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ORIGINAL ARTICLE

Incipient radiation within the dominant Hawaiian tree *Metrosideros polymorpha*

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Although trees comprise a primary component of terrestrial species richness, the drivers and temporal scale of divergence in trees remain poorly understood. We examined the landscape-dominant tree, *Metrosideros polymorpha*, for variation at nine microsatellite loci across 23 populations on young Hawai'i Island, sampling each of the island's five varieties throughout its full geographic range. For four varieties, principal coordinate analysis revealed strong clustering of populations by variety across the 10 430 km^2 island, indicating partitioning of the species into multiple evolutionarily significant units. The single island-endemic form, riparian var. *newellii*, showed especially strong differentiation from other varieties despite occurring in sympatry with other varieties and likely evolved from a bog form on the oldest volcano, Kohala, within the past 500 000 years. Along with comparable riparian forms on other Pacific Islands, var. *newellii* appears to represent parallel incipient ecological speciation within *Metrosideros*. Greater genetic distance among the more common varieties on the oldest volcano and an inverse relationship between allelic diversity and substrate age appear consistent with colonization of Hawai'i Island by older, partially diverged varieties followed by increased hybridization among varieties on younger volcanoes. This study demonstrates that broad population-level sampling is required to uncover patterns of diversification within a ubiquitous and long-lived tree species. Hawaiian *Metrosideros* appears to be a case of incipient radiation in trees and thus should be useful for studies of divergence and the evolution of reproductive isolating barriers at the early stages of speciation.
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INTRODUCTION

The Hawaiian biota is a popular model for understanding evolution and speciation. Although phylogenetic and phylogeographic studies of native Hawaiian taxa have shed light on the patterns and drivers of divergence in plants (for example, Silverswords (Baldwin, 1997)), little insight has been gained for Hawaii's canopy trees. The causes of divergence, and the spatial and temporal scales over which divergence occurs in trees, remain poorly understood. Although rapid local adaptation is possible in tree populations experiencing novel environments even in the presence of gene flow (Petit and Hampe, 2006), neutral genetic divergence, a measure of isolation of gene pools, is typically weak between populations of trees even over expansive scales (Loveless and Hamrick, 1984; Petit and Hampe, 2006). Genetic differentiation is greater among populations of tropical trees relative to temperate tree species; however, this trend disappears when wind-pollinated species and species influenced by strong biogeographic barriers are excluded (Dick *et al.* 2008).

For dominant, continuously distributed tree species, significant differentiation at neutral genetic loci is expected only where reproductive isolating barriers have evolved between parapatric populations spanning environmental gradients or ecotones or where strong selection acts against migrants from foreign ecotypes (Endler, 1977). The evolution of prezygotic reproductive barriers between diverging populations of a tree species is expected to be slow, as evident in the

hybridization frequently observed between closely related species (for example, Howard *et al.*, 1997). Weak reproductive barriers in trees are attributed in large part to the tremendous fecundity of trees. The production of large numbers of seeds over many reproductive seasons may decrease the consequences of occasional hybridization (through lost gametes) for individual life-time fitness (Calvo and Horvitz, 1990). As a result, selection against unfit hybrid offspring (Petit and Hampe, 2006) and migrants from ecologically diverged populations (Le Corre and Kremer, 2003) may be the primary agent promoting phenotypic, and ultimately neutral genetic, differences between closely related woody taxa.

The dominant and highly variable tree species, *Metrosideros polymorpha* ('ōhi'a lehua), comprises several varieties that together span seven of Hawaii's ten terrestrial climate zones (Juvik *et al.*, 1978; Dawson and Stemmermann, 1990) and allow studies of the patterns and causes of divergence in trees. Hawaiian *Metrosideros* is a complex of woody taxa with an approximately 4-million-year history in the Hawaiian Islands (Percy *et al.*, 2008) that likely derives from a single colonist on Kaua'i from the South Pacific by way of the Marquesas Islands (Wright *et al.*, 2001; Percy *et al.*, 2008). The genus has spread to all of the main Hawaiian Islands and diversified into five species, including the widespread and hypervariable *M. polymorpha* Gaud. whose eight named varieties are distinguished by vegetative characters (Dawson and Stemmermann, 1990). All *Metrosideros* taxa are

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pollinated by insects and birds and dispersed by wind (Dawson and Stemmermann, 1990).

Weak or ambiguous neutral genetic differentiation observed among the varieties of *M. polymorpha* (James *et al.*, 2004; Percy *et al.*, 2008; Wright and Ranker, 2010) has been ascribed to hybridization, extreme phenotypic plasticity and even extensive inter-island dispersal of seeds or pollen (Harbaugh *et al.*, 2009), and further has led to the suggestion that varieties be dissolved (James *et al.*, 2004, Harbaugh *et al.*, 2009). In contrast, the first microsatellite study of *Metrosideros* to use population-level sampling revealed significant partitioning among the three most common varieties on east Hawai'i Island (DeBoer and Stacy, 2013), and other studies have documented heritable morphologic (Corn and Hiesey, 1973; Stemmermann, 1983; Cordell *et al.*, 1998), ecological (Morrison and Stacy, 2014) and physiological (Hoof *et al.*, 2008) differences among varieties.

Metrosideros on Hawai'i Island, the youngest, largest (10 430 km^2) and tallest (4205 m) of the islands, comprises only *M. polymorpha*, yet this species dominates the native landscape from sea level to nearly 2500 m in elevation (Dawson and Stemmermann, 1990), occurring continuously where rainfall exceeds ~50 cm per year (Stemmermann and Ihsle, 1993). Hawai'i island comprises five volcanoes, Kohala (0.6 ma), Hualalai (0.4 ma), Mauna Kea (0.4 ma), Mauna Loa (0.1 ma) and Kilauea (0.05–0.07 ma; (U.S. Geological Survey 2007)). Thus, *M. polymorpha* on Hawai'i Island is younger than ~600 000 years old, deriving from one or more older islands (Percy *et al.*, 2008). On Hawai'i Island the species comprises just five varieties that, to varying degrees, parse out the striking environmental gradients created by young volcanoes, including active Kilauea and Mauna Loa (Dawson and Stemmermann, 1990; Stemmermann, 1983). Whereas one of the island's five varieties is endemic and presumed to have arisen on the island, the origin of the four other varieties (multiple colonizations from older island(s) versus *in situ* origin from a single colonist) is not known. The common varieties of *M. polymorpha* on Hawai'i Island share a single common chloroplast haplotype (Percy *et al.*, 2008), but this may be explained by hybridization (and chloroplast capture) among varieties that colonized the island independently followed by selective sweeps at chloroplast loci (Percy *et al.*, 2008), rather than by coalescence of all Hawai'i Island forms to a single island colonist. Regardless of the exact history of colonization, the initial colonizer(s) of Hawai'i island presumably landed on Kohala (Figure 1), the oldest volcano and once vast northwest region that has experienced significant erosion and loss of area over the past 500 000 years (Price and Clague, 2002). Given the ability of *M. polymorpha* to colonize new lava flows (Stemmermann, 1983), the species likely spread to each of the four younger volcanoes shortly after subaerial surfaces appeared. As such, we might expect an examination of neutral genetic variation in *M. polymorpha* across Hawai'i Island's well documented, 0.5-million-year substrate age gradient to reveal a signature of repeated population bottlenecks or expansion.

In this study, we examine the distribution of variation at nine microsatellite loci within *M. polymorpha* across the youngest Hawaiian island. We use neutral markers and sample each variety throughout its full geographic range on the island to address the following questions: is genetic variation across the island partitioned largely by variety, indicating that varieties are valid evolutionary units with partially isolated gene pools? Or is genetic variation partitioned more by geography, indicating that local hybridization among varieties and isolation by distance determine genetic structure in this species? In addition, because one of the island's five varieties is endemic to the island, we examined this form to estimate the degree of genetic
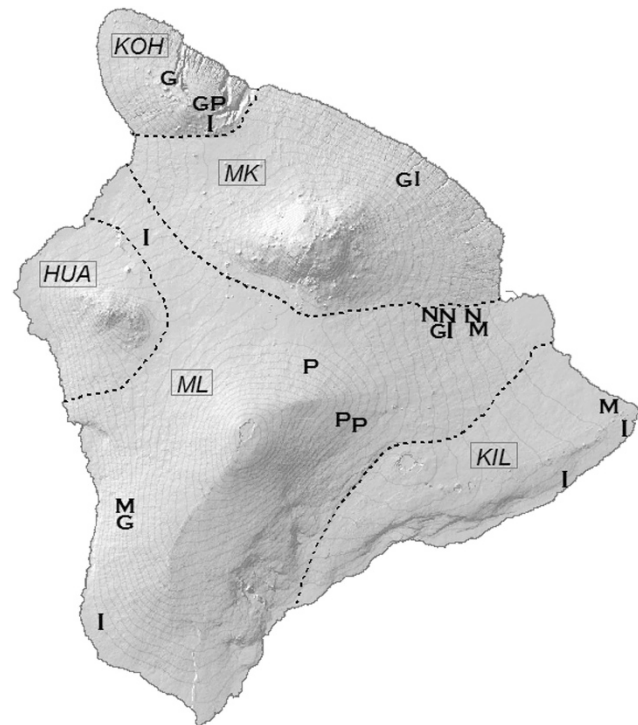
Figure 1 Locations of 23 populations of *M. polymorpha* sampled from four volcanoes: Kohala, Mauna Kea, Mauna Loa and Kilauea on Hawai'i Island. Dotted lines depict the approximate boundaries between surface flows from adjacent volcanoes. No sampling was done from Hualalai.

differentiation possible within a long-lived, continuously distributed and highly dispersible tree species within ~500 000 years. Lastly, we examine genetic variation and structure across the island for signatures left behind from the colonization of a new island and subsequent spread to its younger volcanoes.

MATERIALS AND METHODS
Hawai'i Island varieties of *M. polymorpha*

Metrosideros polymorpha in its many forms dominates Hawai'i island below ~2500 m. Although monotypic stands of each variety exist, because of the continuous distribution of the species on the island, zones of overlap are common. In such zones, hybridization among varieties occurs, especially at middle elevations (Corn and Hiesey, 1973; E. Stacy, pers. obs.). The two most common varieties of *M. polymorpha* on Hawai'i island are pubescent var. *incana* and glabrous var. *glaberrima*, successional forms that dominate early successional or otherwise dry areas and late successional or otherwise wet areas, respectively (Stemmermann, 1983; Dawson and Stemmermann, 1990) below ~1200 m elevation. Variety *polymorpha* dominates high elevations (>1400 m) and is characterized by a more compact growth form, darker pigmentation, stronger pubescence and a revolute leaf margin (Dawson and Stemmermann, 1990; E. Stacy, pers. obs.). Variety *macrophylla* is a glabrous mid-elevation, wet-forest form characterized by long strips of flaking bark and large leaves. All four varieties above, as defined by morphology, occur also on the older main Hawaiian Islands (var. *incana* and var. *glaberrima* on all islands; var. *polymorpha* on Maui and Moloka'i; var. *macrophylla* on Maui). The fifth variety is endemic to Hawai'i Island; var. *newellii* is a glabrous, more darkly pigmented, form endemic to waterways on the island's windward (east) coast below ~1000 m elevation (Dawson and Stemmermann, 1990; E. Stacy, pers. obs.). Because of its highly restricted range, var. *newellii* is listed as vulnerable in the IUCN Red List (World Conservation Monitoring Centre 1998). All varieties may be large trees (>70 cm dbh), except riparian var. *newellii* and var. *polymorpha* at very high elevations, which are often stunted.

Sampling
Leaf buds were sampled from 404 adult trees (10–20 per population; mean: 17.5) from 23 populations comprising the five varieties currently recognized on Hawai'i Island (Dawson and Stemmermann, 1990; Table 1). Each variety was sampled from its full geographic/elevational range within the island (Table 1, Figure 1) and typically sampled from within monotypic stands (where >95% of trees are of a single variety) within the island's near-continuous population of *M. polymorpha*. Where sampling was done from mixed-variety stands, apparent hybrids were avoided (Table 1). Within each population, sampled trees were well dispersed (minimum distance between trees was ∼20 m). Leaf buds were transported to UH Hilo within 6 h of collection and stored at −80 °C before DNA extraction.

DNA extraction
Frozen leaf material was homogenized with Lysing Matrix A tubes in the FastPrep-24 Instrument for 40 s at 4 m s^{-1} (MP Biomedicals, Santa Ana, CA, USA). DNA was extracted from the lysate using a Qiagen DNeasy Plant Mini Kit following the manufacturer's protocol with the following modifications: extension of cell lysis incubation time to 15 min, a second centrifuging added to completely dry the membrane after washing and a decrease in the elution buffer to 75 μl. Extracted DNA was precipitated with 100% ethanol and 3 M Na-acetate (pH 5.2), vacuum dried, washed with 70% ethanol and resuspended in elution buffer (Qiagen, Valencia, CA, USA).

Microsatellite genotyping and haplotype sequencing
Nine previously published microsatellite loci were amplified: MePo501, MePo503, MePo506, MePo508, MePo511, MePo512, MePo513, MePo514 and MePo515 (Crawford *et al.*, 2008). PCR was performed in a 10-μl volume (1× GoTaq Flexi buffer, 2 mM MgCl$_2$, 25 μM each dNTP, 0.05 μl *Taq* polymerase (Promega, Madison, WI, USA), 0.4 μM of a dye-labeled forward primer and an unlabeled reverse primer and 1.5 μl DNA) following Crawford

et al. (2008), with the exceptions of an initial annealing temperature of 55 °C and a final extension step at 72 °C for 10 min. PCR products were separated in 1.5% agarose gels, visualized by SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) and sized on a Beckman-Coulter (Fullerton, CA, USA) CEQ8000. Genotypes were determined using CEQ8000 Software, version 8.0. Four of the plastid genes investigated by Percy *et al.* (2008) are sufficient to discriminate the different islands and were sequenced using the same protocols in one accession of var. *newelli* (Genbank accession numbers: *trnD-trnT*: KJ659776, *trnS-trnG*: KJ659782, *rps16*: KJ659770, *rpl16*: KJ659764).

Genetic diversity
Each locus was tested for the presence of null alleles using Micro-checker (Oosterhout *et al.*, 2004). Standard measures of genetic diversity within populations and varieties (populations pooled) were calculated using Genepop (Raymond and Rousset, 1998; Rousset, 2008), and inbreeding (F_{IS}) was calculated with INEst (using the Individual Inbreeding Model), which estimates population-level F_{IS} and null-allele frequencies simultaneously (Chybicki and Burczyk, 2009). The modified Garza-Williamson index was calculated as (total number of unique alleles)/(allele size range + 1) averaged across loci (Garza and Williamson, 2001). To compare allelic richness (AR) and private allele richness (PAR) among unevenly sampled populations and varieties, we conducted rarefaction of alleles using ADZE 1.0 (Szpiech *et al.*, 2008). To examine variation in genetic diversity across the island, we examined population-level genetic variation and inbreeding across lava flows of different ages through regression and analysis of covariance (ANCOVA) tests treating log substrate age as a covariate. Where populations spanned more than one lava flow, the age of the most recent flow was used.

Genetic structure
Pairwise F_{ST} values were calculated among populations using Genepop and then used for principal coordinate analysis in PAST (Hammer *et al.*, 2001) and

Table 1 Characteristics of 23 populations of five varieties of *M. polymorpha*: var. *glaberrima* (G), var. *incana* (I), var. *macrophylla* (M), var. *newellii* (N) and var. *polymorpha* (P) from four volcanoes: ML = Mauna Loa (ML), Mauna Kea (MK), Kohala (KOH) and Kilauea (KIL) on Hawai'i Island

Population	Number of adults sampled	Variety	Stand composition	Volcano	Flow age (1000 years)	Elevation (m above sea level)	Forest Type	GPS coordinates
Alakahi	20	P	M	KOH	120–230	1222	Bog	20.0677–155.6713
Alakahi	20	G	M	KOH	120–230	1222	Bog	20.0677–155.6713
Kukui'opae	20	G	M	ML	1.5–3	1050	Wet	19.3079–155.8382
Kukui'opae	15	M	M	ML	1.5–3	1050	Wet	19.3079–155.8382
Saddle Rd.	20	I	M	ML	0.156	894	Wet	19.6999–155.2322
Saddle Rd.	20	G	M	ML	0.156	894	Wet	19.6999–155.2322
Wailuku-A[a]	20	N	M	ML	>10	904	Wet	19.7094–155.2336
Wailuku-D	8	N	M	ML	3–5	729	Wet	19.7038–155.1977
Kaiwilahilahi	15	G	M	MK	65–25	539	Wet	19.9513–155.2403
Kaiwilahilahi	14	I	M	MK	65–250	539	Wet	19.9513–155.2403
Mauna Loa-A	20	P	D	ML	1.5–3	2104	Subalpine Dry	19.4942–155.3866
Mauna Loa-B	20	P	D	ML	1.5–3	2104	Subalpine Dry	19.4923–155.3840
Kalohewahewa	15	N	M	ML	3–5	620	Wet	19.9513–155.1847
Halepua'a	15	M	M	KIL	0.75–1.5	98	Wet	19.5215–154.8713
Kaumana	16	M	M	ML	130	450	Wet	19.6819–155.1536
Pu'u Pelu	20	I	D	KOH	120–230	1068	Wet	20.0406–155.6785
S. Kohala	20	I	P	ML	1.5–3	721	Dry	19.8336–155.8035
Manuka	15	I	D	ML	1.5–3	210	Dry	19.1057–155.8725
Hilea	20	I	P	ML	0.2–0.75	65	Dry	19.1274–155.5231
Kaimu	15	I	P	KIL	0.2–0.4	3	Mesic	19.3745–154.9469
Kapoho	16	I	P	KIL	0.4–0.75	31	Mesic	19.4803–154.8329
Kanea'a	20	G	D	KOH	120–230	997	Cloud	20.1374–155.7476
'Aina Hou	20	P	D	ML	0.13	2458	Subalpine Dry	19.5963–155.4624

Stand composition describes the relative dominance of the targeted variety at the site: pure (P) = one variety present, dominant (D) = primarily (>95% of stems) a single variety, or mixed (M) = two varieties abundant at the site.
[a]The Wailuku-A population of riparian var. *newellii* is adjacent to the Saddle Rd. populations of vars. *incana* and *glaberrima*.

to draw a neighbornet network in Splitstree (Huson and Bryant, 2006). STRUCTURE version 2.3.1 was used to determine the number of genetic groups (K) using the default settings with an admixture model; a series of analyses was performed from K = 1 to 20, by setting 10 000 burn-in and 100 000 repetitions with five iterations per K (Pritchard et al., 2000). The delta K method (Evanno et al., 2005) was used to determine the most likely number of clusters. Genetic differentiation among varieties was tested by AMOVA using Arlequin 3.5.

Isolation by distance
Isolation by distance tests using 1000 permutations were done for all 23 populations using Genepop. Follow-on isolation by distance tests were done for the most widespread variety, early-successional var. incana, for which eight populations were sampled from throughout the island, and for the eight combined populations of late-successional var. glaberrima and var. macrophylla. Lastly, to help distinguish the relative importance of variety and geographic distance between populations in the partitioning of genetic variation, we compared isolation by distance patterns for pairs of populations of the same and different varieties.

RESULTS
Null alleles and within-population genetic variation
Significant null alleles were detected at two of the nine loci: 11% at MePo503 and 22% at MePo506. These loci contributed disproportionately to estimates of F_{IS}; thus, F_{IS} values were calculated using INEst. In contrast, these loci did not contribute disproportionately to estimates of F_{ST}; thus, F_{ST} values are reported as averages across all nine loci. The frequency of null alleles did not vary significantly across populations or varieties.

With the 23 populations pooled, the number of alleles per locus ranged from 7 to 50 (mean = 25.2). Each locus showed significant linkage disequilibrium with at least one other locus; however, associations between loci were not consistent across populations. Allelic richness (AR) averaged across loci ranged widely across populations from 3.45 (var. newellii-Wailuku-D) to 7.25 (var. macrophylla-Halepua'a), and private allelic richness (PAR) from 0.022 (var. newellii-Wailuku-A) to 0.412 (var. macrophylla-Halepua'a; Supplementary Table S1). Average expected heterozygosity (H_E) was moderate to high, ranging from 0.498 (var. newellii-Wailuku-A) to 0.775 (var. macrophylla-Halepua'a; Supplementary Table S1). Average inbreeding coefficients calculated using INEst ranged from 0.016 (var. polymorpha-Alakahi) to 0.059 (var. incana-Kapoho; Supplementary Table S1). Low F_{IS} values were consistent with the outcrossed breeding system and high genetic load expected for abundant tree species. The modified Garza-Williamson index averaged across populations (0.66) was slightly below the threshold used to detect population bottlenecks (0.68; Garza & Williamson, 2001), ranging from 0.57 to 0.58 for var. newellii-Wailuku-D and adjacent var. glaberrima-Saddle Rd. to 0.75 for var. polymorpha-Alakahi in Kohala

and var. glaberrima-Kukui'opae in the southwest of the island (Supplementary Table S1). Fifteen of the 23 populations had M-values less than 0.66, consistent with slight population bottlenecks (Supplementary Table S1).

Within-variety genetic variation
With populations pooled by variety, the average number of alleles per locus per variety ranged from ~9 to 20, AR and PAR from 7.1 to 12.6 and 0.5 to 1.7, respectively, and H_O and H_E from 0.52 to 0.66 and 0.63 to 0.78, respectively (per 43 individuals; Table 2). All measures of genetic variation were lowest for the endemic, riparian var. newellii (Table 2). Inbreeding coefficients calculated using INEst and averaged across populations were similar across varieties (0.029–0.039; Table 2). Averaged across loci and varieties, the modified Garza-Williamson Index was moderate (0.78), highest for the two most common varieties, var. incana and var. glaberrima (> 0.82), and lowest for the island-endemic var. newellii (0.70; Table 2).

Genetic structure among varieties
With the exception of var. macrophylla, the principal coordinate analysis of mean pairwise F_{ST} values revealed a strong grouping of populations by variety regardless of sampling location. Further, most F_{ST} values for same variety pairs of populations were lower than most F_{ST} values for different variety pairs of populations regardless of geographic distance between populations (Figure 2). The three populations of the endemic, glabrous form, var. macrophylla, failed to cluster, with one population grouping with var. incana (Figure 3). The principal component analysis of individual multilocus genotypes (not shown) revealed overlap among varieties, with the greatest overlap between the common successional varieties incana and glaberrima. With the exception of one population of var. macrophylla, populations of the pubescent varieties clustered on one side and populations of glabrous forms grouped on the other (Figure 3); the presence of leaf pubescence explained a small but significant amount of variation (AMOVA: 2.4% (P < 0.001) with 5.6% of variation among populations within groups). The glabrous, late-successional var. glaberrima appeared in the center of the distribution (Figure 3). Overall, there was low, but significant genetic differentiation among the five named varieties on Hawai'i Island (3.9%, P < 0.001). Pooling var. macrophylla with the more common and ecologically indistinguishable glabrous form, var. glaberrima, from which it is discerned only by bark morphology and was not differentiated at microsatellite loci (analysis of variance (AMOVA): 0.86% variation between var. macrophylla and var. glaberrima with 3.25% variation among populations within varieties) yielded low, but significant genetic differentiation among the four remaining varieties on Hawai'i Island (4.83%, P < 0.001). Given its lack of differentiation from var. glaberrima, we pooled var. macrophylla with var. glaberrima for subsequent analyses.

Table 2 Variety-level statistics ($\pm$ s.d.) for five varieties of *M. polymorpha* on Hawai'i Island

Variety	N	A	AR	PAR	H_O	H_E	F_{IS}	M
newellii	43	8.78 ± 5.56	7.10 ± 1.42	0.48 ± 0.14	0.524 ± 0.176	0.632 ± 0.225	0.029	0.702
glaberrima	95	19.67 ± 14.78	12.23 ± 2.73	1.14 ± 0.35	0.609 ± 0.197	0.742 ± 0.24	0.039	0.829
macrophylla	46	16.33 ± 11.34	12.64 ± 2.68	1.69 ± 0.44	0.660 ± 0.197	0.780 ± 0.203	0.031	0.778
incana	140	19.78 ± 13.39	11.54 ± 2.56	1.02 ± 0.32	0.604 ± 0.191	0.720 ± 0.241	0.032	0.826
polymorpha	80	17.22 ± 13.74	11.10 ± 2.68	1.10 ± 0.50	0.638 ± 0.173	0.725 ± 0.237	0.029	0.748

All genetic diversity statistics are calculated with populations pooled within variety, and values are averaged across nine nuclear microsatellite loci. Inbreeding coefficients (F_{IS}) are calculated using INEst, which accounts for the presence of null alleles; F_{IS} values are averaged over both populations and loci. N is the total number of individuals sampled, and A is the average number of alleles observed. Average allelic richness (AR) and private allelic richness (PAR) per 43 individuals were calculated using ADZE, observed (H_O) and expected heterozygosity (H_E) were calculated using Genepop, and the modified Garza-Williamson Index (M) was calculated as $r/(k+1)$, such that r = the number of unique alleles and k = the allele size range (in number of repeats).
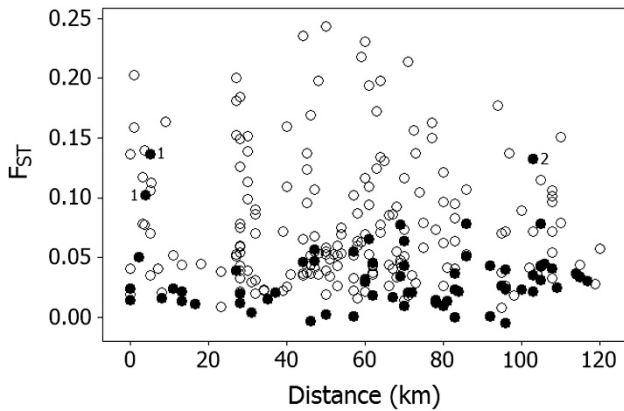
Figure 2 Relationship between geographic distance and pairwise F_{ST} for 23 populations of *M. polymorpha* on Hawai'i Island. F_{ST} values for same variety population pairs (closed circles) are typically lower than those for different variety population pairs (open circles) regardless of the geographic distance between populations. Exceptions include unusually high F_{ST} values[1] among the three small populations of the riparian endemic var. *newellii* likely due to genetic drift, and a high F_{ST} value[2] between var. *glaberrima*-Alakahi in Kohala and the var. *macrophylla* (*glaberrima*, see text)-Halepua'a on the southwest side of the island.
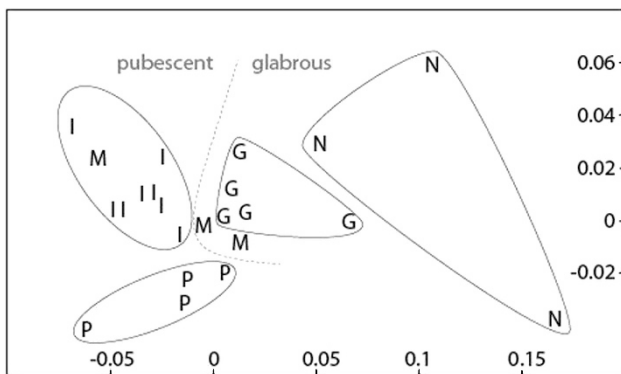
Figure 3 Principal coordinate analysis of pairwise F_{ST} values of 23 populations of five varieties of *M. polymorpha* on Hawai'i Island, using PAST software. Variety codes: G = var. *glaberrima*, I = var. *incana*, M = var. *macrophylla*, N = var. *newellii*, P = var. *polymorpha*. All populations, except those of M, cluster by variety. With one exception (M), the dashed curve separates pubescent populations from glabrous populations.

Lastly, the STRUCTURE analyses revealed weaker clustering of populations by variety. On the basis of the ΔK method, the two most likely numbers of clusters were $K = 19$ and $K = 6$ (Supplementary Figure S1); only the riparian endemic var. *newellii* appears as a distinct gene pool under $K = 6$. STRUCTURE may be less suitable for assessing relationships among populations compared with the other methods above, especially when the degree of differentiation varies across population pairs (Kalinowski, 2011).

The three populations of the Hawai'i Island endemic, var. *newellii*, showed strong differentiation from the other varieties (mean pairwise F_{ST} between var. *newellii* populations and other populations = 0.13, range = 0.04 to 0.25; Figure 4). The populations of this riparian variety, which were sampled from two rivers, comprised substantial genetic variation (pairwise F_{ST} values between var. *newellii* populations were 0.05, 0.102 and 0.137). The splitstree network analysis revealed the Kohala bog population of var. *glaberrima* at the base of
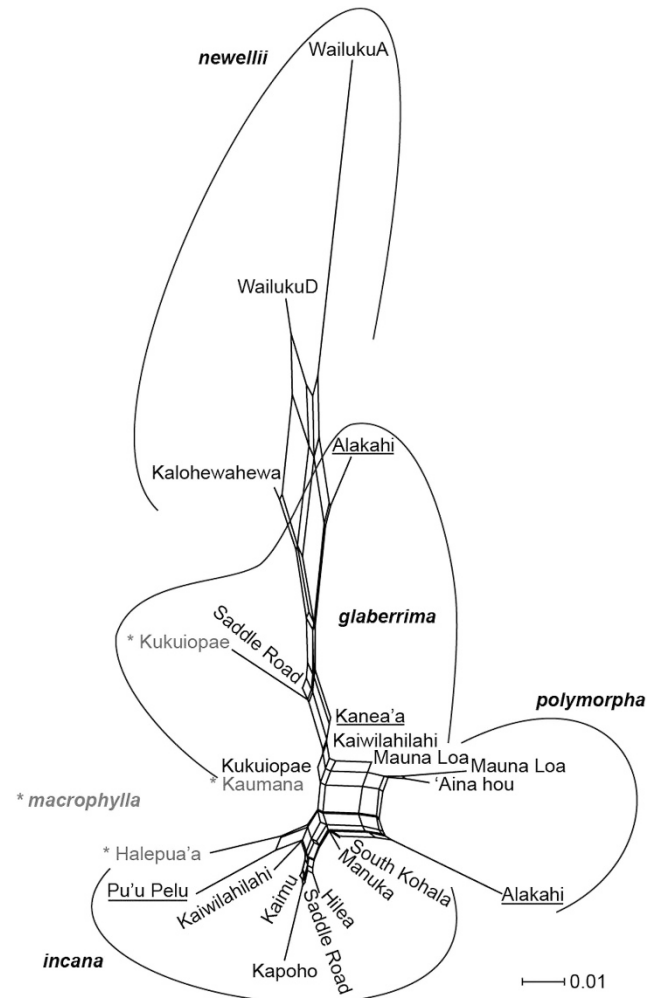
Figure 4 Splitstree network of 23 populations of five varieties of *M. polymorpha* from across Hawai'i Island. The positions of populations from the four supported varieties are indicated by curved lines and labels. The four populations from Kohala are underlined.

the branch comprising the three var. *newellii* populations and the southernmost up-river population (Wailuku-A) at the distal end of the branch (Figure 4). The Kohala bog population of var. *glaberrima* has the morphology of var. *glaberrima* and is positioned intermediately on the network between var. *newellii* and the remaining populations of var. *glaberrima*, suggesting a possible pathway for the origin of riparian var. *newellii* from a glabrous form on the oldest volcano, Kohala, followed by its spread southeastward to progressively more distant waterways along the windward coast of the island.

Kohala volcano effect

Of the three common varieties for which populations were sampled on both old and young volcanoes, among-variety divergence was greater on the oldest volcano, Kohala ($F_{ST} = 0.079$, $F_{ST} = 0.117$, $F_{ST} = 0.137$ for var. *incana*-var. *polymorpha*, var. *incana*-var. *glaberrima* and var. *polymorpha*-var. *glaberrima*, respectively) than on the younger volcanoes, where average between-variety F_{ST} values were 0.051, 0.029 (0.037 with var. *macrophylla* included) and 0.040 (0.042 with var. *macrophylla* included) for the same three comparisons (Figure 4). This pattern may result from the independent colonization

of Hawai'i Island by these three varieties followed by increased hybridization on young volcanoes.

Variation across substrate age

All population-level diversity statistics, including F_{IS} and the modified Garza-Williamson Index, were examined for a relationship with substrate age using the three varieties that occurred across the full range of substrate ages on Hawai'i Island (var. *incana*, var. *polymorpha* and var. *glaberrima* inclusive of var. *macrophylla*). With varieties pooled, allele size range decreased significantly with log substrate age (linear regression: $P = 0.028$; $R^2 = 20\%$; Supplementary Figure S2). The follow-on ANCOVA did not improve the model fit. Covariate analysis of allelic richness, however, revealed a significant inverse relationship with substrate age for all three common varieties ($P = 0.02$) and a greater allelic richness for var. *glaberrima* than for var. *incana* ($P = 0.001$; $R^2 = 52.4\%$; Figure 5). The increases in both measures of genetic diversity with decreasing substrate age are consistent with increased hybridization among the common varieties on young volcanoes. Covariate analysis of the modified Garza-Williamson index also revealed an inverse relationship with substrate age, though not significant ($P = 0.13$), a trend that may be explained by the decrease in allele size range with substrate age. Lastly, no relationship with substrate age was found for H_E, private allelic richness, or INEst-based F_{IS} values for varieties pooled or individually.

Isolation by distance

Because of the influence of variety on genetic structure, there was no pattern of isolation by distance in the analysis of all 23 populations sampled across the island (Mantel test: $P = 0.77$; Figure 2). However, analysis of the most widely distributed variety, var. *incana*, for which eight populations were sampled island-wide, revealed a significant pattern of isolation by distance (Mantel test: 1000 permutations; $P = 0.016$; Supplementary Figure S3a). Furthermore, after removal of the Kohala population (Pu'u Pelu), which showed relatively high differentiation from all other populations regardless of distance (mean F_{ST}: Kohala x other = 0.04 compared with mean F_{ST}: all other = 0.02), a stronger pattern of isolation by distance emerged ($P = 0.003$; Supplementary Figure S3b). Analysis of the eight populations of

Figure 5 Relationship between log substrate age and population-level allelic richness for the three varieties that occur across a broad range of substrate ages: var. *incana* (squares and dashed line), var. *glaberrima* (including var. *macrophylla*; circles and solid line) and var. *polymorpha* (diamonds and dotted line). ANCOVA: variety: F = 13.82, df = 2, $P = 0.001$; log minimum substrate age (covariate): F = 10.02, df = 1, $P = 0.007$; $R^2 = 61.5\%$.

var. *glaberrima* and var. *macrophylla* combined revealed a non-significant trend toward isolation by distance $P = 0.178$ Supplementary Figure S3c) that was not improved by the removal of the Kohala populations of var. *glaberrima* ($P = 0.237$).
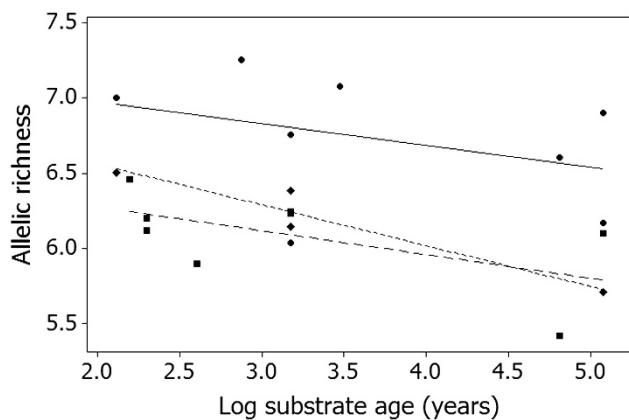
Haplotype of var. *newellii*

The sequencing of the four noncoding plastid regions (*rps16*, *rpl16*, *trnS-trnG* and *trnD-trnT*) revealed that the chloroplast haplotype of var. *newellii* is the most commonly found haplotype on Hawai'i Island and is endemic to that island according to Percy *et al.* (2008).

DISCUSSION

Evidence for incipient radiation in *M. polymorpha*

While the evolutionary significance of the many varieties of *M. polymorpha* has been questioned (James *et al.*, 2004; Harbaugh *et al.*, 2009), the results of this study of neutral genetic variation across 23 populations on Hawai'i Island indicate that four of the five varieties recognized on Hawai'i Island are real evolutionary entities. With the exception of var. *macrophylla*, populations grouped by variety regardless of sampling location on the island, indicating that cohesion within a variety is sufficiently strong to resist the homogenizing effects of hybridization in zones of sympatry. These findings are consistent with a recent study that found low but significant genetic differentiation among the three most common varieties on east Hawai'i Island (DeBoer and Stacy, 2013). The genetic differentiation observed among the common varieties in both studies is among the lower levels observed among populations of temperate ($F_{ST} = 0.116$) and tropical ($F_{ST} = 0.177$) tree species over regional scales (> 50 km; F_{ST} range: 0.007–0.55; Dick *et al.*, 2008). Low genetic differentiation is expected within wind-dispersed, landscape-dominant species with tremendous population sizes, such as *M. polymorpha* on Hawai'i Island (Loveless and Hamrick, 1984). The majority of tropical tree species, in contrast, occur at low population density, a condition that likely promotes genetic differentiation through drift (Dick *et al.*, 2008). Both studies of Hawai'i Island *Metrosideros* also revealed considerable variation among populations within varieties, with divergence among varieties principally through allele frequencies rather than private alleles. Sharing of alleles is expected among diverged populations of the same tree species. In oaks, allele sharing is common even above the species level (Howard *et al.*, 1997), and species-diagnostic alleles are rare at microsatellite loci (Muir and Schlötterer, 2005). Both common ancestry and hybridization among taxa likely explain the preponderance of shared alleles in oaks (Muir and Schlötterer, 2005) and *Metrosideros*.

The support for varieties in this study, coupled with results of earlier common garden studies, is consistent with divergence of *M. polymorpha* across Hawaii's heterogeneous landscape. Three common garden studies have demonstrated heritable phenotypic differences among trees sampled across substrate age and elevation gradients (Corn and Hiesey, 1973; Stemmermann, 1983; Cordell *et al.*, 1998). Although physiological and leaf anatomical traits showed high plasticity, and variance in morphological traits was greater among natural populations than within the garden (Cordell *et al.*, 1998), the characters used to discern varieties, namely the size, margin and pubescence of leaves and internode and petiole lengths, were retained in common garden settings (above references). Further, the morphological characters that define each variety appear to confer adaptation to that form's preferred environment. Moreover, it is possible that the varieties differ physiologically in ways that were not examined in the above studies. For example, Hoof *et al.* (2008) showed that sympatric trees of pubescent var. *polymorpha* and glabrous var. *glaberrima* at

2040 m elevation on Hawai'i Island differed in leaf nutrient composition, water status and stomatal traits, as well as instantaneous gas exchange rates. In addition, significant adaptive differences were observed between the two successional varieties in young seedlings that had not yet developed the morphological differences that are used to define these varieties (including pubescence in var. *incana*; Morrison and Stacy, 2014). The failure of earlier neutral genetic studies to identify differences among varieties is most likely explained by insufficient sampling of populations and/or poor resolution of the molecular markers used (that is, allozymes). In sum, the evidence accumulated to date does not support the extreme phenotypic plasticity inferred previously for *M. polymorpha*. Rather, the combined heritable phenotypic variation and low to moderate partitioning of neutral genetic variation among varieties on Hawai'i island indicate that significant divergence has occurred among four of the island's five named varieties. As the exception, var. *macrophylla* was not supported by our data. Given the lack of neutral genetic differentiation between var. *glaberrima* and var. *macrophylla*, their overall phenotypic similarity and indistinguishable habitat, both are found in late-successional, wet forests, we consider them here as a single taxon.

Independent colonization by varieties and introgression on young volcanoes

The site of origin of the more widespread varieties of *M. polymorpha* is an open question (Percy et al., 2008). Four of the named varieties included in this study occur also on older islands. The presence of similar forms on different islands could result from parallel emergence of analogous forms through the predictable recurring selective regime of young volcanic islands, or from origin on a single island (old or young) followed by spread to other islands. The significantly greater differentiation among varieties on the oldest volcano, Kohala, relative to that on the rest of the island is consistent with the independent colonization of Hawai'i Island by each of its three widespread varieties following their origin on an older island(s). The most likely source of colonists is the neighboring island of Maui, which is separated from Kohala by just 50 km today. During glacial periods when sea levels were lower, the distance between these islands was even less (Price and Clague, 2002).

Increased introgression among varieties on young volcanoes may explain the lower differentiation observed among the common varieties on the three younger volcanoes relative to that on Kohala in spite of the vast area of the younger volcanoes (and thus large distances between some of the sampled populations). The increases in population-level allelic richness and allele size range observed with decreasing substrate age are consistent with increased hybridization among these more abundant forms on the open spaces of new volcanoes. Given the observed habitats of these varieties, it is likely that early-successional var. *incana* leads the colonization of new volcanoes, followed by var. *glaberrima* and var. *polymorpha* as late-successional and high-elevation habitats become marginally available. Hybridization among varieties would be expected, given their known cross-fertility (Corn, 1979) and apparent hybridization in nature (Corn and Hiesey, 1973). The sharply reduced competition in the sparse populations on young lava flows would lead to weaker selection on genotypes (Savolainen et al., 2007), permitting more hybrids to reach reproductive maturity than would be expected in more competitive environments. Introgression of forms on young volcanoes may be a recurring theme in the history of Hawaiian *Metrosideros* that broadens the opportunities for, and potential rate of, adaptation on young volcanoes (Rieseberg et al., 2007).

Purifying selection in extreme environments

The results of the principal coordinate and network analyses of pairwise F_{ST} values in this study are consistent with population divergence by strong selection in extreme environments. Both analyses showed populations of the three extreme-habitat varieties on the edges of the distribution with populations of the late-successional var. *glaberrima* in the center. Early-successional var. *incana* and high-elevation var. *polymorpha* likely experience recurring purifying selection on new lava flows, and consistent purifying selection at high elevations, respectively. Both environments are extremely harsh, with low soil moisture and nitrogen availability, and marked diurnal temperature fluctuations. In addition, var. *polymorpha* at high elevation on Hawai'i Island is subjected to low rainfall, high winds, occasional freezing and poor soil development for tree establishment and growth. Such extreme environments are powerful drivers of population divergence (Lexer and Fay, 2005). In contrast, late-successional var. *glaberrima* may experience weaker abiotic selection throughout the majority of its range compared with the other varieties. The soils of late-successional wet forests on Hawai'i Island are high in organic matter and water-holding capacity, and not limited in nitrogen (Vitousek et al., 1992). Relative to the extreme-habitat varieties, var. *glaberrima* likely experiences stronger biotic selection through competition in closed-canopy, late-successional wet forests where population densities are high.

The central position of var. *glaberrima* in the principal coordinate and network analyses, coupled with its considerable phenotypic breadth, suggest that this variety is well connected to each of the other varieties through hybridization. Universal connectivity of var. *glaberrima* is consistent with the broader elevational range and late-successional habit of this variety that interfaces with early-successional, high-elevation and riparian habitats. In contrast, var. *newellii* rarely co-occurs with var. *incana* and never with var. *polymorpha*, at least under the current interglacial climate, and var. *polymorpha* may be less likely to mix with var. *incana* than with var. *glaberrima*, as var. *incana* appears to prefer low elevations (as indicated by the distribution of monotypic stands of this variety). Across the island var. *glaberrima* comprises considerable phenotypic variation in terms of leaf size and shape as well as habit (bush to tall tree). Further, inclusive of var. *macrophylla*, var. *glaberrima* shows greater allelic richness than the other varieties. Greater neutral genetic variation may correlate with greater functional genetic variation as well, given the broad elevation/climatic distribution of var. *glaberrima* relative to all other varieties. Variety *glaberrima* on Hawai'i island is a highly heterogeneous taxon.

Recent emergence of a riparian variety

The greatest divergence within Hawai'i Island *Metrosideros* involves small populations restricted to one of Hawaii's most extreme environments. The endemic, riparian var. *newellii* showed surprisingly high neutral genetic divergence (mean $F_{ST} = 0.13$; max = 0.25) from other populations, indicating the presence of some barrier to gene flow between this and other varieties despite their contiguous distributions (Loveless and Hamrick, 1984). Differentiation of var. *newellii* was evident through moderate to high F_{ST} values at all nine loci, consistent with genome-wide divergence of a historically small population through drift. The low within-population genetic variation and high among-population variation observed in this form further underlines the rapid neutral evolution that is possible in small populations of trees through drift (Loveless and Hamrick, 1984). The restriction of var. *newellii* to Hawai'i island and its possession of the island-endemic plastid haplotype suggest that this form originated

in situ. Also, all measures of genetic variation and the modified Garza-Williamson Index were lowest for var. *newellii*, consistent with the recent emergence of this form. The position of a Kohala bog population of var. *glaberrima* at the base of the network branch comprising var. *newellii* suggests that this form derived from a glabrous population on the island's oldest volcano and emerged through adaptation to the extreme riparian habitat on the windward coast. Plants in the riparian zone on east Hawai'i Island experience periodic submergence and severe mechanical stress by water after prolonged heavy rains. Such stresses are known to drive divergence in plants (Van Steenis, 1981; Lexer and Fay, 2005), giving rise to novel herbaceous species within 30 000 years (Mitsui and Setoguchi, 2012). Given the high likelihood that var. *newellii* evolved on Hawai'i Island, significant differentiation of this form has occurred within ∼ 0.5 my, as the riparian habitats on Hawai'i Island to which var. *newellii* is restricted are no older than 0.5 my (Moore and Clague, 1992).

The strong divergence of var. *newellii* is surprising because its riparian habitat is fully sympatric with the wet forests that support var. *glaberrima* today, and it seems likely that this variety emerged in sympatry or narrow parapatry with one or more other forms. Given the role of *M. polymorpha* as the first tree to colonize new lava flows and its dominance on Hawai'i Island today, this species has likely always occurred at high density there, a condition that does not tend to promote speciation (Price and Wagner, 2004). Variety *newellii* appears to have arisen from within a landscape-dominant tree species through strong selection in marginal habitat and has maintained or even increased its isolation over time from the more abundant forms of *M. polymorpha*. Today, var. *newellii* is distributed in small linear populations along narrow waterways embedded within wet forest on the windward coast of the island (except where recent deforestation has converted forest to cropland). In this setting, var. *newellii* occurs sympatrically or very narrowly (that is, on a scale of feet) para-patrically with the late-successional var. *glaberrima*. Flowering times of these two forms overlap (J. Johansen, pers. obs.), and apparent hybrids can be found. Even if gene flow were one way between var. *newellii* and the more abundant var. *glaberrima*, we would expect F_{ST} values to be lower than those observed (for example, $F_{ST} = 0.16$ between the upstream Wailuku River var. *newellii* population (Wai-luku A) and the adjacent var. *glaberrima* population (Saddle Rd.)). The high differentiation between these varieties may therefore reflect strong selection against hybrid offspring and migrants in both the riparian and wet-forest habitats (Petit and Hampe, 2006). Further study of the ecological differences between var. *glaberrima* and var. *newellii* and the fitness of their hybrids in parental habitats is needed to identify the nature of the selection underlying the differences between these forms. If var. *newellii* has emerged in sympatry, it would be an uncommon example of incipient sympatric speciation in trees (Savolainen et al., 2006), and also within the Hawaiian flora generally where speciation has most often occurred between allopatric or parapatric populations (Price and Wagner, 2004).

The evolution of the riparian habit may be a recurring theme in *Metrosideros*. Although parallel ecological speciation is considered relatively uncommon in plants (Ostevik et al., 2012), the medium-sized genus *Metrosideros* (calculated 50 species) has produced at least four independent rheophytes. The single South African species, *M. angustifolia*, grows along rivers (Van Steenis, 1981) and is phylogenetically isolated within the subgenus *Mearnsia*. *Metrosideros operculata* is a rupicolous shrub endemic to New Caledonia (Dawson, 1992) and is nested within a local radiation of the subgenus *Mearnsia* (Wright et al., 2000). *Metrosideros sclerocarpa* (Dawson in Green, 1990) is a species endemic to Lord Howe island that likely evolved

in situ (Papadopulos et al., 2011) and is most commonly found near creeks. This species belongs to the subgenus *Metrosideros*, which also contains the Hawaiian species. The rupicolous habit has evolved at least four times within *Metrosideros* in four distant regions, associated each time with the evolution of narrow leaves, a trait typical of rheophytes (Van Steenis, 1981).

Distance-dependent gene flow
Despite the strong potential for long-distance dispersal in *M. polymorpha*, gene flow in this species is likely more restricted than inferred in previous studies. Ample long-distance gene flow within and even among islands was inferred from poor genetic differentiation among populations at cpDNA loci (Percy et al., 2008) and nuclear microsatellite loci (Harbaugh et al., 2009). In contrast, an ISSR-based study of bog populations found a pattern of isolation by distance across the archipelago, which implied distance-dependent dispersal among islands (Wright and Ranker, 2010). The current study, using population-level sampling across the spatial scale of Hawai'i Island, revealed a significant pattern of isolation by distance in the early-successional var. *incana* and a similar, but non-significant trend in the late-successional var. *glaberrima*. These patterns indicate that gene flow across the island is distance dependent and that gene flow across broader spatial scales is infrequent. This result suggests a role for genetic drift in the divergence of distant populations of this dominant tree whether on the same or different islands.

CONCLUSIONS
Metrosideros polymorpha appears to be a case of incipient adaptive radiation in trees on the scale of the Hawaiian archipelago. This species comprises several varieties that are defined by heritable vegetative characters, are non-randomly distributed across Hawaii's heterogeneous landscape and that show significant genetic differentiation on young Hawai'i Island. Greater genetic distance among the more common varieties on the oldest volcano and an inverse relationship between allelic diversity and substrate age appear consistent with the colonization of Hawai'i Island by older, partially diverged varieties followed by increased hybridization among populations on younger volcanoes. The modest differentiation observed among the common varieties is expected in a within-tree-species analysis and appears to be associated with differential adaptation along the successional and elevation gradients created by active volcanoes, facilitated by distance-dependent gene flow. Nonetheless, divergence of varieties through geographic isolation during the course of the species' ∼ 4-my history in the Hawaiian Islands cannot be ruled out. The strong differentiation of the island-endemic riparian var. *newellii* in particular supports a model of divergence via adaptation to extreme environments. Variety *newellii* likely evolved from a Kohala bog form within ∼ 0.5 my due to strong selection in marginal habitat and along with analogous riparian forms on other Pacific islands and in South Africa appears to represent parallel (incipient) ecological speciation within *Metrosideros*. This study demonstrates that broad population-level sampling is required to uncover patterns of diversification within a ubiquitous and long-lived tree species. Because examples of incipient radiation in trees are rare, Hawaiian *Metrosideros* should be useful for understanding speciation in trees, offering opportunities to examine population divergence and the evolution of reproductive isolating barriers at the early stages of speciation.

DATA ARCHIVING
Genotype data available from the Dryad Digital Repository: doi:10.5061/dryad.267kc.

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