



Symposium Article

Isolation of *Metrosideros* (ʻOhiʻa) Taxa on Oʻahu Increases with Elevation and Extreme Environments

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Abstract

Species radiations should be facilitated by short generation times and limited dispersal among discontinuous populations. Hawaii's hyper-diverse, landscape-dominant tree, *Metrosideros*, is unique among the islands' radiations for its massive populations that occur continuously over space and time within islands, its exceptional capacity for gene flow by both pollen and seed, and its extended life span (ca. >650 years). *Metrosideros* shows the greatest phenotypic and microsatellite DNA diversity on Oʻahu, where taxa occur in tight sympatry or parapatry in mesic and montane wet forest on 2 volcanoes. We document the nonrandom distributions of 12 taxa (including unnamed morphotypes) along elevation gradients, measure phenotypes of ~6-year-old common-garden plants of 8 taxa to verify heritability of phenotypes, and examine genotypes of 476 wild adults at 9 microsatellite loci to compare the strengths of isolation across taxa, volcanoes, and distance. All 8 taxa retained their diagnostic phenotypes in the common garden. Populations were isolated by taxon to a range of degrees (pairwise F_{ST} between taxa: 0.004–0.267), and there was no pattern of isolation by distance or by elevation; however, significant isolation between volcanoes was observed within monotypic species, suggesting limited gene flow between volcanoes. Among the infraspecific taxa of *Metrosideros polymorpha*, genetic diversity and isolation significantly decreased and increased, respectively, with elevation. Overall, 5 of the 6 most isolated taxa were associated with highest elevations or otherwise extreme environments. These findings suggest a principal role for selection in the origin and maintenance of the exceptional diversity that occurs within continuous *Metrosideros* stands on Oʻahu.

Keywords: cliffs, Hawaiʻi, microsatellites, sympatry, wind, woody species

Species radiations are most likely to involve discontinuous populations with relatively limited dispersal, where divergence can proceed unimpeded by gene flow (Dobzhansky 1937; Felsenstein 1981;

Slatkin 1987; Rice and Hostert 1993). Short generation time is another factor that should facilitate speciation, and thus species radiations, through its association with faster rates of molecular

evolution (Martin and Palumbi 1993; Petit and Hampe 2006). Some of the most spectacular species radiations known involve groups characterized by at least 2 of these features: discrete populations and limited dispersal (e.g., Hawaiian *Bidens*, Carr 1987), short life span and limited dispersal (e.g., Hawaiian *Laupala* crickets, Otte 1994), or all 3 (e.g., Hawaiian *Drosophila*, Carson and Kaneshiro 1976). Trees, in contrast, are characterized by large population sizes even in species-rich tropical forests (Pitman et al. 2001), and long-distance gene flow, which can occur over several km for both pollen (Kaufman et al. 1998; Nason et al. 1998; White et al. 2002; Bacles et al. 2005; Robledo-Arnuncio and Gil 2005) and seeds (Godoy and Jordano 2001; Gaiotto et al. 2003; Bacles et al. 2006). The high gene flow characteristic of trees is facilitated by their predominantly outcrossed breeding system (Hamrick and Godt 1996), which likely results from the high genetic load typical of long-lived woody species (Klekowski 1988). Trees are also unique for their long generation times and tremendous fecundity that extends over many reproductive seasons (Van Valen 1975).

Long generation times, extended fecundity, and/or widespread mixing of genes over large areas do not impede local adaptation within species (Petit et al. 2004); however, they likely contribute to the lower speciation rates observed for trees (Dodd et al. 1999; Larcombe et al. 2015) and other perennial plants (Archibald et al. 2005). Surveys of purportedly neutral genetic variation in trees routinely reveal high levels of genetic diversity and weak population structure relative to herbaceous plants (Hamrick and Godt 1989, 1996; Nybom 2004). Weak population structure, however, is commonly accompanied by high local differentiation at quantitative traits associated with local adaptation (McKay and Latta 2002). Differentiation within and between tree populations at functional traits associated with local adaptation is likely a result of high seed production followed by strong selection at juvenile stages (Le Corre and Kremer 2003). While adaptive divergence between populations in contrasting environments is expected to lead to the evolution of reproductive isolating barriers (i.e., ecological speciation; Rundle and Nosil 2005; Baack et al. 2015), limited data suggest that isolating barriers between closely related, ecologically diverged trees are modest (Larcombe et al. 2015; Stacy et al. 2017), and introgressive hybridization can be extensive (McKinnon et al. 2004; Nevill et al. 2014). Greater interspecific hybridization in trees is consistent with the weak neutral genetic differentiation commonly observed between closely related tree species (Howard et al. 1997; Muir and Schlötrerer 2005). As a result morphology is likely to be more reliable than genetic criteria for the delineation of closely related tree taxa.

Metrosideros (Myrtaceae) is a long-lived and highly dispersible woody genus that dominates the main Hawaiian Islands where it occurs in massive, continuous stands. In Hawai'i, the genus comprises many vegetatively distinct, largely infraspecific forms that are isolated by varying degrees and that appear to represent multiple stages of an incipient radiation (DeBoer and Stacy 2013; Stacy et al. 2014; Stacy and Sakishima 2019). Incipient radiations allow insights into the process and mechanisms of speciation through examination of patterns of molecular and phenotypic divergence, and ultimately their associations with fitness and reproductive isolating barriers (Via et al. 2000; Ramsey et al. 2003). *Metrosideros* is thought to have colonized the Hawaiian Islands between 3.1 (2.5–3.7) million (Dupuis et al. 2019) and 3.9 (1.4–6.3) million (Percy et al. 2008) years ago, followed by colonization of, and diversification within, each of the younger islands roughly following the progression rule (Percy et al. 2008; Stacy and Sakishima 2019). As a result, *Metrosideros* has a longer history on the older islands of Kaua'i (4.7–5.1 myo) and

O'ahu (3.0–3.7 myo) than on younger Maui nui (1.3–1.9 myo) and Hawai'i Island (<0.5 myo, Clague 1996). Colonization of islands is facilitated by the group's tiny, wind-borne seeds, and widespread gene flow by pollen is promoted by birds and insects attracted to the plant's large and showy inflorescences (Corn and Hiesey 1973; Carpenter 1976). Phylogeographic and population genetic analyses at the scale of the Hawaiian archipelago reveal a strong tendency of *Metrosideros* populations to cluster within islands and a significant pattern of isolation by distance across islands (Percy et al. 2008; Stacy and Sakishima 2019). Across the archipelago, up to 25 predominantly single-island-endemic morphotypes (13 formally recognized) have been identified (Sur et al. 2018; Stacy and Sakishima 2019). Early-successional, pubescent varieties of *Metrosideros polymorpha* are the first trees to colonize new lava flows on active volcanoes, and these forms are replaced by late-successional, glabrous *M. polymorpha* var. *glaberrima* over time wherever rainfall exceeds 500 mm annually (Stemmermann and Ihle 1993). In addition to dominating both nascent and mature forests in Hawai'i, *Metrosideros* has an extended life span reaching >650 years on the islands' richest soils (Hart 2010).

The *Metrosideros* community on the island of O'ahu is the most diverse of any Hawaiian Island (Stacy and Sakishima 2019), and its diversity is unlikely to be equaled by that of *Metrosideros* communities elsewhere. The exceptional diversity of *Metrosideros* on O'ahu may be attributable to the group's extended history there, coupled with Oahu's highly heterogeneous topography and climate. In particular, the Ko'olau backbone, which was created from the slumping of the volcano into the ocean likely between 2.1 and 3.0 MYA (Clague D, personal communication), is oriented perpendicular to the predominant northeast trade winds, resulting in fine-scale variation in water availability and wind across the island (Lau and Mink 2006; Argüeso and Businger 2018). Annual rainfall along the Ko'olau leeward ridges ranges from 6000 mm/year along the backbone to 1000 mm/year at their base (Giambelluca et al. 2013). Extensive field observations on O'ahu suggest the presence of 12 *Metrosideros* taxa, comprising 4 species, including 3 varieties of the ubiquitous *M. polymorpha* and 6 unnamed morphotypes that we treat here as provisional races of *M. polymorpha* (Figure 1). All taxa are consistently diagnosable in the field through leaf macromorphological characters, including the presence (4 taxa) or absence (8 taxa) of leaf pubescence. The majority of these taxa, including all of the extreme phenotypes, are endemic to the island, presumably resulting from in situ diversification (Aradhya et al. 1991; Percy et al. 2008) with the possible exception of *Metrosideros macropus* (Stacy and Sakishima 2019). High cross-fertility among taxa (Stacy E and Ekar J, unpublished data) and the presence of morphologically intermediate individuals throughout the Ko'olau Range (Stacy E, personal observation; Lau J, personal communication) suggest that hybridization among forms is not uncommon. Eight taxa are recorded from both of Oahu's volcanoes, Wai'anae (3.7 myo) and Ko'olau (2.6 myo, Clague 1996), while 4 appear to be endemic to individual volcanoes (Table 1). Within volcanoes, field observations suggest that taxa are distributed nonrandomly within continuous *Metrosideros* communities. For example, on Ko'olau Volcano, 10 taxa occur with overlapping ranges in a predictable sequence from low to high elevation across the volcano's many leeward ridges with one taxon, *Metrosideros tremuloides*, restricted predominantly to steep, windy slopes. Micromorphology (i.e., stomata size and density) of field-collected adult leaves varied among these 10 taxa in a pattern consistent with differential adaptation along the elevation gradient and allowed discrimination of 4 of the 5 unnamed taxa examined (Sur



Figure 1. Leaves of each of 12 *Metrosideros* taxa on O`ahu arranged by relative elevation: low (bottom row) to high (top row). Pubescent taxa are indicated with asterisks. Full taxon names are given in [Table 1](#). Photo credit for A, B, F, M, and Z (Joel Lau).

[et al. 2018](#)). Heritability of the phenotypic traits that distinguish Oahu's *Metrosideros* taxa has yet to be documented.

Oahu's *Metrosideros* community also captures the greatest neutral genetic diversity of any Hawaiian Island. In an archipelago-wide study of nuclear microsatellite variation ([Stacy and Sakishima 2019](#)), mean pairwise F_{ST} between taxa within islands was greatest on O`ahu, and the greatest genetic distance between any 2 taxa from anywhere in the archipelago was observed for 2 O`ahu-endemic taxa (*M. macropus* and *M. polymorpha* race S). Bayesian analysis ([Pritchard et al. 2000](#)) grouped O`ahu taxa into 2 hybridizing clusters comprising, with a single exception, glabrous and pubescent taxa, and SplitsTree network analysis ([Huson and Bryant 2006](#)) further split the glabrous taxa into 2 groups ([Stacy and Sakishima 2019](#)). Patterns of genetic structure within and among O`ahu taxa were not examined.

To gain insights into the origin of the exceptional morphological diversity of *Metrosideros* on O`ahu, we analyzed the geographical distributions of 884 adults of the island's 12 taxa, characterized phenotypes of ~6-year-old individuals of 8 of these taxa raised from seed or air-layered branches in a common garden, and carried out follow-on analyses of microsatellite data from 476 adults representing all 12 taxa ([Stacy and Sakishima 2019](#)). We used the geographical distributions to better define the elevation ranges of individual taxa and the morphological data to assess the degree to which morphologically distinct taxa retain their characteristic phenotypes when grown in a common garden. We used the microsatellite data to address the following questions: Are populations isolated more strongly by taxon

or by volcano? Is gene flow distance-dependent across the spatial scale of the island or across elevation gradients? Lastly, among the many infraspecific taxa of *M. polymorpha*, for which speciation is incomplete, do genetic variation or isolation of individual taxa vary across elevations or volcanoes?

Methods

Adult Distributions in Nature

Between December 2010 and July 2014 we recorded along 11 ridges or other trails across O`ahu ([Figure 2](#)) the waypoints of 884 adults of *Metrosideros* that possessed phenotypes characteristic of the 12 taxa we recognize on the island. A key and descriptions of these 12 taxa are provided in the [Supplementary Appendix](#). The adults mapped using GPS were individuals of "pure-taxon" phenotypes selected for various studies requiring the collection of DNA, RNA, air-layered branches, leaf samples, or open-pollinated seeds, or they were selected to serve as pollen donors or maternal trees for a study of reproductive isolating barriers. The first and second set of herbarium voucher specimens representing the 12 taxa are housed (respectively) at the National Tropical Botanical Garden, Kaua`i, and the T. M. Sperry Herbarium at Pittsburg State University ([Supplementary Table S1](#)). Where multiple adults of interest occurred within <2 m, especially on steep terrain, a single, shared waypoint was recorded for all. Morphological intermediates (apparent hybrids) were avoided. While sampling of waypoints was not done with the aim of rigorous analysis of the distribution of phenotypes across the island,

Table 1. Genetic diversity statistics^a (\pm SE) for each of 12 *Metrosideros* taxa or morphotypes (races) sampled on O'ahu

Taxon	Taxon code	Pubescent?	Volcano	N	A	AR	PAR	H_o	H_e	Mean pairwise F_{ST}
<i>Metrosideros macropus</i>	M	No	K, W	22, 4	7.89 \pm 1.81	3.854 \pm 0.769	0.038 \pm 0.031	0.414 \pm 0.072	0.539 \pm 0.105	0.156 \pm 0.012
<i>Metrosideros polymorpha</i> var. <i>glaberrima</i>	G	No	K, W	35, 13	15.22 \pm 3.26	5.652 \pm 0.809	0.186 \pm 0.064	0.615 \pm 0.080	0.760 \pm 0.079	0.048 \pm 0.011
<i>M. p.</i> var. <i>incana</i>	I	Yes	K, W	27, 2	12.67 \pm 3.00	5.466 \pm 0.874	0.122 \pm 0.064	0.594 \pm 0.070	0.735 \pm 0.086	0.048 \pm 0.012
<i>M. p.</i> race <i>prostrata</i>	A	No	K	39, —	8.44 \pm 1.80	4.496 \pm 0.628	0.066 \pm 0.046	0.574 \pm 0.071	0.706 \pm 0.066	0.098 \pm 0.015
<i>M. p.</i> race B	B	No ^b	K	28, —	9.67 \pm 2.52	4.635 \pm 0.907	0.044 \pm 0.026	0.482 \pm 0.109	0.611 \pm 0.113	0.085 \pm 0.012
<i>M. p.</i> race C	C	Yes	K, W	35, 30	16.00 \pm 3.99	5.468 \pm 0.798	0.103 \pm 0.043	0.639 \pm 0.075	0.763 \pm 0.069	0.053 \pm 0.012
<i>M. p.</i> race F	F	Yes	K, W	21, 39	15.44 \pm 4.04	5.521 \pm 0.808	0.060 \pm 0.020	0.649 \pm 0.048	0.773 \pm 0.064	0.061 \pm 0.014
<i>M. p.</i> race L	L	No	K, W	34, 23	14.56 \pm 3.41	5.372 \pm 0.872	0.136 \pm 0.047	0.571 \pm 0.084	0.713 \pm 0.097	0.052 \pm 0.011
<i>M. p.</i> race S	S	No	W	—, 11	4.89 \pm 1.32	3.441 \pm 0.718	0.050 \pm 0.033	0.384 \pm 0.083	0.512 \pm 0.110	0.137 \pm 0.018
<i>M. p.</i> race Z	Z	No	K, W	20, 0 ^c	9.00 \pm 2.23	4.873 \pm 0.899	0.095 \pm 0.046	0.537 \pm 0.109	0.683 \pm 0.106	0.065 \pm 0.011
<i>Metrosideros rugosa</i>	R	Yes	K	50, —	12.22 \pm 2.63	5.185 \pm 0.732	0.057 \pm 0.026	0.673 \pm 0.065	0.755 \pm 0.067	0.087 \pm 0.015
<i>Metrosideros tremuloides</i>	T	No	K, W	23, 20	11.67 \pm 3.42	4.470 \pm 0.939	0.076 \pm 0.043	0.493 \pm 0.106	0.578 \pm 0.127	0.095 \pm 0.013

Shown also for each taxon or morphotype is the presence/absence of pubescence on the abaxial (bottom) leaf surface, the volcano(es) where it occurs (K = Ko'olau, W = Wai'anae), and the number of adults genotyped (N) from K and W Volcanoes, respectively (total = 476). *M. p.* refers to the dominant species, *Metrosideros polymorpha*, and races C, L, Z, F, B, and S are infraspecific morphotypes not yet recognized in any taxonomic treatment and treated here as provisional races of *M. polymorpha*. Named taxa are described in Dawson and Stemmermann (1990), except here we treat *M. polymorpha* var. *pumila* as *M. polymorpha* race *prostrata*. Vouchers for all taxa are housed at the National Tropical Botanical Garden, Kaua'i and at the T. M. Sperry Herbarium at Pittsburg State University (Supplementary Table S1).

^aAll genetic diversity statistics are calculated with populations pooled within taxon (i.e., volcanoes pooled), and values are averaged across 9 loci. A is the average number of alleles observed. Average allelic richness (AR) and private allelic richness (PAR) per 10 individuals were calculated using ADZE, and observed (H_o) and expected heterozygosity (H_e) were calculated using Genepop. Mean \pm SE pairwise F_{ST} values are calculated for all combinations of the target taxon with the other 11 taxa and represent overall isolation of the target taxon.

^bEmerging leaves of *M. p.* race B are pubescent, but adult leaves are glabrous.

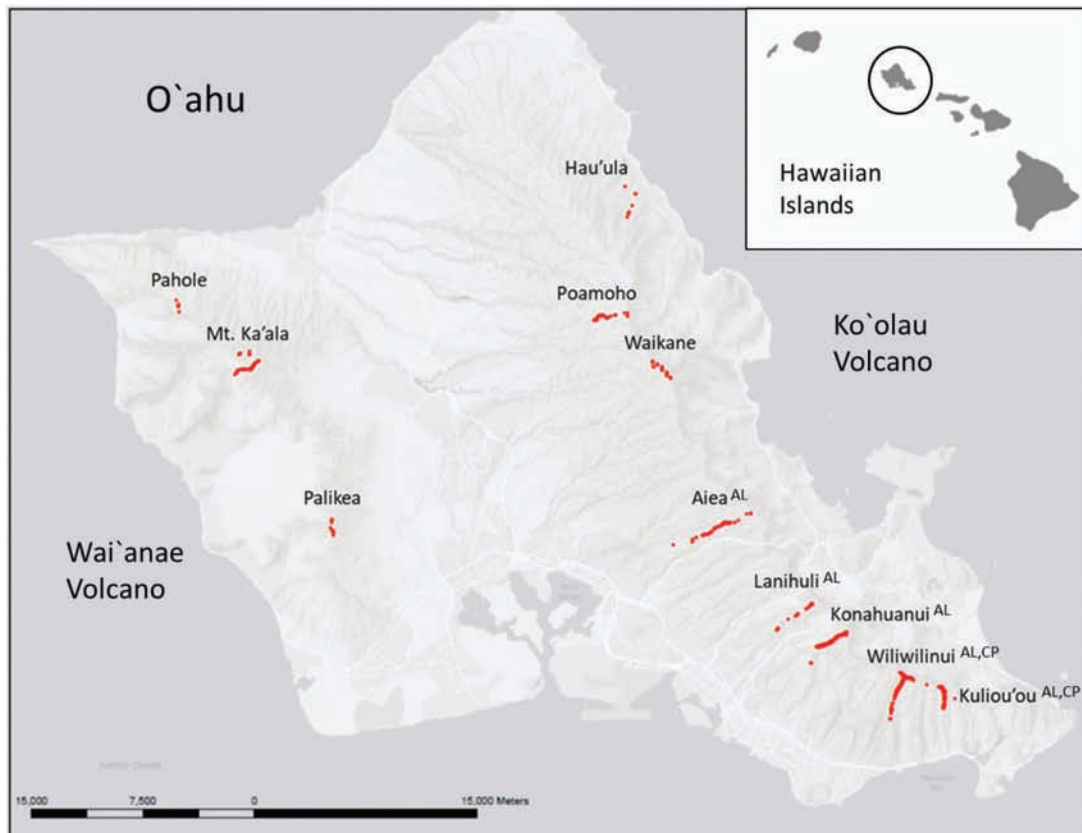
^c*Metrosideros p.* race Z was recorded from Wai'anae Volcano but not sampled.

this large data set is expected to provide a reasonable representation of the elevation range of each taxon sampled on each volcano.

Adult Morphology in a Common Garden

Plants were grown from seeds (7 taxa) and air-layered branches (2 taxa) in a common coldframe environment to minimize the effects of environmentally induced plasticity and permit observation of heritable variation. Seeds were collected from controlled crosses performed along 2 leeward ridges of Ko'olau Volcano from July through November 2011 in a study of reproductive isolating barriers in O'ahu *Metrosideros* (reported elsewhere). Pollinations included independent within-taxon crosses on 32 maternal trees of 5 taxa (3–9 independent families/taxon); taxa included: *Metrosideros rugosa*, *M. polymorpha* var. *incana*, race C, race L, and *M. tremuloides*. Race C and race L are provisional names for morphotypes of *M. polymorpha* (Figure 1). Adults were added to the study as their flowers became available; those with high accessibility and flowering intensity were used as maternal trees, while others were used as pollen donors. Briefly, maternal inflorescences were emasculated just before anthesis of the first flowers and covered with mesh pollinator-exclusion bags. Three days later, whole flowers were transported from individual pollen donors (secured in florist foam inside disposable food storage containers affixed to backpacks) to individual maternal trees, and pollen was transferred directly by touching anthers to stigmas until the latter were covered with pollen. The pollinator-exclusion bags were then replaced and left on the maternal flowers for 14 days. Mature fruits (i.e., fruits with visible cracks) were collected in January through early May 2012. Seed collections were supplemented with open-pollinated

seeds from maternal trees of *M. rugosa* (due to poor seed production from within-species outcrosses with this species), *M. polymorpha* var. *glaberrima* and race B. Seed germination and seedling care followed established protocols (e.g., Morrison and Stacy 2014; Stacy et al. 2016). Briefly, within 2 weeks of collection seeds were sown onto a thin layer of black sand atop well-draining media comprising 3 parts Sunshine Mix #1 (Sunagro Horticulture, Agawam, MA), 2 parts perlite, and 1 part cinder, kept in a misthouse (20 s of mist every 30 min during the day) under low light ($\sim 600 \mu\text{mol}/\text{m}^2/\text{s}$) for 6 weeks to promote germination (February through May 2012), and then transferred to a coldframe on the campus of the University of Hawai'i Hilo under ambient conditions. Seedlings were transferred to individual pots at ~ 6 months in the same media, and subsequently potted up, watered, fertilized, and treated for pests, as needed. Air-layered branches (15–30 cm, depending on taxon) were collected from adults of *M. macropus* and *M. rugosa* in December 2013 (air-layers were prepared May 2013). Rooted branches were transported to the U Hawai'i Hilo greenhouse, placed in individual pots with the above media, and placed in the misthouse (above conditions) for 2–3 weeks before being transferred to the coldframe and maintained as above. Trays with plants were rotated monthly within the cold frame for 2 years, beyond which plants were moved only when reorganization was needed. In spring 2018, the following measurements were taken on each surviving adult derived from either seed or air-layer: leaf length (cm), leaf width (cm), leaf shape (leaf length/leaf width), and petiole length (cm) averaged over 2 leaves; and leaf curvature score (0–3), rugosity score (the degree to which veins protrude from the leaf; 0–2), abaxial pubescence score (0–3), adaxial pubescence score (0–1), and pigmentation score (1–3). Supplementary Figure S1



Site	Volcano	Taxon												Total count	
		A	B	C	F	G	I	L	M	R	S	T	Z		
Aiea	K	20		20		15	11	20	1	2					89
Hau'ula	K					3	5								8
Konahuanui	K		15		20			5	11	11		21	11		94
Kuliou'ou	K		5		1	1	5								12
Lanihuli	K			1		2			2	1		2			8
Mt. Ka'ala	W			1	20	13		20	4		11	20			89
Pahole	W			9			1	3							13
Palikea	W			20	19		1								40
Poamoho	K					5		4	5	2					16
Waikane	K	19									15				34
Wiliwilinui	K		8	14		9	6	5	3	19			9		73
Total count	--	39	28	65	60	48	29	57	26	50	11	43	20		476

Figure 2. Map of O'ahu showing waypoints recorded for adults of *Metrosideros* at 11 sites. Three sites are on Wai'anae Volcano (west side), and 8 are on Ko'olau Volcano (east side). Sites used for controlled pollinations and collection of air-layered branches are indicated with the superscripts, CP and AL, respectively. The attached table shows the distribution across sites of adults sampled for DNA for each taxon. Full taxon names are given in Table 1. The 11 sampled locations span from Pahole, NW O'ahu (21.54191°N, 158.193527°W) to Kuliou'ou, SE O'ahu (21.316488°N, 157.72329°W).

shows a representative example of each level of each qualitative leaf trait. Leaf area (cm^2) using a Licor LI-3100 leaf area meter and leaf dry mass (after drying at 80° for 48 h) were also calculated from 10 mature leaves from each plant (5 for very small plants), and specific leaf area (SLA; cm^2/g) was calculated as leaf area/leaf dry mass.

Data Analysis

The following analyses were done using Minitab 18 (State College, PA). Interval-scale measures were tested for equal variances. Leaf width and SLA were compared across taxa using ANOVA with family as a random variable nested within taxon (to accommodate

the shared ancestry of seedlings from controlled crosses or open-pollinated seeds). Because family was not important in any analysis ($P > 0.05$), follow-on one-way ANOVAs were done, including Tukey's pairwise comparisons using a family error rate of 0.05. Kruskal–Wallis tests were used for among-taxon comparisons of the 4 ordinal-scale measures as well as leaf length, leaf shape, and petiole length, due to unequal variances, followed by pairwise comparisons using the macro %KRUSMC. Using all 11 leaf traits, linear discriminant analysis was done to delineate taxa, and a second discriminant analysis was done using all traits except pubescence (abaxial and adaxial) to delineate taxa into pubescent and glabrous groups.

Pairwise correlations involving all measures were calculated using the Spearman (rho) statistic, and to visualize variation among taxa, 8 measures with pairwise correlations <0.8 were selected for additional multivariate analysis.

Given that multivariate analyses are incompatible with missing data and that for 4 small individuals we elected to not sample leaves for calculation of SLA, a multivariate imputation by chained equations approach (van Buuren and Groothuis-Oudshoorn 2011) was applied to the character matrices using the package Mice (version 3.60) in R. With this method, each missing value is replaced by several (m) values, thus producing m imputed data sets. The differences between these data sets reflect the uncertainty of the missing values. Each imputed data set is analyzed by standard complete-data procedures, which ignore the distinction between real and imputed values. The m resulting analyses are then combined into one final analysis. This approach assumes that, for each incomplete variable, the user specifies a conditional distribution for the missing data given the other data. The advantage of multiple imputations is that it leads to valid statistical inferences in the presence of missing values. The relationship between individuals of each species and the continuous and categorical characters was examined by fitting the characters onto the ordination space using the function “envfit” in the R package vegan (Oksanen et al. 2013), which is a multivariate correlation analysis that partitions the linear component of each predictor on the final PCA axes.

Genetic Isolation across Taxa and Volcanoes

From the archipelago-wide study of microsatellite variation in *Metrosideros* (Stacy and Sakishima 2019), we extracted the 9-locus genotypes of the 476 adults from O`ahu for within-island analyses; microsatellite loci are described in Crawford et al. (2008). Because of the linear distribution of *Metrosideros* along ridges on O`ahu, sampling within a taxon involved a small number of individuals (1–21) from each of multiple ridges; the exception was *M. polymorpha* race S, which is confined to the Mt. Ka`ala summit (Supplementary Figure S2). To obtain reasonable population sizes for population genetic analysis, we pooled individuals within taxa within volcanoes for a total of 19 populations (11 on Ko`olau and 8 on Wai`anae; Table 1); 2 of the latter were excluded from population-level analyses, however, due to low sample size ($n < 10$). We checked for null alleles using Micro-checker (Van Oosterhout et al. 2004). Due to the presence of null alleles at 1 locus (MePo506), inbreeding coefficients (F_{IS}) were calculated using INEst (using the individual inbreeding model; Chybicki and Burczyk 2009); F_{IS} values are reported for individual populations and averaged across loci. We compared population-level inbreeding coefficients between monotypic species (4 populations) and infraspecific taxa of *M. polymorpha* (13 populations) using a Mann–Whitney test. Standard genetic diversity statistics for each taxon (individuals pooled within taxa) and for each taxon on each volcano separately were computed using GENEPOP 4.7.0 (Rousset 2008). Additionally, allelic richness (AR) and private allelic richness (PAR) were estimated using ADZE (Szpiech et al. 2008) to allow comparison among unevenly sampled taxa. Finally, measures of allelic richness and heterozygosity were tested for equal variances and compared among the 12 taxa using ANOVA (allelic richness) and Kruskal–Wallis tests (heterozygosity).

Pairwise F_{ST} values for all pairs of taxa were calculated using GENEPOP 4.7.0 (Rousset 2008). We then used Spearman’s Rho to examine correlations among mean pairwise F_{ST} per taxon and taxon-level measures of genetic diversity, such that mean pairwise F_{ST} is the mean of all between-taxon F_{ST} values involving that taxon and

thus represents the mean strength of isolation from other taxa. To examine isolation by volcano, we ran AMOVA using Arlequin 3.5 (Excoffier and Lischer 2010) with individuals pooled within taxa within volcanoes. To visualize relationships among taxa within and among volcanoes, a NeighborNet network was constructed in SplitsTree (Huson and Bryant 2006) using pairwise F_{ST} values. Lastly, to compare the strength of isolation among taxa on older Wai`anae Volcano to that on younger Ko`olau Volcano we calculated pairwise F_{ST} values among taxa sampled on each volcano separately, first for all taxa sampled and second for just the subset of taxa that were sampled from both volcanoes.

We followed up on the results of the archipelago-wide STRUCTURE analysis (Stacy and Sakishima 2019) that split O`ahu taxa into 2 groups: all glabrous taxa minus *M. polymorpha* race *prostrata*, and all pubescent taxa plus *M. polymorpha* race *prostrata*. We ran STRUCTURE version 2.3.4 for each of these groups using the default settings and an admixture model. We ran a series of $K = 1–12$ and 9 for the glabrous group and pubescent group, respectively, by setting a burn-in of 10 000 and 100 000 repetitions with 20 iterations per K . The most likely number of clusters within each group was estimated multiple ways following Stacy and Sakishima (2019). Briefly, we used the ΔK (Evanno et al. 2005) and posterior probability methods (ln (XIK) (Pritchard et al. 2000), neither of which require a priori knowledge of population structure and thus are not affected by our classification of individuals to taxa. To correct for uneven sampling across populations, we also calculated 2 of Puechmille’s (2016) estimators, MedMedK and MaxMedK, which do require a priori designation of individuals to taxa. We chose estimators based on the median membership coefficient (Q), which is less impacted than mean membership coefficients by the misplacement of individuals into clusters, and Q values of 0.5, 0.6, and 0.7. Lastly, we used STRUCTURE Harvester (Earl 2012) to make graphs of the 2 best K values for each group.

Genetic Isolation across Geographic Distance and Elevation

We used SPAGeDi (Hardy and Vekemans 2002) to test for isolation by distance at several scales: for all individuals across the whole island, for each of 8 widespread taxa across the island, and for the same 8 taxa within Ko`olau Volcano. We also tested for isolation by distance within each of 3 well sampled leeward ridges of Ko`olau Volcano for all taxa on those ridges and again for just the pubescent taxa given the observation that, relative to the glabrous taxa, the pubescent taxa partition the gradient more strongly with less overlap in ranges (see Results). For all isolation by distance tests, we used Queller and Goodnight’s (1989) pairwise relationship coefficient for individuals with standard errors estimated by jackknifing over loci, and 10 000 random permutations of individuals to test for significant relationships with distance. Lastly, to examine patterns of diversity and isolation among the infraspecific varieties of *M. polymorpha*, we removed the 3 monotypic species and calculated Spearman’s Rho between mean elevation, measures of genetic diversity, and mean pairwise F_{ST} (i.e., overall isolation) for each taxon. This was done separately for each volcano.

Results

Adult Distributions in Nature

Waypoints were recorded for an average of 76.4 adults per taxon on Ko`olau [range: 36 (*M. macropus*) to 116 (*M. rugosa*)] and 16.6 adults per taxon on Wai`anae [range: 5 (*M. macropus*) to 28

(*M. polymorpha* race L)]. The taxa represented by waypoints were nonrandomly distributed along individual leeward ridges of both Ko'olau and Wai'anae Volcanoes (Figures 3 and 4; Supplementary Figure S2). On Ko'olau Volcano, typically *M. polymorpha* var. *incana* (and occasionally var. *glaberrima*) were recorded at the lowest elevations, and *M. rugosa* and *M. polymorpha* race B were recorded along the backbone. The distributions of *M. polymorpha* race L and race Z also extended to the backbone at some sites. *Metrosideros* extended to lower elevations on Ko'olau relative to Wai'anae, and for taxa that occur on both volcanoes, elevation ranges were considerably higher on Wai'anae (Figure 4).

Adult Morphology in a Common Garden

The 101 6-year-old plants from 8 taxa raised in the common garden from seeds or air-layered branches developed adult phenotypes characteristic of their taxa in the field. Plants grown from seed developed taxon-diagnostic phenotypes within approximately 1 year [*M. rugosa* (minus pubescence) and *M. tremuloides*] or 3 years (*M. polymorpha* var. *glaberrima*, var. *incana*, race B, race C, race L), with adult pubescence requiring 2–4 years to appear for individuals of pubescent taxa (i.e., *M. polymorpha* var. *incana*, race C, *M. rugosa*). Similarly, all new growth on greenhouse plants derived from air-layered branches was characteristic of the source taxa (i.e., *M. macropus*, *M. rugosa*). For *M. rugosa*, individuals derived from air-layers were indistinguishable from seed-derived plants. Principal components analysis of 8 traits yielded 2 significant axes: PC1 (52.2%), which generally separated taxa with long, smooth, glabrous leaves from

those with pubescent, bullate, curved leaves, and PC2 (14.0%), which was predominantly loaded with leaf width and petiole length (Figure 5). All individual leaf traits varied significantly among taxa (Supplementary Figure S3). Among the 4 pubescent taxa, leaf shape and petiole length were highly uniform (Supplementary Figure S1), while leaf width differed between *M. rugosa* and *M. polymorpha* var. *incana*, leaf curvature score and SLA separated *M. polymorpha* var. *incana* from *M. polymorpha* race C and *M. rugosa*, and rugosity score was unique for *M. rugosa* (Supplementary Figure S3). Among the 8 glabrous taxa, separation was incomplete; leaf width separated *M. macropus* from *M. tremuloides*, pigmentation score separated *M. tremuloides* from *M. polymorpha* var. *glaberrima* and race B, and petiole length separated *M. polymorpha* race B from *M. polymorpha* race L, *M. macropus* and *M. tremuloides*. The discriminant analysis of all 11 measures classified 87 of 97 individuals (89.7%) correctly to taxon (Supplementary Tables S2 and S3). The follow-on discriminant analysis using all characters minus pubescence (Supplementary Table S4) placed 92 of 97 individuals (94.8%) into the correct group (pubescent vs. glabrous); all 5 misplaced individuals were of *M. polymorpha* race B (placed in the pubescent group).

Genetic Isolation across Taxa and Volcanoes

Populations were isolated to a greater or lesser degree by taxon and, with 2 exceptions, not by volcano. The AMOVA with all 476 individuals pooled within taxa within volcanoes revealed 8.15% of total genetic variation partitioned among taxa within volcanoes, no variation (–0.9%) between volcanoes, and 92.75% of variation within

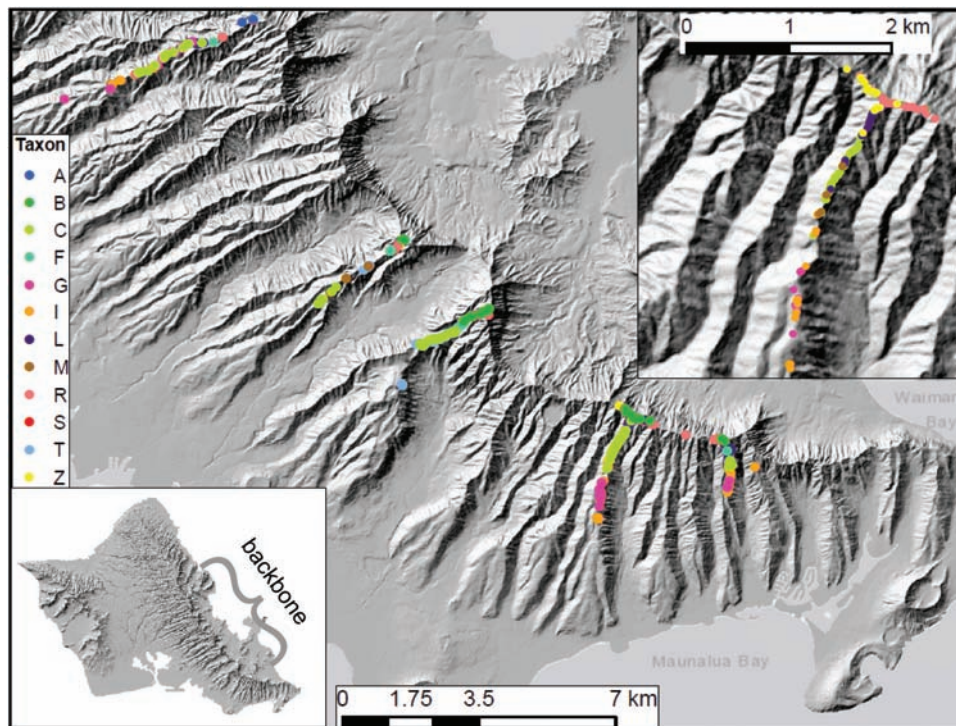


Figure 3. Map of east O'ahu showing GPS locations of all trees sampled at each of 5 of the 11 sampled locations; all 5 locations are leeward ridges of Ko'olau Volcano. Inset (top right): One ridge (Wiliwilinui Trail) is enlarged to show individual locations in better detail, including those along a portion of the Ko'olau backbone perpendicular to the leeward ridge. Inset (bottom left): Island of O'ahu with the Ko'olau backbone oriented perpendicular to the predominant northeast trade winds. Eleven of the island's 12 taxa are shown; 10 occur regularly along the leeward ridges, while *Metrosideros polymorpha* race *prostrata* (A) is recorded from only 7 sites on Ko'olau Volcano (Lau J, personal communication). The 12th taxon, *M. polymorpha* race S, is restricted to the summit of Wai'anae Volcano (Mt. Ka'ala), west O'ahu. Full taxon names are given in Table 1.

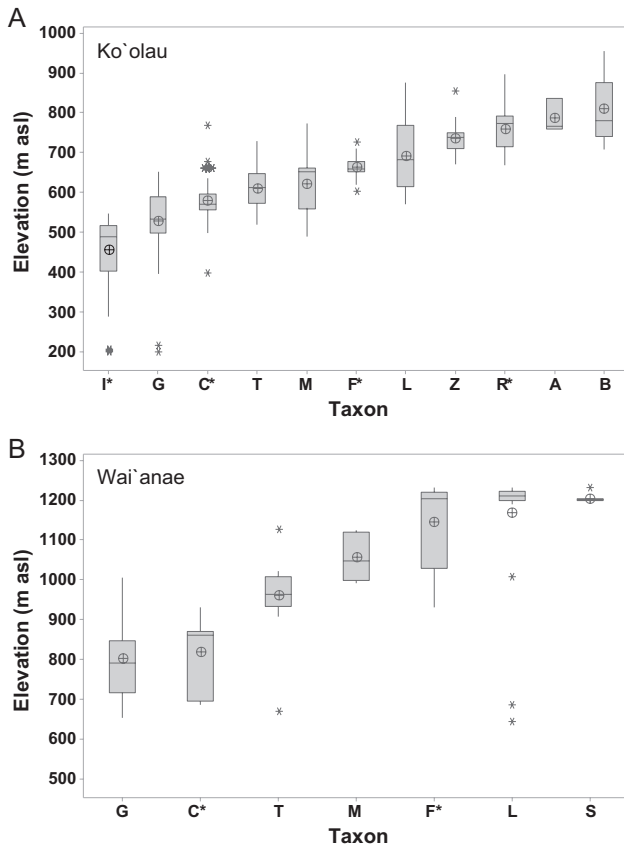


Figure 4. Median and interquartile range values of elevations of 884 adults representing 12 *Metrosideros* taxa on O'ahu, including (A) 767 adults from 11 taxa on Ko'olau Volcano and (B) 117 adults from 7 taxa on Wai'anae Volcano. Pubescent taxa are indicated with asterisks. Outliers and mean values (hatched circles) are also shown. Full taxon names are given in Table 1. Taxa I and Z also occur on Wai'anae Volcano but were not sampled sufficiently to be included here.

taxa within volcanoes (Table 2). Similarly, the population-level SplitsTree network showed close relationships between populations of the same taxa on the 2 volcanoes (Figure 6). In contrast to this general pattern, significant isolation by volcano was observed for the 2 monotypic species that span both volcanoes, *M. tremuloides* (F_{ST} between volcanoes = 0.084) and *M. macropus* (F_{ST} = 0.080). While the sampling of *M. macropus* on Wai'anae was limited ($n = 4$), these 4 individuals contained 3 alleles not present in the Ko'olau population, consistent with isolation.

Given that taxon was the strongest predictor of genetic structure, we contrasted measures of genetic variation among the 12 taxa (Table 1). Variation in AR (per 10 individuals) among taxa was nearly significant ($P = 0.056$), ranging from 3.44 ± 0.72 (SE) for *M. polymorpha* race S to 5.65 ± 0.81 for *M. polymorpha* var. *glaberrima*. PAR also varied nearly significantly among taxa from 0.038 ± 0.031 in *M. macropus* to 0.186 ± 0.064 in *M. polymorpha* var. *glaberrima*. Measures of heterozygosity did not vary across taxa.

The strength of isolation varied markedly across taxa. Pairwise F_{ST} values for pairs of taxa ranged broadly from 0.004 (for *M. polymorpha* var. *glaberrima* and var. *incana*) to 0.267 (for *M. macropus* and *M. polymorpha* race S; Supplementary Table S5), and mean pairwise F_{ST} per taxon, representing overall isolation, ranged from 0.048 ± 0.012 for each of *M. polymorpha* var. *glaberrima* and var. *incana* to 0.137 ± 0.018 and 0.156 ± 0.012 for *M. polymorpha* race

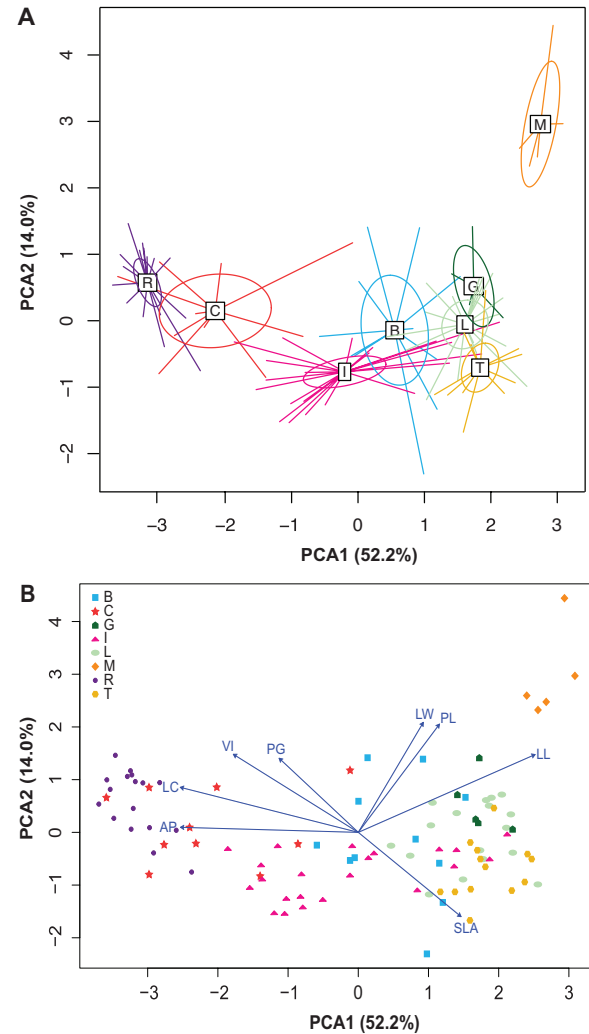


Figure 5. Principal components analysis of 97 common-garden plants of 8 *Metrosideros* taxa from O'ahu: (A) PC1 \times PC2 scores with all points for each variety connected as a spiderplot, with ellipses indicating the 95% CI using standard errors of the points; (B) loading plot, fitting the variables onto the ordination to investigate the relationship between the varieties and each floral trait. The direction of an arrow indicates the direction of the most rapid change in a given trait, and length of the arrows indicates the strength of the correlation between the trait and the ordination. Full taxon names are given in Table 1.

S and *M. macropus*, respectively (Table 1). As expected, the 3 monotypic species (*M. macropus*, *M. rugosa*, and *M. tremuloides*) were on average more isolated than infraspecific taxa of *M. polymorpha* (mean pairwise F_{ST} among species: 0.137–0.183; among varieties: 0.055). Three *M. polymorpha* taxa (race *prostrata*, race B and race S), however, showed sizeable isolation from all other taxa (Table 1; Figure 6). These 3 intraspecific taxa showed strongest affinities with the monotypic species *M. rugosa*, *M. macropus*, and *M. tremuloides*, respectively (Figure 6). AR, PAR, and H_e per taxon were negatively correlated with overall isolation (i.e., mean pairwise F_{ST} per taxon; Figure 7). The inbreeding coefficient was greater for populations of intraspecific taxa of *M. polymorpha* than for populations of the 3 monotypic species (median F_{IS} for varieties: 0.168; for species: 0.087; $W = 17$, $P = 0.036$), and removing the taxon (*M. polymorpha* race S) with an unusually high F_{IS} (0.472 ± 0.087) did not change this result.

Table 2. Results of AMOVA among taxa and volcanoes on O'ahu

Comparison	df	Sum of squares	Variance	% variation
Between volcanoes	1	5.431	-0.01832	-0.89
Among taxa within volcanoes	10	112.708	0.16698	8.15
Within taxa	636	1209.055	1.90103	92.75
Total	649	1327.194	2.04969	

Analysis was restricted to 6 *Metrosideros* taxa that were sampled from both volcanoes (C, F, G, L, M, and T; see Table 1 for taxon names).

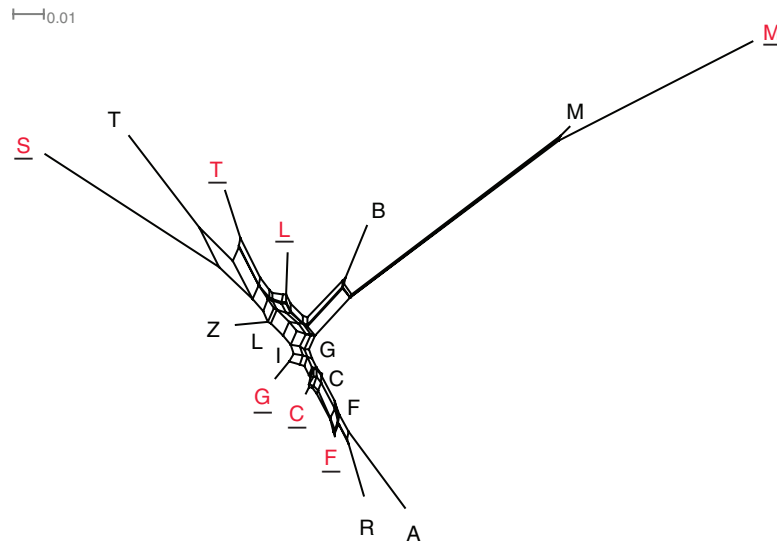


Figure 6. SplitsTree network for 18 populations of 12 *Metrosideros* taxa on O'ahu; individuals are pooled within volcanoes within taxa. Wai'anae populations are underlined. Full taxon names are given in Table 1. Population sizes range from 11 to 50 (mean = 28), except for Wai'anae M ($n = 4$).

The STRUCTURE analyses also revealed a range of strengths of isolation within and among taxa (Figure 8). For the STRUCTURE analysis of all 8 glabrous taxa minus *M. polymorpha* race *prostrata*, the delta K method yielded best $K = 3$, but the posterior probability method and all of Puechmaille's (2016) estimators yielded 6 clusters (Supplementary Figure S4). The 6 weakly to moderately introgressed groups comprised *M. macropus*, *M. tremuloides* from Ko'olau, *M. tremuloides* from Wai'anae, *M. polymorpha* race L and race S from Wai'anae combined, plus 2 highly introgressed groups anchored individually by *M. polymorpha* var. *glaberrima* and *M. polymorpha* race B and mixed with *M. polymorpha* race L and race Z from Ko'olau (Figure 8B). STRUCTURE F -statistics for the 6 groups suggested that the degree to which allele frequencies had diverged from ancestral frequencies due to drift was strong for *M. macropus* ($F_{ST} = 0.237$) and *M. tremuloides* from Ko'olau ($F_{ST} = 0.134$), modest for *M. polymorpha* race S and *M. tremuloides* from Wai'anae ($F_{ST} = 0.061$ – 0.065), and modest to weak for the 2 highly introgressed groups ($F_{ST} = 0.030$ – 0.052).

For the STRUCTURE analysis run separately for the 4 pubescent taxa plus *M. polymorpha* race *prostrata*, both the delta K and posterior probability methods yielded best $K = 5$ (Supplementary Figure S4), and all of Puechmaille's (2016) estimators yielded 3 clusters; the 3 clusters comprised *M. polymorpha* race *prostrata*, *M. rugosa*, and a highly introgressed group comprising *M. polymorpha* var. *incana*, race C and race F (Figure 8C). STRUCTURE F -statistics for the 3 groups suggested that the degree to which allele frequencies had diverged from ancestral frequencies due to drift was substantial in *M. polymorpha* race *prostrata* ($F_{ST} = 0.096$), weak in *M. rugosa* ($F_{ST} = 0.033$), and absent in the introgressed group ($F_{ST} = 0.009$).

The STRUCTURE graph for 5 clusters integrated *M. rugosa* more strongly into the highly introgressed group and split *M. polymorpha* race *prostrata* into 2 groups (Figure 8D).

The strength of isolation among co-occurring taxa did not differ between volcanoes. This held true whether the comparison of pairwise F_{ST} values was done for all taxa occurring on both volcanoes or for just the subset of taxa that are common to both volcanoes (Table 3). The greatest pairwise F_{ST} value observed for any pair of populations within the island occurred between taxa on Wai'anae (i.e., *M. macropus* and *M. polymorpha* race S; 0.342 ± 0.071), but this value may be inflated due to the small sample size for *M. macropus* from Wai'anae.

Genetic Isolation across Geographic Distance and Elevation

There was no relationship between pairwise relatedness between individuals and geographic distance overall ($N = 335$ individuals; distance = 54 km), nor within any of the 8 individual taxa examined across the island (distances: 17.5–51.1 km, depending on taxon; Supplementary Figure S5) or across Ko'olau Volcano; not shown). Similarly, there was no pattern of isolation by distance among individuals along each of 3 leeward ridges (spanning distances of 1.7–2.6 km). Because the 4 pubescent taxa as a group partition the elevation gradient more strongly than the glabrous taxa (i.e., there is less overlap in the distributions of pubescent taxa; Figure 4), we repeated the analyses of isolation by elevation for pubescent taxa only and again found no significant relationship (Supplementary Figure S6).

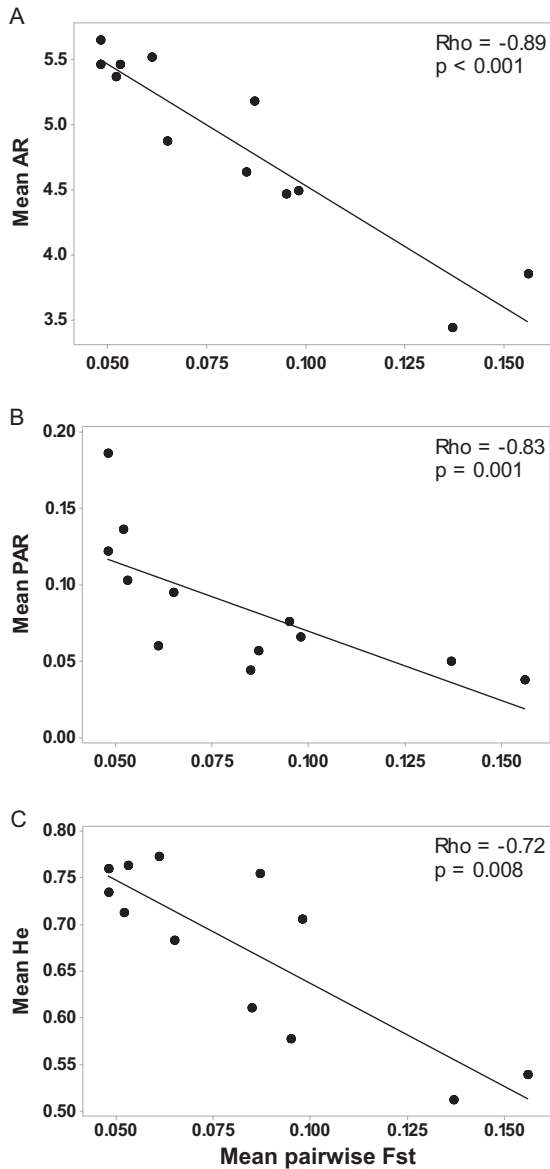


Figure 7. Relationship between strength of isolation (mean pairwise F_{ST}) and (A) mean AR, (B) mean PAR, and (C) mean H_e for 12 *Metrosideros* taxa on O'ahu. Nonparametric Spearman rho values and corresponding P values are also shown.

For the subset of infraspecific taxa of *M. polymorpha* (i.e., with the 3 monotypic species removed), we found significant relationships between mean elevation and measures of both isolation and genetic diversity of taxa on Ko'olau Volcano, where 8 taxa occur. Both AR and PAR per taxon decreased with mean elevation (Figure 9A,B), while mean pairwise F_{ST} per taxon increased (Figure 9C). On Wai'anae Volcano, where only 5 taxa of *M. polymorpha* were genotyped, only mean pairwise F_{ST} was (positively) correlated with elevation (Figure 9E–G). The average taxon-level inbreeding coefficient (F_{IS}) did not vary with elevation on either volcano (Figure 9D,H).

Discussion

Heritable Phenotypic Variation among Taxa

The 6-year-old plants grown from seeds and air-layered branches in the greenhouse developed their taxon-diagnostic adult phenotypes

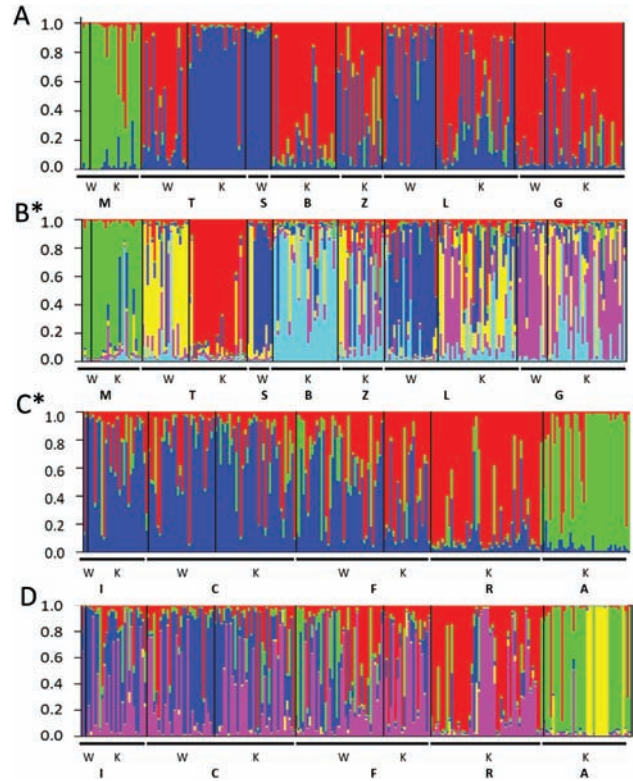


Figure 8. STRUCTURE graphs showing the 2 best K values for all glabrous *Metrosideros* taxa on O'ahu except *Metrosideros polymorpha* race *prostrata* [(A) $K = 3$ and (B) $K = 6$] and all pubescent *Metrosideros* taxa plus *M. polymorpha* race *prostrata* [(C) $K = 3$ and (D) $K = 5$]. Asterisks indicate the K supported by Puechmaille's (2016) MedMedK and MaxMedK estimators. Each population comprises all individuals pooled within each taxon within a volcano (W = Wai'anae, K = Ko'olau). Full taxon names are given in Table 1.

within 2–4 years with seedlings of *M. rugosa* and *M. tremuloides* acquiring taxon-diagnostic juvenile phenotypes within 1 year. As expected, the 3 monotypic species were more readily distinguishable using leaf traits than were the infraspecific taxa of *M. polymorpha*; 100% of individuals of *M. macropus* and *M. rugosa* were classified correctly in the discriminant analysis, as were 11 of 13 individuals of *M. tremuloides* (2 trees grouped with *M. polymorpha* race L). In contrast, discriminant analysis correctly classified just 87.7% of individuals of *M. polymorpha*. In addition to the presence or absence of leaf pubescence, there appear to be multiple leaf traits that in combination differ between pubescent and glabrous taxa. All 5 of the misclassified individuals in the discriminant analysis with just these 2 categories belonged to *M. polymorpha* race B. The misclassification of individuals of this taxon and *M. tremuloides* (above) underscore the limits of delineating O'ahu *Metrosideros* taxa through adult leaf traits alone, as other traits appear to be taxon-diagnostic (e.g., stem diameter and leaf density for *M. tremuloides*; juvenile leaf pubescence for *M. polymorpha* race B).

Barring strong maternal effects in seed-derived taxa (Roach and Wulff 1987), these results suggest that the leaf traits that distinguish *Metrosideros* taxa on O'ahu are heritable. An earlier study of *M. polymorpha* var. *glaberrima* on Hawai'i Island detected modest to strong narrow-sense heritabilities (0.307 ± 0.135 for petiole length and 0.849 ± 0.202 for leaf shape) and slight to modest maternal effect sizes (from 0.075 for petiole length to 0.285 for leaf width), suggesting that phenotypic plasticity, nonadditive genetic variation (Houle 1992), and modest maternal effects may also affect leaf characters in *Metrosideros* (Stacy et al. 2016). Although we

Table 3. Mean, minimum, and maximum pairwise F_{ST} values of *Metrosideros* taxa on Oahu's 2 volcanoes

Volcano	Age (myo)	Taxa	Mean F_{ST}	Min. F_{ST}	Max. F_{ST}
Ko'olau	2.6	ABCFGILMRTZ	0.072	0.005	0.222
		CFGLMT	0.072	0.005	0.222
		CFGLT	0.043	0.005	0.113
Wai'anae	3.7	CFGLMST	0.083	0.021	0.342
		CFGLMT	0.072	0.021	0.241
		CFGLT	0.059	0.021	0.105

Values are shown for each volcano 3 ways: for all taxa sampled, for the 6 sampled taxa that are common to both volcanoes, and for the latter subset of taxa minus M due to weak sampling of M on Wai'anae. Full taxon names are given in Table 1.

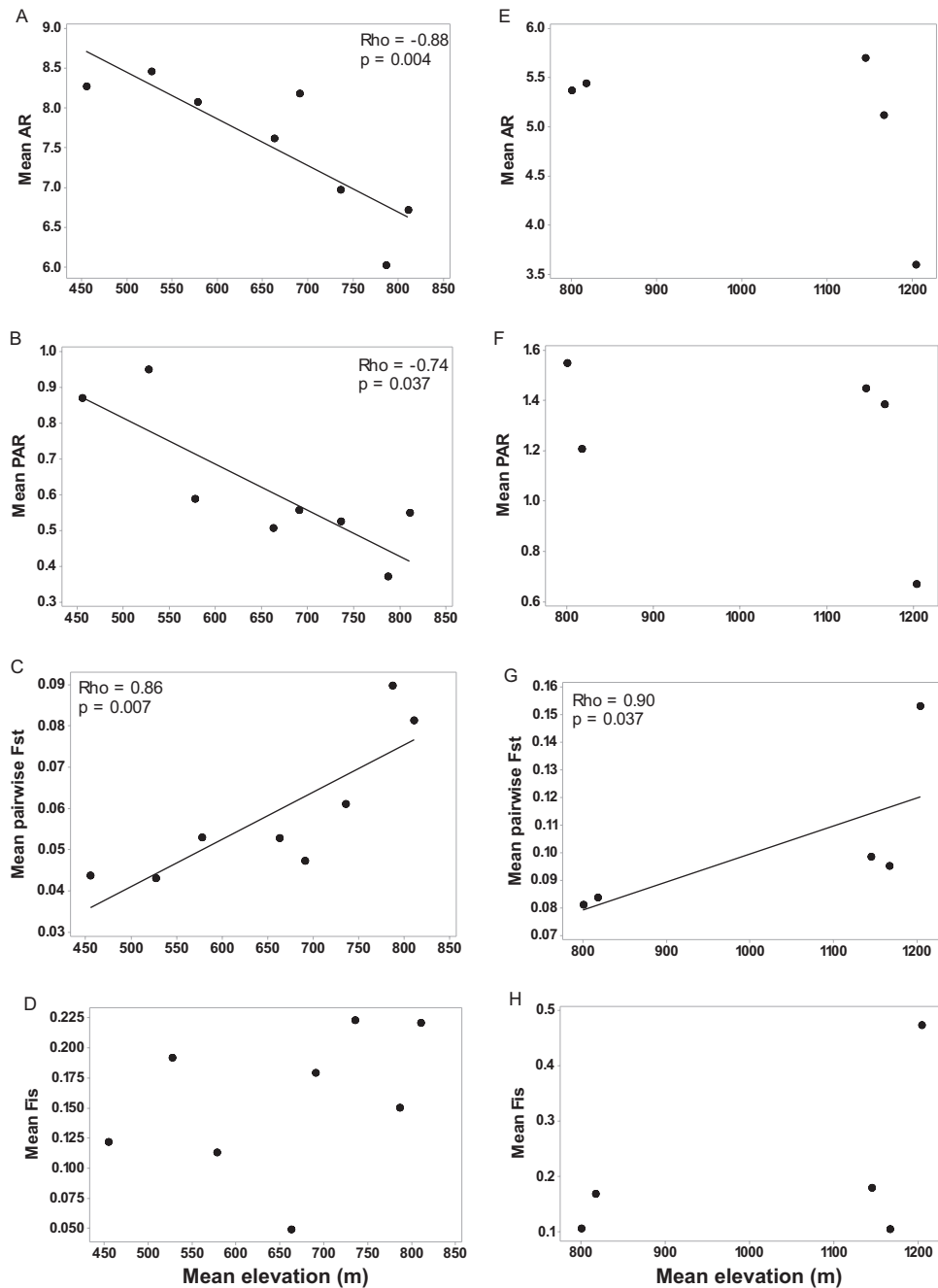


Figure 9. Relationship between mean elevation range and allelic richness (AR), private allelic richness (PAR), mean pairwise F_{ST} , and F_{IS} for 8 infraspecific taxa of *Metrosideros polymorpha* on Ko'olau Volcano (A–D) and 5 infraspecific taxa of *M. polymorpha* on Wai'anae Volcano (E–H). For each volcano, Spearman rho and corresponding P values are shown for all measures that show a significant relationship with elevation.

were unable to include all 12 taxa in the study of morphology, 2 of the 4 taxa excluded (i.e., *M. polymorpha* race *prostrata* and race S) were among the most genetically diverged taxa on O`ahu (see below), and thus heritability of their diagnostic traits is likely. O`ahu *Metrosideros* spans an especially broad spectrum of heritable leaf phenotypes, including variation in shape, curvature, rugosity, and pubescence.

Isolation by Distance across the Island

Across the island there was no relationship between geographic distance and the pairwise relationship coefficient between individuals for the full data set. This result is consistent with the predominant pattern of isolation of populations by taxon to a greater or lesser degree and the routine fine-scale sympatry of genetically diverged taxa across the island (e.g., *M. tremuloides* and *M. polymorpha* var. C at low-to-middle elevations; *M. rugosa* and *M. polymorpha* race B along the Ko`olau backbone; and *M. polymorpha* race F, race L and race S on the summit of Wai`anae Volcano). There was also no pattern of isolation by distance within any of 8 taxa examined across the island or across Ko`olau Volcano, and with 2 exceptions, no evidence of isolation by volcano within taxa, suggesting preliminarily that gene flow is widespread within taxa across the island. Cohesion within taxa across the island might be expected, given the capacity of *Metrosideros* for long-distance seed dispersal by wind (Drake 1992) or pollen dispersal by birds (Carpenter 1976).

The 2 exceptions to the pattern of no isolation by volcano, however, may suggest that gene flow is not rampant between the *Metrosideros* communities on Wai`anae and Ko`olau, which are separated by a span of 13–15 km edge to edge. The 2 exceptions—the monotypic species *M. tremuloides* and *M. macropus*—each showed significant isolation between volcanoes (i.e., $F_{ST} \geq 0.08$) which may be explained by the greater overall isolation of each of these species within the *Metrosideros* community relative to other taxa, coupled with limited gene flow between volcanoes. Stronger isolation of these taxa and restricted gene flow would lead to smaller effective population sizes of *M. macropus* and *M. tremuloides* on each volcano relative to those of all other island-wide taxa, which comprise more strongly introgressed taxa of *M. polymorpha*. Introgression among infraspecific taxa within volcanoes would result in large effective population sizes on each volcano and weak divergence between volcanoes by drift, even with restricted gene flow between volcanoes. In fact, the STRUCTURE plots suggest weak between-volcano differentiation for all of the infraspecific taxa that span both volcanoes (with stronger isolation for *M. polymorpha* race L). Previous work on O`ahu suggested that *Metrosideros* seed dispersal between volcanoes may be limited, at least from Wai`anae to Ko`olau, based on the observation of a cpDNA haplotype group restricted to the former (Percy et al. 2008). Isolation by volcano was also observed for morphologically identical populations of the native *Plantago princeps* var. *princeps* on O`ahu (Dunbar-Co et al. 2008). In the current study, observation of significant between-volcano divergence only in the 2 monotypic *Metrosideros* species suggests that isolation by volcano within O`ahu does not contribute to divergence of *Metrosideros* populations except within taxa that have achieved significant isolation from other taxa.

There was also no pattern of isolation by distance along the leeward ridges of Ko`olau Volcano, representing elevation gradients of ~500–700 m. This result is expected for the analysis that includes all taxa given the co-occurrence of highly diverged taxa at similar elevations (see above), including the occurrence of *M. macropus* and *M. tremuloides* at middle elevations. The lack of isolation by

distance for the subset of pubescent taxa, however, was somewhat surprising given the consistent partitioning of elevations by these 4 taxa and the genetic divergence between taxa at the ends of the gradient (i.e., F_{ST} of 0.059 between *M. polymorpha* var. *incana* and *M. rugosa* separated by ~1.6–2.4 km). The clearly nonrandom distribution of adults of different taxa along the leeward ridges, coupled with the heritability of phenotypes observed in the greenhouse, suggest that seeds dispersed over long distances along leeward ridges will fail at some stage of establishment or later survival. Gene flow among populations along ridges is therefore likely to be achieved predominantly through pollen movement facilitated by the highly overlapping flowering times among many taxa (Stacy E and Ekar J, unpublished data). Similarly high genetic connectivity along 2 longer elevation gradients was observed within *M. polymorpha* on the east slope of Mauna Loa Volcano, Hawai`i Island (DeBoer and Stacy 2013).

While we found no evidence of divergence within taxa by drift across the Ko`olau Volcano, the STRUCTURE analyses revealed a split between the 2 genotyped populations of *M. polymorpha* race *prostrata*. This result is consistent with divergence by drift of the small populations of this taxon, which is restricted to 7, very windy, high-elevation sites across Ko`olau Volcano (Lau J, personal communication). An analogous example from Hawai`i Island is the riparian *M. polymorpha* var. *newellii*, which is restricted to small populations along waterways on the island's windward (wet) coast and is by far the most isolated of the 4 *Metrosideros* taxa on the island ($F_{ST} = 0.13$, Stacy et al. 2014). Despite being embedded in wet forest dominated by *M. polymorpha* var. *glaberrima*, populations of *M. polymorpha* var. *newellii* separated by just 3–5 km are significantly isolated across microsatellite loci (mean pairwise F_{ST} : 0.096), consistent with divergence by drift (Stacy et al. 2014). These 2 examples, coupled with significant isolation by volcano for *M. macropus* and *M. tremuloides*, suggest that divergence by drift within *Metrosideros* taxa occurs only for taxa that have achieved substantial reproductive isolation from all other taxa (i.e., mean pairwise $F_{ST} > \sim 0.095$).

Weak to Strong Isolation by Taxa

All analyses revealed a broad range of strongly isolated to highly introgressed taxa on O`ahu; however, even the highly introgressed taxa of *M. polymorpha* showed evidence of cohesion across volcanoes. Pairwise F_{ST} values between taxa varied from very high (e.g., $F_{ST} = 0.267$ between *M. macropus* and *M. polymorpha* race S) to nonsignificant (e.g., between *M. polymorpha* var. *incana* and var. *glaberrima*). The latter are the 2 most broadly distributed *Metrosideros* taxa in Hawai`i and are genetically diverged only on volcanically active Hawai`i Island (Sakishima and Stacy 2019) where they are partitioned on new and old lava flows (Stemmermann 1983; Morrison and Stacy 2014). While a majority (6) of the taxa of *M. polymorpha* on O`ahu showed little genetic isolation from other taxa despite being morphologically distinct, 3 taxa, *M. polymorpha* race *prostrata*, race S, and to a lesser extent race B, were relatively strongly isolated. The moderate to strong population genetic isolation observed for 50% of Oahu's *Metrosideros* taxa contradict the conclusion of Harbaugh et al. (2009) of “mounting evidence that many *Metrosideros* taxa in the Hawaiian Islands should perhaps be regarded as one hypervariable species, *M. polymorpha*.” Regardless, divergence at neutral genetic markers lags phenotypic divergence in *Metrosideros* on O`ahu and throughout Hawai`i (Stacy and Sakishima 2019). The disparity between neutral genetic divergence and phenotypic divergence is common among Hawaiian plants

(Helenurm and Ganders 1985; Lowrey 1995; Soltis et al. 1996; Okada et al. 1997, 2000; Baldwin and Sanderson 1998; Ganders et al. 2000; Lindqvist and Albert 2002; Howarth et al. 2003; Dunbar-Co et al. 2008; Givnish et al. 2009; Knope et al. 2012; Pillon et al. 2013) and to be expected in long-lived woody species, given the slow evolution of reproductive isolating barriers typical of such species (Petit and Hampe 2006).

Isolation of Taxa by Elevation and Wind

With a single exception (*M. macropus*), the 6 moderately to strongly isolated *Metrosideros* taxa on O`ahu are restricted to the highest elevations (i.e., *M. rugosa*, *M. polymorpha* race *prostrata*, race B, and race S) and steep windy slopes (i.e., *M. tremuloides*). Even among the taxa of *M. polymorpha*, most of which are weakly isolated and presumably represent early stages of divergence, there was a significant pattern of increasing isolation with elevation on both volcanoes. The environments characteristic of almost all of these taxa share the common feature of strong wind: *M. rugosa* and *M. polymorpha* race B along the Ko`olau backbone, which is fully perpendicular to the predominant northeast winds; *M. tremuloides* on the windy sides of the leeward ridges; and *M. polymorpha* race *prostrata*, which grows horizontally at high-elevation sites on leeward ridges down-wind from depressions along the backbone. Windy cliffs were also implicated in the unexpectedly strong isolation of peripheral populations of *M. polymorpha* var. *glaberrima* on each of Moloka`i and Kaua`i (Stacy and Sakishima 2019).

The divergence of taxa restricted to very windy environments within continuous *Metrosideros* stands strongly implicates divergent selection in the origin and possibly maintenance of *Metrosideros* taxa on O`ahu. The high dispersibility and sheer dominance of *Metrosideros* on all islands also point to selection in the origin of the more diverged taxa within this group (Stacy and Sakishima 2019). Divergent or disruptive selection can drive the isolation of gene pools from within panmictic populations under circumstances that are not implausible in nature (Turelli et al. 2001; Gavrilets 2003; Coyne and Orr 2004). On Hawai`i Island, the importance of divergent selection in the evolution of forms is evident through differential adaptation of the island's 4 varieties to early- and late-successional, high-elevation, and riparian environments (Stemmermann 1983; Morrison and Stacy 2014; Ekar et al. 2019; Sakishima et al., in preparation). The current study additionally suggests that cliffs and slopes exposed to strong winds may be important in the origin of *Metrosideros* diversity. Notably, the habitats that support the 4 apparently wind-adapted taxa appear to be bound by sharp ecotones, in contrast to the leeward ridges of Ko`olau Volcano where climate varies gradually with elevation (Lau and Mink 2006; Giambelluca et al. 2013) and presumably allows intermediate genotypes to persist. Finally, small population size may also play a role in the isolation of taxa; 5 of the 6 moderately to strongly isolated taxa (all except *M. tremuloides*) have highly restricted ranges. Divergence at microsatellite loci in such taxa should be accelerated by drift (Ellstrand and Elam 1993).

The tight packing of *Metrosideros* taxa and their apparent hybrids along Oahu's elevation gradients today suggests that these taxa may have arisen when the island was younger and taller and subsequently assumed overlapping ranges upon subsidence and erosion of the island. O`ahu has decreased in size substantially through slumps, whole-island subsidence (~600 m) and erosion (Wagner et al. 1999), changes that would be expected to involve the collapse of broader environmental gradients into narrow ones. Cliffs are features that develop later in the evolution of individual

Hawaiian Islands (Carlquist 1970; Clague 1996), and, interestingly, the 2 Ko`olau backbone specialists, *M. rugosa* and *M. polymorpha* race B, are the least isolated of the 6 moderately to strongly isolated *Metrosideros* taxa on O`ahu, and they are equally diverged (mean pairwise F_{ST} of both: 0.086). More broadly, the rapid rate at which volcanic islands subside and erode (Moore and Clague 1992) relative to the rate of speciation in trees may explain both the observation of 2 apparently niche-free but highly diverged taxa and the presence of multiple weakly diverged yet morphologically distinct taxa on O`ahu. *Metrosideros macropus* occurs as scattered individuals or small groups across a relatively broad elevation range within Oahu's montane wet forest, and *M. polymorpha* race S is restricted to the summit of Wai`anae Volcano, where it is not subjected to strong winds (Argüeso and Businger 2018) or any other apparently extreme conditions today. Given the relatively rapid rate at which volcanic islands evolve, it is possible that these 2 taxa arose along once-sharp ecotones that have since disappeared. According to this reasoning, weak divergence among co-occurring taxa along Oahu's leeward ridges may have arisen through divergent selection that was either too weak or too short-lived to drive strong isolation. Analyses of demographic history and selection using genome-wide variation are needed to uncover the timing and drivers of the origin of Oahu's *Metrosideros* taxa.

Allele Sorting During Isolation of Taxa

The observed patterns of microsatellite variation and isolation among taxa suggest that differentiation of *Metrosideros* taxa proceeds through differential sorting of the standing genetic variation (at least at neutral loci) found in abundance in the weakly diverged taxa of *M. polymorpha*. The 3 monotypic species of *Metrosideros* on O`ahu lacked private alleles at the 9 loci examined, and across all 12 taxa genetic diversity within taxa was negatively correlated with overall isolation. For example, the highly diverged *M. tremuloides* and *M. polymorpha* race S were monomorphic for the same allele at the MePo511 locus. Estimates of the inbreeding coefficient also reflected lower allelic richness in the monotypic species, being close to half the mean inbreeding coefficient for varieties of *M. polymorpha*. Within the subset of *M. polymorpha* taxa on Ko`olau Volcano, both allelic richness and private allelic richness within taxa were negatively correlated with mean pairwise F_{ST} . This scenario may be consistent with the widespread and highly genetically variable *M. polymorpha* var. *glaberrima* acting as a "flexible stem" in the generation of the many island-endemic forms of *Metrosideros* (Stacy and Sakishima 2019), including the riparian *M. polymorpha* var. *newellii* on Hawai`i Island, which also reflects a bottleneck of microsatellite variation within *M. polymorpha* var. *glaberrima* on that island (Stacy et al. 2014).

Conclusions

Metrosideros on O`ahu captures a broad range of genetic distances between taxa, from nonsignificantly differentiated varieties and races to highly differentiated species, and the phenotypic traits diagnostic of at least 8 of the island's 12 taxa appear to be heritable. Our results implicate a role for divergent selection by wind in the origin and perhaps maintenance of a majority of the most isolated taxa within the continuous *Metrosideros* forests on O`ahu. Further, the effects of drift are likely restricted to small populations and relatively strongly isolated taxa. This group appears to offer a rare opportunity to study multiple stages of divergence and speciation within an incipient radiation of woody taxa. Direct tests of differential local

adaptation and reproductive isolating barriers among these taxa are needed. Lastly, additional population sampling (Davis and Nixon 1992; Snow 1997; Snow et al. 2003) for herbarium samples to support taxonomic hypotheses and further enhance taxonomic descriptions are recommended, and a thorough review of type specimens is needed to match published names to specimens with confidence.

Supplementary Material

Supplementary data are available in *Journal of Heredity* online.

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Data Availability

Primary waypoint, morphology, and microsatellite genotype data were deposited in Dryad (doi:10.5061/dryad.pzgmsbcg1).

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