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Isolation of *Metrosideros* (`Ohi`a) taxa on O`ahu increases with elevation and extreme environments

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Running title: Isolation of tree taxa with elevation on O`ahu

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 O`ahu, where taxa occur in tight sympatry or parapatry in mesic and montane wet forest on two 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 eight taxa to verify heritability of phenotypes, and examine genotypes of 476 wild adults at nine microsatellite loci to compare the strengths of isolation across taxa, volcanoes and distance. All eight 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 *M. polymorpha*, genetic diversity and isolation significantly decreased and increased, respectively, with elevation. Overall, five of the six 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 O`ahu.

Keywords: 3-6: cliffs, Hawai`i, microsatellites, sympatry, wind, woody species

INTRODUCTION

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 two 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 three (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 Schlötterer 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; Sakishima and Stacy 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 (Carpenter 1976; Corn and Hiesey 1973). 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 *M. 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 Ihsle 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 (D. Clague, pers.

comm.), is oriented perpendicular to the predominant northeast trade winds, resulting in fine-scale variation in water availability and wind across the island (Argüeso and Businger 2018; Lau and Mink 2006). Annual rainfall along the Ko`olau leeward ridges ranges from 6,000 mm/yr along the backbone to 1,000 mm/yr at their base (Giambelluca *et al* 2013). Extensive field observations on O`ahu suggest the presence of 12 *Metrosideros* taxa, comprising four species, including three varieties of the ubiquitous *M. polymorpha* and six unnamed morphotypes that we treat here as provisional races of *M. polymorpha* (Fig. 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 *M. macropus* (Stacy and Sakishima 2019). High cross-fertility among taxa (E. Stacy and J. Ekar, unpublished data) and the presence of morphologically intermediate individuals throughout the Ko`olau Range (E. Stacy, pers. obs.; J. Lau, pers. comm.) 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 four 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, *M. tremuloides*, restricted predominantly to steep, windy slopes. Micromorphology (i.e., stomate 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 four of the five unnamed varieties (Sur *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 two taxa from anywhere in the archipelago was observed for two O`ahu-endemic taxa (*M. macropus* and *M. polymorpha* race S). Bayesian analysis (Pritchard *et al* 2000) grouped O`ahu taxa into two hybridizing clusters comprising, with a single exception, glabrous and pubescent taxa, and splitstree network analysis (Huson and Bryant 2005) further split the glabrous taxa into two 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 eight 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 (Fig. 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 Supplemental Materials (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 (see Supplemental 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, 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 (Clausen 1940). Seeds were collected from controlled crosses performed along two 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 five taxa (3 – 9 independent families/taxon); taxa included: *M. 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* (Fig. 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 prior to 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., see 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 (Sungro 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 (~600 µmol m− 2 s − 1) 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 two 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 two 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). Supplemental Fig. S1 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^o for 48 hrs) 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 four 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, eight measures with pairwise correlations < 0.8 were selected for additional multivariate analysis.

Given that multivariate analyses are incompatible with missing data and that for four small individuals we elected to not sample leaves for calculation of SLA, a Multivariate Imputation by Chained Equations approach (van Buuren van 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 to 21) from each of multiple ridges; the exception was *M. polymorpha* race S, which is confined to the Mt. Ka`ala summit (Fig. 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); two 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 one locus (MePo506), inbreeding coefficients (F_{IS}) were calculated using INEst (using the Individual Inbreeding Model; Chybicki and Burczyk 2008); 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 2005) 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 two 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 $K = 1$ to 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 ($\ln(X|K)$ (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 two of Puechmaille'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 two 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 eight widespread taxa across the island, and for the same eight taxa within Ko`olau Volcano. We also tested for isolation by distance within each of three 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 three 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 (Figs. 3 and 4; Supplemental Fig. 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 (Fig. 4).

Adult Morphology in a Common Garden

The 101 6-year-old plants from eight 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 one year [*M. rugosa* (minus pubescence) and *M. tremuloides*] or three 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 eight traits yielded two 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 (Fig. 5). All individual leaf traits varied significantly among taxa (Supplemental Fig. S3). Among the four pubescent taxa, leaf shape and petiole length were highly uniform (see Supplemental Fig. 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* (Supplemental Fig. S3). Among the eight 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 (Supplemental Tables S2 and S3). The follow-on discriminant analysis using all characters minus pubescence (Supplemental Table S4) placed 92 of 97 individuals (94.8%) into the correct group (pubescent vs. glabrous); all five 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 two 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 taxa within volcanoes (Table 2). Similarly, the population-level splitstree network showed close relationships between populations of the same taxa on the two volcanoes (Fig. 6). In contrast to this general pattern, significant isolation by volcano was observed for the two 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 four individuals contained three 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; Supplemental 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 S and *M. macropus*, respectively (Table 1). As expected, the three 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; Fig. 6). These three infraspecific taxa showed strongest affinities with the monotypic species *M. rugosa*, *M. macropus* and *M. tremuloides*, respectively (Fig. 6). AR, PAR, and H_e per taxon were negatively correlated with overall isolation (i.e., mean pairwise F_{ST} per taxon; Fig. 7). The inbreeding coefficient was greater for populations of infraspecific taxa of *M. polymorpha* than for populations of the three 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.

The STRUCTURE analyses also revealed a range of strengths of isolation within and among taxa (Fig. 8). For the STRUCTURE analysis of all eight 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 six clusters (Supplemental Fig. S4). The six 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 two 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 (Fig. 8B). STRUCTURE F-statistics for the six 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 two highly introgressed groups (F_{ST} = 0.030-0.052).

For the STRUCTURE analysis run separately for the four pubescent taxa plus *M. polymorpha* race *prostrata*, both the delta K and posterior probability methods yielded best K = 5 (Supplemental Fig. S4), and all of Puechmaille's (2016) estimators yielded three clusters; the three clusters comprised *M. polymorpha* race *prostrata*, *M. rugosa*, and a highly introgressed group comprising *M. polymorpha* var. *incana*, race C and race F (Fig. 8C). STRUCTURE F-statistics for the three 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 five clusters integrated *M. rugosa* more strongly into the highly introgressed group and split *M. polymorpha* race *prostrata* into two groups (Fig. 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 eight individual taxa examined across the island (distances: 17.5 – 51.1 km, depending on taxon; Supplemental Fig. S5) or across Ko`olau Volcano; not shown). Similarly, there was no pattern of isolation by distance among individuals along each of three leeward ridges (spanning distances of 1.7 – 2.6 km). Because the four 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; Fig. 4), we repeated the analyses of isolation by elevation for pubescent taxa only and again found no significant relationship (Supplemental Fig. S6).

For the subset of infraspecific taxa of *M. polymorpha* (i.e., with the three 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 eight taxa occur. Both

AR and PAR per taxon decreased with mean elevation (Fig. 9A,B), while mean pairwise F_{ST} per taxon increased (Fig. 9C). On Wai`anae Volcano, where only five taxa of *M. polymorpha* were genotyped, only mean pairwise F_{ST} was (positively) correlated with elevation (Fig. 9E-G). The average taxon-level inbreeding coefficient (F_{IS}) did not vary with elevation on either volcano (Fig. 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 within 2 – 4 years with seedlings of *M. rugosa* and *M. tremuloides* acquiring taxon-diagnostic juvenile phenotypes within one year. As expected, the three 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* (two 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 five of the misclassified individuals in the discriminant analysis with just these two 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, non-additive genetic variation (Houle 1992), and modest maternal effects may also affect leaf characters in *Metrosideros* (Stacy *et al* 2016). Although we were unable to include all 12 taxa in the study of morphology, two of the four 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 eight taxa examined across the island or across Ko`olau Volcano, and with two 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 two 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 two 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 two 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 four 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 (E. Stacy and J. Ekar, unpub. data). Similarly high genetic connectivity along two 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 two 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 seven, very windy, high-elevation sites across Ko`olau Volcano (J. Lau, pers. comm.). 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 four *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 two 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 two 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 (six) of the taxa of *M. polymorpha* on O`ahu showed little genetic isolation from other taxa despite being morphologically distinct, three 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 (Baldwin and Sanderson 1998; Givnish *et al* 2008; Pillon *et al* 2013; Lindqvist and Albert 2002; Dunbar-Co *et al* 2008; Howarth *et al* 2003; Soltis *et al* 1996; Lowrey 1995; Okada *et al* 1997, 2000; Helenurm and Ganders 1985; Ganders *et al* 2000; Knope *et al* 2012) 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 six 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 four 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 prep.). 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 four 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; five of the six 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 two Ko`olau backbone specialists, *M. rugosa* and *M. polymorpha* race B, are the least isolated of the six 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 two 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 two 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 three monotypic species of *Metrosideros* on O`ahu lacked private alleles at the nine 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 non-significantly differentiated varieties and races to highly differentiated species, and the phenotypic traits diagnostic of at least eight 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.

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Data Availability

Upon acceptance of the manuscript we will deposit primary waypoint, morphology, and microsatellite genotype data in Dryad.

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Table and Figure Captions

Table 1. Genetic diversity statistics[1] (± SE) for each of 12 *Metrosideros* taxa or morphotypes (races) sampled on O`ahu. 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, *M. 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 varieties of *M. polymorpha*. Named taxa are described in Dawson and Stemmerman (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 (see Supplemental Table S1).

Table 2. Results of AMOVA among taxa and volcanoes on O`ahu. Analysis was restricted to six *Metrosideros* taxa that were sampled from both volcanoes (C, F, G, L, M, and T; See Table 1 for taxon names).

Table 3. Mean, minimum, and maximum pairwise F_{ST} values of *Metrosideros* taxa on Oahu's two volcanoes. Values are shown for each volcano three ways: for all taxa sampled, for the six 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 in Table 1.

Fig. 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 in Table 1. Photo credit for B, F and Z (Joel Lau).

Fig. 2. Map of O`ahu showing waypoints (red) recorded for adults of *Metrosideros* at 11 sites. Three sites are on Wai`anae Volcano (west side), and eight 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 in Table 1. The 11 sampled locations span from Pahole, NW O`ahu (21.54191$^{O}$N, 158.193527$^{O}$W) to Kuliou`ou, SE O`ahu (21.316488 ^{O}N, 157.72329^{O}W).

Fig. 3. Map of east O`ahu showing GPS locations of all trees sampled at each of five of the 11 sampled locations; all five 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 *M. polymorpha* race *prostrata* (A) is recorded from only seven sites on Ko`olau Volcano (J. Lau, pers. comm.). The twelfth taxon, *M. polymorpha* race S, is restricted to the summit of Wai`anae Volcano (Mt. Ka`ala), west O`ahu. Full taxon names are in Table 1. Hillshade map of O`ahu courtesy of Forest and Kim Starr.

Fig. 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 seven taxa on Wai`anae Volcano. Pubescent taxa are indicated with asterisks. Outliers and mean values (hatched circles) are also shown. Full taxon names are in Table 1. Taxa I and Z also occur on Wai`anae Volcano but were not sampled sufficiently to be included here.

Fig. 5. Principal components analysis of 97 common-garden plants of eight *Metrosideros* taxa from O`ahu: A) PC1 x 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 in Table 1.

Fig. 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 in Table 1. Population sizes range from 11 to 50 (mean = 28), except for Wai`anae M (n = 4).

Fig. 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.

Fig. 8. STRUCTURE graphs showing the two best K values for all glabrous *Metrosideros* taxa on O`ahu except *M. 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 in Table 1.

Taxon	Taxon code	Pubes-cent?	Volcano	N	A	AR	PAR	H_O	H_E	Mean pairwise F_{ST}
M. macropus	M	No	K,W	22,4	7.89±1.81	3.854±0.769	0.038±0.031	0.414±0.072	0.539±0.105	0.156±0.012
M. p. var. *glaberrima*	G	No	K,W	35,13	15.22±3.26	5.652±0.809	0.186±0.064	0.615±0.080	0.760±0.079	0.048±0.011
M. p. var. *incana*	I	Yes	K,W	27,2	12.67±3.00	5.466±0.874	0.122±0.064	0.594±0.070	0.735±0.086	0.048±0.012
M. p. race *prostrata*	A	No	K	39,--	8.44±1.80	4.496±0.628	0.066±0.046	0.574±0.071	0.706±0.066	0.098±0.015
M. p. race B	B	No[2]	K	28,--	9.67±2.52	4.635±0.907	0.044±0.026	0.482±0.109	0.611±0.113	0.085±0.012
M. p. race C	C	Yes	K,W	35,30	16.00±3.99	5.468±0.798	0.103±0.043	0.639±0.075	0.763±0.069	0.053±0.012
M. p. race F	F	Yes	K,W	21,39	15.44±4.04	5.521±0.808	0.060±0.020	0.649±0.048	0.773±0.064	0.061±0.014
M. p. race L	L	No	K,W	34,23	14.56±3.41	5.372±0.872	0.136±0.047	0.571±0.084	0.713±0.097	0.052±0.011
M. p. race S	S	No	W	--,11	4.89±1.32	3.441±0.718	0.050±0.033	0.384±0.083	0.512±0.110	0.137±0.018
M. p. race Z	Z	No	K,W	20,0[3]	9.00±2.23	4.873±0.899	0.095±0.046	0.537±0.109	0.683±0.106	0.065±0.011
M. rugosa	R	Yes	K	50,--	12.22±2.63	5.185±0.732	0.057±0.026	0.673±0.065	0.755±0.067	0.087±0.015
M. tremuloides	T	No	K,W	23,20	11.67±3.42	4.470±0.939	0.076±0.043	0.493±0.106	0.578±0.127	0.095±0.013

[1] All genetic diversity statistics are calculated with populations pooled within taxon (i.e., volcanoes pooled), and values are averaged across nine 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 ± 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. [2] Emerging leaves of *M. p.* race B are pubescent, but adult leaves are glabrous. [3] *M. p.* race Z was recorded from Wai`anae Volcano but not sampled.

Comparison	d.f.	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	

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

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 in Table 1. Photo credit for B, F and Z (Joel Lau).

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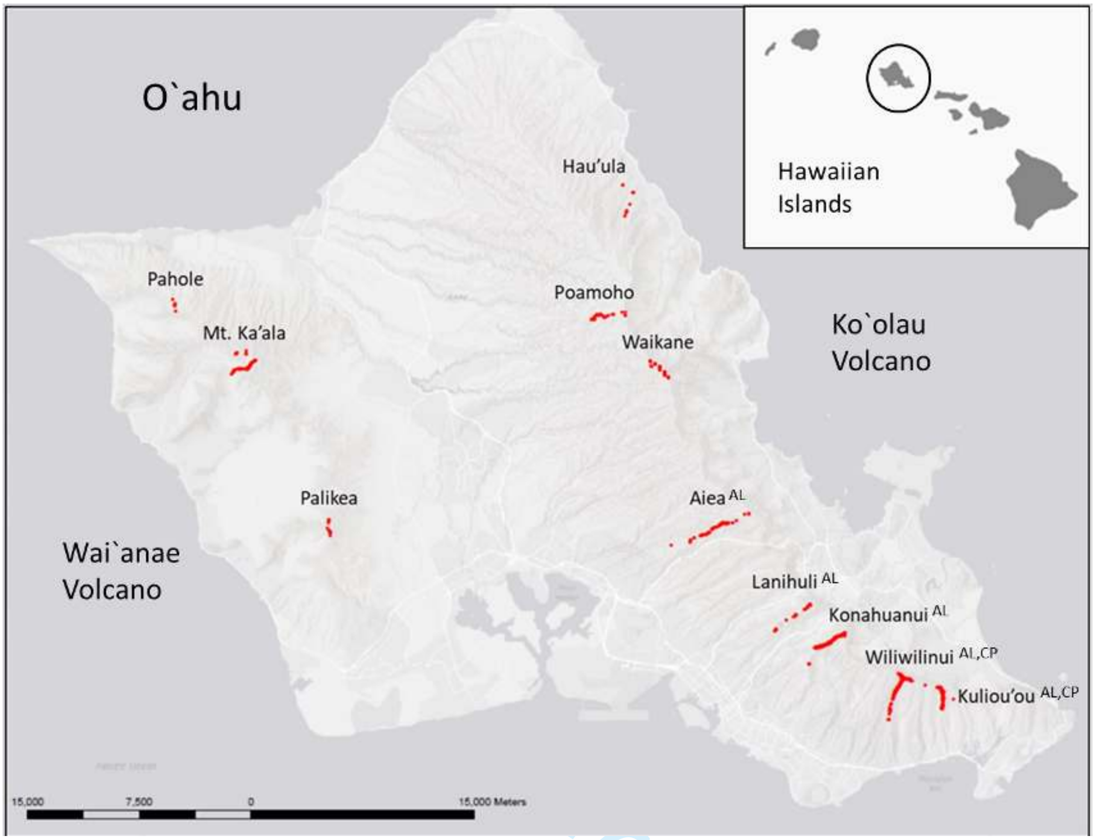
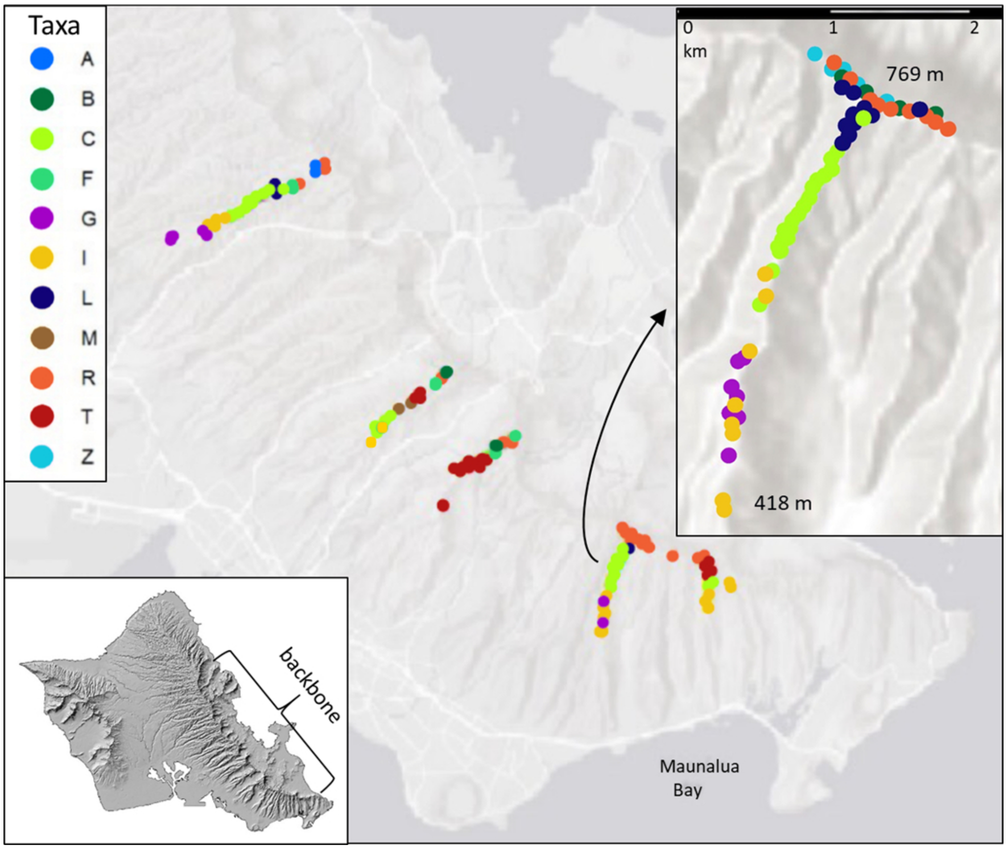


							Taxon							Total
Site	Volcano	A	B	C	F	G	I	L	M	R	S	T	Z	count
Aiea	K	20		20		15	11	20	1	2				89
Hau'ula	K					3	5							8
Konahuanui	K		15		20			5	11	11		21	11	94
Kuliou'ou	K		5		1	1	5							12
Lanihuli	K			1		2			2	1		2		8
Mt. Ka'ala	W			1	20	13		20	4		11	20		89
Pahole	W			9			1	3						13
Palikea	W			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



Map of east O`ahu showing GPS locations of all trees sampled at each of five of the 11 sampled locations; all five 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 *M. polymorpha* race *prostrata* (A) is recorded from only seven sites on Ko`olau Volcano (J. Lau, pers. comm.). The twelfth taxon, *M. polymorpha* race S, is restricted to the summit of Wai`anae Volcano (Mt. Ka`ala), west O`ahu. Full taxon names are in Table 1. Hillshade map of O`ahu courtesy of Forest and Kim Starr.

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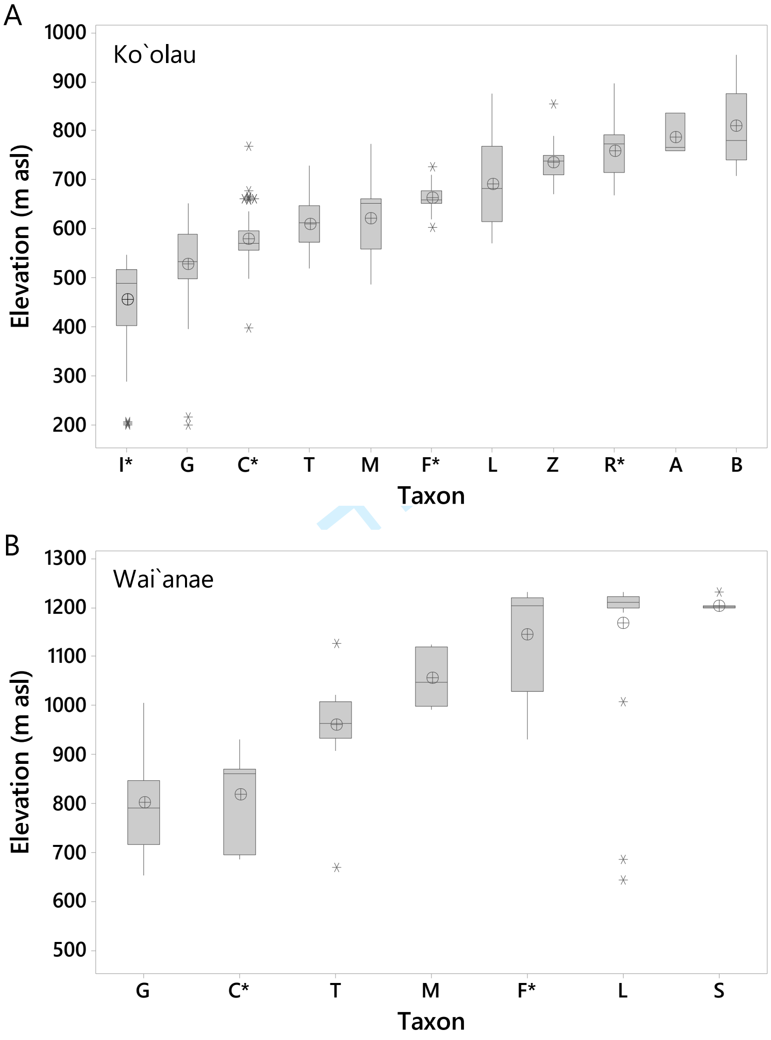
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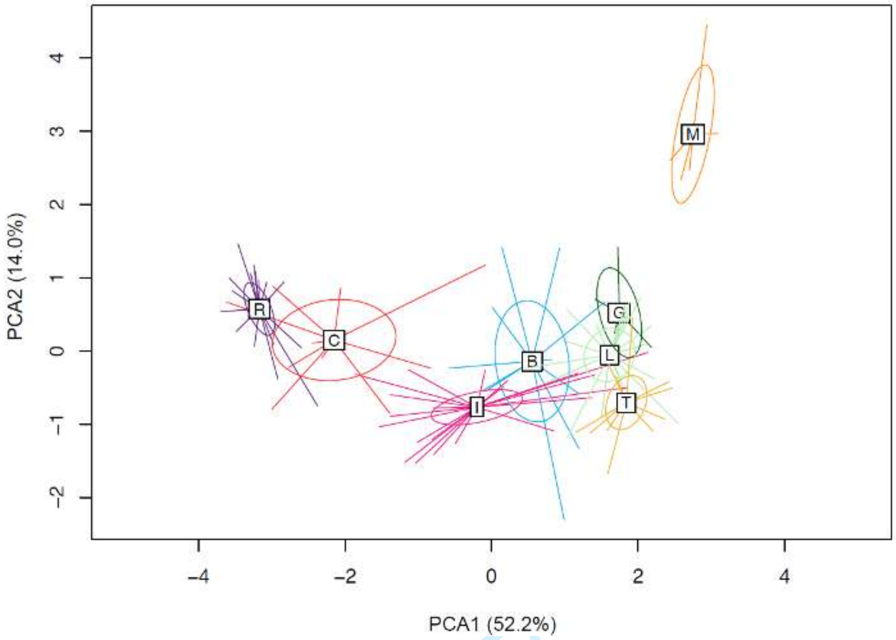
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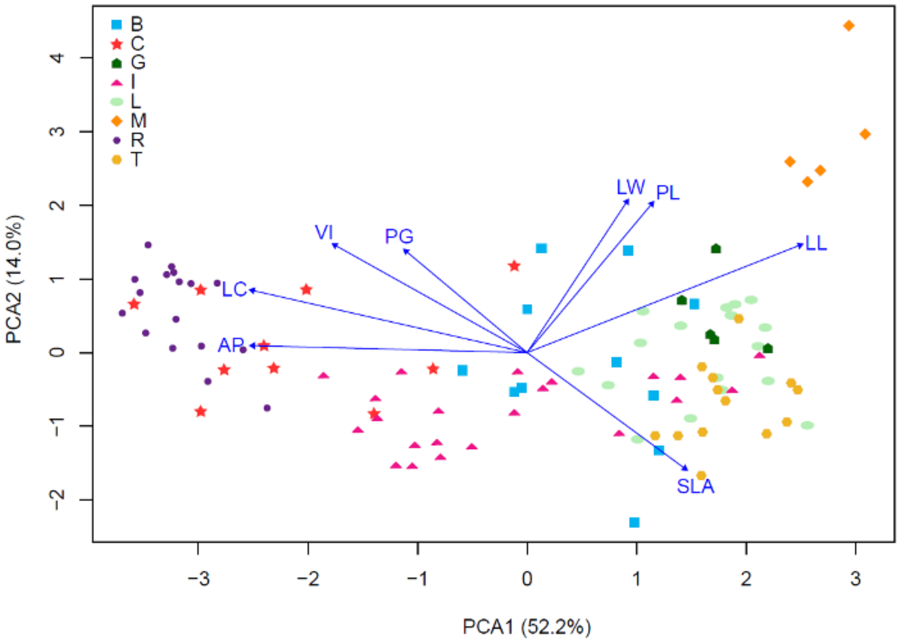
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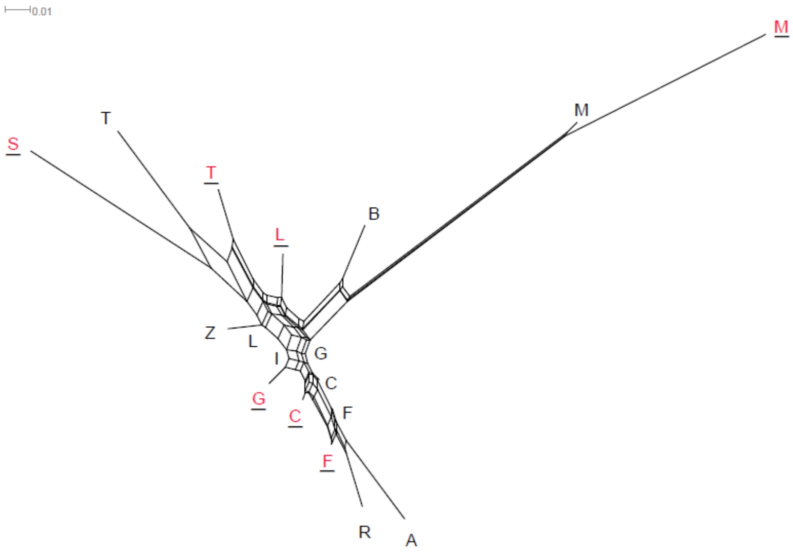
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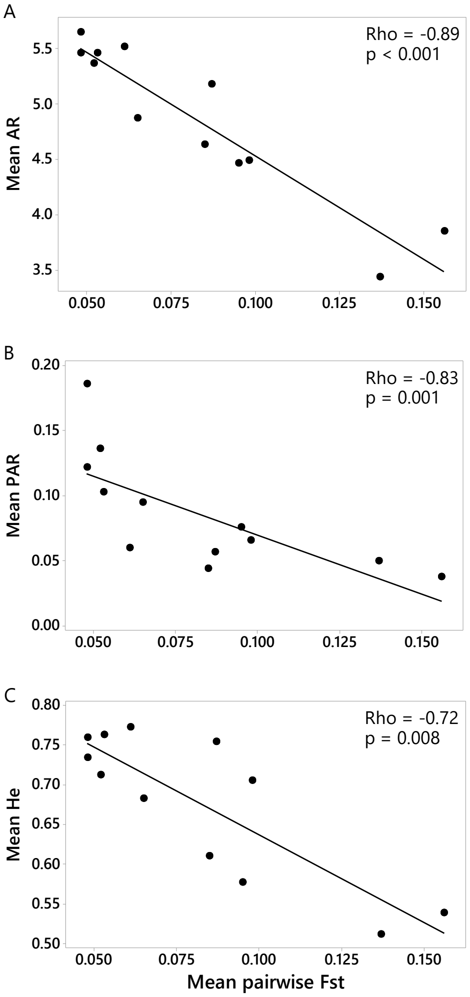
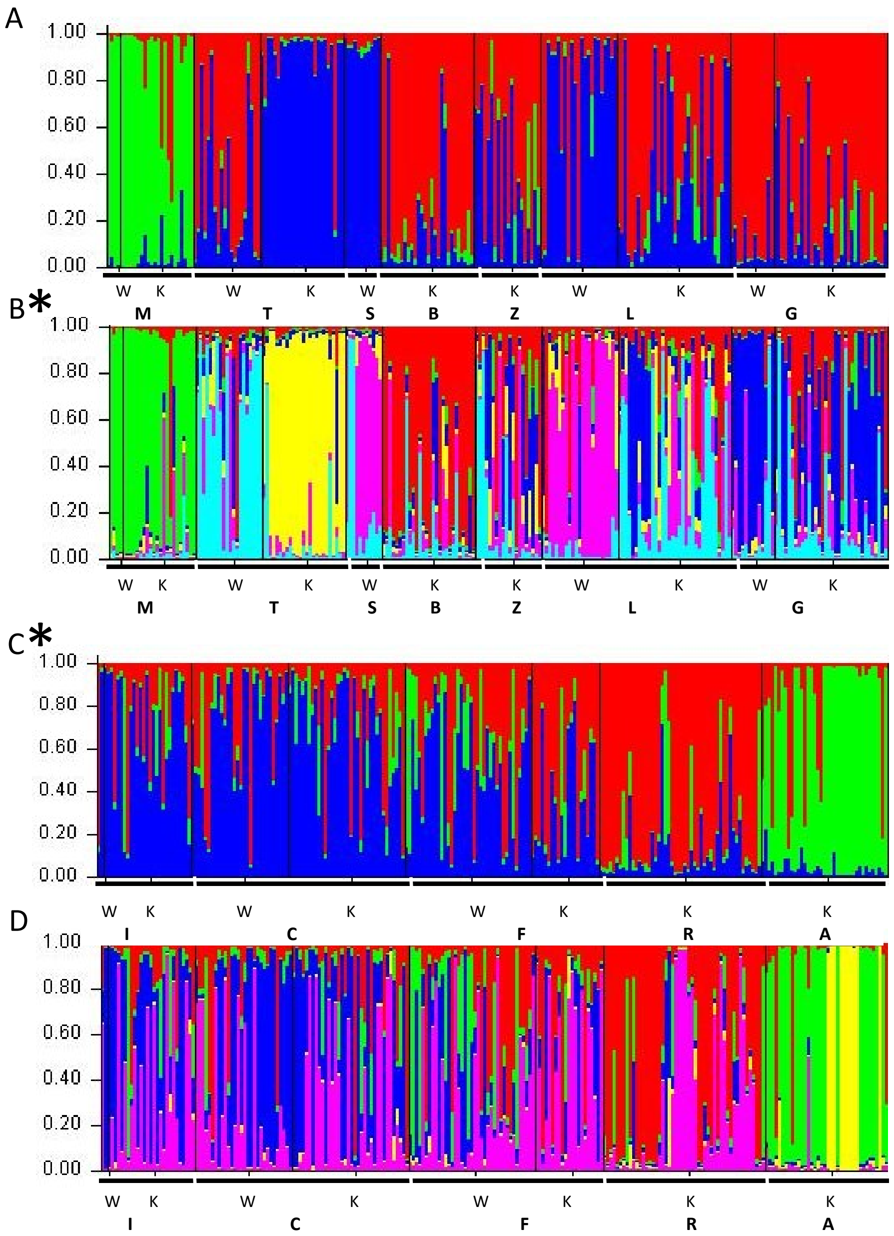
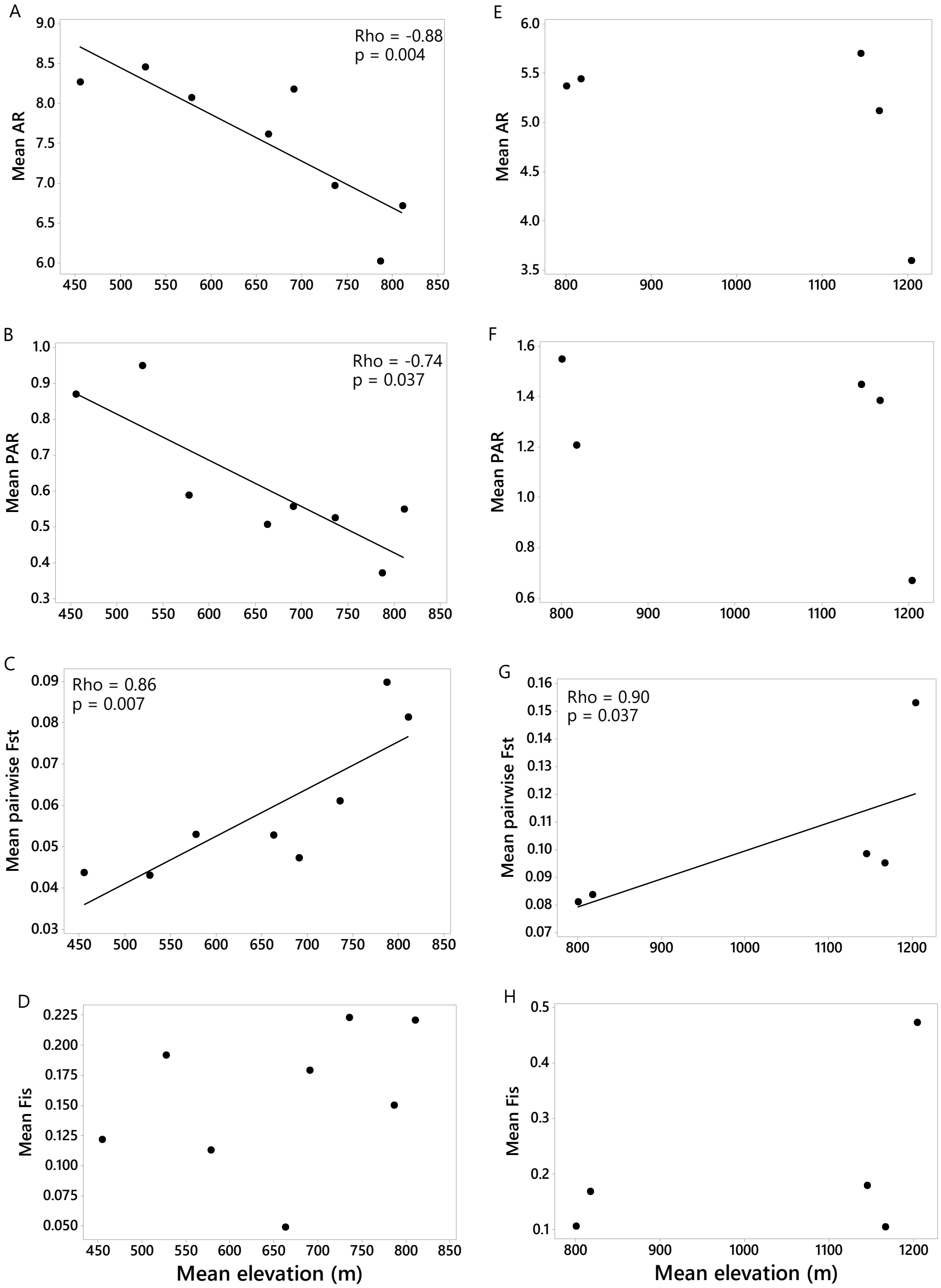
F
F
R
A


A
Rho = -0.89
p < 0.001
Mean AR
5.5
5.0
4.5
4.0
3.5
0.050
0.075
0.100
0.125
0.150
B
Rho = -0.83
p = 0.001
Mean PAR
0.20
0.15
0.10
0.05
0.00
0.050
0.075
0.100
0.125
0.150
C
Rho = -0.72
p = 0.008
Mean He
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.050
0.075
0.100
0.125
0.150
Mean pairwise Fst


A
1.00
0.80
0.60
0.40
0.20
0.00
W K W K W K K W K W K
M T S B Z L G
B*
1.00
0.80
0.60
0.40
0.20
0.00
W K W K W K K W K W K
M T S B Z L G
C*
1.00
0.80
0.60
0.40
0.20
0.00
W K W K W K K K
I C F R A
D
1.00
0.80
0.60
0.40
0.20
0.00
W K W K W K K K
I C F R A


A
Mean AR
9.0
8.5
8.0
7.5
7.0
6.5
6.0
Rho = -0.88
p = 0.004
450 500 550 600 650 700 750 800 850
E
Mean AR
6.0
5.5
5.0
4.5
4.0
3.5
800 900 1000 1100 1200
B
Mean PAR
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Rho = -0.74
p = 0.037
450 500 550 600 650 700 750 800 850
F
Mean PAR
1.6
1.4
1.2
1.0
0.8
0.6
800 900 1000 1100 1200
C
Mean pairwise Fst
0.09
0.08
0.07
0.06
0.05
0.04
0.03
Rho = 0.86
p = 0.007
450 500 550 600 650 700 750 800 850
G
Mean pairwise Fst
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
Rho = 0.90
p = 0.037
800 900 1000 1100 1200
D
Mean Fis
0.225
0.200
0.175
0.150
0.125
0.100
0.075
0.050
450 500 550 600 650 700 750 800 850
Mean elevation (m)
H
Mean Fis
0.5
0.4
0.3
0.2
0.1
800 900 1000 1100 1200
Mean elevation (m)

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

SUPPLEMENTAL MATERIAL

Table S1. Information on 60 voucher specimens of 12 recognized and tentative taxa of *Metrosideros* on O`ahu. 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.

Collection ID	Taxon name	Taxon code	Date collected	Latitude	Longitude	Elevation (m)	Volcano	Leaf type	Collector	Associated collectors
O015	*M. macropus*	M	11/5/2010	21.35507276	-157.83067077	535.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O016	*M. polymorpha* race C	C	11/5/2010	21.35504167	-157.83075753	536.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O017	*M. polymorpha* race B	B	11/5/2010	21.36947053	-157.80942927	854.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O018	*M. polymorpha* var. *glaberrima*	G	11/5/2010	21.36947053	-157.80942927	854.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O019	*M. rugosa*	R	11/5/2010	21.36748385	-157.81177947	717.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O020	*M. tremuloides*	T	11/5/2010	21.36370394	-157.81815282	653.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O021	*M. tremuloides*	T	11/5/2010	21.36266417	-157.81961546	669.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O022	*M. macropus*	M	11/5/2010	21.36063583	-157.82399509	605.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O023	*M. polymorpha* var. *incana*	I	11/6/2010	21.60189877	-157.91619049	205.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O024	*M. polymorpha* var. *glaberrima*	G	11/6/2010	21.60202358	-157.91605379	214.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O025	*M. polymorpha* var. *incana*	I	11/6/2010	21.60195024	-157.91625210	200.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O027	*M. polymorpha* var. *incana*	I	11/6/2010	21.59516182	-157.91819234	289.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O028	*M. polymorpha* var. *glaberrima*	G	11/6/2010	21.59160596	-157.92008783	399.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O029	*M. polymorpha* var. *incana*	I	11/6/2010	21.59132659	-157.92017190	401.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O030	*M. polymorpha* var. *glaberrima*	G	11/6/2010	21.58881303	-157.92111268	463.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O046	M. polymorpha race L	L	11/7/2010	21.41494528	-157.86343513	601.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O070	*M. polymorpha* race C	C	11/7/2010	21.40883613	-157.87427394	527.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O072	*M. polymorpha* var. *incana*	I	11/7/2010	21.40881761	-157.87446538	530.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O075	*M. polymorpha* var. *incana*	I	11/7/2010	21.40838443	-157.87581277	527.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O095	*M. polymorpha* var. *glaberrima*	G	12/4/2010	21.41346194	-157.86615749	531.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O097	*M. macropus*	M	12/4/2010	21.41601825	-157.85622116	678.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O099	*M. rugosa*	R	12/5/2010	21.32816283	-157.75314546	779.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O103	*M. rugosa*	R	12/5/2010	21.32789511	-157.75247893	778.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O116	*M. rugosa*	R	12/5/2010	21.32733990	-157.74930110	792.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O176	*M. polymorpha* race F	F	2/5/2011	21.50687523	-158.14430902	1231.00	Wai`anae	pubescent	J. Johansen	E. Stacy
O211	*M. tremuloides*	T	2/5/2011	21.50240022	-158.15295596	978.00	Wai`anae	glabrous	J. Johansen	E. Stacy

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

O236	*M. macropus*	M	2/6/2011	21.53441682	-157.92289333	749.00	Ko`olau	glabrous	J. Johansen	E. Stacy
O242	*M. rugosa*	R	2/6/2011	21.53279426	-157.92092049	795.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O347	*M. polymorpha* race S	S	5/5/2012	21.50255738	-158.14901328	1199.00	Wai`anae	glabrous	J. Johansen	E. Stacy
O1003	*M. rugosa*	R	6/10/2013	21.32183047	-157.73078749	714.00	Ko`olau	pubescent	J. Johansen	E. Stacy
O350	*M. polymorpha* race S	S	5/6/2012	21.50252360	-158.14933246	1193.00	Wai`anae	glabrous	J. Johansen	E. Stacy
O1001	*M. tremuloides*	T	6/9/2013	21.34533660	-157.80669074	523.08	Ko`olau	glabrous	J. Johansen	E. Stacy
O1000	*M. tremuloides*	T	6/9/2013	21.34533660	-157.80669074	573.08	Ko`olau	glabrous	J. Johansen	E. Stacy
F2	*M. polymorpha* race F	F	10/20/2017	21.34798494	-157.79749042	675.69	Ko`olau	pubescent	E Stacy	
F3	*M. polymorpha* race F	F	10/20/2017	21.34827093	-157.79729093	664.63	Ko`olau	pubescent	E Stacy	
F4	*M. polymorpha* race F	F	10/20/2017	21.34824512	-157.79729714	656.46	Ko`olau	pubescent	E Stacy	
F5	*M. polymorpha* race F	F	10/20/2017	21.34866555	-157.79684611	661.51	Ko`olau	pubescent	E Stacy	
Z1	*M. polymorpha* race Z	Z	10/20/2017	21.32733529	-157.75386957	713.00	Ko`olau	glabrous	E Stacy	
Z2	*M. polymorpha* race Z	Z	10/20/2017	21.32743025	-157.75380386	770.00	Ko`olau	glabrous	E Stacy	
Z3	*M. polymorpha* race Z	Z	10/20/2017	21.32747007	-157.75374946	769.00	Ko`olau	glabrous	E Stacy	
Z4	*M. polymorpha* race Z	Z	10/20/2017	21.32782361	-157.75189362	778.00	Ko`olau	glabrous	E Stacy	
Z5	*M. polymorpha* race Z	Z	10/20/2017	21.32999210	-157.75517832	735.00	Ko`olau	glabrous	E Stacy	
C1	*M. polymorpha* race C	C	10/20/2017	21.41036851	-157.87065094	536.00	Ko`olau	pubescent	E Stacy	
C2	*M. polymorpha* race C	C	10/20/2017	21.41543688	-157.86240089	604.00	Ko`olau	pubescent	E Stacy	
C3	*M. polymorpha* race C	C	10/20/2017	21.41536212	-157.86263240	603.00	Ko`olau	pubescent	E Stacy	
L1	*M. polymorpha* race L	L	10/20/2017	21.41550000	-157.86224222	605.00	Ko`olau	glabrous	E Stacy	
L2	*M. polymorpha* race L	L	10/20/2017	21.41548047	-157.86233593	605.00	Ko`olau	glabrous	E Stacy	
L3	*M. polymorpha* race L	L	10/20/2017	21.41544074	-157.86245051	604.00	Ko`olau	glabrous	E Stacy	
L4	*M. polymorpha* race L	L	10/20/2017	21.41435679	-157.86408464	588.00	Ko`olau	glabrous	E Stacy	
B1	*M. polymorpha* race B	B	10/20/2017	21.41685435	-157.85403902	746.00	Ko`olau	glabrous	E Stacy	
B2	*M. polymorpha* race B	B	10/20/2017	21.32236113	-157.73076536	735.00	Ko`olau	glabrous	E Stacy	
B3	*M. polymorpha* race B	B	10/20/2017	21.32286463	-157.73143223	708.00	Ko`olau	glabrous	E Stacy	
B4	*M. polymorpha* race B	B	10/20/2017	21.32867287	-157.75363203	740.34	Ko`olau	glabrous	E Stacy	
B5	*M. polymorpha* race B	B	10/20/2017	21.32926798	-157.75458757	739.61	Ko`olau	glabrous	E Stacy	
A1	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42034113	-157.84911867	759.00	Ko`olau	glabrous	E Stacy	
A2	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42034113	-157.84911867	759.00	Ko`olau	glabrous	E Stacy	
A3	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42034113	-157.84911867	759.00	Ko`olau	glabrous	E Stacy	
A4	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42088839	-157.84643755	836.00	Ko`olau	glabrous	E Stacy	
A5	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42088839	-157.84643755	836.00	Ko`olau	glabrous	E Stacy	
A6	*M. polymorpha* race *prostrata*	A	10/20/2017	21.42088839	-157.84643755	836.00	Ko`olau	glabrous	E Stacy	

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

Table S2. Summary of classification from discriminant analysis of 97[1] common-garden plants of eight *Metrosideros* taxa from O`ahu. Analysis was done using 11 characters: mean leaf length, mean leaf width, mean leaf shape, mean petiole length, leaf curvature score, rugosity score, abaxial pubescence score, adaxial pubescence score, pigmentation score, leaf area, and specific leaf area. Eighty-seven of 97 (89.7%) plants were correctly classified to taxon.

	True Group							
Put into group	B	C	G	I	L	M	R	T
B	9	0	0	0	0	0	0	0
C	0	8	0	2	0	0	0	0
G	1	0	5	0	1	0	0	0
I	0	2	0	20	0	0	0	0
L	1	0	0	0	15	0	0	2
M	0	0	0	0	0	5	0	0
R	0	0	0	0	0	0	14	0
T	0	0	0	0	1	0	0	11
Total N	11	10	5	22	17	5	14	13
N correct	9	8	5	20	15	5	14	11
Proportion	0.818	0.800	1.000	0.909	0.882	1.000	1.000	0.846

[1] The lower sample size was due to the small size of four plants, from which leaves were not collected for calculation of SLA.

Table S3. Linear discriminant function coefficients for each of eight *Metrosideros* taxa from O`ahu based on 11 morphological traits: mean leaf length (MLL), mean leaf width (MLW), mean leaf shape (MLS), mean petiole length (MPL), leaf curvature score (LCS), rugosity score (RS), abaxial pubescence score (AbPS), adaxial pubescence score (AdPS), pigmentation score (PS), leaf area, and specific leaf area (SLA).

	B	C	G	I	L	M	R	T
Constant	-89.25	-176.66	-87.09	-174.04	-86.44	-144.34	-337.70	-109.41
MLL (cm)	-10.89	-21.81	-14.89	-17.66	-9.02	-15.17	-22.15	-9.58
MLW (cm)	41.43	48.70	42.34	41.93	31.56	45.15	42.99	33.89
MLS	41.54	44.82	42.41	40.67	39.11	41.18	42.45	49.09
MPL (cm)	1.77	11.62	21.51	11.73	21.81	78.85	13.42	26.23
LCS (0-3)	7.97	13.48	5.65	7.35	5.07	5.18	9.98	5.35
RS (0-2)	-12.67	-5.01	-13.91	-7.57	-14.02	-13.34	38.22	-12.65
AbPS (0-3)	-3.99	104.97	-7.85	107.78	-7.57	-2.36	168.01	-6.48
AdPS (0-1)	13.31	-0.22	8.31	-1.15	9.47	8.44	-7.33	12.31
PS (1-3)	6.53	3.44	5.75	2.63	6.26	5.63	-0.31	4.78
LA (cm^2)	-0.10	-0.07	0.15	-0.07	0.15	0.17	-0.13	0.03
SLA (cm^2/g)	0.66	0.38	0.54	0.61	0.66	0.54	0.23	0.73

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

Table S4. Linear discriminant function coefficients for glabrous and pubescent groups of *Metrosideros* taxa from O`ahu based on nine morphological traits: mean leaf length (MLL), mean leaf width (MLW), mean leaf shape (MLS), mean petiole length (MPL), leaf curvature score (LCS), rugosity score (RS), pigmentation score (PS), leaf area (LA), and specific leaf area (SLA).

	Glabrous	Pubescent
Constant	-76.298	-69.973
MLL (cm)	-13.618	-18.862
MLW (cm)	37.422	43.658
MLS	38.838	39.261
MPL (cm)	-0.078	-3.188
LCS (0-3)	7.192	9.511
RS (0-2)	1.201	1.819
PS (1-3)	7.918	6.741
LA (cm^2)	0.101	0.011
SLA (cm^2/g)	0.490	0.510

Table S5. Pairwise F_{ST} values for all combinations of 12 *Metrosideros* taxa on O`ahu. Individuals are pooled within taxa. Full taxon names are in Table 1.

	M	R	T	Z	F	G	I	L	S	B	A
M											
R	0.1561										
T	0.1830	0.1374									
Z	0.1394	0.0807	0.0588								
F	0.1480	0.0235	0.0963	0.0605							
G	0.1234	0.0530	0.0584	0.0223	0.0243						
I	0.1304	0.0594	0.0509	0.0298	0.0206	0.0036					
L	0.1293	0.0789	0.0422	0.0161	0.0436	0.0171	0.0145				
S	0.2668	0.1810	0.1015	0.1030	0.1228	0.0938	0.0979	0.0747			
B	0.1247	0.0898	0.0947	0.0626	0.0759	0.0500	0.0452	0.0488	0.1789		
A	0.1750	0.0542	0.1407	0.0958	0.0429	0.0677	0.0703	0.0775	0.1811	0.1098	
C	0.1381	0.0461	0.0789	0.0459	0.0117	0.0100	0.0061	0.0264	0.1019	0.0585	0.0574

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

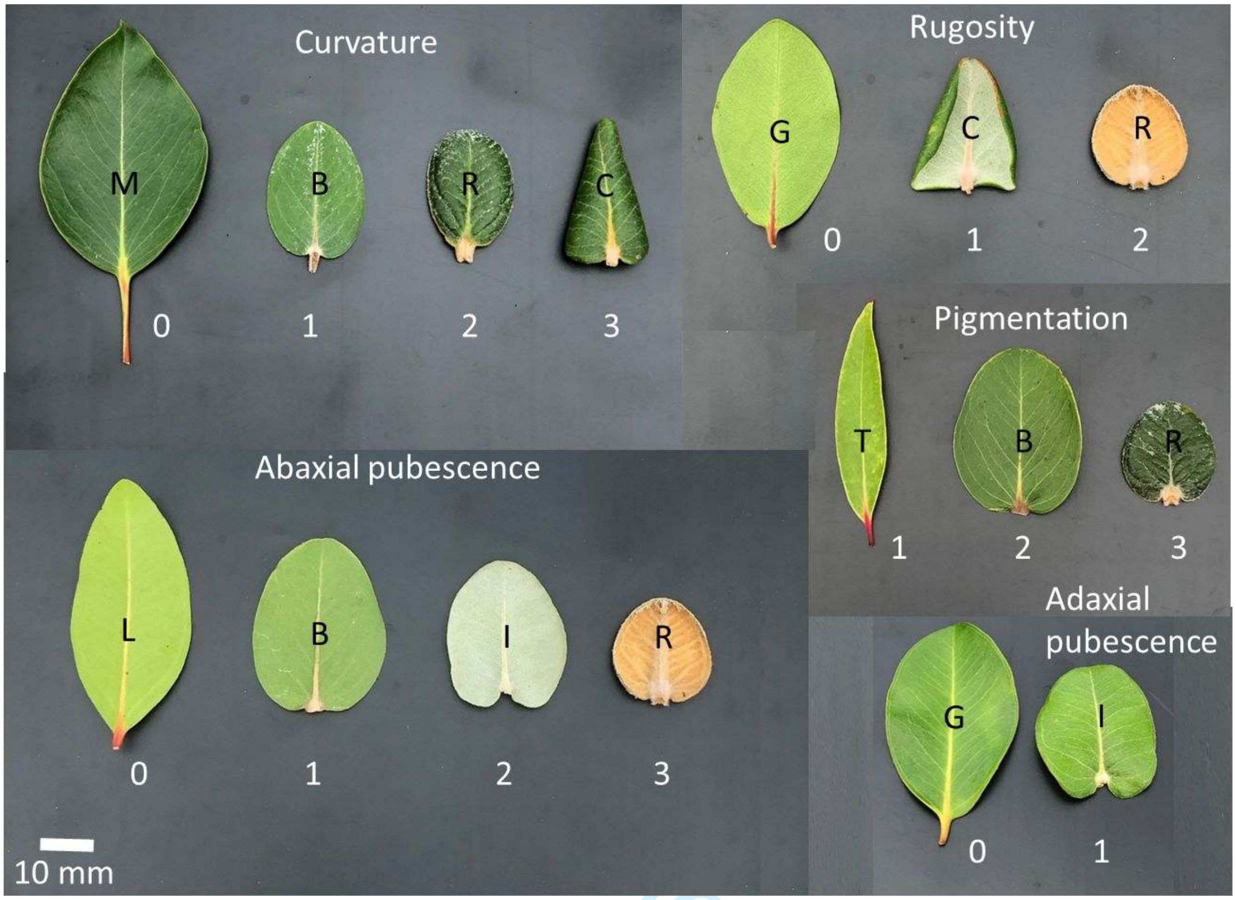


Fig. S1. Representative examples of scores (white text) used for each of five qualitative leaf characters. The taxon code for each example leaf is shown in black text. Leaves of C, I and R are pubescent; all others are glabrous. Full taxon names are in Table 1.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

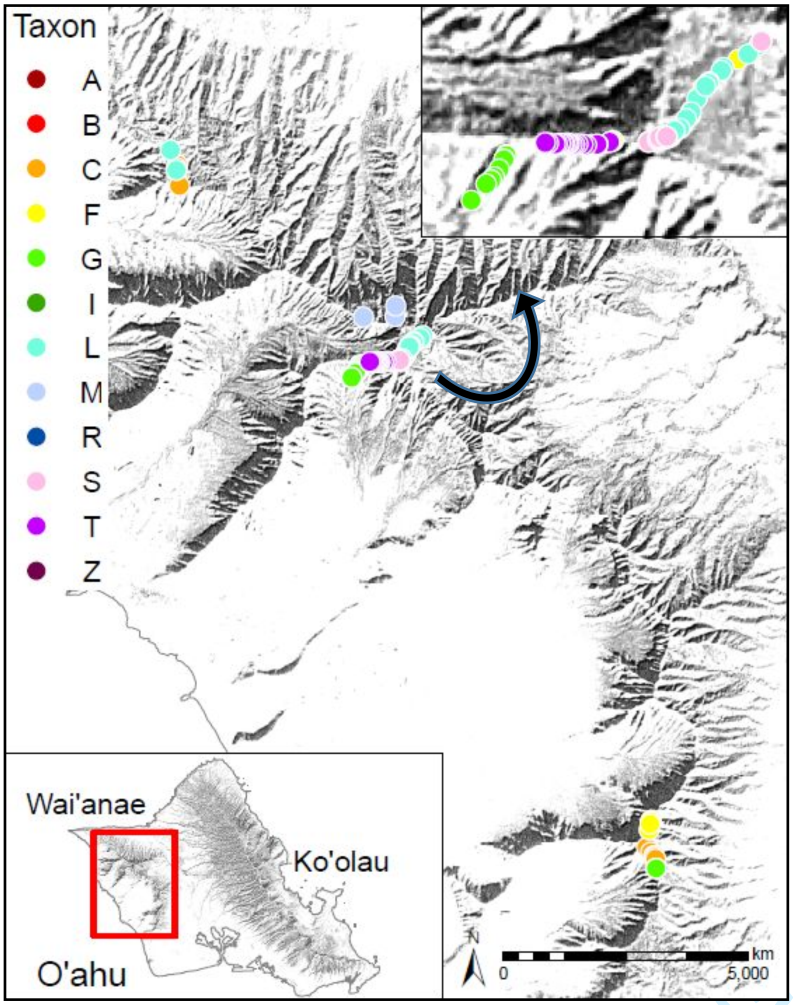


Fig. S2. All waypoints recorded for *Metrosideros* adults on Wai`anae Volcano, O`ahu.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

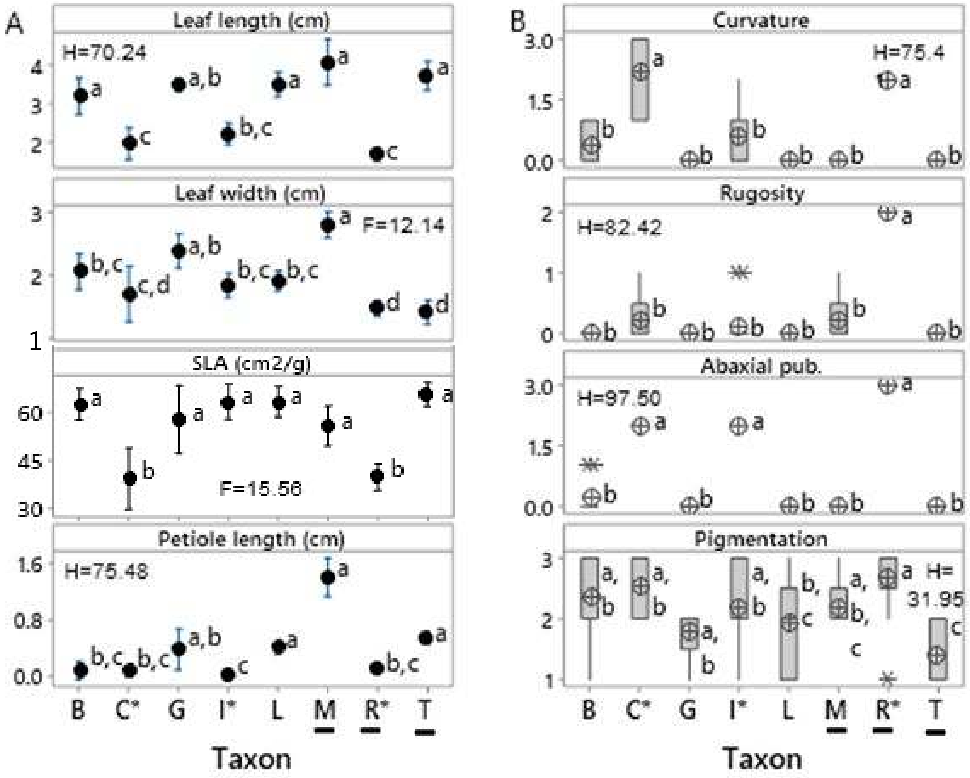


Fig. S3. Morphology of 101 common-garden plants representing eight *Metrosideros* taxa from O`ahu: A) interval-scale measures (mean ± 95% CI), and B) ordinal-scale measures (median, quartiles and range). Pubescent taxa are indicated by *, and monotypic species are underlined. Shared superscripts indicate no statistical difference at family α = 0.2 for Kruskal-Wallis tests and α = 0.05 for one-way ANOVA; df for all Kruskal-Wallis tests = 7; df for leaf width = 7,91; df for SLA = 7,89; p-values for all tests = < 0.001. Full taxon names are in Table 1.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

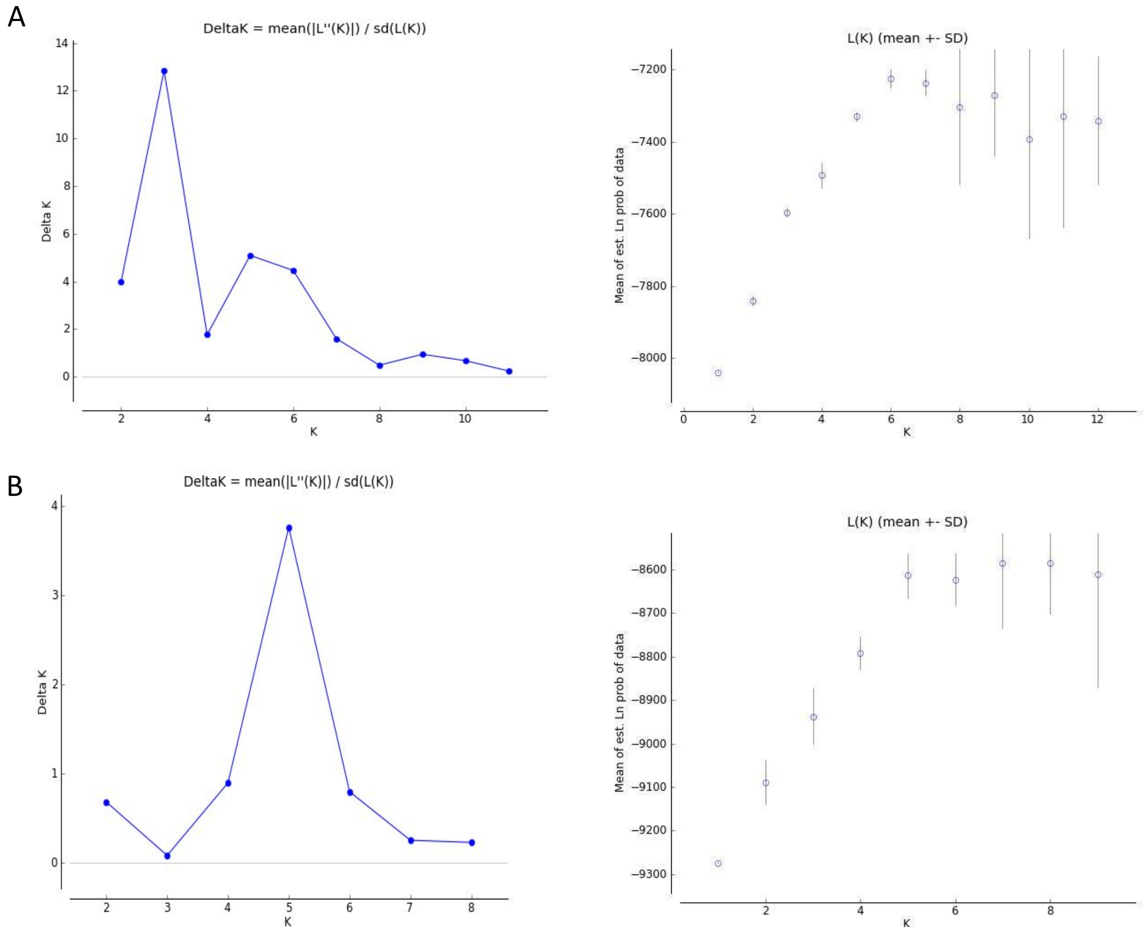


Fig. S4. Plots of delta K (left) and ln Pr(X|K) (mean ± SD; right) for each of K=1 through K_{max} for A) all glabrous *Metrosideros* taxa on O`ahu minus *M. polymorpha* race *prostrata*, and B) all pubescent taxa plus *M. polymorpha* race *prostrata*.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

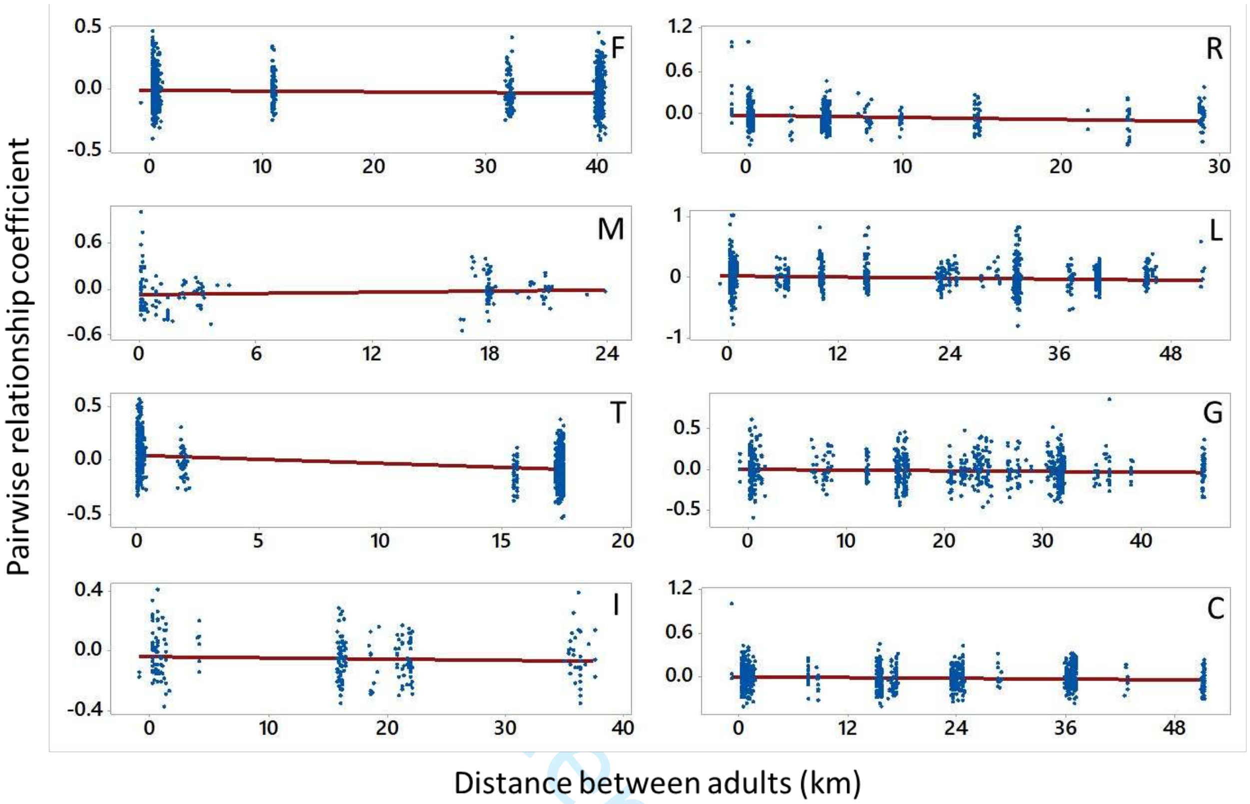


Fig. S5. Relationship between pairwise relationship coefficient and distance for adults of each of eight *Metrosideros* taxa on O`ahu. There is no pattern of isolation by distance for any taxon. Full taxon names are in Table 1.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

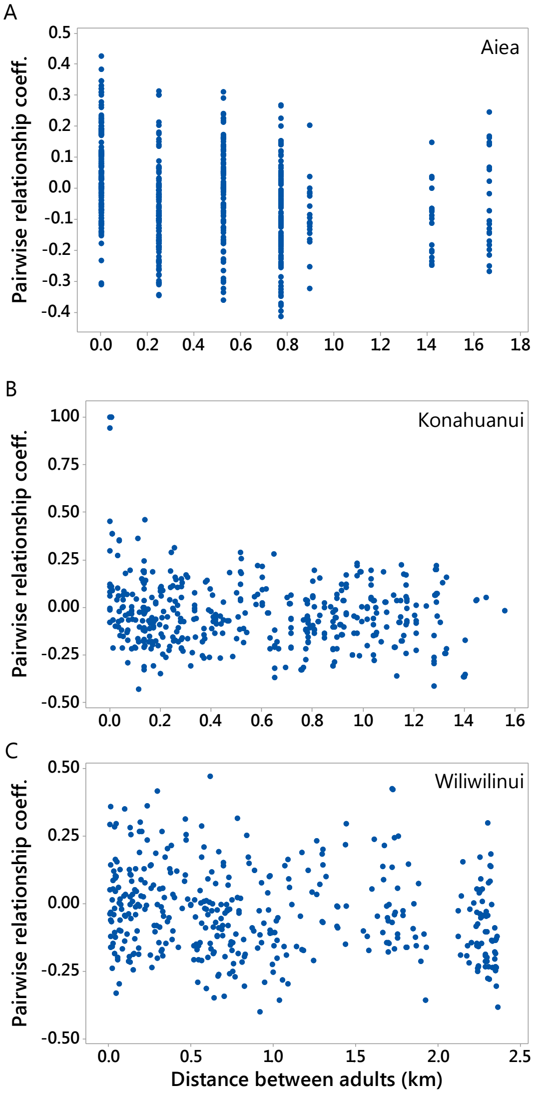


Fig. S6. Relationship between pairwise relationship coefficient (Queller and Goodnight 1989) and geographic distance between adults along each of three Ko`olau leeward ridges (elevation gradients). Shown are data for pubescent taxa only.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

APPENDIX

Tentative key to species, varieties, and morphological forms of *Metrosideros* on O`ahu

This tentative key is based on extended field observations by the first author and associates (Fig. 3). It uses names for taxa that can be confidently matched to current descriptions and type specimens (Dawson and Stemmermann 1990) and the term "race" for those forms for which the appropriate names (based on a type specimen) have not been resolved. The characters indicated here are based on our collections and field observations and may depart to some degree from those recognized for taxa accepted in Dawson and Stemmermann (1990). Unlike many keys, this key includes observations from the field that typically are unavailable, or are cryptic, on dried herbarium specimens (Snow 1997), such as the color of fresh material, predominant elevation range (based on field observations; see Fig. 4 in main text), and position on the landscape. *In sicco* and *in vivo* refer to dried and living material, respectively. The color of flowers (predominantly stamens, also style and petals) is red unless specified otherwise. Descriptive terminology (including those for shapes, indumentum, and colors) follows Beentje (2010).

1. Lower surface of (fully developed) leaves glabrous (=hairless)……………………….……………..……2
1. Lower surface of (fully developed) leaves densely hairy………………...…….………………………….…9

2. Petioles ≥ 6 mm long, narrowly sulcate (=grooved) distally……………………………………..……..…3
2. Petioles 4 mm or less long …..…………………..……………..………………….……………..……4

3. Petioles 6–10 mm long, typically somewhat winged distally; leaf blades 2.5–4.5 x 0.7–1.5 cm, narrowly elliptic, base narrowly cuneate, apex attenuate; occurring on steep windy slopes …………………………………………………………………………………………….. *M. tremuloides*
3. Petioles 15–35 mm long, lacking wings distally; leaf blades elliptic to ovate 5.5–8.0 x 2.0–5.0 cm, base slightly cuneate, apex acute (or sometimes slightly falcate-twisted)…………………….….….. *M. macropus*

4. Plants prostrate with upright stems mostly ≤ 15 cm tall; leaf blades (0.5–)1.2–2.6 x (0.4–)0.8–1.4 cm.……………...…...……………………………………………….. *M. polymorpha* race *prostrata*
4. Plants erect and stems > 15 cm tall; leaf blade dimensions mostly greater than 2.6 x 1.4 cm….………5

5. Inflorescence axis (especially on emergence) and hypanthium (=area below petals) densely silvery pubescent; Ko`olau Volcano only ……………………………………….….. *M. polymorpha* race B
5. Inflorescence axis and hypanthium glabrous (or hypanthium sericeous proximally becoming glabrous in fruit)……………………………………………………………………………...…….………..6

6. Hypanthium sparsely sericeous (=appressed short-hairy) proximally becoming glabrous in fruit; leaf blades 1.3–2.4 x 0.8–2.0 cm, narrowly elliptic to elliptic or obovate….……...... *M. polymorpha* race Z
6. Hypanthium and fruit glabrous; leaf blades mostly greater than 2.5 x 2.0 cm, never narrowly elliptic…7

7. Plants restricted to summit of Wai`anae Volcano (>1,100 m); petioles up to 4 mm long; apex of leaf buds ovoid…………………………………………..………..……………. *M. polymorpha* race S
7. Plants on Wai`anae or Ko`olau Volcano (ca. 400–1,000 m); petioles all ≥3 mm; apex of buds rounded ……………………………………………………………………………………………….……8

8. Emerging leaves deep burgundy or magenta; upper surface of petiole typically with a slight longitudinal ridge proximally (= towards the base); leaf blades (2.0–)2.5–3.7 x (1.0–)1.5–2.4 cm (tending towards the lesser dimensions indicated), elliptic, base mostly cuneate to less frequently

rounded, apex obtuse (mostly) to acute; elevation > ca. 590 m (Ko`olau) and > 1,100 m (Wai`anae) ……………………...………………………..…………………….……..…… *M. polymorpha* race L

8\. Emerging leaves pinkish to lighter magenta; upper surface of petioles typically sulcate (grooved) distally (=near apex); leaf blades (2.0–)2.5–6.5 x 1.1–3.4 cm (tending towards the greater dimensions indicated), ovate to mostly elliptic-oblong, base rounded (infrequently truncate or slightly cordate), apex obtuse to acute; elevation < ca. 640 m on Ko`olau leeward ridges and < ca. 1,000 m on Wai`anae Volcano…………………………………...….…………… *M. polymorpha* var. *glaberrima*

9 (1). Pubescence on midvein of lower leaf surface less frequently distinctly straighter, longer or more erect compared to the more compacted and shorter pubescence of the blades; leaf blades stiffly coriaceous……….………………………………………………………………….…………………10

9\. Pubescence on midvein of lower leaf surface typically straighter, longer and more erect (especially towards the base) compared to the more compacted and shorter pubescence of the blades; leaf blades coriaceous to stiffly coriaceous…………………………………….…………………………………………….………11

10\. Leaf margin (or most of blade from midvein outward) strongly recurved to revolute (or rarely nearly conduplicate); secondary veins (in sicco) of upper leaf surface slightly projecting; indumentum of lower leaf surface pubescent (i.e., hairs mostly straight and more or less erect) on midvein, shorter and lanate on blades; < ca. 620 m on Ko`olau Volcano and < ca. 930 m on Wai`anae Volcano ……………....……………………………………………………………….. *M. polymorpha* race C

10\. Leaf margins stiffly but only slightly recurved; secondary veins (in sicco) of upper leaf surface (typically strongly) impressed (especially the more proximal ones); indumentum of lower leaf surface more or less uniformly sericeous-lanate on midvein and blade and of only slightly greater length towards base of midvein; > ca. 600 m on Ko`olau Volcano and > ca. 940 m on Wai`anae Volcano ………………………………….…………………………….…………………..…… *M. polymorpha* race F

11\. Leaf margin moderately and stiffly recurved; secondary veins (in sicco) of upper leaf surface (typically strongly) impressed (especially the more proximal ones) and projecting prominently to the margins on lower surface; hairs of upper leaf surface dense typically russet (reddish-brown) to buff (dull yellow-brown) in color; plants along the backbone of Ko`olau Volcano, extending slightly down leeward ridges impacted directly by trade winds……………..………………………………*M. rugosa*

11\. Leaf margin (usually) flat to slightly recurved; secondary veins (in sicco) of upper leaf blade surface usually slightly projecting; hairs of lower leaf surface uniformly (on midvein and blade) shortly lanate and grayish; restricted to lower (drier) elevations…….……………………*M. polymorpha* var. *incana*

Key field traits of *Metrosideros* on O`ahu.

Metrosideros macropus: Shrubs or tree-like shrubs 1.5-4(–7) m; leaf blades glabrous; unmistakable by its long petioles (1.5–3.5 cm) and relatively large and broadly ovate, blades (up to 7 x 5 cm), young leaf buds somewhat mucilaginous when crushed; petals typically yellow or occasionally red; uncommon, typically occurring in scattered groups of one to a few individuals in wet forest; ca. 480–780 m on Ko`olau Volcano, predominantly >1,000 m on Wai`anae Volcano.

Metrosideros polymorpha var. *glaberrima*: Shrubs, tree-like shrubs, or trees 1.5–7 m; leaf blades glabrous; emerging leaves and branchlets pinkish to magenta; upper surface of petiole somewhat grooved towards the base; blades up to 6.5 x 3.4 cm, the base rounded (or truncate or cordate), the apex obtuse to acute; inflorescence axis glabrous; ca. 400–640 m on Ko`olau leeward ridges, ca. 650–1,000 m on Wai`anae Volcano.

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

Metrosideros polymorpha var. *incana*: Shrubs, tree-like shrubs, or trees 1.5–7 m; leaf blades densely hairy below; secondary veins of the upper leaf surface flush; the hairs at the base of the midvein on lower surface typically distinctly longer and straighter than the hairs on the adjacent blade; leaf surface flat, the margin flat to slightly recurved; ca. 290–550 m on Ko`olau leeward ridges, known from lower elevations on Wai`anae Volcano but possibly more widespread.

Metrosideros polymorpha race *prostrata*: Prostrate shrubs, the stems growing more or less horizontal and branches growing vertically, mostly less than 15 cm tall, and typically enshrouded in thick moss (the leaves and flowers emerging just above the mossy layers); leaves glabrous, ≤ 1.6 cm long, dark green (in vivo); horizontal stems typically hidden under thick moss; restricted to high-elevation sites on leeward ridges exposed to extreme wind; ca. 740–800 m (or higher; J. Lau, pers. comm.) on Ko`olau Volcano.

Metrosideros polymorpha race B: Shrubs 1.5–2.5 m; emerging leaves pubescent but becoming glabrous at maturity; inflorescence axis and hypanthium silvery-pubescent; flowering predominantly December and January; ca. 680–950 m on Ko`olau Volcano along the backbone, extending down leeward ridges.

Metrosideros polymorpha race C: Trees 3–7 m; leaves hairy (hairs whitish or cream-colored), the margins strongly recurved or upper surface nearly entirely recurved, upper surface often dark green (in vivo), the secondary veins sometimes impressed towards base of blade (less so in sicco); hypanthium densely lanate with whitish hairs; flowers relatively large and firm (not thinly membranous), ca. 480–620 m on Ko`olau leeward ridges, ca. 680–930 on Wai`anae Volcano.

Metrosideros polymorpha race F: A tree or tree-like shrub 1.5–3 m; leaves hairy, the margins stiff but only slightly recurved; secondary veins of upper leaf surface strongly impressed, especially those near base of blade (typically less strongly impressed as in *M. rugosa*); on Wai`anae Volcano young leaves often maroon-magenta, pubescence often extending to upper leaf surface and margin; inflorescence axis densely hairy, with the hairs longer than and the inflorescence axis thicker than that of race B; ca. 600–700 m along Ko'olau leeward ridges and 940 m to summit of Wai`anae Volcano.

Metrosideros polymorpha race L; Shrubs or tree-like shrubs 2–3 m; leaves not or only slightly congested at branch tips (many internodes > 5 mm [compare below with race Z]), leaves glabrous, typically greater than 2.5 cm long, margins flat; emerging leaves deep burgundy or magenta; crown markedly uneven in profile and often carrying a substantial load of psyllid galls; inflorescence axis somewhat thin like that of race B but glabrous and the base of blade cuneate to rounded (vs. rounded to cordate in race B); flowers typically red but may be orange or yellow; ca. 550–850 m on Ko`olau leeward ridges and summit of Wai`anae Volcano (Mt. Ka`ala), occasionally lower.

Metrosideros polymorpha race S: Shrubs or tree-like shrubs 1.5–2 m; leaf bud up to 2.5 cm long, the apex ovoid; petioles up to 4 mm long; leaf blades (1.0–)1.5–4.5 x 1.2–3.1 cm, glabrous, base rounded or slightly cuneate, margins flat; restricted to summit of Wai`anae Volcano (Mt. Ka`ala).

Metrosideros polymorpha race Z: Tree-like shrubs 1–2 m with an even dome-like crown (resembling the top of cultivated broccoli); leaves typically congested at branch tips (many to most internodes < 5 mm), glabrous, less than 2.5 cm long, the margins flat, typically free of galls; emerging leaves green; hypanthium sparsely sericeous but fruits glabrous; flowers red, orange or yellow; ca. 650–780 m, reaching the backbone on Ko`olau Volcano and summit of Wai`anae (Mt. Ka`ala).

Author 1_SupMat_description (e.g., _table, _figure), followed by the article's Running Title and JHered

Metrosideros rugosa: Shrubs or tree-like shrubs 1.5–3 m; upper leaf blade surface often dark green (in vivo), the secondary veins prominent but deeply impressed; leaf blades densely hairy below, the hairs typically reddish-brown to yellowish-brown, and those of the base and lower part of the lower midvein somewhat longer or straighter compared to those of the blade itself; leaf margin moderately and stiffly recurved; ca. 650–860 m on Ko`olau Volcano, along the backbone, extending slightly down leeward ridges impacted directly by trade winds.

Metrosideros tremuloides: Shrubs, tree-like shrubs, or trees 1.5–7 m; branches relatively thin and pendant, often somewhat tangled amongst themselves; leaf blades glabrous; easily distinguished by its relatively long petioles (0.6–1.0 cm) narrowly elliptic, blades with cuneate bases and attenuate apices, leaves larger on Wai`anae; predominantly on steep windy slopes of eroded ridges of both Volcanoes; ca. 500–720 m on Ko`olau Volcano, ca. 900–1,000 m on Wai`anae Volcano.