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GENETIC DIVERGENCE IS DECOUPLED FROM ECOLOGICAL DIVERSIFICATION IN THE HAWAIIAN *NESOSYDNE* PLANTHOPPERS

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Adaptive radiation involves ecological shifts coupled with isolation of gene pools. However, we know little about what drives the initial stages of divergence. We study a system in which ecological diversification is found within a chronologically well-defined geological matrix to provide insight into this enigmatic phase of radiation. We tested the hypothesis that a period of geographic isolation precedes ecological specialization in an adaptive radiation of host-specialized Hawaiian planthoppers. We examined population structure and history using mitochondrial and multiple independent microsatellite loci in a species whose geographic distribution on the island of Hawaii enabled us to observe the chronology of divergence in its very earliest stages. We found that genetic divergence is associated with geographic features but not different plant hosts and that divergence times are very recent and on the same timescales as the dynamic geology of the island. Our results suggest an important role for geography in the dynamics of the early stages of divergence.

KEY WORDS: Adaptive radiation, Delphacidae, Hemiptera, landscape genetics, molecular clock, population genetics, reproductive isolation, speciation.

Adaptive radiations provide exceptional opportunities for understanding processes involved in the formation of species (Lack 1947; Mayr 1963; Schluter 2000; Grant and Grant 2008; Losos 2009). Considerable evidence suggests that ecological opportunity and natural selection play a major role in promoting ecological variety and shaping the tight phenotype–environment correlations observed among the species involved in radiations (Simpson 1949, 1953; Schluter 2000; Nosil and Reimchen 2005; Harmon et al. 2008; Kassen 2009; Parent and Crespi 2009; Schluter 2009; Mahler et al. 2010; Yoder et al. 2010). We also know that geography is involved in promoting many of the well-known species rich, ecologically diversified radiations, including Darwin's finches (Huber et al. 2007; Grant and Grant 2008), Hawaiian spiders (Gillespie 2005), *Anolis* lizards (Losos 2009), and leaf warblers (Price 2010).

Researchers have suggested a significant role of divergence in allopatry for 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 or sea level fluctuations caused fragmentation of habitats and are thought to have allowed for diversification in periods of allopatry. Likewise, habitat fragmentation due to volcanic activity is a dominant factor in the development of landscapes (e.g., ecological succession on the different-aged

lava flows) in remote oceanic archipelagos such as the Hawaiian islands (Vitousek et al. 2009), where many classic adaptive radiations such as the Hawaiian *Drosophila* (Carson and Kaneshiro 1976; Kambysellis et al. 1995; O'Grady et al. 2011), silverswords (Carr 1985; Baldwin 2006), honeycreepers (Pratt 2005), and *Tetragnatha* spiders (Gillespie 2004) emerged. It has been suggested that this fragmenting process due to volcanism provides the conditions under which remote volcanic islands act as "evolutionary crucibles," partially explaining this extraordinary diversity (Carson et al. 1990; Vandergast et al. 2004). This idea is consistent with a classic hypothesis that a period of geographic isolation may be a common phase in the initial stages of adaptive radiations (Lack 1947). The necessity for an allopatric phase, although inferred, has not been demonstrated because of the difficulty of documenting the early stages of radiation in nature. We lack an understanding of how radiations proceed at the earliest stages, in particular what exactly promotes the evolution of reproductive isolation leading to the rapid multiplication of species in radiations (Yoder et al. 2010).

To provide insights into the interaction between geographic isolation, time, and ecological shifts in shaping populations, we study a system in which ecological diversification is found within a chronologically well-defined geological matrix. The study species is an herbivorous insect, which are excellent models for studying the role of ecological shifts in evolutionary differentiation (Ehrlich and Raven 1964; Farrell 1998; Funk et al. 2002; Nyman et al. 2006) because plant diversity is clearly associated with the great diversity of phytophagous insects (Mitter et al. 1988) and host associations represent an important and easily measured ecological variable. *Nesosydne chambersi* (Hemiptera: Delphacidae) is embedded within a lineage in which host plant use is clearly associated with the evolution of this group. Here, we address the following question: what has been the relative importance of ecological shifts and geographic isolation in the initial divergence of populations?

STUDY SYSTEM: A RADIATION ON A RADIATION ON A VOLCANO (*N. CHAMBERSI*, THE SILVERSWORD ALLIANCE AND THE ISLAND OF HAWAII)

Species in the planthopper genus *Nesosydne* are distributed throughout the islands of the eastern Pacific. Their diversification on the Hawaiian islands into more than 80 endemic species (Zimmerman 1948; Fennah 1958; Asche 1997) represents the majority of the diversity in the genus. These native species are unique for study because, although most other delphacids are primarily specialized on monocots (Denno and Roderick 1990), their diversification on the Hawaiian islands corresponds to a remarkable expansion of host use. There, *Nesosydne* use a known total of 28 different plant families (27 dicots, one monocot). Despite this expansion of host range within the genus, species within *Nesosydne* maintain the host specificity characteristic of delphacids, feeding on phloem, and mating and ovipositing on only one or a few closely related host plant species (Giffard 1922; Zimmerman 1948; Wilson et al. 1994; Asche 1997; Roderick 1997; Roderick and Metz 1997; Hasty 2005). Also like other delphacid planthoppers, they use their hosts as a communication channel to send and receive vibratory sexual signals (Claridge 1985; O'Connell 1991; Goodman 2010). This diversity of host plant use, coupled with extreme specialization, strongly suggests that host-related ecological adaptation was paramount in the diversification of this group. The broad evolutionary context of this lineage in the Hawaiian islands has the hallmarks of an adaptive radiation.

The Hawaiian Archipelago provides an exceptional geologic context for both the promotion and study of evolution. It arose as the Pacific plate moved over a hotspot in the central Pacific, generating a series of massive volcanic islands arranged in a linear age progression from youngest in the southeast to oldest in the northwest (Carson and Clague 1995; Price and Clague 2002). Each island formed from shield volcanoes. As new layers of lava cooled during the growth phase of the volcano, they provided substrate for colonization by taxa that were subsequently subject to repeated events of local extirpation and recolonization. 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, Fig. 1) gives us a detailed snapshot of the geographic conditions under which each island formed.

On the Big Island of Hawaii, the single-island endemic planthopper *N. chambersi* is associated with three plants in the Hawaiian silversword alliance, a premier example of an adaptive radiation among plants (Schluter 2000; Baldwin 2006), whose species provide diverse habitats for communities of native invertebrates (Drew and Roderick 2005). *Dubautia ciliolata* and *D. scabra* are closely related, early successional taxa on the island's recently cooled lava flows (Asteraceae, Baldwin and Robichaux 1995). The shrub *D. ciliolata* is divided into two currently recognized subspecies. *Dubautia ciliolata* ssp. *glutinosa* is found on the island's older high volcano, Mauna Kea (MK) (4205 m), where it grows in volcanic soils that date from 14,000 to 65,000 years old (Trusdell et al. 1996). *Dubautia ciliolata* ssp. *ciliolata* is found on the younger high volcano, Mauna Loa (4170 m), where it grows on its newer volcanic substrates (750–3000 years old: Trusdell et al. 1996) down to the low spot bridging Mauna Loa and MK (known as the Saddle). The mat-plant or subshrub *D. scabra* is one of the first colonizing plants to the newest lava flows (Carr 1985; Wagner et al. 1999). In the Saddle, a matrix of different-aged lava flows (Figs. 1, 3) create conditions where *D. ciliolata* ssp. *ciliolata* and *D. scabra* co-occur, each architecturally distinct and edaphically specialized to older and younger flows, respectively (Table 1; for a more in-depth

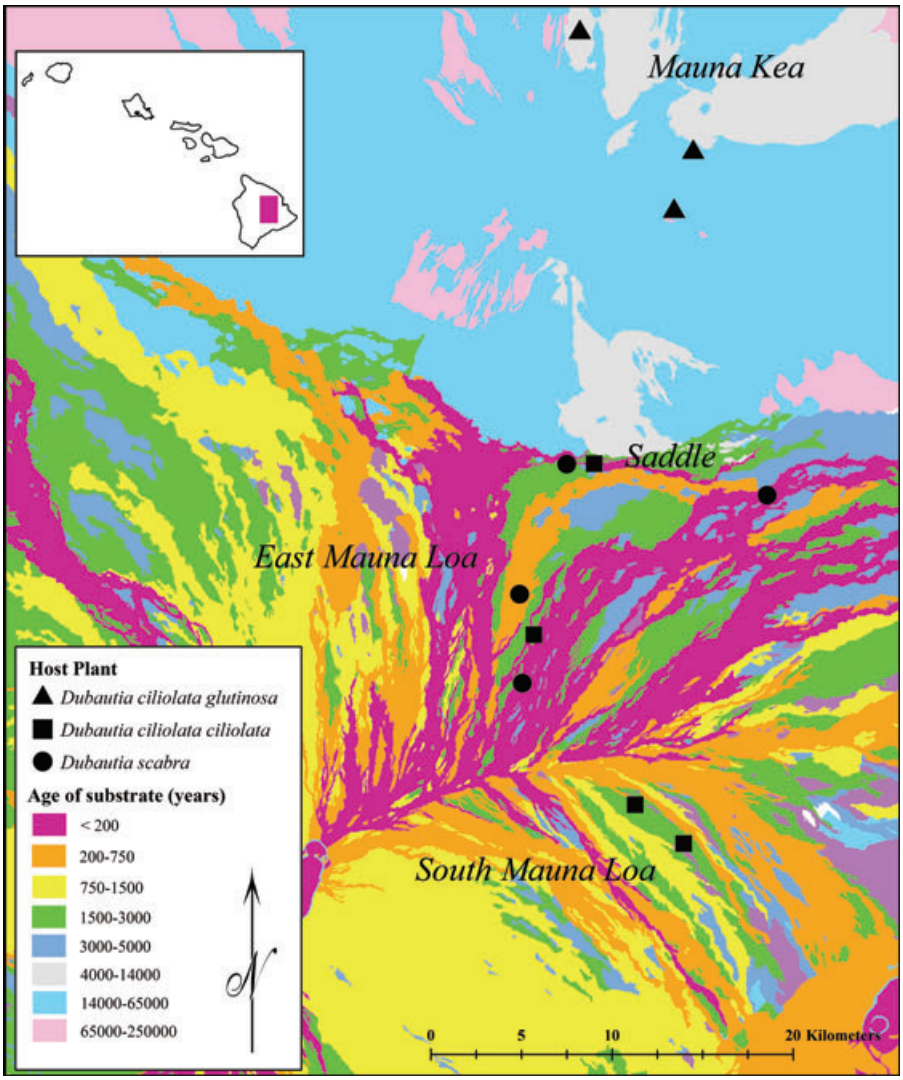

Figure 1. **Collecting localities on the island of Hawaii, showing age of soil substrate in colors (Trusdell et al. 1996) and host plant species in black symbols. Inset map depicts the Hawaiian island chain, with pink box on the island of Hawaii depicting the range of the larger map.**

description of this site, see Robichaux 1984). *Nesosydne chambersi* is distributed across both volcanoes and through the Saddle region on all three plant taxa. It is presently unknown whether *N. chambersi* is evolving diverse adaptations in response to its collection of host plants.

The hypothesis that fragmentation due to volcanic activity on the island of Hawaii is the initial and dominant force initiating divergence in *N. chambersi* makes two testable predictions. First, genetic variation in the species should be associated with geographical features rather than host plant species. Second, the timing of diversification should correspond to within-island geological events. To test these predictions, we performed dense geographic and population-level sampling spanning the geographical range of *N. chambersi*, including both variation in age of substrates and host species. We used mitochondrial and multiple nuclear microsatellite loci to determine how populations were structured across the landscape and test whether it was related more to geography or host plant. We then used coalescent analyses to estimate the timescales of divergence and long-term gene flow between populations. Our results suggest this species is in different stages of population differentiation, having rapidly fractured into multiple genetic pools in association with the dynamic geologic activity of the island rather than with use of different host plants.

Materials and Methods

COLLECTIONS, SAMPLING DESIGN, AND DNA EXTRACTIONS

We collected *N. chambersi* specimens directly into 95% ethanol from 10 localities across the island of Hawaii by beat sampling their host plants (see Drew and Roderick 2005): *D. c. glutinosa*

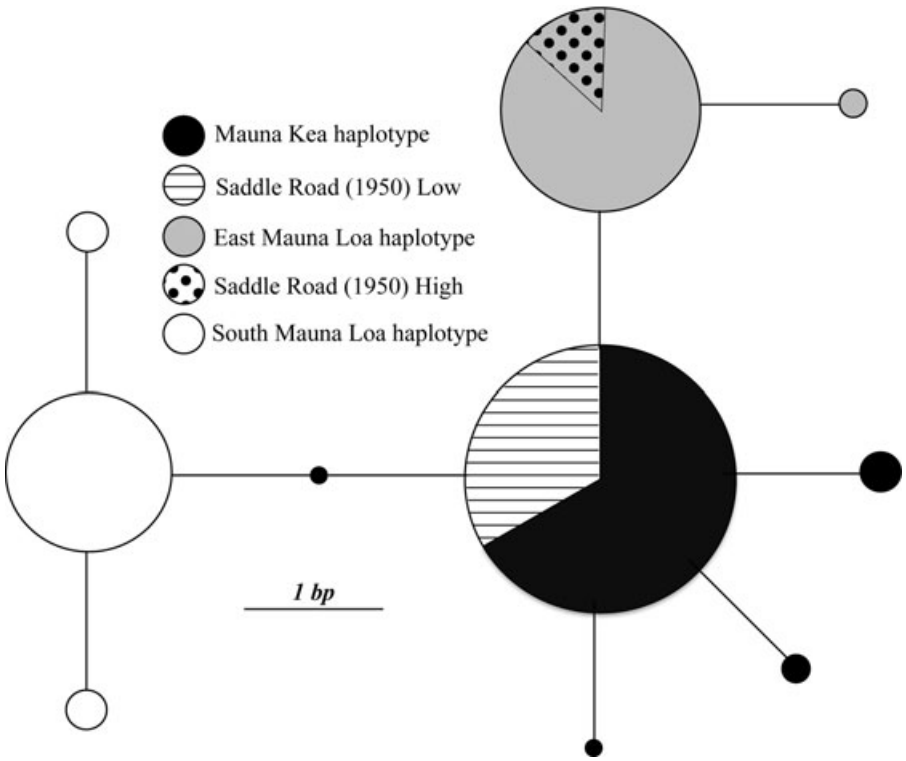

Figure 2. **Mitochondrial haplotype relationships within *Nesosydne chambersi*: cytochrome oxidase I (COI) median—joining network. The dot connecting the South Mauna Loa and the Mauna Kea haplotypes is an individual.**

(three sites), *D. c. ciliolata* (four sites), and *D. scabra* (four sites) (Table 1; Fig. 1). At one site in the Saddle region between the two volcanoes, *D. c. ciliolata* and *D. scabra* co-occur in a patchy matrix of different-aged lava flows (Fig. 3b, Trusdell et al. 1996). Within each site, where possible we sampled only one individual per plant. However, because *N. chambersi*'s distribution is clumped within plant populations and plants are rare at some of the sites, we added additional individuals randomly from plants in some populations to obtain large enough samples. We extracted genomic DNA from multiple individuals (mean $N = 29$, Table 2) from each of the 10 sampling localities using a Qiagen DNeasy DNA extraction kit (Valencia, CA), following the manufacturer's protocol and performing a double elution into a final volume of 100 μl.

MITOCHONDRIAL DNA SEQUENCING AND DIVERSITY STATISTICS

To determine the genealogical relationships among individuals across the island, we 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) (see Supporting information Appendix S1 for details of molecular laboratory protocols). Allelic variation within sampling localities was quantified in terms of number of haplotypes (H_N), number of unique haplotypes (H_U), nucleotide diversity (Π) (Nei and Tajima 1983), and haplotype diversity (H_D) (Nei 1987) in DNAsp v5 (Librado and Rozas 2009; Table 2). To determine relationships among haplotypes across sampling sites, we reconstructed intraspecific relationships using a median—joining network in Network 4.5.1.6 (Bandelt et al. 2000).

MICROSATELLITE GENOTYPING AND DIVERSITY STATISTICS

To assess genetic variation and population structure, we genotyped an average of 29 individuals (range 25–39) (Table 2) from each collecting locality at 14 microsatellite loci: Nc3, Nc4, Nc5, Nc6, Nc7, Nc8, Nc9, Nc10, Nc11, Nc12, Nc13, Nc14, Nc15, and Nc17. We then performed PCR amplification and genotyping according to procedures described previously (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, we checked the data for the presence of clonal genotypes using Gimlet (Valière 2002) prior to running any further analyses. We used Microchecker to check for scoring errors due to the presence of null alleles (Oosterhout et al. 2004).

We tested the loci for linkage disequilibrium and departure from Hardy–Weinberg equilibrium (HWE) using Genepop 3.4 (Raymond and Rousset 1995), assessing significance using

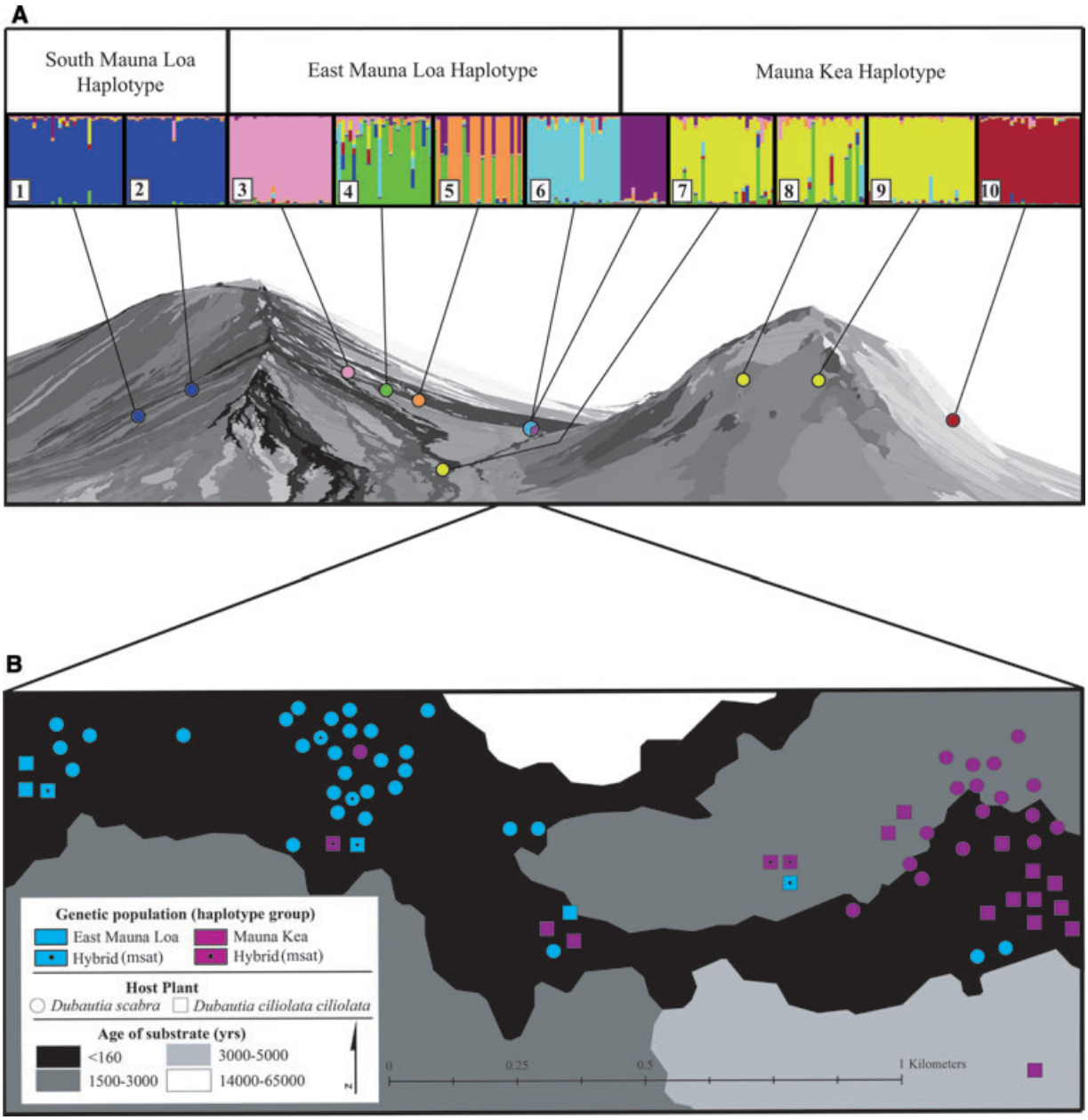

Figure 3. Results from STRUCTURE analysis of 13 microsatellite loci. The base layer is a three-dimensional (3D) image of the island of Hawaii with the age of substrate layer from Figure 1 draped over it (Trusdell, 1996). (a) Collecting sites are as follows: 1-Mauna Loa Trail LOW, 2-Mauna Loa Trail HIGH, 3-Mauna Loa Observatory Road (MLOR) HIGH, 4-MLOR MIDDLE, 5-MLOR Low, 6-Saddle Road (1950 m), 7-Saddle Road (1600 m), 8-Waipahoehoe Gulch, 9-Puu Kanakaleonui, 10-Puu Nau; (b) Close up of collecting site 6-Saddle Road (1950 m), 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 genotype (based on the microsatellite loci) of <75% posterior probability of assignment to that genetic group.

default parameters of the Markov Chain method and correcting for multiple comparisons using the sequential Bonferroni procedure (Rice 1989). We calculated the average number of alleles (A) and expected heterozygosity (H_E) using GENALEX (Peakall and Smouse 2006).

POPULATION STRUCTURE

To determine the extent and pattern of population-level structure within this species, we first estimated F_{ST} at individual loci in FSTAT v1.2 (Goudet 1995), generating standard errors by jackknifing across samples and then computed pairwise F_{ST} over all loci

Table 1. Sampling localities and associated features (See Fig. 1).

Sampling site	Site description	Year Collected	Host plant	Substrate age (years)[1]	Elev (m)	Lat	Long
1. Puu Nau	North side of Mauna Kea, along R1	2007	*Dubautia ciliolata glutinosa*	4000–14,000	2220	19.903	−155.452
2. Puu Kanakaleonui	East side of Mauna Kea, along R1	2006	*Dubautia ciliolata glutinosa*	14,000–65,000	2850	19.847	−155.391
3. Waipahoehoe Gulch	East side of Mauna Kea, along R1	2006	*Dubautia ciliolata glutinosa*	14,000–65,000	2850	19.81	−155.398
4. Saddle Road (1600 m)	Along Saddle Road at 1600 m.	2007	*Dubautia scabra*	<200	1600	19.672	−155.346
5. Saddle Road (1950 m)	South of Puu Huluhulu, along Saddle Road at maximum elev.	2005	*Dubautia ciliolata ciliolata*, *Dubautia scabra*	1500–3000, <200	1950	19.686	−155.447
6. MLOR LOW	Mauna Loa Observatory Road	2008	*Dubautia scabra*	200–750	2270	19.617	−155.472
7. MLOR MIDDLE	Mauna Loa Observatory Road	2008	*Dubautia ciliolata ciliolata*	1500–3000	2340	19.609	−155.47
8. MLOR HIGH	Mauna Loa Observatory Road	2007	*Dubautia scabra*	<200	2650	19.577	−155.474
9. MLT LOW	Mauna Loa Trail, Hawaii Volcanoes NP	2008	*Dubautia ciliolata ciliolata*	1500–3000	2085	19.499	−155.385
10. MLT HIGH	Mauna Loa Trail, Hawaii Volcanoes NP	2007	*Dubautia ciliolata ciliolata*	1500–3000	2475	19.515	−155.412

[1]Trusdell et al. (1996).

between pairs of populations by computing the distance matrix in ARLEQUIN version 3.1 (Excoffier and Schneider 2005), testing significance using 10,000 permutations. We then estimated overall Φ_{ST} using analysis of molecular variance (AMOVA; Excoffier et al. 1992) implemented in ARLEQUIN version 3.1 (Excoffier and Schneider 2005), again testing significance using 10,000 permutations.

Next, we identified genetic clusters in the dataset 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. This analysis resulted in a log-likelihood score that maximized the probability of the data given the models. We averaged the results from five independent runs at each K value and inferred the number of genetic clusters in the data using the ΔK method (Evanno et al. 2005), implemented in STRUCTURE HARVESTER v0.6.5 (Earl 2011). Evanno et al. (2005) demonstrated that this method identifies the highest level structure in the dataset when multiple hierarchical levels of structure exist. Therefore, we performed a nested analysis, following the procedure described in Coulon et al. (2008). Briefly, we included all data in the first analysis. We then split the data into separate datasets, each representing one of the inferred clusters identified by the ΔK method by assigning each individual into the cluster to which it had the highest posterior probability of inferred ancestry. We continued this iteratively until the number of inferred genetic groups for each data subset was one. Because the ΔK method cannot distinguish among groups when the actual number of clusters in the data is one, we determined we had reached that point when the mean log probability of the data was greatest for $K = 1$ and the assignment of individuals to groups at K values greater than 1 was nonsensical.

Each of the nested runs was analyzed using the admixture model, the correlated allele frequency model, and no prior population information. Runs were repeated five times at each K value with different random number starting seeds to test for consistency between runs for one million steps following a burn-in period of 50,000 steps. Consistency between runs and inspection of plots demonstrated convergence of the runs. The entire dataset was tested against models of $K = 1$–15, whereas the number of K values tested for each of the data subsets ranged between K = 1−5 and K = 1−12 (Table S2).

GEOGRAPHY VERSUS ECOLOGY

To test our prediction that genetic variation in *N. chambersi* should be associated with geographical relationships rather than host plant species, we used two approaches. First, we performed two hierarchical AMOVAs in ARLEQUIN version 3.1 (Excoffier and Schneider 2005) using 10,000 permutations to test significance:

(1) with host plant species and (2) with geographic region defined as the uppermost hierarchical level. For (1) host plant species, we used a subset of the data to perform this analysis that included only individuals from East Mauna Loa (EML) and the Saddle Region, which occurred on *D. scabra* and *D. c. ciliolata*. We excluded individuals from the MK and South Mauna Loa (SML) populations from this analysis because in these regions host plant and geography are confounded variables. For (2) geographic region, we included data from all of the sampling sites and grouped the "among geographic region" component into the three major regions: SML, EML, and MK (Fig. 1). Second, we performed a partial Mantel Test in the program IBDWS version 3.15 (Jensen et al. 2005), using 10,000 randomizations to test for the effect of host species on genetic structure while controlling for geographic distance, which allowed us to include the populations from MK. Planthopper population pairs that shared plant hosts were coded with 1 and those that had different hosts were coded with 0 in the indicator matrix.

TIMEFRAME OF DIVERGENCE

To address our prediction that the timing of diversification should correspond to within-island geological events, we fitted our data to a model of isolation with migration, implemented in the coalescent-based software program IM (Hey and Nielsen 2004). This method allowed us 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, averaged over time since divergence. We selected two population pairs to analyze: (a) one pair that represents the relationship between MK and SML (Mauna Loa Trail HIGH and Puu Kanakaleonui) and (b) one pair that represents the relationship between MK and EML (the two genetic populations in the zone of secondary contact at the Saddle Road (1950 m) site [using the 2005 dataset]). We selected these particular comparisons because they represent populations that (a) are genetically distinct, and (b) represent comparisons that span the smallest and largest geographic distances in the dataset. These comparisons allowed us to compare timeframes between the colonization of SML from MK and the colonization of EML from MK. The data we 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 and were analyzed using it as a model. We calibrated the COI locus at a divergence rate of 2.7% sequence divergence per million years. We calculated this rate by averaging sequence divergence between multiple species pairs from within *Nesosydne* (Table S2) using data from Goodman (2010) and applying the maximum date of the island of Hawaii's emergence from the ocean as a conservative calibration (note, many studies of Hawaiian taxa use the date the island reached its maximum height: see Supporting information Appendix S3 and Table S2 for a discussion of how we calculated and evaluated this mutation rate). We did not apply a mutation rate to the microsatellites. We made this decision because we had very good information from within our own lineage to estimate the rate at COI, we did not have such for the microsatellites and would have had to specify an average rate for all microsatellite loci from a very different taxonomic group from the literature.

Prior to performing the final analyses, we ran a series of sensitivity analyses. During these we 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. We performed the final analyses using 100 chains for a total of 100,000 burn-in steps followed by runs of between one and three million post burn-in steps. To ensure that results were similar between runs, we performed three independent runs for each population pair with different random number starting seeds. We selected the geometric heating parameters for the chains following several preliminary runs to achieve sufficient mixing. Finally, we assessed the chains following each run to determine if they were long enough and monitored the evolutionarily stable strategy (ESS) values and the trendlines in the posterior distribution plots to determine whether mixing was sufficient.

Estimates in IM are produced on a per year scale, so for taxa whose generation time is greater or less than one year, it is necessary to scale the results by the number of generations per year. In laboratory conditions, *Nesosydne* have been documented to take approximately six to eight weeks to complete a life cycle (O'Connell 1991). As host plants are available all year and lower latitude conditions maintain relatively similar day lengths throughout the year, breeding is likely to occur throughout the year. Here, we use five generations per year as a conservative estimate of the number of generations per year in field conditions.

GENE FLOW

In the above NETWORK 4.5.1.6 and STRUCTURE 2.3 analyses based on the 2005 dataset, we identified a contact zone of two genetic populations at the Saddle Road (1950 m) site (described below in the Results). To document contemporary gene flow, we repeated collections there in 2008, approximately 15 generations after the original collection and genotyping was performed. We genotyped 37 individuals from the 2008 collections at nine microsatellite loci following laboratory and screening protocols described above. We then created a composite dataset containing the 39 genotyped individuals from the 2005 collections from this site yielding a total of 76 individuals from both years that were genotyped at nine microsatellite loci. To determine whether the two genetic populations identified in 2005 were still present at the site in 2008, we performed a clustering analysis in

STRUCTURE 2.3 (Pritchard et al. 2000) on the pooled dataset, testing the data against models of $K = 1$–6, following the same procedures described above. This analysis indicated that some individuals had mixed ancestry. To determine which populations have contributed to the mixed genetic makeup of these individuals, we created a composite dataset of the 292 individuals used in the STRUCTURE 2.3 (Pritchard et al. 2000) analysis described under "Population Structure" together with the 37 individuals from Saddle Road (1950 m) collected in 2008, yielding a total of 329 individuals. We tested the data against models of $K = 1$–15, following the same procedures described above. To document long-term gene flow, we estimated historical migration using IM (Hey and Nielsen 2004) as described above.

Results

MITOCHONDRIAL DIVERSITY

We sequenced COI (653 bp) from a total of 185 individuals from across all 10 sampling sites (Table 1), within which we recovered 10 unique haplotypes (GenBank accession numbers: JQ771119-JQ771128). Of the 10 substitutions in these haplotypes, we observed nine transitions and one transversion. The relationships among haplotypes correspond to the geographic locations from which they were collected: SML, EML, and MK. Representatives from both the EML and MK groups were collected at the Saddle Road (1950 m) site (Table 2; Fig. 2). The average uncorrected pairwise genetic distance between each mtDNA clade is small (SML/EML = 0.51%; SML/MK = 0.37%; EML/MK = 0.31%: Fig. 2).

MICROSATELLITE DIVERSITY

From 10 sampling localities, we genotyped a total of 292 individuals at 14 microsatellite loci (Table 1). Using GIMLET (Valière 2002), we found all individuals to have unique 14-locus genotypes and thus, we conclude that parthenogenesis is unlikely in this species or at least is sufficiently infrequent as to be undetectable in our dataset. 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). The microsatellite data have been deposited in the Dryad repository under doi:10.5061/dryad.400jc8bj.

One locus (NC15) showed evidence of null alleles at seven of 10 sites and we therefore removed it from all subsequent analyses. We identified some potential null alleles at all loci except NC7, NC8, NC10, NC12, NC17, and at all sites except MLOR HIGH and Puu Nau. For each lows with potential null alleles, 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, Puu Nau), two (Saddle Road [1600 m]), three (MLOR MIDDLE, MLOR LOW, Waipahoehoe Gulch), four (MLT HIGH), and six (Saddle Road [1950 m]).

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 m]—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 m]—NC11, and Saddle Road [1600 m]—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, we rule out locus-specific effects and instead infer that these observations are the result of a biological phenomenon.

We found evidence for departure from linkage equilibrium in two populations after correcting for multiple comparisons using sequential Bonferroni correction. In the Saddle Road (1950 m) 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 we judged the observed linkage disequilibrium to be the result of population substructure within the two sampling sites.

POPULATION STRUCTURE

Estimates of F_{ST} at individual loci ranged from 0.034 to 0.337; 10 of the 13 loci had values above 0.15 (Locus [F_{st}, SE]: NC3 [0.337, 0.107], NC4 [0.231, 0.085], NC5 [0.270, 0.106], NC6 [0.209, 0.053], NC7 [0.34, 0.021], NC8 [0.130, 0.027], NC9 [0.255, 0.079], NC10 [0.168, 0.045], NC11 [0.316, 0.080], NC12 [0.098, 0.028], NC13 [0.272, 0.156], NC14 [0.247, 0.051], NC17 [0.230, 0.089]). Population pairwise estimates of F_{ST} based on sampling localities ranged from 0.048 to 0.468 (Table 3), whereas the overall Φ_{ST} was 0.229 ($P < 0.0001$). All estimates were significantly different from zero. 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. Eight genetic populations within the 10 sampling sites were identified through a series of nested runs using the STRUCTURE algorithm (Fig. 3a; Table S2—see Supporting information for the mean log probabilities of the likelihoods for each K value and ΔK values). 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

Table 2. **Indices of genetic diversity at mitochondrial (COI) and microsatellite loci within sampling localities across Hawaii Island. The number of individuals (*N*) used in the diversity analyses for each type of marker is reported. Sequence-based diversity measures include number of haplotypes (H_N), number of unique haplotypes (H_U), haplotype diversity (H_D) and nucleotide diversity (Π). Measures derived from microsatellite data include the average number of alleles per sampling locality (A) and expected heterozygosity (H_E). Standard deviations (SD) or standard errors (SE) for diversity estimates are in parentheses.**

		Mitochondrial (COI)					Microsatellites, N=13		
Sampling site	MTD NA clade (Fig. 2)	*N*	H_N	H_U	H_D (SD)	Π (SD)	*N*	A (SE)	H_E (SE)
1. Puu Nau	Mauna Kea	19	2	1	0.409 (0.100)	0.00063 (0.00015)	28	3.15 (0.27)	0.31 (0.04)
2. Puu Kanakaleonui	Mauna Kea	16	3	2	0.228 (0.129)	0.00036 (0.00021)	30	4.08 (0.49)	0.43 (0.05)
3. Waipahoehoe Gulch	Mauna Kea	16	2	1	0.282 (0.142)	0.00043 (0.00022)	25	3.69 (0.40)	0.50 (0.06)
4. Saddle Road (1600 m)	Mauna Kea	20	1	0	0.233 (0.126)	0.00036 (0.00019)	29	4.31 (0.60)	0.51 (0.05)
5. Saddle Road (1950 m)	Mauna Kea/ East Mauna Loa	31	2	0	0.490 (0.045)	0.00075 (0.00007)	39	3.00 (0.378)	0.46 (0.07)
6. MLOR LOW	East Mauna Loa	18	1	0	0.000	0.000	25	2.92 (0.37)	0.43 (0.07)
7. MLORMIDDLE	East Mauna Loa	16	2	1	0.233 (0.126)	0.00036 (0.00019)	27	3.92 (0.62)	0.50 (0.06)
8. MLOR HIGH	East Mauna Loa	16	1	0	0.000	0.000	29	2.46 (0.18)	0.40 (0.06)
9. MLTLOW	South Mauna Loa	13	2	1	0.442 (0.087)	0.00068 (0.00013)	28	3.00 (0.39)	0.30 (0.07)
10. MLT HIGH	South Mauna Loa	20	2	1	0.385 (0.132)	0.00059 (0.00020)	32	3.69 (0.50)	0.38 (0.06)

and Mauna Loa Trail LOW cluster together here corresponding perfectly to the SML haplotype group. Likewise, Saddle Road (1600 m), Waipahoehoe Gulch, and Puu Kanakaleonui cluster together in agreement with the MK haplotype group. However, Puu Nau is clearly a distinct population at the microsatellite loci despite its inclusion into the MK haplotype group. Furthermore, the clustering analysis revealed that the EML haplotype group is a dramatically structured set of genetic populations in which four sampling sites contain five genetic populations within the EML haplotype group, extending from the Mauna Loa Observatory Road at 2650 m (MLOR HIGH) down to the Saddle Road at 1950 m (Saddle Road [1950 m]). 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 m) within one collecting site (Fig. 3a and b). Comparison between the mitochondrial haplotypes and the microsatellite clustering assignments at this site showed 100% correspondence between them, indicating that this is a zone of secondary contact.

GEOGRAPHY VERSUS ECOLOGY

Results from the AMOVA addressing whether microsatellite variation is partitioned by host plant species or geographic region demonstrate that host plant species is not associated with genetic structure in *N. chambersi* (Table 4). When planthopper populations were grouped by host plant species, the "among host plant" component explained none of the variation. 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 pairwise F_{ST} calculations (Table 3). Partial Mantel Tests demonstrated that geographic distance had a significant effect on genetic distance among population pairs ($r = 0.49$, $P = 0.005$) whereas host species did not ($r = -0.198$, $P = 0.871$), and that this result was consistent even while considering one while controlling for the other (Table 5).

Table 3. Pairwise F_{ST} between localities.

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

All values are significantly different from zero (Bold: $P < 0.001$, *$P < 0.0001$).

TIMEFRAME OF DIVERGENCE

We examined convergence between the three 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 three replicates yielded posterior distributions with similar values and the values estimated from the longest runs are presented here (Table 6). Based on our estimated divergence rate of 2.7% sequence divergence per million years, we estimated the time since divergence between the two genetic populations in the zone of secondary contact (Saddle Road [1950 m]) as 2643 (95% highest posterior density [HPD]: 1172–35,047) years ago. We estimated the time since divergence between the MK population at Puu Kanakaleonui and the SML population at Mauna Loa Trail LOW as 20,139 (95% HPD: 7418–134,956) years ago (Table 6; see Supporting information Figs. S1 and S3 for graphs of IM posterior probability distributions of each parameter).

GENE FLOW

Contemporary

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, approximately 15 generations later. 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 (Table S4; Fig. S4). This indicates these populations are not an ephemeral phenomenon and are stable in contemporary time. Of the 76 individuals analyzed from this site in both years, 12 have posterior probability assignments to their primary group of less than 0.8, and eight of these have less than 0.75. Although the other genetic population at Saddle Road (1950 m) contributes to the genetic make-up of many of the mixed individuals, other populations from elsewhere on the island do as well, in particular MLORL and Saddle Road (1600 m) (Table S5).

Long term

Estimated gene flow since population separation is low and asymmetrical between both population pairs. From MK to SML and from SML to MK, clear peaks are at $2Nm = 0.146$ (95% HPD: 0.018–4.182) and $2Nm = 0.084$ (95% HPD: 0.010–4.798), respectively. The number of migrants over historical time is even lower and more asymmetrical between the Saddle Road (1950 m) populations. However, the posterior probability distributions never quite reach zero, which implies that while the median estimates are informative, the confidence intervals are not reliable for this parameter: from the Saddle Road (1950 m) Low (MK haplotype) to Saddle Road (1950 m) High (EML haplotype), $2Nm = 0.0004$ (95% HPD: 0.003–6.082), whereas $2Nm = 0.098$ (95%

Table 4. Hierarchical AMOVA for partitioning of genetic variation in *Nesosydne chambersi.*

Uppermost hierarchy level	Source of variation	df	Sum of squares	Covariance component	Percent of molecular variance	*F*-statistics	*P*-value
(1) 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
(2) 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

Va=variance among groups; Vb=variance among populations within groups; Vc=within populations.

HPD: 0.025–14.96) in the reverse direction (Table 6; see Supporting information Figs. S1 and S3 for graphs of IM posterior probability distributions of each parameter).

Discussion

This study was designed to test the hypothesis that geographic isolation via habitat fragmentation is the dominant force promoting initial genetic divergence in *N. chambersi*. Our results demonstrate that in this species, genetic structure has developed rapidly and is strongly associated with geography at multiple evolutionary scales recorded by both mitochondrial and nuclear DNA. Furthermore, for the same populations genetic structure was not associated with host plant use. Together, these results suggest that the significant amount of genetic structure observed among these sites was driven by geological processes, namely recent colonization of the novel habitats created as the lava flows cooled and subsequently became colonized by the host plants.

A HIGHLY STRUCTURED SPECIES

Our results provide a snapshot of different stages of population differentiation across the island of Hawaii. Genetic diversity within and distance between the three mtDNA groups is very small, but is clearly partitioned into three major geographic regions (SML, EML, and MK: Figs. 2, 3), which strongly suggests recent divergence and low vagility. Microsatellite allelic diversity is also low, and our observation of significant isolation by distance at these loci indicates that genetic divergence is consistent with distance-limited dispersal (Slatkin 1997). However, pairwise F_{ST} values are quite high (Table 3), and the clustering analysis (Fig. 3) demonstrates several well-supported clusters that do not follow a simple pattern of isolation by distance. Given the geographic context, it is likely that we are observing populations that have been isolated for longer periods of time and forming strong clusters (the SML group and the MK group), together with population that became separated much more recently as new habitat became available to colonize (such as within the EML group).

The SML haplotype group, which was collected from two sampling sites south of Mauna Loa's North East rift zone, appears 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 low but significant differentiation ($F_{ST} = 0.09$) within this group.

The low-diversity EML haplotype group represented in our sampling is distributed on a matrix of recent lava flows on the east face of Mauna Loa from 2650 m down into the Saddle region at 1950 m (Saddle Road [1950 m]). The nuclear microsatellite loci we examined here exhibited significant structure between each sampling site that we did not observe at the mitochondrial locus, and the presence of individuals with mixed genetic backgrounds in MLOR MIDDLE and MLOR LOW indicate that colonization of these new habitats is likely ongoing.

The MK 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 1250 m in elevation and lives on two different host plant species. This is the largest genetic population sampled and contains individuals collected from *D. c. glutinosa* in Waipahoehoe Gulch, Puu Kanakaleonui and from the Saddle road site at 1600 m in elevation (Saddle Road [1600 m]), collected from *D. scabra*. A likely explanation for this surprisingly large distribution is that the Saddle Road (1600 m) 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, which appears likely to be on MK. A second genetic population within this haplotype group was collected along Saddle road at the 1950 m site (Saddle Road HIGH), collected from both *D. scabra* and *D. c. ciliolata*. The final genetic population belonging to this haplotype group was collected at Puu Nau from a stand of *D. c. glutinosa* that is isolated in a patchy landscape and is strongly defined as its own genetic population at the nuclear loci.

Table 5. Partial Mantel Tests for partitioning of genetic variation in *Nesosydne chambersi.*

Correlation	*R*	*P*
Genetics with host plant	−0.198	0.871
Genetics with geographic distance	0.492	0.005
Genetics with host plant, controlling for geographic distance	−0.112	0.716
Genetics with geographic distance, controlling for host plant	0.471	0.007

MECHANISM BEHIND POPULATION STRUCTURE

We predicted that if volcanic activity were the initial and dominant force initiating divergence in this species, the timeframe of divergence would be consistent with periods of fragmentation due to volcanic activity. The clear position of the likelihood peaks for our divergence time estimates indicate that the genetic populations at the Saddle Road (1950 m) site diverged approximately 2650 years ago and the populations situated on either volcano (SML and Puu Kanakaleonui on MK) diverged approximately 20,150 years ago (Table 6). Because the geology of this island has been studied in detail, it is possible to compare our divergence time results to a known framework of within-island geologic events, and both estimates are consistent with recent divergence in a landscape dominated by patchy island-like habitats, which are covered and recolonized by the stochastic activity of a growing volcano.

The Saddle Road (1950 m) populations were collected from a matrix of flows primarily dating to less than 200 and 1500–3000 years old (Fig. 1: 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 over evolutionary time. Our divergence time estimate of 2650 years is consistent with the idea that the insect populations colonized these flows at different times after their host plants established in the new substrate and recently have come back into physical contact.

Divergence times between the Mauna Loa (Mauna Loa Trail LOW) and MK (Puu Kanakaleonui) populations are estimated to be older, but are still recent (20,150 years). The Mauna Loa population was collected from a lava flow that dates to between 1500 and 3000 years old and is situated south of Mauna Loa's northeast rift zone, whereas the MK population was collected from older, eroded soils dating to between 14,000 and 65,000 years (Fig. 1, Trusdell et al. 1996). The rift zones are a dominant feature in the Mauna Loa landscape, and periodic flows over the last several thousand years of island building (Macdonald et al. 1983) would have repeatedly extirpated adjoining habitat between the two volcanoes. The patterns in our data are consistent with a model in which *N. chambersi* colonized the SML landscape and became isolated there by the rift zone activity.

Table 6. Maximum-likelihood estimates of gene flow and divergence parameters. Conversions to demographic units are provided using 2.7% sequence divergence per million years (in gray), calculated from *Nesosydne* (Table S3). Ranges represent the 95% highest posterior density (HPD). See also Figure S1–4.

Parameter estimates	Mauna Loa (South)	Mauna Kea	Saddle Road (1950 m), High (East Mauna Loa haplotype)	Saddle Road (1950 m), 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	20,139 (7,418–134,956)		2643 (1172–35,047)	
N_E	1.21×10^5 (5.67×10^4–4.06×10^5)	3.31×10^5 (1.58×10^5–8.11×10^5)	4.54×10^4 (2.01×10^4–3.6×10^5)	5.6×10^3 (3.16×10^3–7.97×10^4)
N_E, ancestral	8.92×10^6 (4.33×10^6–1.45×10^8)		8.73×10^6 (4.27×10^6–1.9×10^8)	
m rate	6.02×10^{-7} (1.58×10^{-7}–5.15×10^{-6})	1.27×10^{-7} (3.22×10^{-8}–2.96×10^{-6})	4.79×10^{-9} (7.18×10^{-8}–8.44×10^{-6})	8.72×10^{-6} (4.02×10^{-6}–9.38×10^{-5})
2N*m*	0.146 (0.018–4.182)	0.084 (0.010–4.798)	0.0004 (0.003–6.082)	0.098 (0.025–14.96)

Given the documented pattern of genetic variation, the estimated dates of divergence and the extremely young geologic setting of the island, it is reasonable to conclude that divergence in *N. chambersi* species has been quite recent. The Hawaiian islands are full of species-rich radiations, and the estimation of divergence times by placing date calibrations on nodes of molecular phylogenies is becoming routine. The date most commonly used is when Hawaii Island is estimated to have reached its maximum height, 0.5 million years ago (Price and Clague 2002). In this study, we used the most conservative calibration possible—the estimated date the island emerged from the ocean, 0.09 ($\pm$0.1) million years ago (D.A. Clague, pers. comm.; based on a new age date of 1.15 million years for the earliest submarine Kohala lavas from Lipman and Calvert 2011, see Supporting information Appendix S3 for more detail and comparison to other Hawaiian arthropods). Using the more commonly applied calibration date results in much faster mutation rates (see Table S2) and even younger date estimates. Still, the divergence times we present here are extremely recent. Our results, obtained using coalescent methods and a very conservative calibration to calculate the mutation rate, demonstrate that within-species divergence times may be orders of magnitude younger than the calibrations commonly applied in phylogenetic studies of groups in these islands.

GEOGRAPHY OR ECOLOGY

It is clear that geographic isolation plays a role in promoting divergence in many adaptive radiations (Gillespie 2005; Grant and Grant 2008; Losos 2009; Thorpe et al. 2010), and the idea that it promotes divergence leading to speciation is considered a null hypothesis for how species form (Mayr 1942; Coyne and Orr 2004). However, speciation in allopatry is typically thought to be a slow process, so the notion that it could be responsible for the increased rates of speciation observed in adaptive radiations tends to be considered unconvincing or incomplete (Coyne and Orr 2004). Several researchers have now observed genetic divergence occurring in response to relatively rapid geologic events that are common in islands and lakes where radiations often occur, for example: fragmentation due to volcanism (Carson et al. 1990; Excoffier et al. 1992; Pestano and Brown 1999; Malhotra and Thorpe 2000; Beheregaray et al. 2003; Vandergast et al. 2004; Gubitz et al. 2005; Bloor et al. 2008), landslides (Moya et al. 2004; Brown et al. 2006), and sea level rise or fall (Sturmbauer et al. 2001; Glor et al. 2004), which is suggestive that these or other processes may be common forces that promote rapid divergence in allopatry in these settings.

Natural selection and ecological opportunity also clearly play a role in promoting diversification in adaptive radiations (Lack 1947; Simpson 1953; Schluter 2000). However, we still lack an understanding of how exactly geographic isolation interacts with natural selection and ecological opportunity to generate reproductive isolation and create the sort of rapid speciation we observe (Yoder et al. 2010). Rundell and Price (2009) suggested that it may occur via a process that proceeds through multiple phases that are simply difficult to observe. They propose that speciation and diversification may commonly be decoupled in radiations—that nonecological forces such as geographic isolation may first generate multiple reproductively or partially reproductively isolated gene pools that are then primed to radiate when presented with ecological opportunity. The difficulty with testing this hypothesis is being able to observe the earliest stages of divergence.

The planthopper-*Dubautia* study system provides an unusual opportunity to examine a species we know to be in the early stages of divergence to test whether divergence is related to ecological (host plant) or geographical forces. Throughout EML and the Saddle Region, the area of the island where two host plant species are distributed alternately throughout the landscape, our results indicate that genetic variation is not related to host plant use, suggesting that ecological selection is not driving the observed genetic divergence at this stage (sensu Rundle and Nosil 2005). Our findings are instead consistent with an ecological fitting scenario (e.g., Janzen 1980) whereby the insects reach the newly developing habitat and use whichever of the hosts they encounter, later becoming isolated in the patchy matrix of lava flows. These results clearly motivate future studies that focus on divergence in phenotypic traits to understand whether diverse adaptations are evolving within the genetic populations.

From the perspective of this system, the tip of a tree within a large ecologically specialized radiation, it appears that early genetic divergence is decoupled from ecological adaptation. This study provides a model for visualizing how within-island colonization of a dynamic, fragmenting landscape may enable radiation by rapidly providing multiple genetically distinct populations that are ready to subsequently diversify if presented with ecological opportunity, as suggested by Rundell and Price (2009). Our data suggest that what we are observing is an initial period of allopatry among populations within a species embedded in a lineage that demonstrates extreme host-associated differentiation within the genus. In the context of the youth of the island, the data presented here demonstrate clearly that the earliest steps in the divergence of this species are related to geographic isolation.

THE EVOLUTION OF REPRODUCTIVE ISOLATION?

The data we present here provide a way to visualize how populations of a species may be rapidly broken up on a dynamically forming landscape. They demonstrate a considerable amount of genetic clustering among populations, which may indicate progression toward speciation (e.g., Mallet 1995; Feder 1998; Rosenblum and Harmon 2010). However, if geography is involved in speciation, it must be also associated with reproductive

isolation in addition to genetic divergence. The presence of a contact zone between ecologically similar populations at Saddle Road (1950 m) (Figs. 2, 3a and b) allowed us to evaluate whether gene flow is occurring between genetically distinct populations within cruising range of one another, which would be a first step toward providing support for the idea that reproductive isolation has developed (Coyne and Orr 2004; Sobel et al. 2010). In each year sampled, both genetic groups were present and we identified mostly pure individuals that were clearly assigned to either one of the two genetic groups at the site. However, in each year a few individuals had mixed genetic assignments (Table S5). This indicates that mating does occur between the populations, but it does not appear to be occurring at a high enough frequency to create a hybrid swarm. Despite evidence of a small amount of mating between the two genetic populations, long-term estimates of gene flow are quite low (less than 0.1 individual per generation; Table 6), which indicates that the effect of any mixed mating on either gene pool is negligible.

Using field measurements, we have demonstrated that gene flow is very restricted between ecologically similar populations that are in physical contact with one another. Maintenance of these populations in the absence of ecological isolation may be due to shifts in behavior associated with sexual signaling between these sites and across its geographic range, and is the subject of further study (Goodman 2010; Goodman et al., unpubl. ms.). A next step in assessing the extent of reproductive isolation among populations of varying genetic divergences will be to measure pre- and postzygotic isolation by performing mating trials in a controlled experimental setting.

Conclusions

This system offers a rare vantage point into an enigmatic early phase of adaptive radiation, providing an unusually clear example in which genetic divergence is decoupled from ecological diversification in a lineage of host-specialized insects. Our results reveal a species that has fractured into multiple genetic pools in association with the dynamic geologic activity of the island of Hawaii rather than host associations. This result supports a model of evolution in which genetic divergence driven by geographic isolation occurs first, providing isolated genetic pools that may rapidly become reproductively isolated. These new lineages would then be set on independent evolutionary trajectories (Rundell and Price 2009), where they have the potential to go extinct, diverge by genetic drift, or diverge by natural or sexual selection when faced with ecological opportunity.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Laboratory methods for mitochondrial DNA.
Appendix S2. Nested structure analysis.
Appendix S3. Discussion of divergence time estimation assumptions.
Appendix S4. IM effective sample sizes and posterior probability distributions.
Appendix S5. Temporal analysis of the Saddle Road (1950 m) site.
Table S1. Nested analysis of populations using STRUCTURE 2.3.
Table S2. Cytochrome oxidase I (COI) rates of mutation.
Table S3. Effective sample sizes for each parameter from each independent IM run.
Table S4. Results of the clustering analysis that included individuals from 2005 to 2008 at Saddle Road (1950 m).
Table S5. Assignment to populations of individuals with mixed ancestry collected from Saddle Road (1950).
Figure S1. South Mauna Loa–Mauna Kea IM graphs: (A) m1 = migration rate into South Mauna Loa from Mauna Kea; (B) m2 = migration rate into Mauna Kea from South Mauna Loa; (C) t = maximum time of population splitting (unconverted).
Figure S2. South Mauna Loa–Mauna Kea IM graphs: (D) q1 = scalar for South Mauna Loa theta; (E) q2 = scalar for Mauna Kea theta; (F) qA = scalar for ancestral population theta.
Figure S3. Saddle Road (1950 m) High–Saddle Road Low (1950 m): (A) m1 = migration rate into Saddle Road (1950 m) High from Saddle Road Low (1950 m); (B) m2 = migration rate into Saddle Road Low (1950 m) from Saddle Road (1950 m) High; (C) t = maximum time of population splitting (unconverted).
Figure S4. Saddle Road (1950 m) High–Saddle Road Low (1950 m): (D) q1 = scalar for Saddle Road (1950 m) High theta; (E) q2 = scalar for Saddle Road Low (1950 m) theta; (F) qA = scalar for ancestral population theta.
Figure S5. Delta K values for the temporal clustering analysis.

Supporting Information may be found in the online version of this article.

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