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# PHYLOGEOGRAPHIC PATTERNS AND DEMOGRAPHIC HISTORY OF *SCHIEDEA GLOBOSA* (CARYOPHYLLACEAE) ON THE HAWAIIAN ISLANDS[1]

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Geomorphological changes have been demonstrated to have had profound impacts on biodiversity, often leading to demographic expansions and contractions and allopatric divergence of taxa. We examined DNA sequence variation at two nuclear and one maternally inherited plastid locus among 10 populations of *Schiedea globosa* on the Hawaiian Islands to assess the primary factors shaping genetic structure, phylogeographic patterns, and the importance of geographic isolation to population divergence. *Schiedea globosa* has characteristics that may promote gene flow, including wind pollination and rafting of plants in ocean currents. However, we detected significant differentiation among populations on all islands except Hawaii, with the maternally inherited plastid locus having the greatest genetic structure ($F_{ST}$ = 0.81). Migration rates across all loci are less than one migrant per generation. We found evidence of growth in several populations and on the islands of Molokai and Maui, which supports population expansion associated with the formation of Maui Nui during the last glacial maximum. Similar to data for many other Hawaiian taxa, these data suggest *S. globosa* originated on Oahu and subsequently colonized Molokai, Maui, and Hawaii in progression. Given the high level of genetic structure, allopatric divergence will likely contribute to further divergence of populations.

**Key words:** ancestral polymorphism; Caryophyllaceae; gene coalescence; DNA sequence; intraspecific variation; migration; phylogeography; *Schiedea globosa*.

Oceanic island systems are natural laboratories for studies of organismal evolution because their isolation, age, and size allow for acceleration and exaggeration of evolutionary processes relative to mainland populations (Carlquist, 1974; Simon, 1987; Wagner and Funk, 1995; Price and Wagner, 2004). Numerous phylogenetic studies have demonstrated the evolutionary histories of various adaptive radiations (e.g., reviewed in Emerson, 2002; Price and Clague, 2002; Price and Wagner, 2004) and elucidated important biogeographic processes contributing to diversification on islands, but basic questions about the processes by which new insular species arise are still unanswered (e.g., Wagner and Funk, 1995; Roderick and Gillespie, 1998; Givnish, 1998; Nepokroeff et al., 2003; Price and Wagner, 2004; Wagner et al., 2005). In particular, the evolutionary history and fate of species occupying multiple islands has not received adequate attention in the literature, yet these species may exhibit the earliest stages of allopatric speciation (e.g., Roderick and Gillespie, 1998; Hormiga et al., 2003). Thus, full understanding of factors important in the diversification of taxa requires examination of genotypic patterns across and within species within the context of historical geography.

Periodic climatic oscillations over the Pleistocene are believed to have greatly influenced the amount and distribution of genetic variation in many species (e.g., Hewitt, 1999; Avise, 2000; Soltis et al., 2006). On the Hawaiian Islands, Pleistocene climate change caused sea levels to drop, perhaps by as much as 120 m (Matthews, 1990), which altered island size and shape. Most notably, the islands of Molokai, Maui, Lanai, and Kahoolawe were connected for more than 75% of the past 1.2 million years (Myr) into Maui Nui, a large island that reached a maximum size of 14 000 km$^2$ (Carson and Clague, 1995; Clague, 1996; Price and Elliott-Fisk, 2004). At the glacial maximum, 0.02 Myr ago (Ma), Maui Nui was ca. 5900 km$^2$ in size. Prior to the completion of Maui Nui, Molokai was also briefly connected to Oahu (i.e., for 0.3 Myr) shortly after its formation ca. 2.0 Ma via a high connection through Penguin Bank and a bridge northwest of Molokai (Price and Elliott-Fisk, 2004). An increase in suitable habitat would likely have allowed species to migrate between islands and to expand their ranges. By contrast, during interglacial periods, oceanic channels are generally expected to have caused reductions in gene flow between islands (Wagner and Funk, 1995; Roderick and Gillespie, 1998; Craddock, 2000). Each of these events should leave a demographic signature that can be inferred by examination of genetic variation within and across populations. For example, if populations on Oahu and Molokai were connected through Penguin Bank, then moderate rates of gene flow are expected in taxa inhabiting these areas. Similarly, if the extensive area of Maui Nui allowed for range expansion, then populations should grow at positive rates. Other studies have revealed that long-lasting demographic effects vary dramatically across taxa, depending on where populations were located during glacial episodes, their ability to adapt to changing environmental conditions, and their dispersal ability (e.g., Campbell et al., 2006). In this study, we used DNA sequence data to evaluate the impact of historical

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events and geography on genetic structure in an endemic and widespread species of the Hawaiian Islands, *Schiedea globosa* H. Mann (Caryophyllaceae).

*Schiedea* is a Hawaiian endemic genus, which comprises the fifth largest radiation (i.e., 32 extant species and two extirpated species) of Hawaiian angiosperms from a single colonization (Wagner et al., 2005). *Schiedea globosa* is a perennial, wind-pollinated species with a subdioecious breeding system (i.e., primarily staminate or pistillate individuals with occasional hermaphroditic flowers in staminate inflorescences; Wagner et al., 2005). The most widespread species in the genus, *S. globosa* occurs on the eastern and southeastern coasts of Oahu, the northern coast of Maui, the northern coast of Molokai, and in the Waipio Valley on the north coast of Hawaii. One historical collection is also known from Lanai (Wagner et al., 2005). Population sizes vary, and populations are restricted to steep, north-facing coastal rocky slopes or cliffs from 0–460 m above sea level. Previous studies of *S. globosa* based on allozymes (Nei's $I = 0.75$ between islands vs. Nei's $I = 0.934$–$0.943$ within islands; Weller et al., 1996) and a nuclear locus ($F_{ST} = 0.57$; Filatov and Burke, 2004) demonstrated significant genetic structure between populations on Oahu and Maui, suggesting that ocean channels restrict gene flow between islands. However, no previous study has examined the full range of populations. Populations on Molokai may have historical importance to intraspecific divergence in *S. globosa*, given that Molokai was connected to Oahu via Penguin Bank approximately 1.7–2.0 Ma and was part of the Maui Nui complex until approximately 0.3–0.4 Ma (Carson and Clague, 1995). Molokai, which is geographically intermediate between Oahu and Maui, may provide a corridor for gene flow between populations on these islands. The ability of vegetative portions of plants (and possibly seeds) to disperse in water currents via rafting (Wagner et al., 2005) suggests that this species may be capable of gene flow over long distances.

We compared levels and patterns of DNA sequence variation among populations of *S. globosa*, tested for population growth, and quantified migration rates between populations using Bayesian methods to assess the primary factors shaping genetic variation in *S. globosa* and to test the following hypotheses: (1) range expansion of *S. globosa* is consistent with the progression rule such that ancestral haplotypes occur on Oahu, and more recently derived haplotypes are found on Molokai, Maui, and Hawaii, respectively; (2) oceanic channels are significant barriers to gene flow such that migration rates between any two pairs of islands will be low, and there will be significant genetic structure, as revealed by an analysis of molecular variance, among the islands; and (3) populations on Molokai and Maui underwent demographic expansion associated with a large and contiguous habitat on Maui Nui during the last glacial maximum.

## MATERIALS AND METHODS

***Sampling and DNA sequencing***—We sampled 1–10 individuals from 10 populations of *S. globosa* on Oahu, Molokai, Maui, and Hawaii. Although sample sizes for some populations are small, all islands where *S. globosa* is known to occur are represented in the data set (Fig. 1). Genomic DNA was extracted from frozen or dried leaf tissue using a modified CTAB protocol (Doyle and Doyle, 1987) or the DNeasy Plant Mini Extraction Kit (Qiagen, Valencia, California, USA). DNA sequences were generated for three markers: the intergenic chloroplast region between *psbM* and *trnD* genes (*psbM-trnD*), the fourth intron of the nuclear gene phosphoenolpyruvate carboxylase (*PepC*), and a portion of the nuclear gene chloroplast-expressed glutamine synthetase (*ncpGS*). The *psbM-trnD* and *ncpGS* regions were amplified in 25-μL reactions containing 1× Thermophilic DNA buffer (ProMega, Madison, Wisconsin, USA), 2 mM $MgCl_2$, 200 μM each dNTP, 0.4 μM each primer, 1 μL BSA (100×), 0.5 U *Taq* DNA polymerase (ProMega), and 1 μL template DNA. *PepC* was amplified in 50-μL reactions containing the same concentrations of reagents as for *psbM-trnD* and *ncpGS*, except 2 μL BSA (100×), 1.0 U *Taq* DNA polymerase, and 5.0 μL template DNA were used. The *psbM-trnD* region was amplified with primers psbM1 and trnD (Lee and Wen, 2004), *PepC* was amplified with primers PPCL1 and PPCL2 (Gaskin and Schaal, 2002), and *ncpGS* was amplified with primers GScp687f and GScp994r (Emshwiller and Doyle, 1999). The thermal cycler protocol used to amplify *psbM-trnD* and *PepC* was 35 cycles of 95°C for 1 min, 50°C for 1 min, 65°C for 4 min; with a 1°C/8 s ramp from the annealing temperature to the extension temperature (Small et al., 1998). The *ncpGS* region was amplified with the following thermal cycler conditions: 1 cycle of 95°C for 5 min, 35 cycles of 95°C for 1 min, 50°C for 1 min, 72°C for 2 min; 1 cycle of 72°C for 7 min. A negative control, including all reagents except template DNA, was included with every set of reactions to check for contamination. Samples of PCR products were electrophoresed in 1× tris-borate-ethylenediaminetetraacetic acid (TBE) agarose gels to check for amplification of a band of the expected size. Following successful amplification, *psbM-trnD* products were purified directly with the QIAquick PCR Purification Kit (Qiagen). The nuclear regions, for which multiple bands amplified, were purified by cutting bands out of agarose gels followed by purification with the QIAquick Gel Extraction Kit (Qiagen). Purified templates were sequenced bi-directionally using the dideoxy chain termination method with the Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and Better Buffer (Gel Co., San Francisco, California). Each 15-μL reaction contained 4 μL Better Buffer, 1 μL Big Dye Terminator mix, 0.07 μM primer, and ca. 20 ng of purified template DNA. Reactions were cleaned with an ethanol/sodium acetate precipitation method, and samples were subjected to capillary electrophoresis on an Avant-3100 Genetic Analyzer (Applied Biosystems) following manufacturer's instructions. Individually sequenced strands were manually edited and used to create a consensus sequence in the program Sequencher (GeneCodes, Ann Arbor, Michigan, USA). For individuals with simple heterozygosity at the *PepC* and *ncpGS* loci (i.e., a maximum of two heterozygous sites in the total sequence) identities of the two alleles were inferred using haplotype subtraction (Clark, 1990). For those individuals with more than two heterozygous sites or whose allelic identities could not be determined by haplotype subtraction, we cloned *PepC* and *ncpGS* with the pGEM-T Easy Vector System II (Promega) and sequenced 2–5 individual clones using the protocol outlined. All sequences were manually aligned using the program Se-Al (Rambaut, 1996). Sequences have been deposited in GenBank (accessions: *psbM-trnD*, FJ496357–FJ496425; *ncpGS*, FJ496426–FJ496541; *pepC*, FJ496542–FJ496647).

We checked the *ncpGS* and *PepC* data sets for recombinant alleles using two statistical tests, the RDP method (Martin and Rybicki, 2000) and the MaxChi method (Maynard Smith, 1992; Posada, 2002) implemented in the software RDP version 2.0 (Martin and Rybicki, 2000). Under the RDP test, we analyzed the sequences at window sizes of 5, 10, 50, and 100, using internal references only. The MaxChi test was run by considering triplets of sequences and all sequences simultaneously with gaps removed and a variable window size of 0.013. The significance of $\chi^2$ statistics was tested using a permutation test of 1000 iterations.

***Analysis of genetic diversity and structure***—Measures of haplotypic and nucleotide diversity were estimated for each of the gene regions using DNAsp version 4.10 (Rozas et al., 2003). We examined genetic structure among islands ($F_{CT}$) and populations ($F_{SC}$, $F_{ST}$) for each of the markers based on pairwise differences in analyses of molecular variance (AMOVA; Excoffier et al., 1992) implemented in the program ARLEQUIN version 3.11 (Excoffier et al., 2005). The three populations containing a single individual were not included in these analyses. *P*-values for the variance components were derived by comparing the observed value with values obtained through 50 000 random permutations of the data.

A phylogenetic network of individuals based on the combined data were generated using the algorithm of Joly and Bruneau (2006). This method allows multiple loci to be combined in a phylogenetic analysis and represents patterns among individuals rather than alleles or haplotypes as in traditional gene trees. Additionally, networks more accurately represent intraspecific relationships characterized by recombination and gene flow (Joly and Bruneau, 2006; Linder and Rieseberg, 2004). Parsimony and Bayesian phylogenetic analyses produced qualitatively similar results to those presented here. To construct the phylogenetic network, we coded gaps for each gene region as present/absent and

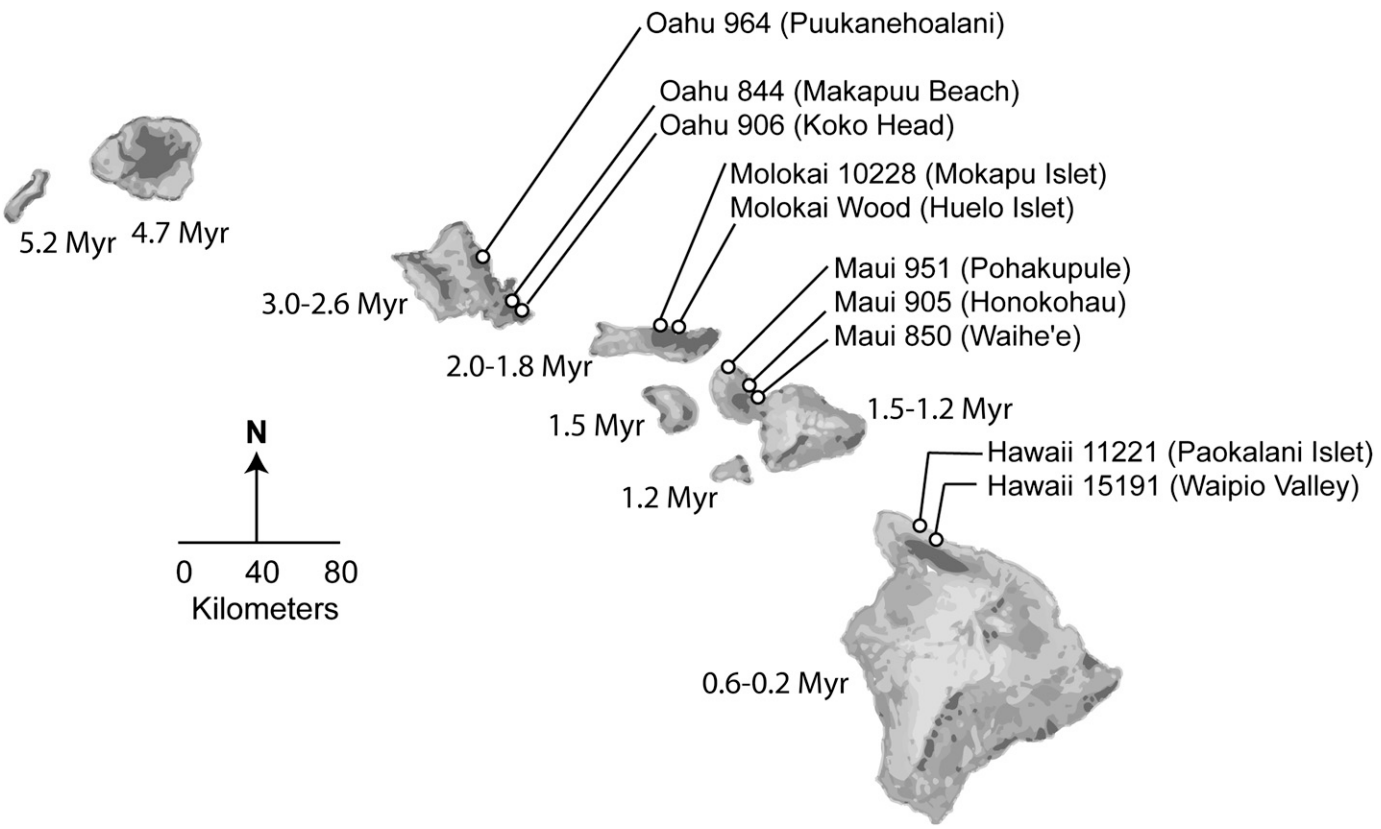


Fig. 1. Locations of sampled populations of *Schiedea globosa* on the Hawaiian Islands. Ages of the islands based on Clague (1996).

calculated average uncorrected pairwise distances between chloroplast haplotypes or nuclear alleles in the program PAUP* version 4.0b10 (Swofford, 2001). The three resulting distance matrices served as the input for the calculation of standardized pairwise distances between individuals in the software POFAD (Joly and Bruneau, 2006). The three gene matrices were standardized into a single distance matrix by assigning a distance of 1 to the largest distance of the matrix and scaling the other distances appropriately. The standardized interindividual distances were then used as input for the NeighborNet analysis (Bryant and Moulton, 2004), conducted using the software SplitsTree4 (Huson and Bryant, 2006), to produce a network of relationships among individuals. Four closely related species in section *Schiedea* (*Schiedea haleakalensis*, *S. hookeri*, *S. mannii*, *S. sarmentosa*) were included as outgroups and used to root the network.

***Demographic analyses***—The potential for historical demographic expansion to have occurred in populations on the islands of Oahu, Molokai, and Maui was quantified with Fu's $F_S$ (1997), and the $R_2$ statistic of Ramos-Onsins and Rozas (2002) for each gene region in the program DNAsp (Rozas et al., 2003). Significant negative $D$ and $F_S$ statistics can indicate nonneutrality or population expansion; the latter is more likely when large negative values are observed across multiple loci. By contrast, the $R_2$ statistic of Ramos-Onsins and Rozas (2002) was developed specifically to detect population growth and may have greater power than $F_S$ when sample sizes and the number of segregating sites are small. The significance of $D$, $F_S$, and $R_2$ was tested using 1000 permutations based on a coalescent model, without recombination for the cpDNA locus and with an intermediate level of recombination allowed for the nuclear loci, and a fixed population mutation rate, θ, estimated from the data. We also used a Markov chain Monte Carlo approach implemented in the program FLUCTUATE version 1.3 (Kuhner et al., 1998) to assess population growth or decline. In this method, the exponential population growth rate, $g$, and population mutation rate, θ, are estimated. In each run, we used 10 short chains and 10 long chains with sampling increments of 10 and 1000 and 20 000 steps per chain, respectively, a random starting tree, and empirically determined locus-specific transition to transversion ratios of 0.5 for *psbM-trnD*, 2.26 for *ncpGS,* and 1.71 for *PepC*. Theta was estimated using Watterson's (1975) method, and the starting value of $g$ was set to 1. Convergence was checked by conducting replicate runs with different seed numbers and varying the number and length of chains. Because values of $g$ may be biased upward and standard deviations are only approximate, we conservatively used $g$ to indicate population growth if the estimated value of $g$ was >3 standard deviations (SD), similar to the method used by Lessa et al. (2003).

Migration rates among all islands, except Hawaii, were estimated using combined analyses of the three loci and Bayesian methods implemented in the programs IM (Hey and Nielsen, 2004) and Migrate-n version 2.03 (Beerli and Felsenstein, 1999, 2001; Beerli, 2004b). Both methods allow for heterogeneity in mutation rates among loci. IM implements the isolation with migration model of Nielsen and Wakeley (2001) and derives demographic parameters for two samples at a time. By contrast, Migrate-n simultaneously estimates migration rates among all populations and is useful for evaluating potential ghost populations associated with individual pairwise analyses conducted with IM (Beerli, 2004a; Slatkin, 2005).

Using IM, estimates of the population mutation rate ($\theta = 4N_e\mu$ where $N_e$ is the effective population size and μ is the mutation rate), migration rate ($m = m/\mu$ where $m$ is the rate of migration for each gene copy), and divergence time ($t = t\mu$, with time in generations since divergence) were derived from Bayesian posterior distributions generated through Monte Carlo Markov chain (MCMC) simulations. After initial tests of starting parameters, we conducted coalescent simulations for each comparison using the following starting parameters: an HKY model of substitution, a burn-in period of 500 000 generations, 10 chains with five chain swap attempts per step, a linear heating mode, and heating parameter = 0.005. Following the recommendations of the authors (Hey and Nielsen, 2004), we conducted simulations for a minimum of 10 million generations until the shape of the posterior distribution stabilized. Initial analyses were conducted in duplicate as an additional test of convergence of the Markov chain.

Migrate-n analyses were conducted for Maui, Molokai, and Oahu on the combined data set using the DNA sequence model and incorporating empirically determined locus-specific transition to transversion ratios of 0.5 for *psbM-trnD*, 2.26 for *ncpGS*, and 1.71 for *PepC*. Uniform priors were used in which θ was bound between 0.00001 and 20 and migration rate was bound between

0.00001 and 5000. We conducted searches using three long chains with 1 million sampled genealogies set at a sampling increment of 20. The first 100 000 trees were discarded as a burn-in period before computing posterior probabilities.

The conversion of model parameters into demographic parameters permits one to evaluate divergence time, population migration rate, and population mutation rate using biologically meaningful values. Because the model parameters estimated by IM and Migrate-n are scaled by the mutation rate, μ, their conversion into demographic parameters requires an understanding of the mutation rate for the sampled loci. We are unaware of published mutation rates for any of the gene regions studied in *S. globosa*. Therefore, we used genetic distance to an outgroup for the entire *Schiedea* lineage and the geological history of the Hawaiian Islands to derive locus-specific mutation rates based on the formula $D = 2Kt$, where $D$ is the mean number of substitutions, $K$ is the mutation rate, and $t$ is the time of divergence. We tested for the presence of a constant molecular clock using maximum likelihood (ML) heuristic searches of the data sets in PAUP* (Swofford, 2001) with and without the constraint of a molecular clock and based on the best fitting model of sequence evolution with tree-bisection-reconnection (TBR) branch swapping and 10 random addition replicates. The likelihood values for trees found under the constraint of a molecular clock and without it, as determined in a likelihood ratio test (Huelsenbeck and Rannala, 1997), were not significantly different for any of the gene regions. Thus, we computed a mean pairwise distance between *Honckenya peploides*, one of the closest sister species to the *Schiedea* lineage (Wagner et al., 2005), and a sample of *Schiedea* species based on parameters of the model of sequence evolution used in the ML tree searches. Mean pairwise distance ($p$) is related to $D$ by the formula $D = -0.75 \cdot \ln(1 - 4p/3)$ (Hartl and Clark, 1997). $D$ was estimated to be 0.0740, 0.3189, and 0.1199 for *psbM-trnD*, *ncpGS*, and *PepC*, respectively. The time at which the *Schiedea* lineage began to diverge from *H. peploides* on the Hawaiian main islands was estimated to be approximately 5 Myr based on reports of divergence times for other endemic Hawaiian lineages and geologic evidence presented by Price and Clague (2002) as well as the distribution of extant *Schiedea* species. Preliminary studies of molecular dating for the Hawaiian *Schiedea* lineage using soft-bounds Bayesian approaches indicate that the lineage is approximately 5 million years old or slightly less (A.Willyard et al., University of South Dakota, unpublished data). With this estimate of divergence time, the mutation rates for each of the gene regions are $5.36 \times 10^{-6}$ substitutions·sequence$^{-1}$·year$^{-1}$ for *psbM-trnD*, $2.30 \times 10^{-5}$ substitutions·sequence$^{-1}$·year$^{-1}$ for *ncpGS*, and $7.04 \times 10^{-6}$ substitutions·sequence$^{-1}$·year$^{-1}$ for *PepC*. The geometric mean mutation rate across markers is $9.54 \times 10^{-6}$ substitutions·sequence$^{-1}$·year$^{-1}$. Notably, our estimated mutation rate for *S. globosa* is within the range of estimated rates for plant genomes based on the work of Wolfe et al. (1987). Using a mean range of $1–3 \times 10^{-9}$ and $5–30 \times 10^{-9}$ substitutions·sequence$^{-1}$·year$^{-1}$ across chloroplast and nuclear genes, respectively, we estimate that the geometric mean mutation rate for *psbM-trnD*, *ncpGS*, and *PepC* is $6.199 \times 10^{-6}$ substitutions·sequence$^{-1}$·year$^{-1}$.

## RESULTS

***Genetic diversity***—Amplification with the *ncpGS* and *PepC* primers occasionally resulted in two or three bands, but only one band was consistently present across all individuals. This band was sequenced for all samples included in this study. Several inconsistently amplified bands contained strongly divergent sequence in the noncoding portions of the genes (data not presented). No more than two nucleotides were observed at a site for any given individual, suggesting the presence of allelic variation rather than orthologous loci for these markers. Additionally, cloned sequences of *ncpGS* from 12 individuals and of *PepC* from 18 individuals revealed no additional variation compared to the original sequence chromatograms. Thus, the loci used for this study appear to be present in single or at most low copy in *Schiedea*, similar to reports in other taxa (e.g., Emshwiller and Doyle, 1999; Gaskin and Schaal, 2002). Aligned lengths of the three gene regions were 724 bp for *psbM-trnD*, 720 bp for *ncpGS*, and 587 bp for *PepC*. Length variants were found in each of the gene regions, and many individuals were heterozygous for indels in *ncpGS* and/or *PepC*, which is common at low taxonomic levels (Britten et al., 2003). We found no evidence of recombination for *ncpGS* or *PepC* with any variation of the RDP or MaxChi test ($P > 0.05$).

The nuclear loci had more allelic variation than the chloroplast locus, and many individuals were heterozygous at one or both nuclear loci. Including coded insertions/deletions, we observed 10 plastid haplotypes, 31 *PepC* alleles, and 48 *ncpGS* alleles across the 59 sampled individuals. The two nuclear loci contained nearly twice as much nucleotide diversity (mean $\pi_{species} = 0.0042$) compared to the plastid locus ($\pi_{species} = 0.0027$; Table 1). Oahu and Maui contained twice as many plastid haplotypes compared to Molokai. Maui contained the largest number of *ncpGS* alleles, and Molokai contained more *PepC* alleles than the other islands (Table 1). Inbreeding coefficients ($F_{IS}$) for the two nuclear loci were estimated to be –0.12 for *PepC* ($P > 0.05$) and 0.15 for *ncpGS* ($P > 0.05$; Table 2).

The pattern of genetic structure differed across loci. Most of the variation at the nuclear loci resided within individuals or populations, although the loci differed in the distribution of variation among islands (Table 2). For example, only 3% ($P > 0.05$) of the variation in *PepC* was attributed to interisland differences ($F_{CT} = 0.03$; Table 2), whereas 19% ($F_{CT} = 0.19$, $P < 0.05$) of the variation in *ncpGS* resided among islands. In contrast, the chloroplast gene *psbM-trnD* had strong genetic structure among the islands ($F_{CT} = 0.79$, $P < 0.05$).

Phylogenetic patterns of nuclear alleles and plastid haplotypes are concordant with the results of AMOVA. The plastid haplotypes are distinguishable by island with the exception of three individuals on Molokai that share a haplotype with individuals on Maui (data not shown). The samples from Hawaii cluster with haplotypes from Maui. Considerably less genetic structure by island was found in the nuclear gene trees (data not shown). By contrast, the phylogenetic network, which is based on the combined data sets, indicates a high level of structure that is not visible in any single data set. In this network, three major splits can be seen, corresponding to Oahu, Molokai, and Maui + Hawaii (Fig. 2). Two exceptions to this general pattern are the clustering of the single individual from 964 on Oahu with individuals from Maui and the clustering of three individuals from Molokai (10213, 10224, 10228–39) within the Maui + Hawaii cluster.

***Demographic analyses***—Significant departures from neutrality were detected using Fu's $F_S$ statistic for *psbM-trnD* on Maui; *ncpGS* in population 951, on Maui and Molokai, and in the species; and *pepC* on Oahu and in the species (Table 3). A single significant $R_2$ value was found at *psbM-trnD* on Maui. Maximum likelihood estimates of growth rates suggest rapid expansion of the species as well as populations on Oahu, Molokai, and Maui (Table 3). However, not all loci supported positive growth on all islands. Whereas positive growth is suggested by all loci on Oahu, only the nuclear loci had evidence of growth on Molokai and Maui. In general, the nuclear loci had stronger growth patterns than the chloroplast locus, and *ncpGS* had the strongest evidence of growth, which is similar to results from $F_S$ tests. In addition to population expansion on the islands, several individual populations (844, Wood-Huelo, 905, and 951) had evidence of rapid expansion in at least one locus.

Coalescent-based estimates of the migration rate ($m$) for pairwise comparisons of the islands are presented in Table 4. Estimates of migration rates were between 0.015 and 1.965 per generation. The generation time of *S. globosa* is estimated at approximately 5 years (S. Weller and A. Sakai, personal observations). Based on a mutation rate of $10^{-5}$ to $10^{-8}$ substitutions$^{-1}$·

TABLE 1. Diversity statistics by population and island for three gene regions, *psbM-trnD*, *ncpGS*, and *PepC*, examined in *Schiedea globosa*. The unique identifier of the population, the number of individuals sampled (*N*), the number of haplotypes (*n*), and nucleotide diversity ($\pi$) ± SD are shown. Values for islands represent total diversity across pooled populations.

| | | *psb*M-*trn*D | | *ncpGS* | | *PepC* | |
|---|---|---|---|---|---|---|---|
| Population | *N* | *n* | $\pi$ | *n* | $\pi$ | *n* | $\pi$ |
| 844-Oahu | 3 | 2 | 0.0018 ± 0.0009 | 5 | 0.0030 ± 0.0006 | 5 | 0.0036 ± 0.0008 |
| 906-Oahu | 11 | 3 | 0.0012 ± 0.0008 | 10 | 0.0043 ± 0.0007 | 8 | 0.0034 ± 0.0008 |
| 964-Oahu | 1 | 1 | 0 | 1 | 0 | 2 | 0.0017 ± 0.0008 |
| Oahu island | 15 | 5 | 0.0019 ± 0.0006 | 15 | 0.0050 ± 0.0004 | 13 | 0.0035 ± 0.0005 |
| Wood-Huelo-Molokai | 10 | 2 | 0.0010 ± 0.0004 | 10 | 0.0034 ± 0.0007 | 11 | 0.0037 ± 0.0004 |
| 10228-Molokai | 10 | 2 | 0.0006 ± 0.0004 | 8 | 0.0022 ± 0.0003 | 14 | 0.0062 ± 0.0016 |
| Molokai island | 20 | 2 | 0.0007 ± 0.0003 | 14 | 0.0028 ± 0.0004 | 17 | 0.0050 ± 0.0009 |
| 850-Maui | 2 | 2 | 0.0014 ± 0.0007 | 4 | 0.0063 ± 0.0017 | 4 | 0.0009 ± 0.0005 |
| 905-Maui | 10 | 1 | 0 | 12 | 0.0044 ± 0.0006 | 4 | 0.0016 ± 0.0004 |
| 951-Maui | 10 | 2 | 0.0003 ± 0.0002 | 15 | 0.0054 ± 0.0008 | 8 | 0.0027 ± 0.0004 |
| Maui island | 22 | 4 | 0.0005 ± 0.0002 | 26 | 0.0051 ± 0.0005 | 13 | 0.0025 ± 0.0003 |
| 11221-Hawaii | 1 | 1 | 0 | 2 | 0.0014 ± 0.0007 | 2 | 0.0018 ± 0.0009 |
| 15191-Hawaii | 1 | 1 | 0 | 2 | 0.0028 ± 0.0014 | 2 | 0.0017 ± 0.0009 |
| Hawaii island | 2 | 1 | 0 | 4 | 0.0031 ± 0.0007 | 4 | 0.0029 ± 0.0008 |
| Species | 59 | 10 | 0.0027 ± 0.0003 | 52 | 0.0047 ± 0.0003 | 37 | 0.0042 ± 0.0004 |

sequence$^{-1}$·generation, gene flow among the islands is estimated to be less than 1 migrant per generation, but 95% confidence intervals exclude zero. Estimates of migration rate with Migrate-n were higher than from the IM analyses, but still indicated little gene flow between islands (less than 0.013 migrants per generation; Table 4). Although estimated migration rates varied somewhat by locus, gene flow was not greater than one migrant per generation for any locus (data not shown).

## DISCUSSION

***Genetic variability within Schiedea globosa***—*Schiedea globosa* has nearly two times more diversity in the nuclear loci compared to the plastid locus (Table 1), a finding that is consistent with the nonrecombining nature and smaller effective sizes of plastid loci. Nucleotide diversity at *ncpGS* ($\pi$ = 0.0045) and *PepC* ($\pi$ = 0.0039) are substantially higher than previous estimates based on *SgXY1* for *S. globosa* ($\pi$ = 0.0025) sampled from Oahu and Maui (Filatov and Burke, 2004). Our estimates of nucleotide diversity at the species level are similar, though, to sequence diversity in noncoding regions of the *ASAP* locus reported for endemic species of *Dubautia* and *Argyroxiphium* on the Hawaiian Islands (Lawton-Rauh et al., 2003; Friar et al., 2006). In these studies, nucleotide diversity averaged between 0.002 and 0.004 across species.

In previous studies of *S. globosa* by Weller et al. (1996) and Filatov and Burke (2004), populations on Maui had lower levels of variability than those on Oahu. We found a similar pattern for the plastid locus but not for the nuclear loci. Molokai and Maui frequently contained more unique *ncpGS* and *PepC* alleles than did Oahu (Table 1). This apparent difference in patterns of genetic structure among the islands and across studies may be due to differences in sampling sizes and sampling locations. Previous studies were based on five (Weller et al., 1996) and three (Filatov and Burke, 2004) populations on two islands, Oahu and Maui, whereas the current study, including populations from all islands where the species occurs, provides a more extensive view of the evolutionary history of this species. Another possible reason for this difference is the use of different markers across studies. Filatov and Burke (2004) studied DNA sequence variation in *S. globosa*, but they considered a single locus and used a gene that may experience purifying selection. Weller et al. (1996) used data for allozymes, which may also be under selection (Hudson et al., 1994; Filatov and Charlesworth, 1999). Differential selection and genetic drift could have generated

TABLE 2. (A) Hierarchical structure of genetic variation in *Schiedea globosa* on Oahu, Molokai, and Maui based on analyses of molecular variance. The total amount of variation residing at each level as well as (B) *F*-statistics (with 95% confidence intervals) are reported for each locus.

| | *psb*M-*trn*D | *ncpGS* | *PepC* |
|---|---|---|---|
| A) Hierarchical level | | | |
| Among islands | 78.9 | 18.9 | 3.2 |
| Among populations/islands | 3.2 | 2.8 | 15.3 |
| Among individuals/populations | 17.9 | 11.8 | –9.9 |
| Within individuals | NA | 66.5 | 91.4 |
| B) *F*-statistic | | | |
| $F_{IS}$ | NA | 0.15 (0.068–0.249) | –0.12 (–0.221–0.005) |
| $F_{IT}$ | NA | 0.33* (0.259–0.426) | 0.08 (–0.026–0.215) |
| $F_{SC}$ | 0.15* (–0.010–0.405) | 0.03 (0.003–0.078) | 0.16* (0.135–0.177) |
| $F_{CT}$ | 0.79* (0.681–0.851) | 0.19* (0.154–0.218) | 0.03 (–0.023–0.097) |
| $F_{ST}$ | 0.82* (0.761–0.863) | NA | NA |

*Notes:* * Significant *F*-statistic ($P < 0.05$), determined by comparison to distributions generated from 50 000 permutations. $F_{IS}$ = inbreeding coefficient, $F_{IT}$ = variation in individuals relative to total variation, $F_{SC}$ = variation in populations relative to island-wide variation, $F_{CT}$ = variation on islands relative to total variation, $F_{ST}$ = variation in populations relative to total variation.

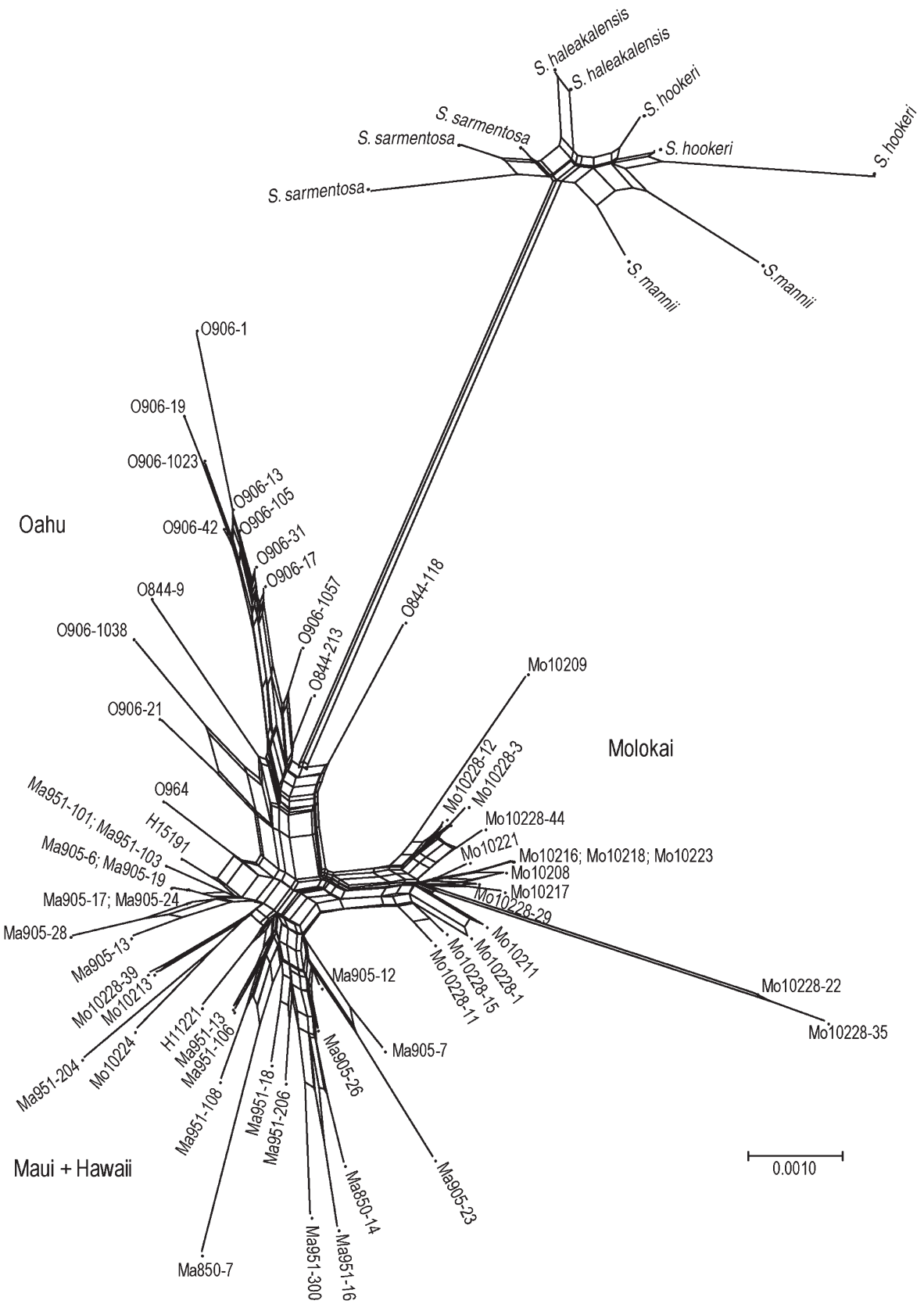


Fig. 2. Phylogenetic network produced from a NeighborNet analysis of genetic distances among individuals of *Schiedea globosa*. Tips of the network represent unique genotypes. The tips are labeled by island (O = Oahu, Mo = Molokai, Ma = Maui, H = Hawaii) and population name. Note that individuals from the Wood-Huelo population are indicated by accession numbers 10208–10224.

the patterns observed in earlier studies, whereas sequence variation in introns, which comprise most of the data in this study, may be more variable. Consequently, small losses associated with founder events may not have reduced overall levels of diversity at nuclear introns in *S. globosa*. Villablanca et al. (1998) also reported significant differences in the amount of genetic variation in founder populations of a medfly species when mitochondrial markers, allozymes, or nuclear intron sequences were used to estimate diversity. Greater diversity of intron regions is attributed to the lack of strong selection on these regions and the larger effective population size of nuclear vs. cytoplasmic genes. Finally, the outcrossing breeding system of *S. globosa* is also likely to contribute to the retention of high levels of diversity at nuclear loci (Sakai et al., 1989). Both nuclear loci do not indicate significant levels of inbreeding in *S. globosa* (Table 2), which is consistent with this expectation.

***Island phylogeography***—Given the progressive colonization from older to younger islands (Wagner and Funk, 1995) found for many other Hawaiian lineages, we hypothesized that the most ancestral genotypes would be found on Oahu, where *S. globosa* likely evolved and that the highest degree of similarity would be between Maui and Hawaii, representing the most recent colonization event. Several patterns from these data support this hypothesis. First, in the phylogenetic network, genotypes from Oahu occupy the basalmost positions within *S. globosa* relative to the outgroup species, and individuals on Molokai and Maui have more derived genotypes. With these data, we were not able to determine the actual number of colonization events from Oahu to Maui Nui, but the high level of genetic diversity in populations on Molokai and Maui suggests multiple colonization events and/or large founder populations, rather than a loss of diversity often associated with genetic drift in founder populations. The clustering of three individuals from Molokai populations Wood-Huelo and 10228 within the Maui + Hawaii group is consistent with progressive colonization from Molokai to Maui, perhaps directly from these populations. Last, we found strong similarity between genotypes on Hawaii and those on Maui. The presence of Hawaii genotypes in different areas of the network also suggests that Hawaii received multiple colonists from Maui or that the colonizing population was quite large and ancestral polymorphism has been maintained on Hawaii. The only inconsistent pattern in these data, with regard to the progression rule, is the high similarity between the individual in population 964 on Oahu with genotypes on Maui. Such a pattern could indicate a backward colonization to Oahu, given that the three data sets independently demonstrate this pattern of relationship.

***Gene flow between islands***—The high level of diversity observed in populations on Molokai and Maui could also indicate ongoing gene flow with populations on Oahu. Indeed, *S. globosa* is capable of dispersing its genes in wind currents via pollen and in ocean currents via rafting plants. However, previous studies found significant differentiation between populations on Oahu and Maui (Weller et al., 1996; Filatov and Burke, 2004). We also found strong structure among the islands of Oahu, Molokai, and Maui in the chloroplast region ($F_{CT} = 0.79$; Table 2), but substantially less genetic structure at the nuclear loci. Little is known about specific mechanisms of seed dispersal for most *Schiedea* species, but patterns of seedling growth suggest that seeds fall from dehisced capsules beneath the maternal plant (Wagner et al., 2005). If one assumes that the chloroplast genome is maternally inherited in *Schiedea,* then the limited degree of seed dispersal is expected to generate substantial genetic structure among populations and islands at chloroplast loci. By contrast, dispersal of pollen and vegetative plants largely determine genetic structure at nuclear loci. The greater dispersal abilities of pollen in wind currents and vegetative plants in ocean currents are expected to result in less structure at the nuclear loci. Dioecious breeding systems can also alter gene flow by seed and pollen if functional differences in the frequency and fitness of males and females occur. Hence, variation in the proportion of males and females in *S. globosa* from year to year (Sakai and Weller, 1991) could contribute to historical differences in gene flow estimated from chloroplast and nuclear loci if migration between islands occurs inconsistently as is expected for *S. globosa*.

Alternatively, differential patterns of structure between chloroplast and nuclear loci could be explained by incomplete lineage sorting. In the absence of natural selection, coalescence of alleles to a common ancestor (i.e., complete lineage sorting) at nuclear loci takes nearly four times longer than cytoplasmic markers because nuclear loci are recombining, biparentally inherited, and have larger effective population sizes (Rosenberg and Nordborg, 2002). Thus, if populations have been separated long enough that nuclear alleles have fully sorted, then contemporary gene flow most likely explains the lack of genetic structure, whereas recent population divergence is congruent with incomplete lineage sorting. Given the recent origin of taxa on the Hawaiian main islands within the last 5 million years (Price and Clague, 2002; Price and Wagner, 2004), it is likely that incomplete lineage sorting has influenced patterns of genetic structure observed in *S. globosa.* This leaves unanswered the question of whether gene flow is ongoing among the islands. Bayesian estimates of gene flow between populations indicate less than one migrant per generation between all pairs of islands (Table 4). Although the IM and Migrate-n methods differed in absolute rates of migration, they are consistent in indicating an extremely low rate of migration. Nevertheless, given that confidence intervals surrounding estimated migration rates exclude zero, low levels of gene flow between islands are predicted to occur infrequently. Such low migration rates, based on the combined data set as well as on analyses of individual loci (not reported), suggest that lineage sorting is a more likely explanation for a lack of strong structure at the nuclear loci. These rare migration events between islands may be due to pollen dispersal in wind currents or oceanic dispersal of rafting plants.

The formation of Penguin Bank between Oahu and Molokai may have provided a means of initial dispersal to the latter island, as did Maui Nui for colonization to Maui from Molokai. However, subsequent to these colonization events, the ocean appears to be a formidable barrier to gene flow except in rare cases. The evolutionary impact of low migration rates is ongoing differentiation of *S. globosa*, which is ultimately likely to produce distinct taxa. Some morphological differences have been noted among populations of *S. globosa* (Wagner et al., 2005), indicating that natural selection may be shaping phenotypic variation as well. The patterns observed in *S. globosa* may reflect a common mechanism of speciation in the *Schiedea* lineage, given that most species are endemic to a single island and sister species frequently occur on different islands.

***Demographic expansion***—Low sea stands ca. 0.5 Ma effectively eliminated ocean channels and produced land connections (e.g., Maui Nui) that may have allowed organisms to

TABLE 3. Indications of demographic expansion across three genes in populations and on islands where *Schiedea globosa* occurs based on Fu's $F_S$, Ramos-Onsins and Rozas $R_2$ statistic, and the population growth rate ($g$). Single-sample populations were not tested. Significant values of $F_S$ and $R_2$ are indicated by * ($P < 0.05$) and were derived from 1000 permutations of the data. The standard deviation of the growth rate is indicated in parentheses. Significant population growth was determined from $g$ if the estimated value of $g$ was >3 standard deviation units.

| | *psbM-trnD* | | | *ncpGS* | | | *pepC* | | |
|---|---|---|---|---|---|---|---|---|---|
| Island/population | $F_S$ | $R_2$ | $g$ (SD) | $F_S$ | $R_2$ | $g$ (SD) | $F_S$ | $R_2$ | $g$ (SD) |
| **Oahu** | −0.736 | 0.121 | 1927 (431) | −4.415 | 0.138 | 584 (177) | −5.48* | 0.100 | 1244 (169) |
| 844 | NA | 0.471 | 7289 (1286) | −1.974 | 0.151 | 1636 (309) | −0.439 | 0.186 | 4033 (511) |
| 906 | 0.401 | 0.231 | 191 (358) | −2.430 | 0.142 | 350 (173) | −1.995 | 0.107 | 399 (253) |
| **Molokai** | 1.524 | 0.134 | −484 (623) | −5.826* | 0.075 | 759 (194) | −5.479 | 0.075 | 125 (71) |
| Wood-Huelo | 1.523 | 0.178 | 72 (1130) | −1.858 | 0.106 | 521 (145) | −3.123 | 0.124 | 1439 (465) |
| 10228 | 0.586 | 0.300 | 7404 (2889) | −3.049 | 0.155 | 952 (380) | −3.133 | 0.101 | 88 (71) |
| **Maui** | −2.320* | 0.087* | 10000 (4105) | −11.617* | 0.075 | 773 (99) | −1.877 | 0.116 | 1317 (199) |
| 850 | NA | 0.500 | NA | 1.343 | 0.205 | 533 (225) | 0.172 | 0.433 | 10000 (3894) |
| 905 | NA | NA | NA | −3.694 | 0.139 | 687 (223) | −0.197 | 0.153 | 557 (1061) |
| 951 | −0.339 | 0.300 | 10000 (6453) | −6.463* | 0.149 | 761 (168) | −1.034 | 0.186 | 765 (367) |
| **Species** | −1.824 | 0.096 | 850 (282) | −31.043* | 0.075 | 708 (34) | −16.483* | 0.048 | 752 (44) |

freely colonize large contiguous habitats. We predicted that populations on Maui and Molokai would contain a signature of population expansion dating back to the formation of Maui Nui. While we did not find consistent evidence of expansion at all loci or across all populations on Maui Nui, these data support our hypothesis. Lack of evidence for positive growth across loci has been reported in other studies (Lawton-Rauh et al., 2007) and may reflect stochasticity associated with loci not under strong selection and differential genetic patterns of cytoplasmic and nuclear markers. Additionally, the lack of positive growth rates for individual populations on Maui Nui may be due to low sample sizes.

Support for a hypothesis of population expansion is available across the loci. Significant $F_S$ values were detected at the *ncpGS* locus on both islands and for the entire species. Additionally, significant values were detected for Maui at the *psbM-trnD* locus. It was surprising that we did not find evidence of population expansion with the $R_2$ test because this test has been demonstrated to have greater power to detect demographic expansion than $F_S$ with small sample sizes (<50) and few segregating sites (<20; Ramos-Onsins and Rozas, 2002; Pilkington et al., 2008). Maximum likelihood estimates of the growth rate also support rapid expansion on Molokai and Maui. Although the method implemented in FLUCTUATE shows an upward bias in the estimation of $g$, our assumption of population growth only when the estimated parameter exceeded three SD units, provides a conservative assessment of the potential for population expansion. Indeed, growth rates on Molokai and Maui are at least 3.6 times larger than the SD (Table 3). Lastly, comparisons of haplotype and nucleotide diversity have also been used to assess the potential for population expansion (Grant and Bowen, 1998). High haplotype diversity in conjunction with low nucleotide diversity has been associated with recent population growth (Grant and Bowen, 1998). The data presented in this study are consistent with this pattern and provide further evidence of population growth on Maui Nui.

***Conclusions***—*Schiedea globosa,* the most widely distributed species within *Schiedea*, is a small-scale model of evolutionary divergence within this group. Divergence in allopatry is a primary factor in the evolutionary history of *S. globosa,* as it is for many other Hawaiian taxa (Wagner and Funk, 1995). These data demonstrate an origin of *S. globosa* on Oahu with subsequent colonization of younger islands in progression. Once isolated, diversification throughout *Schiedea* has been accompanied by morphological changes associated with shifts in breeding system (Sakai and Weller, 1991; Wagner et al., 2005; Sakai et al., 2006). The presence of morphological differences among populations of *S. globosa* (Wagner et al., 2005) suggests that similar changes may be taking place on a small scale. The low levels of migration found with these data suggest a continued path of diversification within *S. globosa* among the islands. The collection of additional molecular data will help to refine divergence times for populations of *S. globosa* throughout the islands, and phylogenetic analyses of the *Schiedea* lineage will help to clarify the sister taxa of *S. globosa* and the nature of its origin on Oahu.

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TABLE 4. Bayesian posterior probability estimates of island migration rate per generation ($M$) based on the geometric mean mutation rate of 9.54 x $10^{-6}$ substitutions$^{-1}$·sequence$^{-1}$·year across the three loci and the migration rate inferred from combined analysis of the three loci in the program IM or Migrate-n. A generation time of 5 years was assumed. The 95% confidence intervals surrounding the point estimates are reported in parentheses. M1 and M2 represent migration rates per generation *into* the first and second island in the comparison, respectively.

| | Posterior probability (95% confidence interval) | | |
|---|---|---|---|
| Method | Oahu vs. Molokai (individuals per generation) | Oahu vs. Maui (individuals per generation) | Molokai vs. Maui (individuals per generation) |
| M1-IM | $4.55 \times 10^{-5}$ ($7.22 \times 10^{-7}$ to $9.46 \times 10^{-5}$) | $7.22 \times 10^{-7}$ ($7.20 \times 10^{-7}$ to $3.10 \times 10^{-5}$) | $9.46 \times 10^{-5}$ ($3.83 \times 10^{-5}$ to $1.84 \times 10^{-4}$) |
| M2-IM | $7.94 \times 10^{-6}$ ($7.22 \times 10^{-7}$ to $6.86 \times 10^{-5}$) | $2.09 \times 10^{-5}$ ($2.17 \times 10^{-6}$ to $6.14 \times 10^{-5}$) | $7.22 \times 10^{-7}$ ($7.20 \times 10^{-7}$ to $2.53 \times 10^{-5}$) |
| M1-Migrate | $2.96 \times 10^{-2}$ ($1.58 \times 10^{-2}$ to $4.97 \times 10^{-2}$) | $2.89 \times 10^{-2}$ ($1.01 \times 10^{-2}$ to $4.89 \times 10^{-2}$) | $6.13 \times 10^{-2}$ ($4.42 \times 10^{-2}$ to $8.23 \times 10^{-2}$) |
| M2-Migrate | $4.54 \times 10^{-3}$ ($1.13 \times 10^{-3}$ to $1.18 \times 10^{-2}$) | $5.29 \times 10^{-3}$ ($1.64 \times 10^{-3}$ to $1.23 \times 10^{-2}$) | $2.68 \times 10^{-3}$ ($4.45 \times 10^{-4}$ to $1.75 \times 10^{-2}$) |

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