation. Considerable evidence suggests that natural selection plays a major role in shaping the classic examples of tight phenotype-environment correlations we observe among species in adaptive radiations (Schluter, 2009, Schluter, 2000) – for example, Caribbean *Anolis* lizard limb length and perch size (Losos, 2009); Hawaiian *Tetragnatha* spiders body color and habitat background color (Gillespie, 2004); and Darwin's Galapagos finches beak size and seed size (Grant and Grant, 2008). However, an alternative, and one that would produce the same signature of ecological diversification at the phylogenetic level, is that the disruption of gene flow and subsequent speciation may occur prior to diversification into divergent selective environments as a result of geographic isolation or some other non-adaptive force (Rundell and Price, 2009) – or non-ecological speciation followed by adaptive differentiation. Distinguishing these alternatives is difficult in practice, and requires a study system that offers an ecologically diversified lineage in which species are presently in a variety of stages of diversification.

A significant role for divergence in allopatry has been documented among some of the best-studied examples of adaptive radiations. For example, in both African rift-lake cichlid fish (Sturmbauer et al., 2001) and Cuban *Anolis* lizards (Glor et al., 2004), respective lake and sea level fluctuations due to historical periods of climate caused fragmentation of habitats, are thought to have allowed for diversification in periods of allopatry. Similarly, volcanic activity is a powerful source of habitat fragmentation in remote oceanic archipelagos. It has been suggested that this fragmenting process provides the conditions under which remote volcanic islands act as "evolutionary crucibles" (Carson et al., 1990), promoting diversification through isolation in allopatry and providing at least a partial explanation for the extraordinary diversity observed in many island radiations (Gillespie and Roderick, 2002, Ricklefs and Bermingham, 2007, Roderick and Gillespie, 1998, Wagner and Funk, 1995).

Here, I examine the forces acting on populations in the very early stages of divergence – specifically, I ask whether geographic isolation via habitat fragmentation promotes diversification in the initial stages of speciation within an ecologically specialized radiation of Hawaiian planthoppers in the genus *Nesosydne*. I use the known geological history of the island of Hawaii to provide critical dates of habitat formation. The Hawaiian Archipelago arose in a time series as the Pacific plate moved over a hotspot in the central Pacific, generating a series of

massive volcanic islands that are arranged in a linear age progression from youngest in the southeast to oldest in the northwest (Wagner and Funk, 1995, Price and Clague, 2002). Each island is a composite of several shield volcanoes that formed sequentially. As new layers cooled, they provided substrate for colonization by biological taxa, which were then subject to repeated events of local extirpation and recolonization as the volcanoes grew. This process can be observed today on the youngest island, the island of Hawaii, where the still-flowing and highly fragmented landscape of Mauna Loa (Trusdell et al., 1996, Figure 1) gives us a snapshot of the geographic conditions under which each island formed. In this way, the real-time observations of conditions on Hawaii offer a realistic picture of the fragmented conditions under which many classic adaptive radiations originated.

The hypothesis that fragmentation due to volcano building activity on the island of Hawaii is the initial and dominant force promoting initial diversification in adaptive radiation makes two testable predictions: first, genetic variation in the species should be associated with geographical relationships rather than ecological features such as host plants; and second, the temporal scale of diversification should correspond to within-island geological events. To test these predictions, I use dense geographic and population-level sampling, temporal sampling in a zone of secondary contact, and a mitochondrial locus together with multiple independent nuclear loci to infer current population structure as well the timescales of divergence between populations in this species. My results reveal a species that has fractured into multiple genetic pools via geographic isolation due to the dynamic geologic activity of the island. This novel perspective provides a rare window into the forces acting on populations in the earliest stages of diversification in an adaptive radiation.

Materials and Methods

Study system: A radiation on a radiation on a volcano – Nesosydne chambersi*, the silversword alliance and the Big Island of Hawaii*

The planthopper genus *Nesosydne* (Delphacidae: Hempitera) is distributed throughout islands in the eastern Pacific. Eighty-two of its described species, the majority of its diversity, are endemic to the Hawaiian Islands (Zimmerman, 1948, Fennah, 1958, Asche, 1997). Like other delphacids, species of *Nesosydne* are highly host specific, feeding on phloem, and mating and ovipositing on only one or a couple of closely related host plant species. The majority of the species in the Hawaiian *Nesosydne* are specialized to a different host plant species – representing a total of 20 different plant families (Zimmerman, 1948, Wilson et al., 1994, Roderick and Metz, 1997, Hasty, 2005, Fennah, 1958, Asche, 1997). This incredible host plant diversity suggests a role for resource specialization in the diversification of this group.

The Hawaiian silversword alliance is the premier example of an adaptive radiation among plants (Schluter, 2000, Baldwin, 2006), within which the species provide divergent habitats for communities of native invertebrates (Drew and Roderick, 2005). On the Big Island of Hawaii, the single-island endemic planthopper, *Nesosydne chambersi* (Kirkaldy, 1908), is associated with two of the plants in the silversword alliance, *Dubautia ciliolata* (DC) D. Keck and *Dubautia scabra* (DC) D. Keck (Asteraceae). These two plant species are early colonists to the island's recently cooled lava flows. *Dubautia ciliolata* is undergoing a divergence event that appears to

be associated with the island's volcanic history. The species is currently classified into two subspecies: *D. ciliolata glutinosa* is found on the island's older high volcano, Mauna Kea (restricted to decomposed lava flows that date to 14,000-65,000 year-old) while *D. ciliolata ciliolata* is found on the younger high volcano, Mauna Loa (restricted to newer lava flow substrate 750 – 3000 years old) (Trusdell et al., 1996). The east face of Mauna Loa from the summit down to the area bridging Manua Loa and Mauna Kea (known as the Saddle region) is a matrix of different age lava flows (Figure 1), and *D. scabra* (Carr, 1985) is one of the first colonizing plants to the newest of these flows. *N. chambersi* is distributed across both volcanoes and through the Saddle region and feeds on all three plant types. Both the phylogenetic (Chapter 2, Hasty et al 2005) and dynamic geographic context of the new Big Island landscape suggests this species is in the early stages of diversification.

Collections and Sampling Design:

I collected *Nesosydne chambersi* specimens directly into 95% ethanol from 10 sampling sites across the island of Hawaii by beat sampling the plants: *Dubautia ciliolata glutinosa* (3 sites), *Dubautia ciliolata ciliolata* (4 sites) and *Dubautia scabra* (4 sites) (Table 1, Figure 1). At one site in the Saddle region between the two volcanoes, *Dubautia ciliolata ciliolata* and *Dubautia scabra* co-occur in a patchy matrix of different aged lava flows (Figure 4b, Trusdell et al., 1996).

Laboratory Methods:

DNA extractions: I extracted genomic DNA from multiple individuals (mean N=29, Table 2) from each of the 10 sampling sites using a QIAGEN DNeasy DNA extraction kit, following the manufacturer's protocol and eluting into a final volume of 100 µL.

Mitochondrial DNA sequencing: To determine the genealogical relationships among individuals across the island, I sequenced 653 base pairs of the mitochondrial gene region Cytochrome Oxidase I (COI) using the primers LCO 1490 and HCO 2198 (Simon et al., 1994). I performed all PCR reactions in 25 µL volumes with 2 µL DNA, 2.5µL of 10X PCR Buffer (Applied Biosystems), 5 µL Betaine (Sigma), 2 µL of 10 mM dNTPs (Promega), 1.25 µL of each primer (1:9 dilution), 2 µL of 25 mM $MgCl_2$ (Applied Biosystems), 0.2 µL of 5U/µL AmpliTaq® (Applied Biosystems) and 8.8 µL ddH_2O. I performed thermal cycling using a touchdown protocol, with an initial activation cycle at 96° C for 2.5 min. This was followed by 25 cycles of 30 s denaturing at 96° C, 30 s annealing through a touchdown series starting from 55° C (or 60° C) and stepping down 0.4° C per cycle, with 45 s extension at 72° C. This was followed by 15 cycles of 30 s denaturing at 96° C, 30 s annealing at 45° C and 45 s extension at 72° C, and was completed by a final extension for 7 min at 72° C. I purified sequencing products using *ExoSAP-IT* (USB Corporation, Cleveland, OH) and cycle sequenced using the primers described above. I cycle sequenced each PCR product in both directions in 10 µL volume reactions: 2 µL of the cleaned PCR product, 1 µL BigDye v3.1 (Applied Biosystems) 1.5 µL 5X sequencing buffer, 0.4 µL primer, and 5.1 µL ddH20, purified them using Sephadex (GE

Healthcare, Piscataway, NJ) and visualized the product on an ABI 3730. Finally, I edited raw sequences and aligned forward and reverse sequences using Sequencher 4.0 (Gene Codes Corporation, Ann Arbor, MI).

Microsatellite genotyping: To assess genetic variation and population structure, I genotyped an average of 29 (range: 25 – 39) individuals (Table 2) from each collecting site at 14 of the microsatellite loci: Nc3, Nc4, Nc5, Nc6, Nc7, Nc8, Nc9, Nc10, Nc11, Nc12, Nc13, Nc14, Nc15 and Nc17 (Goodman et al., 2008). I then performed PCR amplification and genotyping according to procedures described in Goodman et al (2008). Although sperm-dependent parthenogenesis has been documented within the family Delphacidae (Denbieman and Devrijer, 1987), it is not known whether any of the *Nesosydne* has a parthenogenic lifestyle. Therefore, I checked the dataset was checked for the presence of clonal genotypes using GIMLET (Valiére, 2002) prior to running any further analyses. I used MICROCHECKER to check for scoring errors due to the presence of null alleles (Oosterhout et al., 2004).

Statistical Analyses:

Mitochondrial Diversity: I used ARLEQUIN version 3.1 (Excoffier and Schneider, 2005) to calculate diversity statistics. I identified redundant sequences using MacClade (Maddison and Maddison, 2000) and removed sequences to create an alignment containing only unique haplotypes. To determine relationships among haplotypes across sampling sites, intraspecific relationships were reconstructed using a median – joining network using Network 4.5.1.6 (Bandelt et al., 2000). Finally, I calculated average uncorrected pairwise sequence divergence between each haplotype group in PAUP* (Swofford, 2002).

Microsatellite Diversity: I tested the loci for linkage disequilibrium and departure from Hardy-Weinberg equilibrium (HWE) using GENEPOP 3.4 (Raymond and Rousset, 1995), assessing significance using default parameters of the Markov Chain method and correcting for multiple comparisons using the sequential Bonferroni procedure (Rice, 1989). I calculated allelic richness and expected heterozygosities using ARLEQUIN version 3.1 (Excoffier and Schneider, 2005).

Population Structure: To determine the levels of genetic differentiation among sampling sites, I calculated overall and pairwise F_{ST} , averaging across loci between sampling sites using an Analysis of Molecular Variance (AMOVA) performed in ARLEQUIN version 3.1 (Excoffier and Schneider, 2005), testing significance using 10,000 permutations. To determine relationships among populations based on the microsatellite markers, I used the program POPULATIONS v. 1.2.30 (Langella, 1999) to construct an unrooted neighbor-joining tree based on chord distance, D_C (Cavalli-Sforza and Edwards, 1967), among populations using information from the full dataset and assessed support for groupings with 1000 bootstraps. D_C does not make the assumption of constant population size or constant mutation rates among loci (Chapuis and Estoup, 2007) and performs better than other genetic distances in recovering correct tree topologies (Takezaki and Nei, 1996).

To determine if microsatellite molecular variation is partitioned by host plant or by geographic site, I performed two hierarchical AMOVAs in ARLEQUIN version 3.1 (Excoffier and Schneider, 2005) using 10,000 permutations to test significance: a) with *host plant* and b) with *geographic region* defined as the uppermost hierarchical level. For a) *host plant*, I used a subset of the data to perform this analysis that included only individuals from East Mauna Loa and the Saddle Region, which occurred on *Dubautia scabra* and *D. ciliolata ciliolata*. I excluded individuals from the Mauna Kea and South Mauna Loa populations from this analysis because in these regions host plant and geography are confounded variables. For b) *geographic region*, I included data from all of the sampling sites and grouped the "among geographic region" component into the three major regions: South Mauna Loa, East Mauna Loa and Mauna Kea (Figure 1).

Population Assignments: I identified genetic clusters in the data set using STRUCTURE version 2.3 (Pritchard et al., 2000), a software program that uses a Bayesian model-based clustering approach to group individuals based on genotype frequencies into populations by testing the data against various models of possible population numbers (K: 2, 3, 4, …15). Runs were repeated five times at each K value at different random number starting seeds to test for consistency between runs with a burnin period of 15,000 steps and a run length of 50,000 steps. Consistency between runs and inspection of plots demonstrated convergence of the runs. This analysis resulted in a log-likelihood score that maximized the probability of the data given the models. I selected the lowest K-value that maximized the structure in the data following the method of Evanno et.al. (2005), which minimizes both the mean log-likelihood score and the variance between runs.

Temporal stability of genetic populations: The mitochondrial and microsatellite analyses above revealed that there is a zone of secondary contact between two genetic groups at the site along the Saddle Road (1950) site (Table 1) between Mauna Kea and Mauna Loa. In order to assess whether these two populations are stable in time or represent an ephemeral phenomenon and whether the number of hybrid individuals changed dramatically between years, I repeated collections at the site in 2008, three years after the original collecting and genotyping was performed (approximately 15 generations). I genotyped 37 individuals from the 2008 dataset at ten of the microsatellite loci following laboratory and screening protocols described above. I then created a composite dataset containing the 39 genotyped individuals from the 2005 collection data from this site to result in a total of 76 individuals from both years that were genotyped at ten microsatellite loci. To assess the stability of these two genetic populations and identify if the two genetic populations identified in 2005 were still present at the site in 2008, I performed a clustering analysis in STRUCTURE (Pritchard et al., 2000) on the pooled dataset, testing the data against models of K=1 – 5, following the procedures described above.

Population Histories: To estimate divergence times, historical migration rates and effective population sizes, I fitted my data to a model of isolation with migration, implemented in the coalescent-based software IM (Hey and Nielsen, 2004). I analyzed two population pairs: a) Mauna Loa Trail HIGH and Pu'u Kanakaleonui and b) the two genetic populations in the zone of secondary contact at the Saddle Road (1950) site (using the 2005 dataset). The data I used for

each pairwise analysis consisted of the mitochondrial locus, analyzed using the infinite alleles model together with eight microsatellite loci that conform well to the model of stepwise mutation.

Prior to performing the final analyses, I ran a series of sensitivity analyses. During these I adjusted the prior parameter values depending on the results of the posterior distributions from the previous run, using them to select the appropriate upper bounds for each parameter. I performed the final analyses using 100 chains for a total of 100,000 burn-in steps followed by runs of between 1 and 3 million steps. To ensure that results were similar between runs, I performed two complete independent runs for each population pair with different random number starting seeds. I selected the geometric heating parameters for the chains following several preliminary runs to achieve sufficient mixing. Finally, I assessed the chains following each run to determine if they were long enough and monitored the ESS values and the trendlines in the posterior distribution plots to determine whether mixing was sufficient.

This method allowed me to simultaneously estimate several parameters about each population pair: (1) θ in each contemporary population as well as the ancestral population, (2) time of divergence and (3) migration rates between each population. In order to convert the parameter estimate of *t* to real time (t), it is necessary to calibrate at least one locus with an estimated mutation rate (μ) for that gene region – if possible, based on data from within the study lineage of interest. I estimated the mutation rate μ for the COI locus by first calculating the average percent uncorrected sequence divergence (p) in PAUP* (Swofford, 2002) between 5 *Nesosydne* sister pairs (including *N. chambersi* and it's sister species, *N. bridwelli* (Muir, 1919)) that are situated with one taxa on Maui and the other on Hawaii. I then averaged the amount of divergence and calibrated it using the geologic age estimate of the island of Hawaii, 0.5 million years (Price and Clague, 2002). I corroborated my estimated rate by examining divergence rates for Hawaiian arthropods from a variety of independent lineages that straddle the same biogeographic setting as *N. chambersi* and *N. bridwelli*: in each sister taxon pair, one species or population is from Maui and the other is from Hawaii. Thus, I could calculate pairwise sequence divergence as described above using the same calibration of 0.5 million years for the age of the island of Hawaii to estimate a rate. I obtained COI sequences from published examples from GenBank as well as from several unpublished examples, which I received directly from the researchers cited in Table 5.

Finally, estimates in IM are produced on a per year scale. For taxa whose generation time is less or more than one year, it is necessary to scale the results by the number of generations per year. In laboratory conditions, *Nesosydne* have been documented as taking approximately 6 to 8 weeks to complete a life cycle (O'Connell, 1991). As host plants are available all year and tropical conditions maintain relatively similar day lengths throughout the year, breeding is likely to occur throughout the year. Here I use 5 generations per year as a conservative estimate of the number of generations per year in field conditions.

Results

Mitochondrial diversity: I sequenced COI (653 bp) from a total of 185 individuals from across all 10 sampling sites (Table 1), within which I recovered 10 unique haplotypes. Of these, 9 were transitions and 1 was a transversion. There are 3 major haplotype clades apparent in the

haplotype network (Table 2, Figure 2) which correspond very well to the geographic regions from which they were collected: South Mauna Loa (SML: Mauna Loa Trail High+Mauna Loa Trail Low), East Mauna Loa (EML: East Mauna Loa High+East Mauna Loa Middle+ East Mauna Loa Low+ Saddle Road (1900) High) and Mauna Kea (MK: Puu Kanakaleonui+Saddle Road (1600)+Waipahoehoe Gulch+Puu Nau+Saddle Road (1900) Low). The average uncorrected pairwise genetic distance between each mtDNA clade is small (SML/EML= 0.51%; SML/MK=0.37%; EML/MK=0.31%: Figure 2). Only two sites show exceptions to perfect correspondence of haplotype group to geographic region, and both occur along the Saddle Road between Mauna Loa and Mauna Kea. First, the Saddle Road (1600m) site was collected from a new lava flow originating from Mauna Loa, yet the mitochondrial haplotype corresponds to that of Mauna Kea, suggesting this area was recently colonized from Mauna Kea. Second, the Saddle Road (1950m) site between the two volcanoes contains mitochondrial haplotypes from the EML and MK haplotype groups, which suggests that the site was recently colonized by members of both haplotype groups and now forms a zone of secondary contact.

Microsatellite diversity: From 10 sampling sites, I genotyped a total of 292 individuals at 14 microsatellite loci (Table 1). Using GIMLET (Valiére, 2002), I found all individuals to have unique 14-locus genotypes and thus, I conclude that parthenogenesis is unlikely in this species or at least is sufficiently infrequent as to be undetectable in my data set. All 292 individuals were included in the full analysis. Microsatellite diversity was low to moderate across populations with the number of alleles per population averaging between 2.6 and 4.5 and expected heterozygosities ranging between 0.308 and 0.531 (Table 2).

One locus (NC15) showed evidence of null alleles at 7 of 10 sites and I therefore removed it from all subsequent analyses. I identified some potential null alleles at all loci except NC7, NC8, NC10, NC12, NC17 and at all sites except MLOR HIGH and Pu'unau. For these loci, the number of sites identified as having null alleles at that locus was: one (NC3), two (NC4, NC5, NC9, NC11, NC14), three (NC6), four (NC12). For each site with potential null alleles, the number of loci implicated was: one (MLT LOW, Pu'unau), two (Saddle Road (1600)), three (MLOR MIDDLE, MLOR LOW, Waipahoehoe Gulch), four (MLT HIGH) and six (Saddle Road (1950).

There was no evidence of departure from HWE with any marker from any population with three exceptions (MLT HIGH – NC11, MLOR LOW – NC4 and Saddle Road (1950) – NC9). No loci in any population showed evidence of heterozygote excess. However, tests for heterozygote deficiency by population revealed four populations to each have one locus (MLT HIGH – NC11, MLOR LOW – NC4, Saddle Road (1950) – NC11 and Saddle Road (1600)– NC9) that shows evidence of heterozygote deficiency. Departures from HWE involved three different loci (two of the loci in one population each, one locus in two populations), but because they behave normally in the other populations, I rule out locus specific effects and instead infer that these observations are the result of a biological phenomenon.

I found evidence for departure from linkage equilibrium in two populations after correcting for multiple comparisons using sequential Bonferroni correction. In the Saddle Road (1950) population, one pairwise combination of loci (NC4 + NC11) and in population MLOR LOW, three pairwise combinations (NC3+NC14, NC3+NC17, and NC5+NC14) were found to be significantly in linkage disequilibrium. Because loci did not show the same patterns across

multiple sites, they were inferred to be physically independent and I judged the observed linkage disequilibrium to be the result of population substructure within the two sampling sites.

Population Structure: Population pairwise estimates of F_{ST} range from 0.048-0.468 (Table 3), while the overall F_{ST} was 0.229 ($p<0.0001$). All estimates are significantly different from zero. The neighbor-joining tree based on microsatellites recovered two well-supported groups, both of which correspond well to their mitochondrial haplotype groupings: Mauna Loa Trail High+Mauna Loa Trail Low, and Saddle Road (1600)+Waipahoehoe Gulch+Puu Kanakaleonui. The other populations are clearly very differentiated from one another, but support values are too low to provide much information about the relationships among them (Figure 3).

Results from the AMOVA addressing whether microsatellite variation is partitioned by host plant or geographic region demonstrate that host plant is not associated with genetic structure in this species (Table 4). When planthopper populations were grouped by *host plant*, the among host plant component explained -1.88% of the variance in the data (negative values for this analysis are due to sampling error and can be interpreted as zero). 18.25% of the variation was explained by the among sites, within host plant groups component and the remainder of the variation was contained within sites. In contrast, when the planthopper populations were grouped by *geographic region*, 9.34% of the variance in the data was explained by the among geographic region component, with another 15.9% explained by the among sites within geographic region component. The remainder of the variance was contained within sites (Table 4). Both analyses indicate that the "within groups, among sites" component explains a similar amount of the variation, indicating that genetic variation is significantly structured among sampling sites – a result corroborated by the F_{ST} calculations (Table 3). These results indicate that host plant is not playing a role in the structuring of microsatellite variation in this species, and that geography, at both the regional and among sites scales has a lot more explanatory power.

Population Assignments: The clustering analysis of all individuals from all collection sites revealed a highly structured set of subpopulations that are distributed in a complex manner across the sampled region (Figure 4a). Eight genetic populations within the 10 sampling sites were identified using the STRUCTURE algorithm. Assignments of individuals to genetic populations corresponded well to the major haplotype groups defined by the mitochondrial analysis in most places. Mauna Loa Trail HIGH and Mauna Loa Trail LOW cluster together here corresponding perfectly to the South Mauna Loa haplotype group. Likewise, Saddle Road (1600), Waipahoehoe Gulch and Pu'u Kanakaleonui cluster together in agreement with the Mauna Kea haplotype group. However, Pu'u Nau is clearly a distinct population at the microsatellite loci despite its inclusion into the Mauna Kea haplotype group. Furthermore, the clustering analysis revealed that the East Mauna Loa haplotype group is a dramatically structured set of genetic populations in which four sampling sites contain five genetic populations within the East Mauna Loa haplotype group, extending from the Mauna Loa Observatory Road at 2,650 m (MLOR HIGH) down to the Saddle Road at 1,950 m (Saddle Road (1950)). MLOR MIDDLE and MLOR LOW, although they contain a high proportion of individuals assigned to their own unique genetic population, also contain many individuals of mixed ancestry from other genetic

populations, indicating new dispersal into these sites. Two genetic populations were documented at Saddle Road (1950) within one collecting site (Figure 4a and 4b). Comparison between the mitochondrial haplotypes and the microsatellite clustering assignments at this site showed 100% correspondence between them, supporting the previous inference of a zone of secondary contact.

Temporal stability of genetic populations: The temporal clustering analysis demonstrated that the two genetic populations identified in the 2005 samples are still present and strongly assigned in the 2008 samples. The optimum K value for the pooled dataset of individuals collected from both 2005 and 2008 is clearly K=2, with individuals from both sampling periods grouped into each genetic population. The number of individuals with mixed genetic backgrounds between sampling periods was similar (5 of 39 individuals in 2005, 3 of 37 individuals in 2008). This indicates that genetic populations observed in the 2005 samples are not an ephemeral phenomenon and instead represent relatively stable populations that have not undergone fusion since the initial sampling period.

Population Histories:

COI mutation rates in Hawaiian arthropods: My empirical calculation of COI divergence rate in *Nesosydne* resulted in an average divergence rate of 8.9% sequence divergence between the two species per million years (a lineage specific mutation rate of 4.45%), which yielded my estimated mutation rate (u) for the locus of 5.81×10^{-5} substitutions/site/year. Compared with published estimates of arthropod divergence rates, my estimated rate is quite high. Brower (1994) performed a review of seven studies that provided divergence rate estimates for a variety of taxa in several different geographic locations and concluded that 2.3% is a reasonable standard estimate. However, the seven studies included used a variety of different gene regions and are therefore mostly not applicable to this study. The two studies included that did use COI were later incorporated into Quek et al's (2004) review of published estimates, in which the authors concluded that 1.5% appears to be a fairly good standard divergence estimate for recently diverged species at the COI locus. This was based on 5 different studies, again from a variety of arthropod taxa from several different geographic locations. Each study averaged divergence rates across several taxon pairs with each lineage, which obscured the high amount of variance indentified in some of them.

So how reasonable is this empirically derived estimated divergence rate of 8.9% per million years, given that we are accustomed to seeing rate calibrations of much lesser magnitudes? My survey of divergence rates of the COI locus in Hawaiian arthropod taxon pairs that are subject to the same environmental and geographic conditions, situated with one member on Maui and the other on Hawaii, resulted in an average divergence rate of 6.4% per million years (SD 1.3 – 11.7, Table 5). The estimated rate of 8.9% per million years for *Nesosydne chambersi/N. bridwelli* is higher than the mean average divergence rate for the Hawaiian arthropods included in the sample, but is well within the standard deviation. I therefore conclude that it is a reasonable mutation rate to apply to this locus in this system. However, because the discrepancy between 8.9% and 1.5% sequence divergence per million years is so large, I provide all of my converted IM parameter estimates in units based on both rates, as well as the unconverted estimates, in Table 6.

IM estimates: I examined convergence between the two independent IM runs for each pairwise analysis by verifying whether each replicate converged to similar parameter values and whether the chains mixed well within each run. In each case, the two replicates yielded posterior distributions with similar values and the values estimated from the longest runs are presented here (Table 6). Based on my estimated mutation rate of 8.9% sequence divergence per million years and a generation time of 0.2 years, I estimated the time since divergence between the two genetic populations in the zone of secondary contact (Saddle Road (1950)) as 396 (175 – 5,254) years ago. I estimated the time since divergence between the Mauna Kea population at Pu'u Kanakaleonui and the south Mauna Loa population at Mauna Loa Trail LOW as 3,019 (1,112 - 20,231) years ago. In comparison, the estimated divergence times based on the more general rate of 1.5% sequence divergence per million years for arthropods and a generation time of 0.2 years were higher, but still quite low on an absolute scale: Saddle Road (1950) High vs. Saddle Road (1950) Low – 2,370 (1,050-31,426); Mauna Kea vs. south Mauna Loa – 18,058 (6,651-121,010). In both cases, the posterior probability distributions of the estimates are clustered towards the lower values (Figures 5a and 5b).

Estimated gene flow since population separation is low and asymmetrical between both population pairs. From Mauna Kea to South Mauna Loa and from South Mauna Loa to Mauna Kea are low, with clear peaks at $2Nm = 0.146$ (0.018-4.182) and $2Nm = 0.084$ (0.010-4.798) respectively. The number of migrants over historical time are even lower and more asymmetrical between the zone of secondary contact populations, although the posterior probability distributions never quite reach zero – implying that while the median estimates are informative, the confidence intervals are not reliable: from the Mauna Kea haplotype to the East Mauna Loa haplotype, $2Nm = 0.0004$ (0.003-6.082), while $2Nm = 0.098$ (0.025-14.96) in the other direction (Table 6). Estimates of $2Nm$ do not need to be converted using a mutation rate.

Discussion

This study was designed to test the hypothesis that geographic isolation via habitat fragmentation is the dominant force promoting divergence in *Nesosydne chambersi* in the early stages of diversification. It revealed a species that has been highly structured by the geology of the newly forming Big Island landscape. From the perspective of this study, within the tip of a tree within a large ecologically specialized radiation, it appears that early genetic divergence is decoupled from ecological adaptation – providing isolated genetic pools that are then available to proceed on independent evolutionary trajectories.

Population structure

Genetic distance between the three mtDNA clades is very small, but is clearly partitioned into three major geographic regions (Figure 2), which strongly suggests recent divergence and low vagility. Microsatellite variation is also low and calculations of F_{ST} (Table 3), along with the clustering analysis (Figure 2), reveal a highly structured set of subpopulations whose arrangement across the sampling area suggest we are glimpsing a snapshot of diversification of in various stages across the island.

The *South Mauna Loa haplotype group*, which was collected from two sampling sites south of Mauna Loa's North East rift zone, appear to be acting more or less as one genetic population. Although STRUCTURE consistently and strongly supports them as a single cluster, the F_{ST} analysis reveals some low but significant differentiation (F_{ST}=0.09). This discrepancy in results is like ly simply due to the difficulty STRUCTURE has discerning low amounts of structure in data sets that also contain populations with much greater levels of divergence (Pritchard *pers com*).

The *Mauna Kea haplotype group* contains a more complicated set of subpopulations that inhabit stable, older habitats as well as the newest lava flow sampled in the Saddle region. Three genetic populations were identified in the clustering analysis, one of which spans a range of 1,250 m in elevation and lives on two different host plant species. This is the largest genetic population sampled and contains individuals collected from *Dubautia ciliolata glutinosa* in Waipahoehoe Gulch and Pu'u Kanakaleonui and from the Saddle road site at 1,600 m in elevation (Saddle Road (1600)), collected from *Dubautia scabra*. A likely explanation for this surprisingly large distribution is that the Saddle Road (1600) site was recently colonized and has not yet achieved much differentiation (F_{ST} values between this site and both of the higher elevation sites = 0.05), or continues to be swamped by ongoing migrants into the site from the parent population. A second genetic population within this haplotype group was collected along Saddle road at the 1,950 m site (Saddle Road HIGH), collected from both *Dubautia scabra* and *Dubautia ciliolata ciliolata*. The final genetic population belonging to this haplotype group was collected at Pu'u Nau from a stand of *Dubautia ciliolata glutinosa* that is isolated in a patchy landscape and is strongly defined as its own genetic population at the nuclear loci.

The low diversity *East Mauna Loa haplotype* group represented in my sampling is entirely distributed on a matrix of recent lava flows (155-3,000 yrs old) on the east face of Mauna Loa from 2,650 m down into the Saddle region at 1,950 m (Saddle Road (1950)). Nuclear loci examined here uncovered profound structure between each sampling site. The presence of individuals with mixed genetic backgrounds in MLOR MIDDLE and MLOR LOW indicate that colonization of these new habitats is likely ongoing.

Stable zone of secondary contact between the two volcanoes

Results presented here reveal a zone of secondary contact between a population with a Mauna Kea mitochondrial haplotype and a population with an East Mauna Loa mitochondrial haplotype at the Saddle Road (1950) (Figure 4a and 4b). To assess whether these are populations that are stable in time, if the populations are fusing, or if I happened to have collected an ephemeral population, I repeated collections 3 years (approximately 15 generations) following the original collections. Both genetic populations were clearly present in a similar spatial distribution in the later sample and hybridization had not increased between sampling periods, indicating that the populations are stable in contemporary time. This conclusion is reinforced by results I obtained by fitting my data to a model of isolation with migration, in which I demonstrated that migration has been extremely low between these populations over historical time – although migration is strongly asymmetrical, the highest amount of migration (from Saddle Road (1950) High into Saddle Road (1950) Low) still amounted to less than 0.1

individual per generation (Table 6). Together, these results illustrate two populations that have achieved at least partial reproductive isolation.

Geography or Ecology?

Two different host plant species inhabiting the flows on the east and south face of Mauna Loa (Figure 1) provided an unusual opportunity to test if genetic variation at the nuclear loci is more strongly associated with host plant or geography, and results indicate that genetic variation is not related to host plant use in this species. This indicates that the significant amount of genetic structure observed among these sites is likely to have been driven by recent colonization of the novel habitats created as the lava flows cooled and subsequently became colonized by the host plants. This implies an ecological fitting scenario (Janzen, 1980), whereby the insects reach the newly developing habitat and utilize whichever of the two hosts they encounter in the patchy matrix of lava flows, rather than a tracking of either of the hosts across the landscape. Thus, genetic divergence in geographic isolation acts prior to strict ecological specialization in this species.

Mechanism behind population structure

In this study, I asked the questions: when did divergence occur between these populations and is that timeframe is consistent with the idea that divergence was driven by fragmentation due to volcanic activity? Because the geology of this island has been studied in detail, it is possible to evaluate these divergence dating results within a known framework of external date calibrations. The two volcanoes examined in this study are much younger than the maximum age determined for the island of Hawaii of 0.5 million years old (Price and Clague, 2002) – Mauna Kea completed shield building approximately 130,000 years ago, while Mauna Loa is still at the end of that process today (Moore and Clague, 1992). Given my mutation rate estimate (see *Materials and Methods*), the clear position of the peaks for the divergence time estimates span the relatively recent timescales of 396 years for the zone of secondary contact populations and 3,019 years for the allopatric populations situated on either volcano (Table 6). These estimates are indeed on similar timescales as the recent within-island geologic events related to Mauna Loa's volcanic activity. Populations in the zone of secondary contact populations at the Saddle Road (1950) site were collected from a matrix of flows that have been dated to be from 155 – 3,000 years old (Trusdell et al., 1996). These flows cover layer upon layer of older flows that have been building on each other throughout the volcano's development, allowing patches of suitable habitat to blink in and out of existence. Comparison between the Pu'u Kanakaleonui and Mauna Loa Trail LOW populations revealed an older (but still recent) divergence of approximately 3,019 years, which is still consistent with a model of divergence that is driven by geologically – induced fragmentation. These populations likely became separated following the colonization of the southern Mauna Loa landscape, after which gene flow ceased between it and the Mauna Kea population. The South Mauna Loa population is situated south of Mauna Loa's northeast rift zone, whose periodic flows over the last several thousand years (Macdonald et al., 1983) would have continually extirpated adjoining habitat.

Since multiple assumptions are necessary order to generate such estimates, they must always be taken as a heuristic at best. In this study, I took one of the major sources of uncertainty in generating parameter estimates, divergence rate, and examined my results using a more generally applied divergence rate for arthropods (see *Materials and Methods*). I found that even if my empirically determined rate of divergence for COI in this lineage (8.9%) is relaxed to the more general rate of 1.5% (Quek et al., 2004), the date estimates are still consistent with recent divergence in a landscape dominated by patchy habitat islands that get covered and re-colonized by the stochastic activity of a growing volcano (Table 6) – thus, my conclusions do not change, regardless of the rate applied. Although the holy grail of exact divergence dates are beyond reach for the foreseeable future, it is reasonable to conclude that divergence has been quite recent, given the documented pattern of genetic variation, the estimated dates of divergence and the extremely recent geologic setting of the island.

The populations examined here provide an excellent example of the early stages of the fracturing of a species into distinct gene pools in a highly dynamic landscape, which is instructive as to the initial conditions for radiations found in volcanic landscapes. The Hawaiian islands are full of speciose radiations, and the estimation of divergence times using molecular phylogenies calibrated by maximum island ages is becoming commonplace. This study indicates that divergence times may be orders of magnitude younger than the maximum age estimates for islands, and provides a model for visualizing how within island colonization of a dynamic, fragmenting habitat can generate such conditions.

Implications for the study of host-associated insects:

Host-associated insects are excellent models for the study of ecological selection and sympatric divergence (Funk et al., 2002), but similar to the study of adaptive radiation, there is a lack of resolution as to the relationship between speciation and adaptive divergence in this area as well. Despite repeated observations of tight host-plant associations among lineages of insects from a wide variety of taxonomic groups and strong selection imposed by divergent host plant relationships, e.g.: the apple maggot fly (Feder, 1998), the pea aphid (Via, 1999) and *Timema* walking sticks (Nosil, 2007), it is difficult to say with certainty the initial causes of these divergences (e.g.: Via, 2009, Lozier et al., 2007). Even the story of the apple maggot fly, the poster child for sympatric speciation, has become more complicated with the accumulation of detailed demographic information (Xie et al., 2007, Feder et al., 2003). This, together with data presented here, suggests that multiple stages may be expected along the diversification pathway (see: Rundle and Nosil, 2005), even among species for which the role of ecological selection to divergence is considered fundamental.

Implications for the study of adaptive radiation

This study system provided an unusual opportunity to test if host plant or geography plays a more prominent role in the early stages of diversification of an ecologically specialized radiation. My results indicate that in this species, genetic structure is strongly associated with geography at multiple evolutionary scales recorded by both mitochondrial and nuclear DNA, but not to host plant use. Furthermore, diversification appears to be acting on the same timescales as

major geologic events in the dynamic landscape of the island of Hawaii. This suggests a model in which genetic divergence driven by geographic isolation occurs first, providing isolated genetic pools that may become reproductively isolated. As such, they are then set on independent evolutionary trajectories whereby they are free to diverge by natural or sexual selection, diverge by genetic drift or go extinct.

How appropriate a model is this for other adaptive radiations? Despite repeated observations of classic cases of adaptive radiations from a wide variety of taxonomic groups, it is difficult to say with certainty the initial causes of divergence. However, it is clear that the speciation process is complex and multiple phases may be the rule rather than the exception (Schluter, 2000, Rundle and Nosil, 2005, Losos, 2009, Grant and Grant, 1997, Grant and Grant, 2008). As such, the study of radiations from different vantage points will be instructive as to what processes dominate divergence at different stages.

Literature Cited

ARNEDO, M. A. & GILLESPIE, R. G. 2006. Species diversification patterns in the Polynesian jumping spider genus *Havaika* Proszynski, 2001 (Aranea, Salticidae). *Molecular Phylogenetics and Evolution,* 41**,** 472-495.

ASCHE, M. 1997. A Review of the Systematics of Hawaiian Planthoppers (Hemiptera: Fulgoroidea). *Pacific Science,* 51**,** 366-376.

BALDWIN, B. 2006. Contrasting Patterns and Processes of Evolutionary Change in the Tarweed - Silversword Lineage: Revisiting Clausen, Keck and Hiesey's Findings. *Annals of the Missouri Botanical Garden,* 93**,** 66-96.

BANDELT, H. J., MACAULAY, V. & RICHARDS, M. B. 2000. Median networks: speedy construction and greedy reduction, one simulation, and two case studies from human mtDNA. *Molecular Phylogenetics and Evolution,* 16**,** 8-28.

BROWER, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences,* 91**,** 6491-6495.

BROWN, J. M. 2010. *RE: Personal communication.* Type to GOODMAN, K. R.

CARR, G. 1985. Monograph of the Hawaiian Madiinae (Asteraceae): *Argyroxiphium, Dubautia, and Wilkesia*. *Allertonia,* 4**,** 1-123.

CARSON, H. L., LOCKWOOD, J. P. & CRADDOCK, E. M. 1990. Extinction and Recolonization of Local Populations on a Growing Shield Volcano. *Proceedings of the National Academy of Sciences of the United States of America,* 87**,** 7055-7057.

CAVALLI-SFORZA, L. L. & EDWARDS, A. W. F. 1967. Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics,* 19**,** 233-257.

CHAPUIS, M. P. & ESTOUP, A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution,* 24.

DENBIEMAN, C. F. M. & DEVRIJER, P. W. F. 1987. True parthenogenesis for the first time demonstrated in planthoppers (Homoptera, Delphacidae). *Annales de la Société Entomologique de France,* 23**,** 3-9.

DREW, A. E. & RODERICK, G. K. 2005. Insect biodiversity on plant hybrids within the Hawaiian silversword alliance (Asteraceae: Heliantheae-Madinae). *Environmental Entomology,* 34**,** 1095-1108.

EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14**,** 2611-2620.

EWING, C. 2010. *RE: Personal communication.* Type to GOODMAN, K. R.

EXCOFFIER, L. G. & SCHNEIDER, S. 2005. Arlequin ver.3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* 1**,** 47-50.

FEDER, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? *In:* HOWARD, D. & BERLOCHER, S. H. (eds.) *Endless Forms: Species and Speciation.* Oxford: Oxford University Press.

FEDER, J. L., BERLOCHER, S. H., ROETHELE, J. B., DAMBROSKI, H., SMITH, J. J., PERRY, W. L., GAVRILOVIC, V., FILCHAK, K. E. & ALUJA, M. 2003. Allopatric

genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proceedings of the National Academy of Sciences,* 100**,** 10314-10319.
FENNAH, R. G. 1958. Fulgoroidea of South-Eastern Polynesia. *Transactions of the Royal Entomological Society of London,* 110**,** 117-220.
FUNK, D. J., FILCHAK, K. E. & FEDER, J. L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica,* 116**,** 251-267.
GARB, J. E. & GILLESPIE, R. G. 2009. Diversity despite dispersal: coloniztion history and phylogeography of Hawaiian crab spiders inferred from multilocus genetic data. *Molecular Ecology,* 18**,** 1746-1764.
GILLESPIE, R. G. 2004. Community assembly through adaptive radiation in Hawaiian spiders. *Science,* 303**,** 356-359.
GILLESPIE, R. G. & RODERICK, G. K. 2002. Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology,* 47**,** 595-632.
GLOR, R. E., M.E., G., LARSON, A., LOSOS, J. B., SCHETTINO, L. R., LARA, A.-R. C. & JACKMAN, T. R. 2004. Parial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proceedings of the Royal Society B,* 271**,** 2257-2265.
GOODMAN, K. R., MORRIS, V. R. F., WELTER, S. C. & RODERICK, G. K. 2008. Isolation and characterization of microsatellite markers in an endemic Hawaiian planthopper (*Nesosydne chambersi*: Delphacidae). *Molecular Ecology Resources,* 8**,** 1436-1438.
GRANT, P. R. & GRANT, B. R. 2008. *How and Why Species Multiply: The Radiation of Darwin's Finches,* Princeton, NJ, Princeton University Press.
GRANT, P. R. & GRANT, R. G. 1997. Genetics and the origin of bird species. *Proceedings of the National Academy of Sciences,* 94**,** 7768 - 7775.
HASTY, G. L. 2005. *Host-plant specialization affects species diversity and vulnerability to endangerment in a group of island-endemic phytophagous insects (Delphacidae, Hawaiian Islands).* PhD, University of California at Berkeley.
HEY, J. & NIELSEN, R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics,* 167**,** 747-760.
HORMIGA, G., ARNEDO, M. A. & GILLESPIE, R. G. 2003. Speciation on a conveyor belt: sequential colonization of the Hawaiian islands by Orsonwelles spiders (Araneae: Linyphiidae). *Systematic Biology,* 52**,** 70-88.
JANZEN, D. H. 1980. On ecological fitting. *Oikos,* 45**,** 308-310.
LANGELLA, O. 1999. Populations 1.2.30: Population genetic software (individuals or population distances, phylogenetic trees). Available at http://bioinformatics.org/~tryphon/populations/.
LOSOS, J. B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* Berkeley, CA, University of California Press.
LOZIER, J. D., RODERICK, G. K. & MILLS, N. J. 2007. Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host plant associated species in the aphid genus *Hyalopterus* (Hemiptera: Aphididae). *Evolution,* 61**,** 1353-1367.
MACDONALD, G. A., ABBOTT, A. T. & PETERSON, F. L. 1983. *Volcanoes in the Sea, The Geology of Hawaii,* Honolulu, Hawaii, The University Press of Hawaii.

MADDISON, W. P. & MADDISON, D. R. 2000. *MacClade version 4: Analysis of phylogeny and character evolution,* Sunderland, Massachusetts, Sinauer Associates.
MAGNACCA, K. N. & DANFORTH, B. N. 2006. Evolution and biogeography of native Hawaiian *Hylaeus* bees (Hymenoptera: Colletidae). *Cladistics,* 22**,** 393-411.
MEDEIROS, M. J., DAVIS, D., HOWARTH, F. G. & GILLESPIE, R. G. 2009. Evolution of cave living in Hawaiian *Schrankia* (Lepidoptera: Noctuidae) with description of a remarkable new cave species. *Zoological Journal of the Linnean Society,* 156**,** 114-139.
MEDEIROS, M. J. & GILLESPIE, R. G. in press. Biogeography and the evolution of flightlessness in a radiation of Hawaiian moths (Xylorycitidae: *Thryocopa*). *Journal of Biogeography*.
MOORE, J. G. & CLAGUE, D. A. 1992. Volcano growth and evolution of the island of Hawaii. *Geological Society of America Bulletin,* 104**,** 1471-1484.
NOSIL, P. 2007. Synthesis: Divergent Host Plant Adaptation and Reproductive Isolation between Ecotypes of *Timema cristinae* Walking Sticks. *The American Naturalist,* 169**,** 151-162.
O'CONNELL, C. 1991. *Species Barriers and Breakdowns in Hawaiian Planthoppers.* Master of Science, University of Hawaii.
OBOYSKI, P. 2010. *Evolution, Systematics and Ecology of Hawaiian Cydia (Lepidoptera: Tortricidae).* PhD Dissertation, University of California, Berkeley.
OOSTERHOUT, C. V., HUTCHINSON, W. F., WILLS, D. P. M. & SHIPLEY, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4**,** 536-538.
PRICE, J. P. & CLAGUE, D. A. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London, B* 269**,** 2429-2435.
PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* 155**,** 945-959.
QUEK, S. P., DAVIES, S. J., ITINO, T. & PIERCE, N. E. 2004. Codiversification in an ant-plant mutualism: Stem texture and the evolution of host use in Crematogaster (Formicidae : Myrmicinae) inhabitants of Macaranga (Euphorbiaceae). *Evolution,* 58**,** 554-570.
RAYMOND, M. & ROUSSET, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity,* 86**,** 248-249.
RICE, W. R. 1989. Analyzing Tables of Statistical Tests. *Evolution,* 43**,** 223-225.
RICKLEFS, R. E. & BERMINGHAM, E. 2007. The Causes of Evolutionary Radiations in Archipelagoes: Passerine Birds in the Lesser Antilles. *American Naturalist,* 169**,** 285-197.
RODERICK, G. K. & GILLESPIE, R. G. 1998. Speciation and phylogeography of Hawaiian terestrial arthropods. *Molecular Ecology,* 7**,** 519-531.
RODERICK, G. K. & METZ, E. C. 1997. Biodiversity of Planthoppers (Hemiptera: Delphacidae) on the Hawaiian Silversword Alliance: Effects of Host Plant Phylogeny and Hybridisation. *Memoirs of the Museum of Victoria,* 56**,** 393-399.

RUBINOFF, D. 2008. Phylogeography and ecology of an endemic radiation of Hawaiian aquatic case-bearing moths (Hyposmocoma: Cosmopterigidae). *Philosophical Transactions of the Royal Society B,* 363**,** 3459 - 3465.
RUNDELL, R. J. & PRICE, T. D. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends In Ecology & Evolution,* 24**,** 394-399.
RUNDLE, H. D. & NOSIL, P. 2005. Ecological speciation. *Ecology Letters,* 8**,** 336-352.
SCHLUTER, D. 2000. *The Ecology of Adaptive Radiation,* Oxford, Oxford University Press.
SCHLUTER, D. 2009. Evidence for Ecological Speciation and Its Alternative. *Science,* 323**,** 737-741.
SHAPIRO, L. H., STRAZANAC, J. S. & RODERICK, G. K. 2006. Molecular phylogeny of *Banza* (Orthoptera: Tettigoniidae), the endemic katydids of the Hawaiian Archipelago. *Molecular Phylogenetics and Evolution,* 41**,** 53-63.
SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America,* 86**,** 651-701.
STURMBAUER, C., BARIC, S., SALZBURGER, W., RUBER, L. & VERHEYEN, E. 2001. Lake Level Fluctuations Synchronize Genetic Divergences of Cichlid Fishes in African Lakes. *Molecular Biology and Evolution,* 18**,** 144-154.
SWOFFORD, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0. Sunderland, MA: Sinauer Associates.
TAKEZAKI, N. & NEI, M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics,* 144**,** 389-399.
TRUSDELL, F. A., WOLFE, E. W. & MORRIS, J. 1996. Digital Database of the Geologic Map of the Island of Hawaii: Data Series 144. *USGS Series IMAP, Report Number 2524-A.*
VALIÉRE, N. 2002. GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes,* 2**,** 377-379.
VIA, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution,* 53**,** 1446-1457.
VIA, S. 2009. Natural selection in action during speciation. *Proceedings of the National Academy of Sciences,* 106**,** 9939-9946.
WAGNER, W. & FUNK, V. 1995. *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago,* Washington DC, Smithsonian Institution Press.
WILSON, W. W., MITTER, C., DENNO, R. F. & WILSON, M. R. 1994. Evolutionary patterns of host plant use by delphacid planthoppers and their relatives. *In:* DENNO, R. F. & PERFECT, T. J. (eds.) *Planthoppers: Their Ecology and Management.* New York: Chapman & Hall.
XIE, X., RULL, J., MICHEL, A. P., VELEZ, S., FORBES, A. A., LOBO, N. F., ALUJA, M. & FEDER, J. L. 2007. Hawthorne-Infesting Populations of Rhagoletis pomonella in Mexico and Speciation Mode Plurality. *Evolution,* 61**,** 1091-1105.
ZIMMERMAN, E. C. 1948. *Homoptera: Auchenorhyncha,* Honolulu, HI, University of Hawaii Press.

Tables

Table 1. Sampling site information (See Figure 1).

Sampling site	Site Description	Year col.	Host Plant	Substrate Age (yrs)*	Elev (m)	Lat	Long
1. Pu'u Nau	north side of Mauna Kea, along R1	2007	*Dubautia ciliolata glutinosa*	4000-14000	2,220	-155.452	19.903
2. Pu'u Kanakaleonui	east side of Mauna Kea, along R1	2006	*Dubautia ciliolata glutinosa*	14000-65000	2,850	-155.391	19.847
3. Waipahoehoe Gulch	east side of Mauna Kea, along R1	2006	*Dubautia ciliolata glutinosa*	14000-65000	2,850	-155.398	19.81
4. Saddle Road (1600)	along Saddle Road at 1,600m.	2007	*Dubautia scabra*	<200	1,600	-155.346	19.672
5. Saddle Road (1950)	South of Pu'u Huluhulu, along Saddle Road at maximum elev.	2005	▪ *Dubautia ciliolata ciliolata* ▪ *Dubautia scabra*	<200, 1500-3000	1,950	-155.447	19.686
6. MLOR, LOW	Mauna Loa Observatory Road	2008	*Dubautia scabra*	200-750	2,270	-155.472	19.617
7. MLOR MIDDLE	Mauna Loa Observatory Road	2008	*Dubautia ciliolata ciliolata*	1500-3000	2,340	-155.47	19.609
8. MLOR HIGH	Mauna Loa Observatory Road	2007	*Dubautia scabra*	<200	2,650	-155.474	19.577
9. MLT, LOW	Mauna Loa Trail, Hawaii Volcanoes NP	2008	*Dubautia ciliolata ciliolata*	1500-3000	2,085	-155.385	19.499
10. MLT, HIGH	Mauna Loa Trail, Hawaii Volcanoes NP	2007	*Dubautia ciliolata ciliolata*	1500-3000	2,475	-155.412	19.515

* *Trusdell et al 1996*

Table 2. Diversity Statistics

Sampling site	mtDNA clade	No. mtDNA sequenced	Total no. haplotypes	No. unique haplotypes	Nucleotide diversity (*pi*)	Haplotype diversity (*h*)	No. Msat individuals	Ave. no. alleles	H_E
1. Pu'unau	Mauna Kea	19	2	1	0.00063	0.4094	28	3.1	0.308
2. Pu'u Kanakaleonui	Mauna Kea	17	3	2	0.00038	0.2417	30	4.2	0.456
3. Waipahoehoe Gulch	Mauna Kea	16	2	1	0.00036	0.2330	25	3.8	0.511
4. Saddle Road (1600)	Mauna Kea	20	1	0	0.00000	0.0000	29	4.5	0.521
5. Saddle Road (1950)	Mauna Kea/ East Mauna Loa	31	2	0	0.00075	0.4903	39	3.3	0.460
6. MLOR LOW	E. Mauna Loa	18	1	0	0.00000	0.0000	25	2.9	0.443
7. MLOR MIDDLE	E. Mauna Loa	16	2	1	0.00036	0.2330	27	3.9	0.512
8. MLOR HIGH	E. Mauna Loa	16	1	0	0.00000	0.0000	29	2.4	0.411
9. MLT, LOW	South Mauna Loa	13	2	1	0.00059	0.3846	28	3.0	0.291
10. MLT, HIGH	S. Mauna Loa	21	2	1	0.00068	0.4421	32	3.7	0.371

Table 3. Pairwise F_{ST} values between sampling sites, all are significantly different from zero (Bold: p<0.001, "": p=0.0000).*

Site Name	MLT, HIGH	MLT, LOW	MLOR, HIGH	MLOR, MIDDLE	MLOR, LOW	Saddle Road, HIGH	Saddle Road LOW	Waipahoehoe Gulch	Pu'u Kanakaleonui	Pu'unau
1. Pu'unau	0.39265*	0.46762*	0.27349*	0.33306*	0.39521*	0.30530*	0.30403*	0.25401*	0.31442*	0.00000
2. Pu'u Kanakaleonui	0.31415*	0.38399*	0.26074*	0.18736*	0.23302*	0.21835*	0.05078*	0.05373*	0.00000	
3. Waipahoehoe Gulch	0.25072*	0.34005*	0.18139*	0.12835*	0.17294*	0.11901*	**0.04844**	0.00000		
4. Saddle Road (1600)	0.30248*	0.37655*	0.23850*	0.17609*	0.21086*	0.19998*	0.00000			
5. Saddle Road (1950)	0.23350*	0.28739*	0.24739*	0.18179*	0.15033*	0.00000				
6. MLOR, LOW	0.25340*	0.29988*	0.26502*	0.12762*	0.00000					
7. MLOR, MIDDLE	0.25220*	0.27017*	0.21427*	0.00000						
8. MLOR, HIGH	0.32843*	0.37746*	0.00000							
9. MLT, LOW	0.09512*	0.00000								
10. MLT, HIGH	0.00000									

Table 4. Results from hierarchical AMOVA

Uppermost hierarchy level	Source of variation	df	S.S.	Covariance component	% of molecular variance	*F*-statistics	*P*-value
(a) Host Plant	Among host plant groups	1	22.42	-0.07 Va	-1.88	F_{CT}=0.02	0.89
	Among site within groups	5	147.93	0.65 Vb	18.25	F_{SC}=0.18	<0.0001
	Within sites	291	860.65	2.96 Vc	83.63	F_{ST}=0.16	<0.0001
(b) Geography	Among geographic regions	2	189.41	0.34 Va	9.34	F_{CT}=0.09	0.01
	Among sites within regions	8	249.1	0.58 Vb	15.9	F_{SC}=0.18	<0.0001
	Within sites	547	1490.07	2.72 Vc	74.76	F_{ST}=0.25	<0.0001

Table 5. Cytochrome oxidase I (COI) rates for arthropod taxon pairs that are situated with one member on the island of Maui and the other on the island of Hawaii, calibrated using Price and Clague's (2002) date estimate for the emergence of the island of Hawaii (0.5 million yrs. ago). In the case of unpublished data, I obtained sequences directly from the researchers cited. In the case of published data, I downloaded sequences from GenBank. For all comparisons, I calculated uncorrected pairwise sequence divergence in PAUP. Grey indicates multiple sister comparisons within the same genus.

Order	Taxon pairs (Maui vs. Hawaii)	#base pairs	ave. uncorrected sequence divergence (p)	% divergence per million years	Source
Hemiptera	*Nesosydne bridwelli* vs. *N. chambersi*	653	0.0203	4.1	Goodman, Chapter 2
Hemiptera	*Nesosydne vs. N. umbratica*	451	0.0672	13.5	Goodman, Chapter 2
Hemiptera	*Nesosydne rubescens pele vs. N. ?*	451	0.0067	1.3	Goodman, Chapter 2
Hemiptera	*Nesosydne raillardicola vs. N. ?*	451	0.0846	16.9	Goodman, Chapter 2
Hemiptera	*Nesosydne ? vs. N. geranii*	451	0.0455	9.1	Goodman, Chapter 2
			***Nesosydne* AVERAGE = 8.9 (SD=2.39 – 15.41)**		
Orthoptera	*Banza "pillimauiensis"/B. mauiensis* vs. *B. nitida*	1254	0.0532	10.6	(Shapiro et al., 2006)
Lepidoptera	*Thryocopa indecora Maui* vs. *T. indecora Hawaii*	500	0.0480	9.6	(Medeiros and Gillespie, in press)
Lepidoptera	*Shrankia altivolans Maui* vs. *S. altivolans Hawaii*	407	0.0111	2.2	(Medeiros et al., 2009)
Lepidoptera	*Cydia olinda Maui* vs. *Cydia olinda Hawaii*	658	0.0198	4.0	(Oboyski, 2010)
Lepidoptera	*Cydia rufipennis* vs. *Cydia montana*	658	0.0144	2.9	(Oboyski, 2010)
Lepidoptera	*Hyposcoma sp. 2 W.Maui* vs. *H. sp. 9 Hawaii*	762	0.0387	7.7	(Rubinoff, 2008)
Coleoptera	*Prosopeus celatus* vs. *P. rudis*	609	0.0181	3.6	(Ewing, 2010)
Coleoptera	*Prosopeus celatus* vs. *P. blackburnii*	609	0.1057	21.1	(Ewing, 2010)
Coleoptera	*Eupetinus nsp.scimivertus* vs. *E. nsp.puupilli*	609	0.0361	7.2	(Ewing, 2010)
Hymenoptera	*Hylaeus anthracinus Maui* vs. *H. anthracinus Hawaii*	584	0.0736	14.7	(Magnacca and Danforth, 2006)
Hymenoptera	*Hylaeus kukui Maui* vs. *H. kukui Hawaii*	654	0.0110	2.2	(Magnacca and Danforth, 2006)
Hymenoptera	*Hylaeus coniceps Maui* vs. *H. coniceps Hawaii*	654	0.0000	0.0	(Magnacca and Danforth, 2006)
Diptera	*Trupanea arboraeae vs. T. cratericola*	784	0.0209	4.1	(Brown, 2010)
Araneae	*Orsonwelles falstafius* vs. *O. graphica*	439	0.0410	8.2	(Hormiga et al., 2003)
Araneae	*Tetragnatha quasimodo Maui* vs. *T. quasimodo Maui*	408	0.0421	8.4	(Gillespie, 2004)
Araneae	*Tetragnatha restricta Maui* vs. *T. restricta Hawaii*	423	0.0213	4.3	(Gillespie, 2004)
Araneae	*Havaika sp. "morphotype D"* vs. *H. cruciata*	472	0.0244	4.9	(Arnedo and Gillespie, 2006)
Araneae	*Mecaphesa anguiliventris Maui* vs. *M. anguiliventris Hawaii*	820	0.0085	1.7	(Garb and Gillespie, 2009)
			non-*Nesosydne* HAWAIIAN ARTHROPOD AVERAGE = 6.5 (SD=1.33 – 11.71)		

Table 6. Maximum-likelihood estimates of gene flow and divergence parameters, conversions to demographic units are provided using 2 mutation rates: using 8.9% sequence divergence per million years (in grey), calculated from *Nesosydne* (Table 5), and 1.5% sequence divergence per million years, an average of a wide variety of insect taxa (Quek et al 2004). Ranges represent the 95 Highest Posterior Density. Time in years is calculated assuming 5 generations per year (see Methods). See also Figures 5a and 5b.

Parameter estimates	**Mauna Loa (South)**	**Mauna Kea**	**Saddle Road (1950) High** *(East Mauna Loa haplotype)*	**Saddle Road (1950) Low** *(Mauna Kea haplotype)*
t	0.216 (0.079-1.448)		0.012 (0.005-0.153)	
theta	0.208 (0.097-0.698)	0.569 (0.2713-1.392)	0.032 (0.014-0.251)	0.004 (0.002-0.056)
theta, ancestral	15.318 (7.440-249.028)		6.078 (2.974-132.548)	
m	1.403 (0.368-11.993)	0.295 (0.075-6.895)	0.028 (0.413-48.483)	50.1 (23.1-539.1)
Parameter Estimates Converted into Demographic Units				
t, years (8.9%)	3,019 (1,112 – 20,231)		396 (175-5,254)	
t, years (1.5%)	18,058 (6,651 – 121,010)		2,370 (1,050-31,426)	
N_E (8.9%)	1.82E+04 (8.5E+03 – 6.09E+04)	4.97E+04 (2.37E+04 – 1.22E+05)	6.8E+03 (3.01E+03-5.4E+04)	8.4E+02 (4.74E02-1.2E+04)
N_E (1.5%)	1.09E+05 (5.08E+04 – 3.64E+05)	2.97E+05 (1.42E+05 – 7.27E+05)	4.07E+04 (1.8E+04-3.23E+05)	5.02E+03 (2.83E+03-7.15E+04)
N_E, ancestral (8.9%)	1.34E+06 (6.5E+05 – 2.17E+07)		1.31E+06 (6.4E+05-2.85E+07)	
N_E, ancestral (1.5%)	8.0E+06 (3.89E+06 – 1.3E+08)		7.83E+06 (3.83E+06-1.71E+08)	
m rate (8.9%)	4.02E-06 (1.05E-06 – 3.43E-05)	8.45E-07 (2.15E-07 – 1.97E-05)	3.19E-08 (4.79E-07 – 5.63E-05)	5.82E-05 (2.68E-05 – 6.26E-04)
m rate (1.5%)	6.71E-07 (1.76E-07 – 5.74E-06)	1.41E-07 (3.59E-08 – 3.3E-06)	5.34E-09 (8.01E-08 – 9.41E-06)	9.72E-06 (4.48E-06 – 1.05E-04)
2N*m* (4.1% = 1.5%)	0.146 (0.018-4.182)	0.084 (0.010-4.798)	0.0004 (0.003-6.082)	0.098 (0.025 – 14.96)

Figures

Figure 1. Map of Collecting Locations on the island of Hawaii, showing age of soil substrate in colors (Trusdell et al., 1996) and host plant in black symbols. Inset map depicts the Hawaiian island chain, with pink box on the island of Hawaii depicting the range of the larger map.

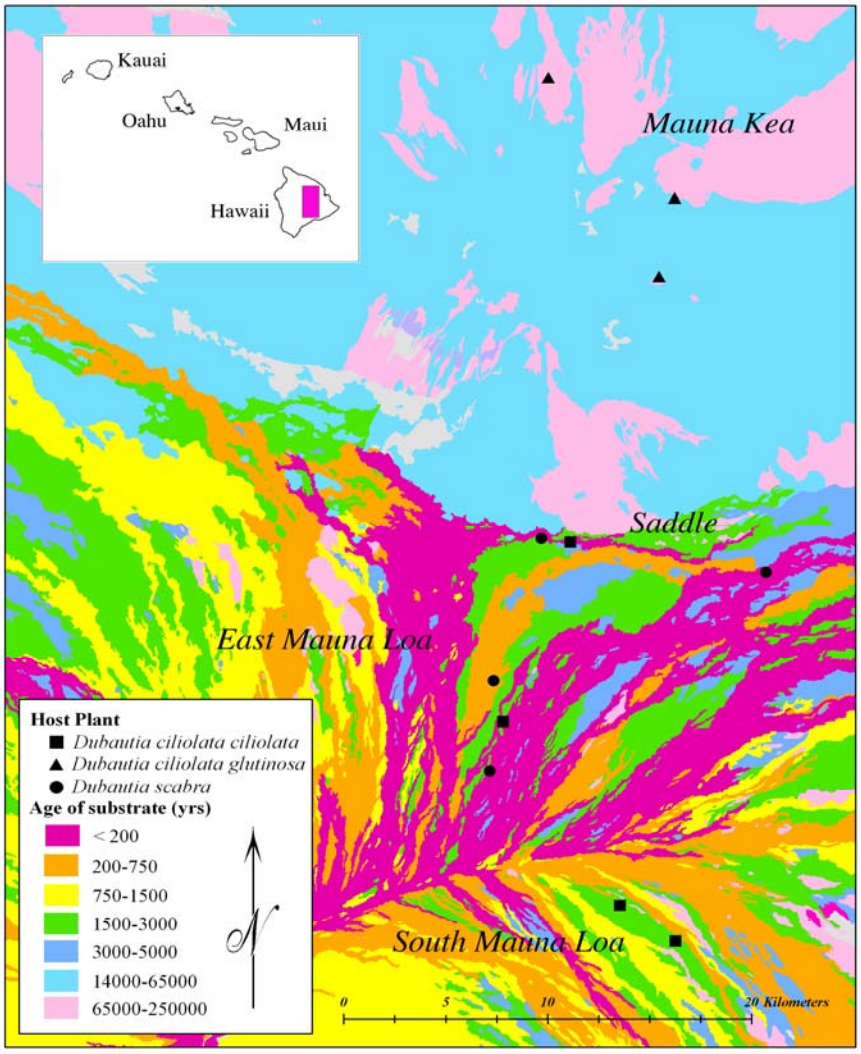


Figure 2. Cytochrome Oxidase I (COI) haplotype network. Pie slices in the Mauna Kea and East Mauna Loa haplotypes indicate individuals collected from the zone of secondary contact in the Saddle Road (1950) site. Mauna Kea = (Pu'u Nau; Pu'u Kanakaleonui; Waipahoehoe Gulch; Saddle Road (1600), Saddle Road (1950) Low), East Mauna Loa=(MLOR LOW; MLOR MIDDLE; MLOR HIGH; Saddle Road (1950) High), South Mauna Loa=(MLT LOW; MLT HIGH)

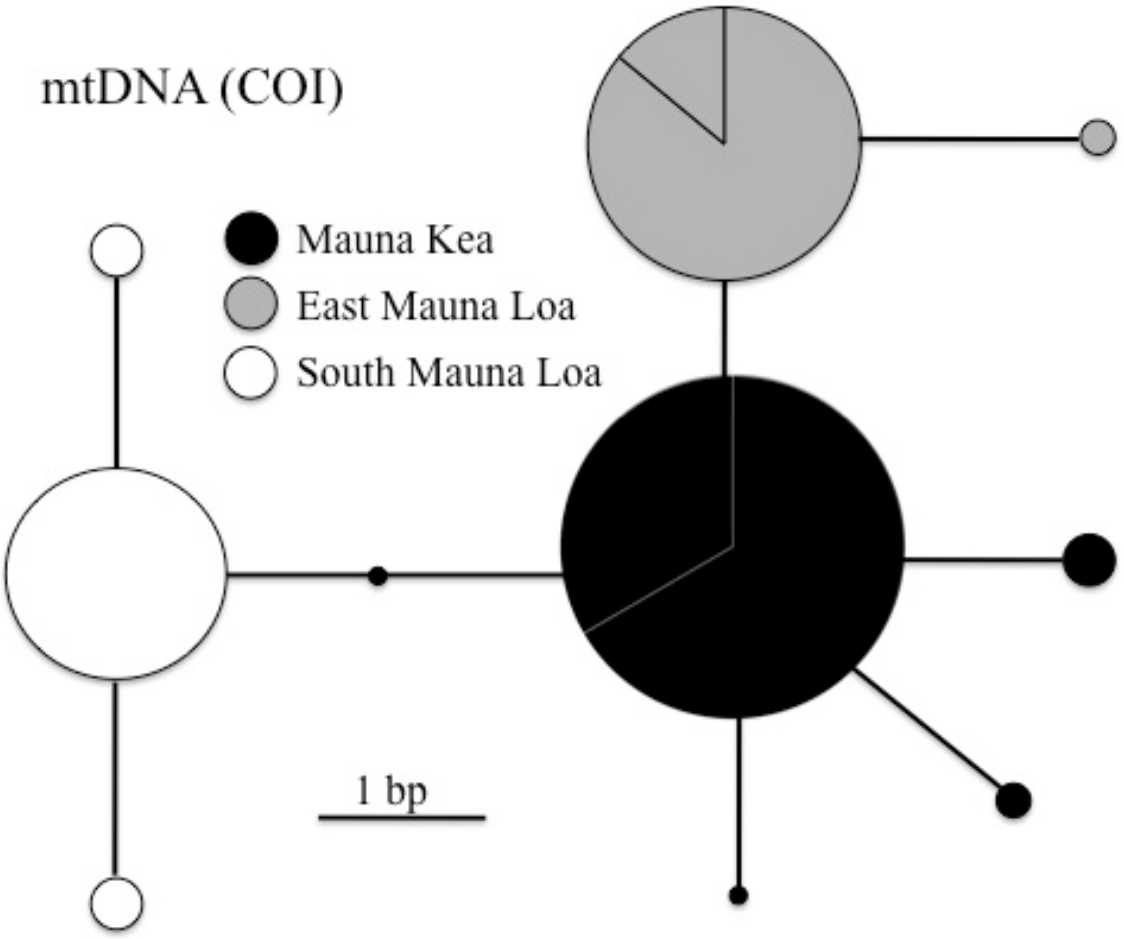


Figure 3. Unrooted neighbor-joining tree of populations based on microsatellites. Numbers indicate bootstrap support.

Figure 4. Results from Structure analysis of 13 microsatellite loci. The base layer is a 3D image of the island of Hawaii with the age of substrate layer from Figure 1 draped over it (Trusdell, 1996). (**a.**) Collecting sites are as follows: 1-Mauna Loa Trail LOW, 2-Mauna Loa Trail HIGH, 3-Mauna Loa Observatory Road (MLOR) HIGH, 4-MLOR MIDDLE, 5-MLOR Low, 6-Saddle Road 1950, 7-Saddle Road 1600, 8-Waipahoehoe Gulch, 9-Pu'u Kanakaleonui, 10-Pu'u Nau; (**b.**) Close up of collecting site 6-Saddle Road 1950, showing individuals collected in 2005 and 2008 forming a zone of secondary contact between two genetic groups. Hybrid individuals (indicated with a dot) were assigned to their genetic group (indicated by color) if their mitochondrial haplotypes match that group and results from the Structure analysis indicated a mixed genotype (based on the microsatellite loci) and >50% posterior probability of assignment to that genetic group.

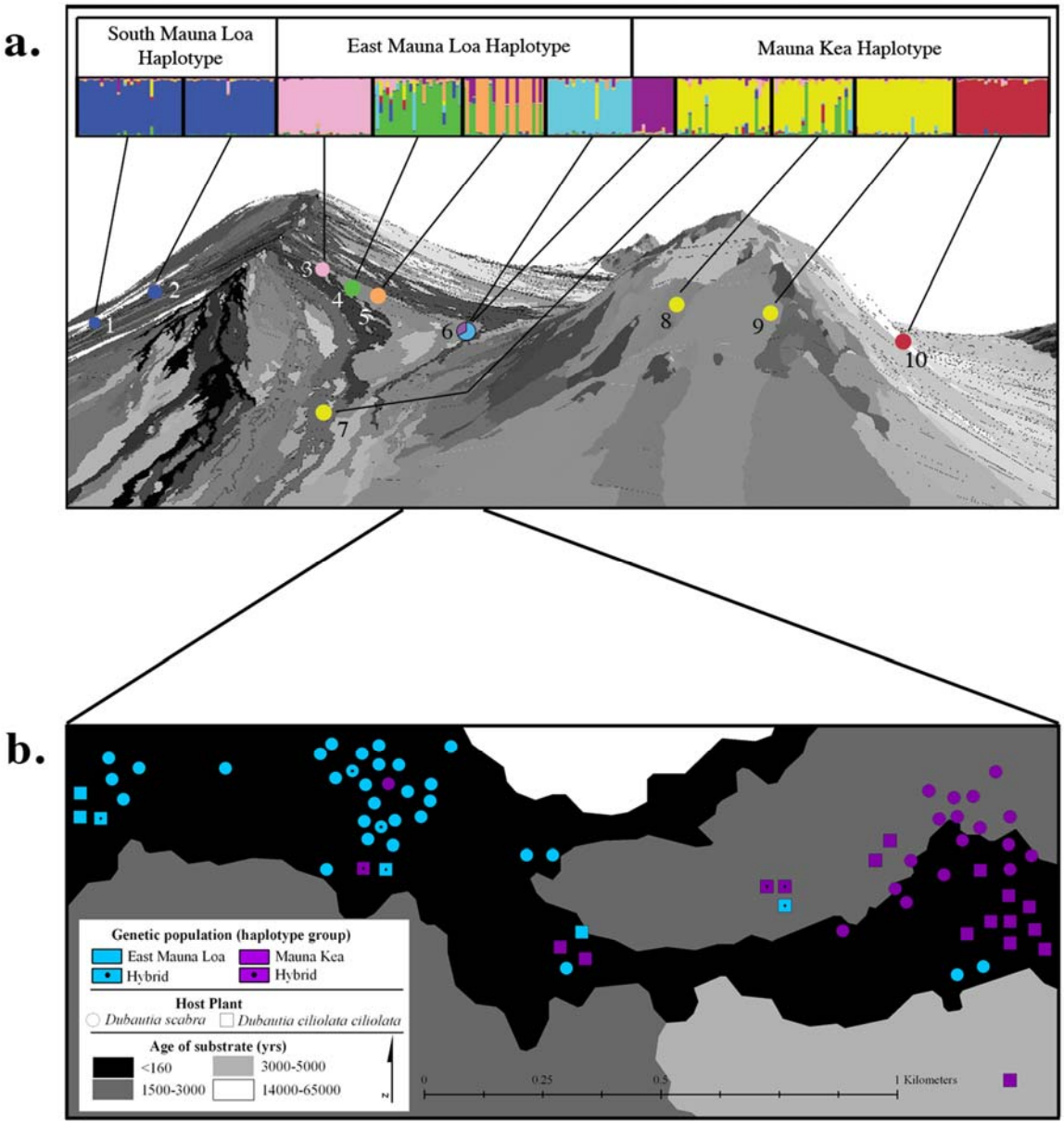

a.
South Mauna Loa Haplotype
East Mauna Loa Haplotype
Mauna Kea Haplotype
1
2
3
4
5
6
7
8
9
10
b.
Genetic population (haplotype group)
East Mauna Loa
Mauna Kea
Hybrid
Hybrid
Host Plant
Dubautia scabra
Dubautia ciliolata ciliolata
Age of substrate (yrs)
<160
3000-5000
1500-3000
14000-65000
N
0
0.25
0.5
1 Kilometers

Figure 5a. South Mauna Loa – Mauna Kea IM graphs.

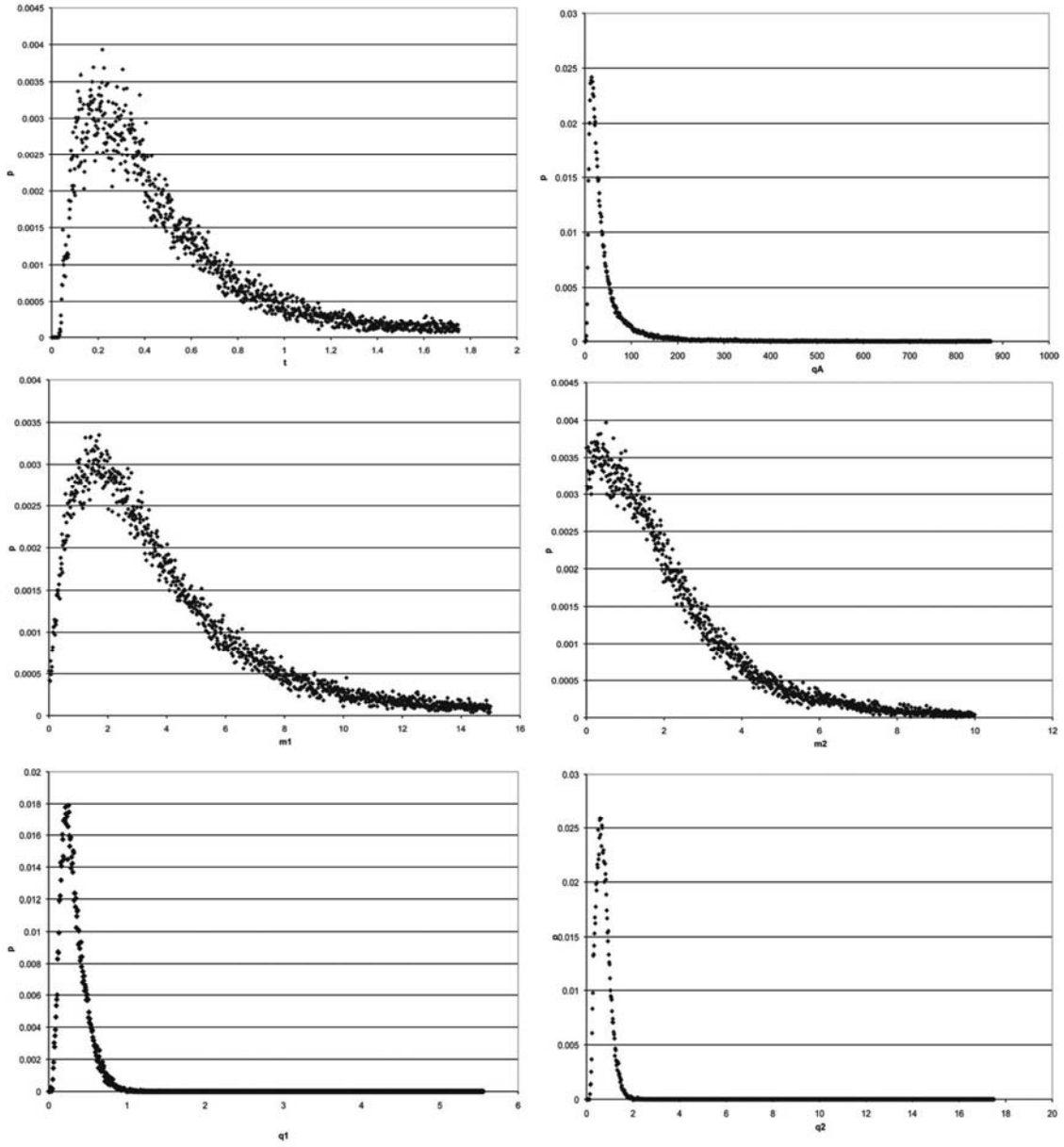
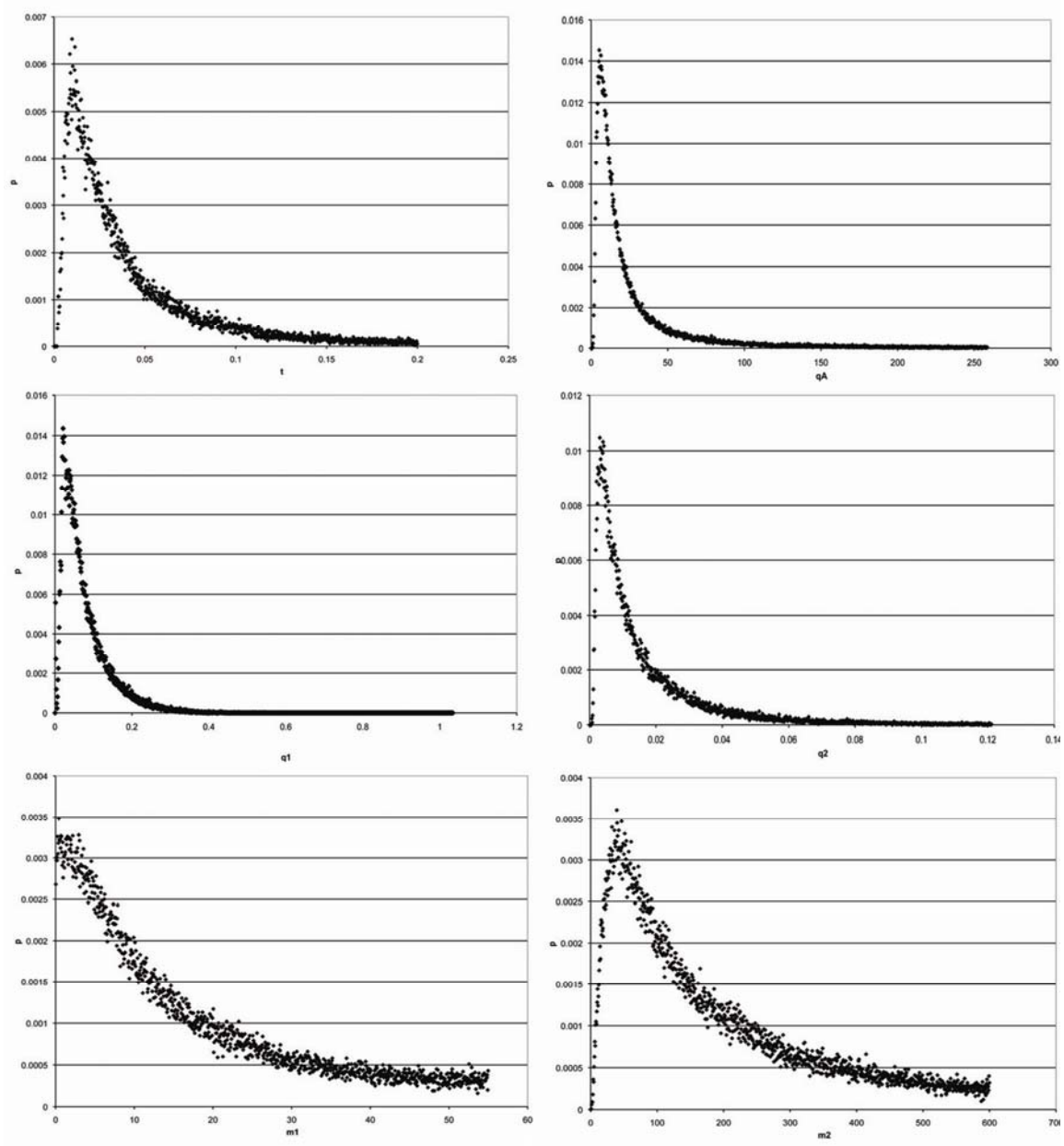


Figure 5b. Saddle Road (1950m) High – Saddle Road Low (1950m) IM graphs



Chapter 4. Rapid diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera: Delphacidae) in both the presence and absence of ecological divergence

Introduction

Many forces may act to promote speciation. Evidence from a variety of taxonomic systems and theoretical work implicates geographic isolation (Dobzhansky, 1937; Mayr, 1963; Slatkin, 1987), natural selection (Berlocher and Feder, 2002; Kirkpatrick and Ravigne, 2002; Rundle and Nosil, 2005; Schluter, 2000; Via, 2001) and sexual selection (Panhuis et al., 2001; Ritchie, 2007; West-Eberhard, 1983) in the evolution of reproductive isolation. However, what is less well understood is at what stage in the divergence process do these mechanisms appear and how do they interact?

Understanding the order of appearance of various mechanisms is a major goal of speciation studies, but a difficult question to approach (Sobel et al., 2010). One empirical method has been to examine phylogenies of adaptive radiations to understand what promotes divergence at various stages of the radiation, and some authors have hypothesized that radiations may follow a predictable sequence across taxonomic groups. There is some discrepancy among results so far (reviewed in: Ackerly et al., 2006; Losos, 2010; Streelman and Danley, 2003). Part of the problem may be due to the fact that these studies have used only phylogenetic approaches that may not be able to detect rapidly evolving forces acting early in diversification (Ackerly et al., 2006; Losos, 2010; Oakley and Cunningham, 2000; Schluter et al., 1997), which makes understanding if different taxonomic groups have followed the same sequence difficult. However, it has been suggested based on empirical studies of vertebrates that diversification occurs 1st in habitat, 2nd in trophic morphology and 3rd in communication (Streelman and Danley, 2003). Theoretical work on speciation also indicates there may be predictable stages to diversification, whereby taxa diversify: 1st between macrohabitats, 2nd between microhabitats, 3rd between magic traits (traits that control both local adaptation and nonrandom mating), and 4th between traits that control survival and reproduction (Gavrilets, 2004). In other words, empirical and theoretical work both suggests that holding all else constant, forces associated with ecology should be relatively more important early in radiations with those associated with sexual behavior following (Gavrilets and Losos, 2009). Empirical data from a variety of taxonomic groups incorporating non-phylogenetic approaches are needed in order to evaluate the ubiquity of a predictable sequence to diversification.

Many animals communicate with sexual signals that are used in species recognition and mate choice (Bradbury and Vehrencamp, 1998). Changes in the production or processing of sexual signals have the potential to promote rapid divergence and eventually reproductive isolation between groups (West-Eberhard, 1983). One proposed mechanism for promoting change in signals is "sensory drive" (Boughman, 2002; Endler, 1992), in

which changes in an animal's ecological setting drive rapid divergence in sexual signals due to differential signal propagation in each environment. Under this hypothesis, ecological shifts precede signal divergence. However, shifts in ecology may not necessarily promote signal divergence, for example: if animals are generalists and inhabit diverse ecological settings, if signaling environments and ecology are not equivalent (Elias et al., 2004), or if novel ecological settings do not show strong differences in signal propagation. Finally, signal divergence also has the potential to occur in the absence of any observable ecological differentiation and has been documented in several phylogenetic studies (Arnegard et al., in press; Masta and Maddison, 2002; Mendelson and Shaw, 2005); in these cases, non-ecological mechanisms are likely to be involved.

While multiple factors may influence the evolution of sexual signals, they are not necessarily involved in speciation unless they lead to some reproductive isolation (West-Eberhard, 1983). One way to measure reproductive isolation is in the currency of gene flow. Fine-scale study of sexual signal diversification coupled with detailed population genetic and phylogeographical analysis may lead to significant insights into the timing of factors associated with the speciation process (Panhuis et al., 2001; Ritchie, 2007). For many species with large geographic ranges, it can be difficult to assess how diversification proceeds. Indeed, the near-ubiquitous presence of population substructure within species and the variation in ecological factors across a species' range guarantees that dynamics of diversification will vary throughout a single species (Thompson, 2005). For this reason, radiations of ecologically specialized lineages on oceanic islands are well-suited to address issues associated with the stages of divergence – they contain populations and species in various stages of evolutionary divergence in an explicit and time-calibrated geographic framework (Gillespie and Roderick, 2002; Roderick and Gillespie, 1998; Roderick and Percy, 2008). In this study, I assess signal diversification in *Nesosydne chambersi* (Kirkaldy, 1908) (Hemiptera: Delphacidae), an insect from the island of Hawaii, testing two hypotheses: that ecological specialization precedes divergence in sexual behavior, and that divergence in sexual behavior is associated with the maintenance of reproductive isolation.

Study system: Planthoppers in the Delphacidae family use substrate-borne signals as a central component of their communication systems to locate and court mates (Claridge, 1985a, b; Claridge and de Vrijer, 1985; de Vrijer, 1984; Ichikawa, 1976; Ossiannilsson, 1949). Signals in planthoppers, like other acoustic signals, are multidimensional (Gerhardt and Huber, 2002) and share a similar form that consists of repetitive patterns of low frequency whines and pulses repeated in a complex temporal pattern and transmitted as waves through their host plant substrates. Among delphacid species studied to date, signals have been demonstrated to be species-specific (Claridge, 1985a, b) and several temporal traits in the male call have also been shown to vary among geographic populations (Butlin, 1993; Claridge et al., 1984, 1985; O'Connell, 1991). Although the genetics underlying signal traits have not been studied extensively, some temporal traits (including inter-pulse interval and pulse repetition frequency) have a significant heritable (additive genetic) component and thus should have the ability to

change rapidly in response to selection (Butlin, 1996; De Winter, 1995; Heady and Denno, 1991).

Within Delphacidae, *Nesosydne* is a genus that is distributed throughout islands in the eastern Pacific, with a large adaptive radiation of 82 described species endemic to the Hawaiian Islands (Asche, 1997; Fennah, 1958; Zimmerman, 1948). Species in this genus specialize on a wide variety of host plants from 20 different families (Asche, 1997; Drew and Roderick, 2005; Fennah, 1958; Hasty, 2005; Roderick, 1997; Roderick and Metz, 1997; Wilson et al., 1994; Zimmerman, 1948), and like other members of their family, have a sexual communication system that relies on vibrations transmitted through their host plants (O'Connell, 1991). *Nesosydne chambersi* is endemic to the island of Hawaii, the youngest of the island chain. The island of Hawaii is geologically complex, and harbors stable habitats on the older volcanoes (including Mauna Kea) as well as ephemeral habitats that have been subject to repeated lava flows over the last several thousand years (on the still-forming Mauna Loa, see Figure 1). *N. chambersi* feeds on three closely related but architecturally distinct host plants in the silversword alliance: *Dubautia ciliolata glutinosa*, *Dubautia ciliolata ciliolata*, and *Dubautia scabra* (Asteraceae). *Dubautia ciliolata glutinosa* is restricted to Mauna Kea, while *D. ciliolata ciliolata* and *D. scabra* are each edaphically specialized to lava flows of different ages and are distributed in a patchwork across the Mauna Loa landscape. The genetic structure and phylogeography of *N. chambersi* suggests that Mauna Kea harbors the ancestral population to those on Mauna Loa, and that it is a species in the process of diversification whose large amount of genetic structure is driven by natural fragmentation due to volcanic activity and not to divergent host plant use (Chapter 4). Divergence among these populations is quite recent, on the order of a few hundred to a few thousand years (Chapter 4, Table 6).

A zone of secondary contact between two populations with mitochondrial haplotype and microsatellite signatures that are distinct from one another is located in the Saddle region between Mauna Loa and Mauna Kea (Figure 1, "Saddle Road (1950)" from Chapter 4) at a site that contains two different host plant species (*D. ciliolata ciliolata* and *D. scabra*). My sampling has revealed a pair of genetic populations with a parapatric distribution, each of which is situated along one end of an approximately 2 km transect. Individuals from each genetic population have been documented in the opposite population and hybrids are scattered throughout (Chapter 4, Figure 4b). Observation of contemporary hybridization indicated that between temporal sampling periods separated by approximately 15 generations, populations did not increase their level of hybridization. Estimation of historical migration (migration averaged over time since the most recent common ancestor) revealed that gene flow has been very low (<0.1 individual/generation; Chapter 4). Together, these data describe genetic populations that are stable in both contemporary and historical time – indicating that something is acting to maintain isolation between them.

Here I examined the evolution of sexual signals in *N. chambersi*, testing two hypotheses: (1) that ecological specialization precedes divergence in sexual behavior, and (2) that changes in sexual signaling are associated with the maintenance of reproductive isolation between populations in secondary contact. My data indicate that signal traits diverge quickly both in the presence and absence of ecological shifts and that they are

associated with reproductive isolation among ecologically similar populations in secondary contact.

Materials and Methods

Collections: Four genetically distinct populations of *N. chambersi* were selected for this study: one from Mauna Kea that feeds exclusively on *D. ciliolata glutinosa*, and three other "non-Mauna Kea" populations: Mauna Loa (collected from *D. ciliolata ciliolata*), Saddle Road High (collected from *D. scabra*) and Saddle Road Low (collected from *D. ciliolata ciliolata* and *D. scabra*) (Table 1, Figure 1). Each of the three non-Mauna Kea populations represents an independent colonization to the newly formed habitat on Mauna Loa, which extends into the Saddle region between the two volcanoes (Chapter 4). *N. chambersi* individuals were collected live from the four populations described above, and males and females were separated upon returning to the laboratory, maintained on cuttings of the host plant species from which they were collected from in the field.

Recording procedures: In order to obtain recordings of *Nesosydne chambersi's* vibrational signals, a recording studio was created at the USGS Biological Resource Division laboratory facilities at 1220 m on Mauna Loa in Hawaii Volcanoes National Park in May and September of 2009. Experimental chambers were created from insect rearing cages; briefly, the cages were positioned on boxes and plant cuttings were placed inside with the stems emerging from a hole in the bottom. The cuttings were stabilized in sand and the space around the hole was sealed with cotton. One or two field-collected males were released into each cage and allowed to settle onto the vegetation. After approximately 10-30 minutes, one female was released into the cage and activity was monitored until calling began. If calling did not commence, an additional one or two females were released into the cage. Only calls from one male were analyzed per plant. If multiple males were introduced to each plant, males were distinguished on the basis of call intensity. All recordings were made of insects calling on the host plant species from which they were collected in the field.

Two recording methods were used to obtain mating signals: an accelerometer and a laser vibrometer. For accelerometer recordings, the device (USB Powered-Dual Channel ICP Sensor Signal Conditioner, Model 485B36) was secured to a wire clip and then attached to the plant stem below the cage. It was connected to a pre-amplifier (iMic, Griffin Technology), and recordings were made onto a Mac PowerBook G4. Signals were recorded using Audacity software (version 1.2, 44.1 kHz sampling rate). For laser vibrometer recordings, the laser beam was focused onto reflective tape at the center of the plant using a Portable Digital Vibrometer (PDV-100, Polytec) connected to a High Resolution Audio Recorder (Sound Devices 722, 48kHz sampling rate). Laser recordings were resampled to a 44.1 kHz sampling rate prior to trait scoring. Prior to performing statistical analyses, all analyzed characters (see below) were tested for differences between recording methods in the means of each signal trait using t-tests – none were found. Since some signal traits are known to be influenced by temperature (Gerhardt and Huber, 2002), temperature was noted at the time of each recording and corrected post-hoc when needed. To test for potential temperature affects, each variable (see below) was tested for

correlation (linear regression, JMP, SAS Institute), and those found to be significantly correlated with temperature were corrected to 22.5 °C (the mean observed for the recording period).

Sound analysis:

Temporal traits: The structure of male *Nesosydne chambersi* songs are comprised of several components – the fundamental unit of which is the whine, which itself is made up of a series of rapid pulses. A signal bout is composed of several whines, and whines are sometimes followed by several distinct pulses that are stronger and slower than those in the whine. Temporal characteristics of signals were visualized and measured using Audacity software (audacity.sourceforge.net). For each trait, multiple samples were scored for each individual male and then averaged to obtain a single score per male. Temporal traits that have been demonstrated to be variable among treehoppers and planthoppers (e.g. Claridge et al., 1985; Rodriquez et al., 2006; Sattman and Cocroft, 2003) were selected for scoring in this study and included (n=number of individual males, x=mean number of samples scored per individual): whine length (n=78, x=15.1), whine pulse rate (n=67, x= 8.9), pulse rate (n=55, x=9.0), inter-signal interval (n=72, x=12.3), ratio of the number of signals per bout to the inter-bout interval (RSI: n=44, x=6.7) (Figure 2).

Frequency traits: In past research on planthoppers, frequency characters were considered too variable to provide useful information (de Vrijer, 1984) and thus the frequency domain has been ignored. However, O'Connell (1991) noted that in *Neosydne* "there seems to be a frequency pattern that is distinct among species" (O'Connell, 1991, p.14) although this pattern was not quantified. de Vrijer's (1984) observation that frequency is highly variable in delphacids is borne out by my observations of this species. *N. chambersi* calls are generated using strong fundamental frequency with a significant amount of additional signal energy concentrated in harmonics (Figure 2). I examined frequency characteristics by comparing fundamental frequency among populations. All frequency spectra were measured using normalized power spectra calculated in Matlab (The Mathworks).

To obtain measurements of the fundamental frequency of whines for each individual, power spectra were calculated for each individual (n=59) by averaging one second segments from the center of five separate whines thus avoiding the frequency modulations found at the beginning and end of the whines. Peak intensities were normalized to the maximum intensity within each measurement, and peak positions identified using custom written Matlab scripts. Background noise was strong between 0-95Hz hence any energy in this bandwidth was ignored. In treehoppers (the most closely related family on which frequency analysis has been performed), signal energy is concentrated at higher frequencies (100-450 Hz: McNett and Cocroft, 2008; Rodriguez et al., 2004; Sattman and Cocroft, 2003) hence the most important frequency elements were likely captured using these methods. Future work is needed to verify this assumption.

Statistical analysis:

The distributions of each variable were first tested for normality using the Shapiro-Wilks test; significantly skewed traits were log transformed for subsequent analyses. Because field conditions prevented recording planthoppers signaling on each host plant in each location, I did not attempt to build a model including both site and host plant. Instead, I first examined whether each signal trait varied between Mauna Kea and non-Mauna Kea populations using t-tests. Then, I examined whether each signal trait varied by population by performing an overall analysis of variance (ANOVA), and if differences were observed, I proceeded to test for pairwise differences between each population using Tukey's HSD post-hoc test. Finally, I tested whether host plant influences male signal traits in one genetic population known to use two host plant species (Saddle Road Low) using t-tests. All statistical analyses except when noted were conducted in R (R Development Core Team, 2009).

Results

Trait diversification between Mauna Kea vs. non-Mauna Kea populations

Mauna Kea differed from the non-Mauna Kea populations in both spectral (fundamental frequency) and temporal elements (whine pulse rate, inter-signal interval): fundamental frequency, (Mauna Kea vs. non-Mauna Kea, $p=0.0048$), whine pulse rate (Mauna Kea vs. non-Mauna Kea, $p<0.0001$) and inter-signal interval (Mauna Kea vs. non-Mauna Kea:, $p=0.0004$) (Table 2, 3).

Trait diversification among all populations

Trait diversification overall: The overall ANOVAs indicated that there are significant differences among populations in all of the signal traits examined here except for pulse rate (Table 3), thus the application of Tukey's HSD was justified to assess how trait diversification is distributed in pairwise contrasts (below).

"Same ecological setting" contrasts (including Saddle Road zone of secondary contact): In the contact zone, differences in the means of one temporal trait (RSI) are greater in magnitude than between comparisons of either Saddle Road population with the allopatric Mauna Loa (Saddle Road High vs. Saddle Road Low, $p_{adj}=0.0002$, Table 3, Figure 3). Two additional temporal traits are weakly differentiated between the two Saddle Road populations (whine length and inter-signal interval, Table 3), indicating that at least one and possibly several traits are acting in tandem to prevent gene flow in this area. In addition, Mauna Loa and Saddle Road High differed in two traits: whine length (Mauna Loa vs. Saddle Road High, $p_{adj}=0.0004$) and the RSI (Mauna Loa vs. Saddle Road High, $p_{adj}=0.03$).

"Different ecological setting" contrasts: Mauna Kea and each of the non-Mauna Kea populations varied in several comparisons: whine pulse rate (Mauna Kea vs. Mauna

Loa, $p_{adj}<0.001$; Mauna Kea vs. Saddle Road High, $p_{adj}<0.05$; Mauna Kea vs. Saddle Road Low, $p_{adj}<0.0001$); inter-signal interval (Mauna Kea vs. Mauna Loa, $p_{adj}<0.01$; Mauna Kea vs. Saddle Road Low, $p_{adj}<0.001$); and RSI (Mauna Kea vs. Saddle Road Low, $p_{adj}<0.01$) (Table 3).

Trait diversification among host plants in the Saddle Road Low population

Within the Saddle Road Low population, I found no significant differences ($p>0.05$) between the means of any of the signal traits recorded from the two different host plant species (*D. ciliolata ciliolata* and *D. scabra).*

Discussion

Ecological shifts are known to be of fundamental importance in the speciation of herbivorous insects, (Berlocher and Feder, 2002; Rundle and Nosil, 2005), as is signal evolution in Hemiptera and other acoustic animals (Claridge, 1985b; Cocroft et al., 2007; Gerhardt and Huber, 2002). In this study, I demonstrate that signals are diverging in a temporal trait between populations in the same ecological setting (Saddle Road zone of secondary contact). I also demonstrate that signals are diverging in both frequency and temporal traits between populations in ecologically different settings (Mauna Kea vs. non-Mauna Kea). Together, my results reveal a communication system that is rapidly diversifying under the influence of a variety of forces and whose divergence is pronounced among reproductively isolated populations in geographic contact.

Signal divergence in the absence of an ecological shift: a natural experiment in the zone of secondary contact

Regions of secondary contact are of great interest to evolutionary biologists because they provide a way to examine the efficacy of reproductive isolating factors in their natural setting (Barton and Hewitt, 1985). Saddle Road Low and Saddle Road High form a stable zone of secondary contact with clear genetic differences that have been estimated to have diverged quite recently – 400 (175-5,250) years. This suggests that although individuals are within range of one another and hybrids can be observed (Chapter 4), something is acting to prevent the populations from fusing. In this study, I tested the hypothesis that the reduced gene flow observed between the populations is associated with divergence in sexual signaling traits and found that it is. I demonstrated a strong difference in the means of the temporal trait "RSI" between the two Saddle Road populations. This difference is stronger in magnitude between the populations in the zone of secondary contact than among populations in allopatry (Figure 3), a pattern that is consistent with reproductive character displacement (Brown and Wilson, 1956; Howard, 1993). However, whether the trait RSI diverged to the extent observed in allopatry prior to secondary contact (West-Eberhard, 1983) or whether its magnitude was amplified via reproductive character displacement (Howard, 1993) cannot be distinguished from these data. Either way, it is very clear that this behavioral trait is pliable and is subject to rapid change in populations without a shift in ecology.

I found no relationship between the two host plant species available at the site (*D. ciliolata ciliolata* and *D. scabra*) and genetic (Chapter 4) or signal variation. This indicates that strict ecological specialization to host plants has not occurred in the Saddle Road region and is thus is not what is acting to promote this pattern. Although other ecological forces such as differences in wind or predators among sites have the potential to provide ecological selection, my observations suggest these are very unlikely to be different between these two parapatric Saddle Road populations – thus they cannot explain the temporal trait differences observed there. Temporal traits of acoustic signals (such as RSI) are predicted to be less constrained by signaling environment than frequency traits (Elias and Mason, in press; Elias et al., 2010) and consequently I would expect that if changes are to take place in signals in the absence of ecological shifts, they would be in the temporal domain. What non-ecological factors might be responsible for initiating and then driving changes among sexual traits? Two potentially complimentary hypotheses that may be especially relevant to this system are sexual selection by female choice and genetic drift.

Sexual selection, particularly selection acting directly on courtship signals, may be an important force in driving male signal trait diversification (Boul et al., 2007; Rodriquez et al., 2006; West-Eberhard, 1983). Sexual selection has been observed acting in the apparent absence of ecological selection, for example, in Hawaiian *Laupala* crickets (Mendelson and Shaw, 2005), Hawaiian *Drosophila* (Carson, 1997), and *Habronattus* jumping spiders of the Sky Islands of the western United States (Elias et al., 2006a; Elias et al., 2006b; Masta and Maddison, 2002). A strong relationship has been demonstrated in *Enchenopa* treehoppers between female preferences and several male signal traits (frequency, whine length, pulse number, pulse rate, signal number; Rodriquez et al., 2006), many of which I also studied here. Given the similarities in lifestyle and communication systems between treehoppers and planthoppers, is plausible that female preference plays a strong role in trait evolution in this system as well.

Although evolution by drift is typically thought to act slowly and therefore not be a major force in evolution (Coyne and Orr, 2004; Sobel et al., 2010), this may not be true in populations of limited size and it may be a more likely factor in diversification of island groups (Carson, 1978; Kaneshiro, 1980; Wagner and Funk, 1995). Recent modeling work has shown that sexual isolation may evolve quite rapidly (in as few as 1000 generations) due at least in part to the stochastic forces of drift with population sizes in the range of 1000-5000 (Uyeda et al., 2009). Together, the estimated demographic parameters of the Saddle Road populations are consistent with those that would be needed for drift to play a role in trait evolution and reproductive isolation according to this model. The estimated divergence between the two Saddle Road populations occurred 1,980 (875 – 26,270) generations ago. Furthermore, the Saddle Road Low population has an estimated effective population size of 840 (474 – 12,000) (Chapter 4). Interestingly, signal trait divergence between the Mauna Kea population and the Saddle Road Low population is strong at almost all signal traits, despite the fact that they share a mitochondrial haplotype. It could be that we are witnessing the effects of drift immediately after colonization of this site.

It may very well be that a combination of drift and sexual selection (or other factors) may be at play in this system. Sexual selection, even if it is important to this system, may not be acting alone as it needs variability to act upon. Initial changes in male

traits may be the result of a variety of factors, including drift (reviewed in: Kirkpatrick and Ryan, 1991). For example, drift may initiate changes in mean signal phenotypes between populations that are then driven apart via sexual selection by female preference. Likewise, finite population sizes and strong female preferences may together drive reproductive isolation (Uyeda et al., 2009). Future work will focus on disentangling these possible factors and documenting the predicted association between trait divergence and female preferences.

Other partially non-ecological explanations may explain the data as well. For example, the observed patterns may arise by genotype by environment interactions. Recent studies by Rodriguez et al (2008) experimentally demonstrated that an herbivore's shift to a novel host plant can promote the expression of extant genetic variation in the newly colonized environment, and the authors suggest that this might underlie the initial divergence of signal traits among populations immediately after the colonization of a novel environment. In a similar vein, each colonized population (Saddle Road High and Saddle Road Low) is likely to contain different pools of standing variation that was present in the ancestral population (Mauna Kea) by chance. Ecological selection, although similar in the Saddle Road populations, may then act to fix different traits from this variation among populations (Mani and Clarke, 1990; Schluter, 2009).

Signal divergence in the presence of an ecological shift: Mauna Kea vs. non-Mauna Kea populations

Here I have also shown that a change in the mean fundamental frequency coincides with a shift from Mauna Kea (with *D. ciliolata glutinosa*) to the non-Mauna Kea populations, a novel environment that contains two new (*D. ciliolata ciliolata* and *D. scabra*), but not the old host plant species. This observation is interesting in light of the fact that the non-Mauna Kea populations each have a distinct mitochondrial haplotype (Chapter 4) – indicating that similarities in this trait are due to shared environment, not shared history. There are a variety of aspects of the environment that are different between the Mauna Kea and non-Mauna Kea populations (e.g., elevation, substrate, host plant, population densities). However, the most likely environmental factor influencing fundamental frequency is the shift in host plant from *D. ciliolata glutinosa* to *D. ciliolata ciliolata* and *D. scabra*. Such a change in fundamental frequency associated with a shift in host plants may be consistent with two different hypotheses. First, the filtering properties of the novel plants may provide immediate and strong selection on what frequencies can be effectively transmitted (Cokl, 2003; Michelsen et al., 1982). This is consistent with Endler's (1992) sensory drive hypothesis—as substrate-borne environments strongly constrain signal propagation, host plant shifts would lead to signal divergence (as novel environments distort male signals) before any preference divergence. Alternatively, it may point to genotype by environment interactions such as were found with membracid treehoppers – adults recorded on their own versus alternative host plants did not alter the fundamental frequency of their signal on either host plant, but offspring that were reared on alternative host plants did (Rodriguez et al., 2008). Future work will investigate how

differential signal propagation in the various host plant species may or may not constrain signal form.

Conclusions

In conclusion, *N. chambersi* offers a rare window into the early stages of signal evolution and speciation from a natural system. These data show that signal traits diverge quickly both in the absence and presence of ecological shifts and that they are associated with reproductive isolation among ecologically similar populations in secondary contact. While any of a variety of non-ecological forces may be at work in this system to produce this pattern, an explanation based on an ecological shift alone is insufficient to explain the patterns observed in the data. In this relatively simple system, it is clear that ecological divergence does not have to precede divergence in sexual behavior.

Literature Cited

Ackerly, D.D., Schwilk, D.W., and Webb, C.O., 2006, Niche Evolution and Adaptive Radiation: Testing the Order of Trait Divergence: Ecology, v. 87, p. S50-S61.

Arnegard, M.E., McIntyre, P.B., Harmon, L.J., Zelditch, M.L., Crampton, W.G.R., Davis, J.K., Sullivan, J.P., Lavoue, S., and Hopkins, C.D., in press, Sexual Signal Evolution Outpaces Ecological Divergence during Electric Fish Species Radiation: The American Naturalist.

Asche, M., 1997, A Review of the Systematics of Hawaiian Planthoppers (Hemiptera: Fulgoroidea): Pacific Science, v. 51, p. 366-376.

Barton, N.H., and Hewitt, G.M., 1985, Analysis of Hybrid Zones: Annual Review of Ecology and Systematics, v. 16, p. 113-148.

Berlocher, S.H., and Feder, J.L., 2002, Sympatric speciation in phytophagous insects: Moving beyond controversy?: Annual Review of Entomology, v. 47.

Boughman, J.W., 2002, How sensory drive can promote speciation: Trends In Ecology & Evolution, v. 17, p. 571-577.

Boul, K.E., Funk, W.C., Darst, C.R., Cannatella, D.C., and Ryan, M.J., 2007, Sexual selection drives speciation in an Amazonian frog: Proceedings of the Royal Society B, v. 274, p. 399-406.

Bradbury, J.W., and Vehrencamp, S.L., 1998, Principles of Animal Communication: Sunderland, MA, Sinauer Associates, Inc. .

Brown, W.L.J., and Wilson, E.O., 1956, Character displacement: Systematic Zoology, v. 5, p. 49-64.

Butlin, R.K., 1993, The variability of mating signals and preferences in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae): Journal of Insect Behavior, v. 6, p. 125-140.

—, 1996, Co-ordination of the sexual signalling system and the genetic basis of differentiation between populations in the brown planthopper, *Nilaparvata lugens*: Heredity, v. 77, p. 369-377.

Carson, H.L., 1978, Speciation and sexual selection in Hawaiian *Drosophila*: New York, Springer-Verlag.

—, 1997, Sexual Selection: A Driver of Genetic Change in Hawaiian *Drosophila*: Journal of Heredity, v. 88, p. 343-352.

Claridge, M.F., 1985a, Acoustic behavior of leafhoppers and planthoppers: Species problems and speciation, *in* Nault, L.R., and Rodriguez, J.G., eds., The Leafhoppers and Planthoppers: New York, John Wiley and Sons, p. 103-125.

—, 1985b, Acoustic Signals in the Homoptera: Behavior, Taxonomy, and Evolution: Annual Review of Entomology, v. 30, p. 297-317.

Claridge, M.F., and de Vrijer, P.W.F., 1985, Reproductive Behavior: The Role of Acoustic Signals in Species Recognition and Speciation, *in* Denno, R.F., and Perfect, J.T., eds., Planthoppers: Their Ecology and Management: New York, Chapman & Hall.

Claridge, M.F., Den Hollander, J., and Morgan, J.C., 1984, Specificity of acoustic signals and mate choice in the brown planthopper *Nilaparvata lugens*: Entomologia exp appl,, v. 34, p. 221-226.

—, 1985, Variation in courtship signals and hybridization between geographically definable populations of the rice Brown planthopper, *Nilaparvata lugens* (Stal): Biological Journal of the Linnean Society, v. 24, p. 35-49.
Cocroft, R.B., Rodriguez, R.L., and Hunt, R.E., 2007, Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* complex of treehoppers, *in* Tilmon, K.J., ed., Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects: Berkeley, CA, University of California Press. .
Cokl, A.J.V.-D., M., 2003, Communication with Substrate-Borne Signals in Small Plant-Dwelling Insects: Annual Review of Entomology, v. 48, p. 29-50.
Coyne, J.A., and Orr, H.A., 2004, Speciation: Massachusetts, Sinauer Associates, Inc., 545 p.
de Vrijer, P.W.F., 1984, Variability in calling signals of the planthopper *Javesella pellucida* (F.) (Homoptera: Delphacidae) in relation to temperature, and consequences for species recognition during distant communication: Netherlands Journal of Zoology, v. 34, p. 388-406.
De Winter, A.J., 1995, Genetic Control and Evolution of Acoustic Signals in Planthoppers (Homoptera: Delphacidae): Researches On Population Ecology, v. 37, p. 99-104.
Dobzhansky, T.G., 1937, Genetics and the origin of species: New York, Columbia Univerisy Press.
Drew, A.E., and Roderick, G.K., 2005, Abundance and Diversity of Nesosydne Planthoppers (Hemiptera: Delphacidae) Across Hybrid Zones of the Hawaiian Silversword Alliance: Pacific Science, v. in press.
Elias, D.O., Hebets, E.A., and Hoy, R.R., 2006a, Female preference for signal complexity/novelty in a jumping spider: Behavioral Ecology, v. 17, p. 765-771.
Elias, D.O., Land, B.R., Mason, A.C., and Hoy, R.R., 2006b, The classification and quantification of dynamic visual signals in jumping spiders: Journal of Comparative Physiology-A, v. 192, p. 785-797.
Elias, D.O., and Mason, A.C., in press, Signaling in variable environments: Substrate-borne signaling mechanisms and communication behavior in spiders, *in* O'Connel-Rodwell, C., ed., The use of Vibrations in communication: Properties, Mechanisms and Function across Taxa.
Elias, D.O., Mason, A.C., and Hebets, E.A., 2010, A signal-substrate match in the substrate-borne component of a multimodal courtship display: Current Zoology, v. 56, p. 370-378.
Elias, D.O., Mason, A.C., and Hoy, R.R., 2004, The effect of substrate on the efficacy of seismic courtship signal transission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae): Journal of Experimental Biology, v. 207, p. 4105-4110.
Endler, J.A., 1992, Signals, signal conditions, and the direction of evolution: The American Naturalist, v. 139, p. s125-s153.
Fennah, R.G., 1958, Fulgoroidea of South-Eastern Polynesia: Transactions of the Royal Entomological Society of London, v. 110, p. 117-220.
Gavrilets, S., 2004, Fitness Landscapes and the Origin of Species: Princeton, N.J., Princeton University Press.

Gavrilets, S., and Losos, J.B., 2009, Adaptive Radiation: Contrasting Theory with Data: Science, v. 323 p. 732-737.
Gerhardt, H.C., and Huber, F., 2002, Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions: Chicago, IL, University of Chicago Press.
Gillespie, R.G., and Roderick, G.K., 2002, Arthropods on islands: colonization, speciation, and conservation: Annual Review of Entomology, v. 47, p. 595-632.
Hasty, G.L., 2005, Host-plant specialization affects species diversity and vulnerability to endangerment in a group of island-endemic phytophagous insects (Delphacidae, Hawaiian Islands): Berkeley, University of California at Berkeley.
Heady, S.E., and Denno, R.F., 1991, Reproductive isolation in *Prokelisia* Planthoppers (Homoptera: Delphacidae): Acoustic differentiation and hybridization failure: Journal of Insect Behavior, v. 4, p. 367-390.
Howard, D., 1993, Reinforcement: Origin, Dynamics, and Fate of an Evolutionary Hypothesis, *in* Harrison, R.G., ed., Hybrid Zones and the Evolutionary Process: New York, Oxford University Press, p. 46-69.
Ichikawa, T., 1976, Mutual communication by substrate vibrations in the mating behavior of planthoppers (Homoptera: Delphacidae): Applied Entomology and Zoology, v. 11, p. 8-23.
Kaneshiro, K.Y., 1980, Sexual selection, speciation and the direction of evolution: Evolution, v. 34, p. 437-444.
Kirkpatrick, M., and Ravigne, V., 2002, Speciation by Natural and Sexual Selection: Models and Experiments: The American Naturalist, v. 159, p. S22-S35.
Kirkpatrick, M., and Ryan, M.J., 1991, The evolution of mating preferences and the paradox of the lek: Nature, v. 350, p. 33-38.
Losos, J.B., 2010, Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism: American Society of Naturalists E.O. Wilson Award Address: The American Naturalist, v. 175, p. 623-639.
Mani, G.S., and Clarke, B.C., 1990, Mutational Order: A Major Stochastic Process in Evolution: Proceedings of the Royal Society B, v. 240, p. 29-37.
Masta, S.E., and Maddison, W.P., 2002, Sexual selection driving diversification in jumping spiders: Proceedings of the National Academy of Sciences, v. 99, p. 4442-4447.
Mayr, E., 1963, Animal species and evolution: Cambridge, MA, Harvard University Press.
McNett, G.D., and Cocroft, R.B., 2008, Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers: Behavioral Ecology, v. 19, p. 650-656.
Mendelson, T.C., and Shaw, K.L., 2005, Rapid speciation in an arthropod: the likely force behind an explosion of new Hawaiian cricket species is revealed: Nature, v. 433, p. 375-376.
Michelsen, A., Fink, F., Gogala, M., and Traue, D., 1982, Plants as transmission channels for insect vibrational songs: Behavioral Ecology and Sociobiology, v. 11, p. 269-281.
O'Connell, C., 1991, Species Barriers and Breakdowns in Hawaiian Planthoppers: Manoa, University of Hawaii.

Oakley, T.H., and Cunningham, C.W., 2000, Independent contrasts succeed where ancestor reconstruction fails in a known bacteriophage phylogeny: Evolution, v. 54, p. 397-405.
Ossiannilsson, F., 1949, Insect Drummers, a study on the morphology and function of the sound-producing organ of Swedish Homoptera Auchenorrhyncha, with notes on their sound production: Opuscula Entomologica Suppl., v. 10, p. 1-145.
Panhuis, T.M., Butlin, R.K., Zuk, M., and Tregenza, T., 2001, Sexual Selection and Speciation: Trends In Ecology & Evolution, v. 16, p. 364-371.
Ritchie, M.G., 2007, Sexual Selection and Speciation: Annual Review of Ecology and Systematics, v. 38, p. 79-102.
Roderick, G.K., 1997, Herbivorous Insects and the Hawaiian Silversword Alliance: Coevolution or Cospeciation?: Pacific Science, v. 51, p. 440-449.
Roderick, G.K., and Gillespie, R.G., 1998, Speciation and phylogeography of Hawaiian terestrial arthropods: Molecular Ecology, v. 7, p. 519-531.
Roderick, G.K., and Metz, E.C., 1997, Biodiversity of Planthoppers (Hemiptera: Delphacidae) on the Hawaiian Silversword Alliance: Effects of Host Plant Phylogeny and Hybridisation: Memoirs of the Museum of Victoria, v. 56, p. 393-399.
Roderick, G.K., and Percy, D.M., 2008, Host plant use, diversification, and coevolution: Insights from remote oceanic islands, *in* Tilmon, K.J., ed., Evolutionary Biology of Plant and Insect Relationships: Berkeley, University of California Press.
Rodriguez, R.L., Sullivan, L.M., and Cocroft, R.B., 2004, Vibrational Communication and Reproductive Isolation in the *Enchenopa binotata* Species Complex of Treehoppers (Hemiptera: Membracidae): Evolution, v. 58, p. 571-578.
Rodriguez, R.L., Sullivan, L.M., Snyder, R.L., and Cocroft, R.B., 2008, Host shifts and the beginning of signal divergence: Evolution, v. 62, p. 12-20.
Rodriquez, R.L., Ramaswamy, K., and Cocroft, R.B., 2006, Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects: Proceedings of the Royal Society B, v. 273, p. 2585-2593.
Rundle, H.D., and Nosil, P., 2005, Ecological speciation: Ecology Letters, v. 8, p. 336-352.
Sattman, D.A., and Cocroft, R.B., 2003, Phenotypic Plasticity and Repeatability in the Mating Signals of Enchenopa Treehoppers, with Implications for Reduced Gene Flow among Host-Shifted Populations: Ethology, v. 109, p. 981-994.
Schluter, D., 2000, The Ecology of Adaptive Radiation: Oxford, Oxford University Press, 288 p.
—, 2009, Evidence for Ecological Speciation and Its Alternative: Science, v. 323, p. 737-741.
Schluter, D., Price, J.P., Moores, A.O., and Ludwig, D., 1997, Likelihood of Ancestor States in Adaptive Radiation: Evolution, v. 51, p. 1699-1711.
Slatkin, M., 1987, Gene flow and the geographic structure of natural populations: Science, v. 236, p. 787-792.
Sobel, J.M., Chen, G.F., Watt, L.R., and Schemske, D.W., 2010, The Biology of Speciation: Evolution, v. 64, p. 295-315.

Streelman, J.T., and Danley, P.D., 2003, The stages of vertebrate evolutionary radiation: Trends In Ecology & Evolution, v. 18, p. 126-131.
Team, R.D.C., 2009, R: A language and environment for statistical computing: Vienna, Austria. , R Foundation for Statistical Computing. ISBN 3-9000-51-07-0, URL http://www.R-project.org.
Thompson, J.N., 2005, The Geographic Mosaic of Coevolution: Chicago, The University of Chicago Press, 400 p.
Uyeda, J.C., Arnold, S.J., Hohenlohe, P.A., and Mead, L.S., 2009, Drift promotes speciation by sexual selection: Evolution, v. 63, p. 583-594.
Via, S., 2001, Sympatric speciation in animals: the ugly duckling grows up: Trends In Ecology & Evolution, v. 16, p. 381-390.
Wagner, W., and Funk, V., 1995, Hawaiian Biogeography: Evolution on a Hot Spot Archipelago: Washington DC, Smithsonian Institution Press, 467 p.
West-Eberhard, M.J., 1983, Sexual selection, social competition, and speciation: Quarterly Review of Biology, v. 58, p. 155-183.
Wilson, W.W., Mitter, C., Denno, R.F., and Wilson, M.R., 1994, Evolutionary patterns of host plant use by delphacid planthoppers and their relatives, *in* Denno, R.F., and Perfect, T.J., eds., Planthoppers: Their Ecology and Management: New York, Chapman & Hall, p. 7-113.
Zimmerman, E.C., 1948, Homoptera: Auchenorhyncha: Honolulu, HI, University of Hawaii Press.

Tables

Table 1. Sampling Locations and Site Characteristics. Host plant indicates the host plants that individuals were collected from in the field and recordings were made in the lab.

Population Name	Host Plant	Elevation (m)	Age of Substrate (yrs)	Latitude	Longitude
Mauna Kea *(Pu'u Kanakaleonui)*	*Dubautia ciliolata glutinosa*	2,850	14,000-65,000	-155.391	19.847
Saddle Road High	*Dubautia scabra*	1,950	<200 – 3,000	-155.452	19.689
Saddle Road Low	*Dubautia scabra* *Dubautia ciliolata ciliolata*	1,950	<200 – 3,000	-155.439	19.686
Mauna Loa Trail *(Hawaii Volcanoes National Park)*	*Dubautia ciliolata ciliolata*	2,085	1,500 – 3,000	-155.385	19.499

Table 2. Summary of signal traits by population: mean, standard error (n).

Population *Host Plant*	*Fundamental Frequency (Hz)*	*Whine Pulse Rate (#/s)*	*Whine Length (s)*	*Pulse Rate (#/s)*	*Inter-Signal Interval (s)*	*Signals per bout/ Interval between bouts (RSI) (#/s)*
Mauna Kea *D. ciliolata glutinosa*	203.9, 2.6 (12)	35.62, 0.45 (14)	2.89, 0.12 (15)	5.86, 0.34 (10)	5.35, 0.22 (15)	0.24, 0.05 (10)
Mauna Loa *D. ciliolata ciliolata*	195.3, 3.5 (15)	33.12, 0.61 (16)	3.25, 0.16 (18)	5.79, 0.44 (14)	6.50, 0.30 (17)	0.15, 0.06 (13)
Saddle Road High *D. scabra*	195.3, 3.5 (14)	33.88, 0.63 (14)	2.59, 0.16 (17)	6.59, 0.42 (14)	5.80, 0.30 (16)	0.36, 0.06 (11)
Saddle Road Low *D. ciliolata ciliolata*	191.2, 3.6 (6)	32.40, 0.45 (12)	3.12, 0.16 (13)	6.82, 0.56 (7)	6.46, 0.33 (11)	0.08, 0.03 (6)
Saddle Road Low *D. scabra*	197.5, 4.4 (11)	33.29, 0.66 (11)	2.77, 0.22 (15)	6.75, 0.73 (10)	6.44, 0.45 (13)	0.11, 0.04 (4)

Table 3. Summary of differences among populations in signal traits, compared with host plant associations and genetic divergence among populations. Genetic data is from Goodman *et al in prep* - (F_{ST} (microsatellites)/% uncorrected pairwise sequence divergence (mitochondrial – cytochrome oxidase I)). Overall ANOVA effect sizes are reported as the *F-value* and *t-values* are reported for pairwise comparisons of populations. Grey boxes indicate statistical significance at the 95% level; pairwise combinations are significant after adjustment for multiple comparisons using Tukey's HSD (ns=not significant). Zone of secondary contact is Saddle Road High vs. Saddle Road Low.

Contrasts	***Host Plants***	***Genetics***	***Frequency Element***	***Temporal Elements***				
		FST / % sequence divergence	*Fundamental Frequency*	*Whine Pulse Rate*	*Whine Length*	*Pulse Rate*	*Inter-Signal Interval*	*Signals per bout/ Interval between bouts (RSI)*
1. Overall ANOVAs								
Overall		0.282/ 0.0044	$2.776_{3,54}$ $p<0.05$	$8.978_{3,63}$ $p<0.001$	$5.984_{3,74}$ $p<0.01$	$1.979_{3,51}$ p=ns	$7.475_{3,68}$ $p<0.001$	$8.064_{3,40}$ $p<0.001$
2. Comparisons between Mauna Kea and non-Mauna Kea populations combined								
Mauna Kea vs. non-Mauna Kea	Different	--	$8.63_{1,56}$ $p<0.01$	$22.91_{1,65}$ p-value: 1.018e-05	$0.255_{1,74}$ p=ns	$0.9254_{1,52}$ p=ns	$13.87_{1,70}$ $p<0.001$	$-1.122_{1,40}$ p=ns
3a. Pairwise tests between Mauna Kea and each of the non-Mauna Kea populations								
Mauna Kea vs. Mauna Loa	Different	0.295/ 0.0048	$-2.433_{1,25}$ $p_{adj}<0.10$	$-4.091_{1,28}$ $p_{adj}<0.001$	$2.213_{1,31}$ p_{adj}=ns	--	$3.899_{1,30}$ $p_{adj}<0.01$	$-1.462_{1,21}$ p_{adj}=ns
Mauna Kea vs. Saddle Road High	Different	0.245/ 0.0035	$-2.331_{1,24}$ p_{adj}=0.10	$-2.764_{1,28}$ $p_{adj}<0.05$	$-1.852_{1,30}$ p_{adj}=ns	--	$1.514_{1,29}$ p_{adj}=ns	$1.283_{1,19}$ p_{adj}=ns
Mauna Kea vs. Saddle Road Low	Different	0.294/ 0.0000	$-2.504_{1,26}$ $p_{adj}<0.10$	$-4.941_{1,35}$ $p_{adj}<0.0001$	$0.257_{1,41}$ p_{adj}=ns	--	$4.023_{1,37}$ $p_{adj}<0.001$	$-3.330_{1,18}$ $p_{adj}<0.01$
3b. Pairwise tests between each of the non-Mauna Kea populations								
Mauna Loa vs. Saddle Road High	Same	0.274/ 0.0035	$0.069_{1,27}$ p_{adj}=ns	$1.295_{1,28}$ p_{adj}=ns	$-4.060_{1,33}$ $p_{adj}<0.001$	--	$2.276_{1,31}$ $p_{adj}<0.10$	$2.913_{1,22}$ $p_{adj}<0.05$
Mauna Loa vs. Saddle Road Low	Same	0.301/ 0.0054	$-0.005_{1,30}$ p_{adj}=ns	$-0.572_{1,37}$ p_{adj}=ns	$-2.198_{1,44}$ p_{adj}=ns	--	$-0.170_{1,39}$ p_{adj}=ns	$-2.111_{1,21}$ p_{adj}=ns
Saddle Road High vs. Saddle Road Low	Same	0.243/ 0.0048	$-0.073_{1,29}$ p_{adj}=ns	$-2.135_{1,35}$ p_{adj}=ns	$2.107_{1,43}$ $p_{adj}<0.10$	--	$2.113_{1,38}$ $p_{adj}<0.10$	$-4.222_{1,19}$ $p_{adj}<0.001$

Figures

Figure 1. Map of collecting locations on the island of Hawaii with area map inset (grey box on inset indicates the extent of the sampling map). The base layer show lava flow and soil substrates of varying ages (Trusdell et al 1996, Table 1). Black symbols indicate host plants (Table 1).

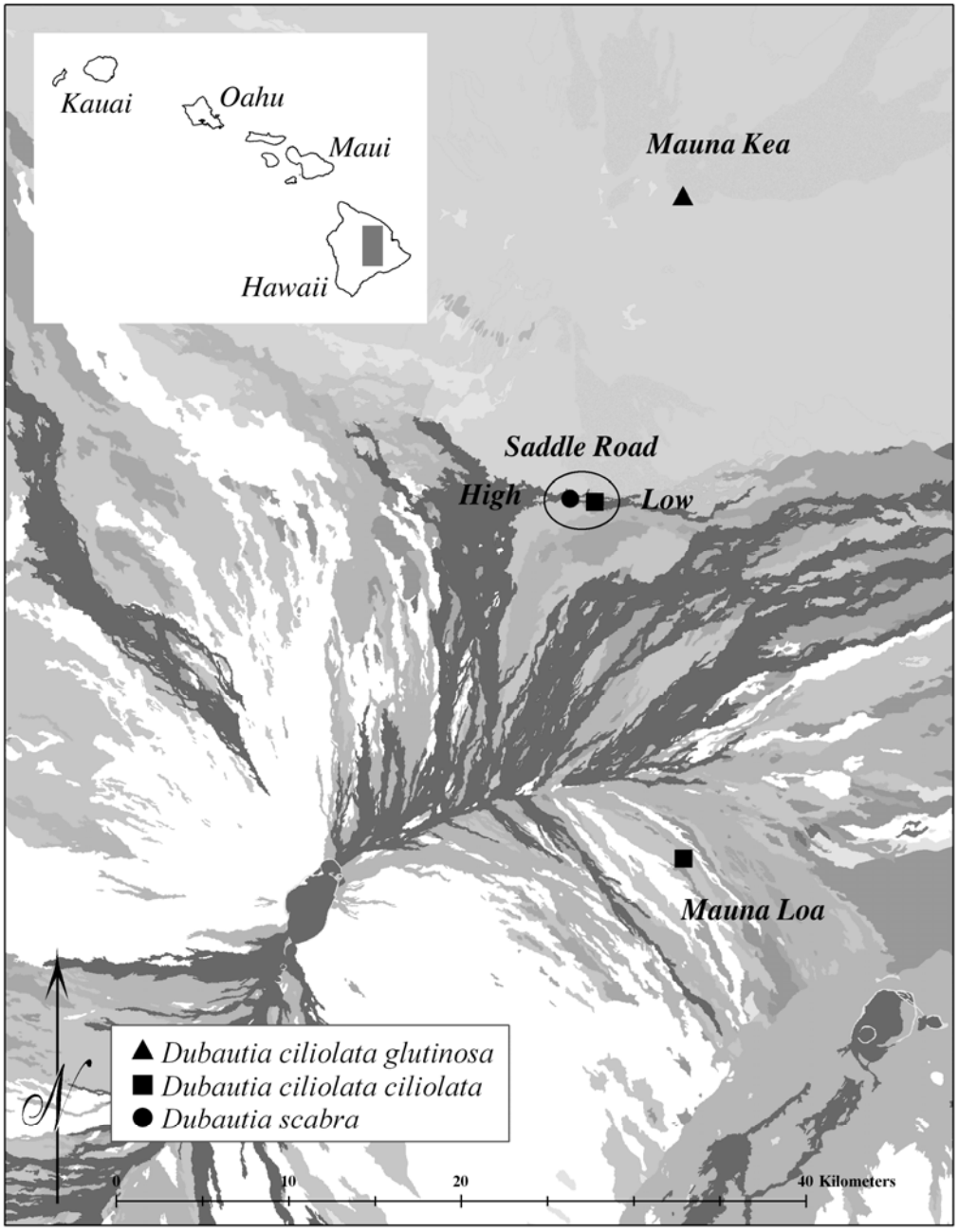


Figure 2. Vibratory signals (signal traits) of male planthoppers measured in this study: (A) a1/a2=ratio of number of whines per bout/interval between bouts (RSI); (B) b=inter-signal interval, c=whine length, (C) d=pulse rate (# of slow pulses/unit time), e=whine pulse rate (# of pulses in whine/unit time), f=fundamental frequency

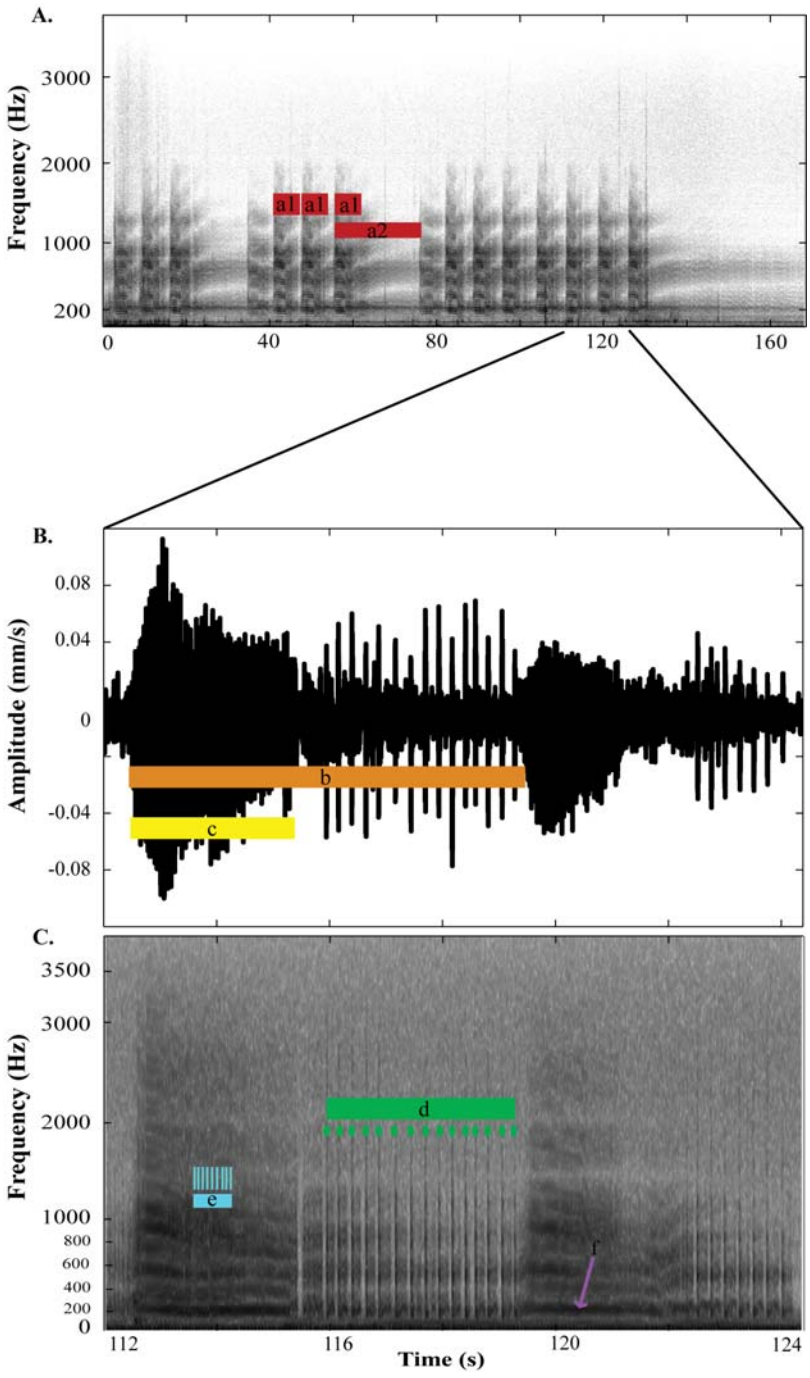


Figure 3. Displacement of the signal trait "RSI" in the zone of secondary contact versus an ecologically similar population in allopatry.