

## ORIGINAL ARTICLE

# Genetic analysis of an ephemeral intraspecific hybrid zone in the hypervariable tree, *Metrosideros polymorpha*, on Hawai‘i Island

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Intraspecific hybrid zones involving long-lived woody species are rare and can provide insights into the genetic basis of early-diverging traits in speciation. Within the landscape-dominant Hawaiian tree, *Metrosideros polymorpha*, are morphologically distinct successional varieties, *incana* and *glaberrima*, that dominate new and old lava flows, respectively, below 1200 m on volcanically active Hawai‘i Island, with var. *glaberrima* also extending to higher elevations and bogs. Here, we use morphological measurements on 86 adult trees to document the presence of an *incana*–*glaberrima* hybrid zone on the 1855 Mauna Loa lava flow on east Hawai‘i Island and parent–offspring analysis of 1311 greenhouse seedlings from 71 crosses involving 72 adults to estimate heritabilities and genetic correlations among vegetative traits. Both the variation in adult leaf pubescence at the site and the consistency between adult and offspring phenotypes suggest the presence of two hybrid classes, F<sub>1</sub>s and var. *incana* backcrosses, as would be expected on a relatively young lava flow. Nine nuclear microsatellite loci failed to distinguish parental and hybrid genotypes. All four leaf traits examined showed an additive genetic basis with moderate to strong heritabilities, and genetic correlations were stronger for the more range-restricted var. *incana*. The differences between varieties in trait values, heritabilities and genetic correlations, coupled with high genetic variation within but low genetic variation between varieties, are consistent with a multi-million-year history of alternating periods of disruptive selection in contrasting environments and admixture in ephemeral hybrid zones. Finally, the contrasting genetic architectures suggest different evolutionary trajectories of leaf traits in these forms.

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## INTRODUCTION

Hybrid zones—‘narrow regions in which genetically distinct populations meet, mate, and produce hybrids’ (Barton and Hewitt, 1985)—are particularly fruitful settings for studies of speciation (Harrison, 1990; Hewitt, 2001). Intraspecific hybrid zones in particular are relatively few in number, yet the parapatric ‘races’ that form them represent important stages of the speciation process (Hewitt, 1988), allowing studies of gene flow, selection and the evolution of isolating barriers at the early stages of speciation (Barton and Hewitt, 1985). Unfortunately, the resolution of hybrid zone studies involving very closely related taxa is often limited by uncertain designation of hybrid genotypes because of weak genetic differentiation between parental forms (Nielsen *et al.*, 2003).

Within species, genetic architecture can be shaped by selection operating on key adaptive traits, and it contributes significantly to the evolutionary trajectories of complex phenotypes (Sinervo and Svensson, 2002; Hansen, 2006). For example, taxa with broad ecological niches (for example, generalists) may have greater phenotypic and genetic variation and lower genetic correlations than specialists in which selection has favored stronger genetic correlations among key traits (Grant and Grant, 1994; Sinervo and Svensson,

2002). Within hybrid zones the genetic architectures of hybridizing taxa can be altered depending on the extent of admixture. Although introgression will generally increase genetic variation within hybridizing taxa (Grant and Grant, 1994; Seehausen, 2004), it is less clear how genetic correlations between traits will be altered through hybridization. For taxa with contrasting genetic correlational structures, introgression is expected to weaken genetic correlations within taxa (Grant and Grant, 1994). Overall, hybridization can increase population evolvability through elevated genetic variation and altered genetic architectures (Hansen, 2006) while facilitating ecological diversification through the generation of novel combinations of adaptive traits (Grant and Grant, 1994; Seehausen, 2004).

*Metrosideros polymorpha* Gaud. (‘ohi‘a lehua or ‘ohi‘a) is a landscape-dominant tree species in Hawaii that offers the opportunity to examine the genetic architecture of key phenotypic traits at the early stages of ecological divergence in trees. This hypervariable species comprises both pubescent and glabrous forms that are differentially distributed across elevation and rainfall gradients on the Hawaiian Islands (Dawson and Stemmermann, 1990; Kitayama *et al.*, 1997). Four of Hawai‘i Island’s five named varieties share the same common chloroplast haplotype (Percy *et al.*, 2008) and show

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significant within-taxon cohesion across the island and isolation from each other at neutral genetic loci, suggesting *M. polymorpha* as an unusual case of incipient radiation in trees (Stacy *et al.*, 2014). All forms have hermaphrodite red (or less often orange or yellow), shaving-brush-shaped flowers arranged in showy inflorescences, but differ from each other in vegetative traits (Dawson and Stemmermann, 1990). Common garden studies reveal heritable differences among varieties (or among populations sampled across environmental gradients) in morphological (Corn and Hiesey, 1973), leaf anatomical and physiological (Stemmermann, 1983; Kitayama *et al.*, 1997; Cordell *et al.*, 1998, 2000) traits, including leaf pubescence (Stemmermann, 1983; Kitayama *et al.*, 1997). Whereas these studies indicate heritability of the traits that are used to distinguish varieties of *M. polymorpha* on Hawai'i Island, heritabilities within varieties are unknown.

The two most abundant varieties of 'ohi'a on Hawai'i Island, early-successional var. *incana* and late-successional var. *glaberrima*, are ecotypes (Turesson, 1922) that dominate new and old lava flows, respectively, anywhere moisture is sufficient below roughly 1200 m above sea level (Mueller-Dombois, 1983; Stemmermann, 1983; Mueller-Dombois, 1987; Kitayama *et al.*, 1997). Var. *glaberrima* is unique among Hawai'i Island varieties in its ecological breadth, ranging from low-elevation stands through to ~1500 m in elevation with scattered individuals occurring higher. Var. *glaberrima* occurs also in bogs on the oldest volcano, Kohala, and hosts the most neutral genetic variation of any variety on the island (Stacy *et al.*, 2014). Replacement of early-successional var. *incana* by late-successional var. *glaberrima* on an aging lava flow occurs over a period of >1400 but <3000 years, depending on lava type and elevation (Drake and Mueller-Dombois, 1993; Drake, 1993; Kitayama *et al.*, 1997). The forms can be distinguished entirely through leaf characters; the appressed (abaxial) pubescent leaves of var. *incana* tend to be smaller than the glabrous leaves of var. *glaberrima* (Dawson and Stemmermann, 1990). Previous studies of vars. *incana* and *glaberrima* on Hawai'i Island documented different leaf nitrogen contents (Vitousek *et al.*, 1995), and the differential adaptation of their seedlings to light and nitrogen (Morrison and Stacy, 2014), both of which vary significantly between new and old substrates on east Hawai'i Island (Crews *et al.*, 1995). Other studies of glabrous and pubescent seedlings or trees from lower elevations (indicative of these two varieties) grown in a common garden revealed differences in water retention (Stemmermann, 1983), cuticle thickness and possibly osmotic potentials and photosynthesis rates (Kitayama *et al.*, 1997). The direction of these differences is consistent with specialization of var. *incana* to the especially harsh abiotic conditions (including drought; Kitayama *et al.*, 1997) of new lava flows or otherwise dry areas at lower elevations versus the more generalist nature of var. *glaberrima*. Despite these functional differences, var. *incana* and *glaberrima* are the most weakly genetically differentiated pair of 'ohi'a varieties on Hawai'i Island (mean population pairwise  $F_{ST}$  = 0.05 ( $P < 0.01$ ) versus  $F_{ST}$  = 0.056–0.151 for other pairwise combinations of varieties; DeBoer and Stacy, 2013; Stacy *et al.*, 2014).

On the 1855 Mauna Loa lava flow below ~1200 m in elevation, adults of both successional varieties are present as well as trees with intermediate phenotypes, suggesting extensive intraspecific hybridization on this intermediate-aged lava flow (Kitayama *et al.*, 1997); above that elevation on the same flow, hybridization with the high-elevation var. *polymorpha* is apparent. To test the hypothesis that there is an intraspecific hybrid zone on the 1855 Mauna Loa lava flow on east Hawai'i Island, we analyzed the vegetative morphology and microsatellite genotypes of 86 trees at ~880 m elevation. We used a classic crossing design and parent–offspring analysis to resolve the parental

and hybrid status of individuals in the hybrid zone based on vegetative traits. Finally, we used this same crossing design to test the hypothesis that the heritabilities of, and genetic correlations among, key leaf traits differ between the two successional varieties representing contrasting niche breadths.

## MATERIALS AND METHODS

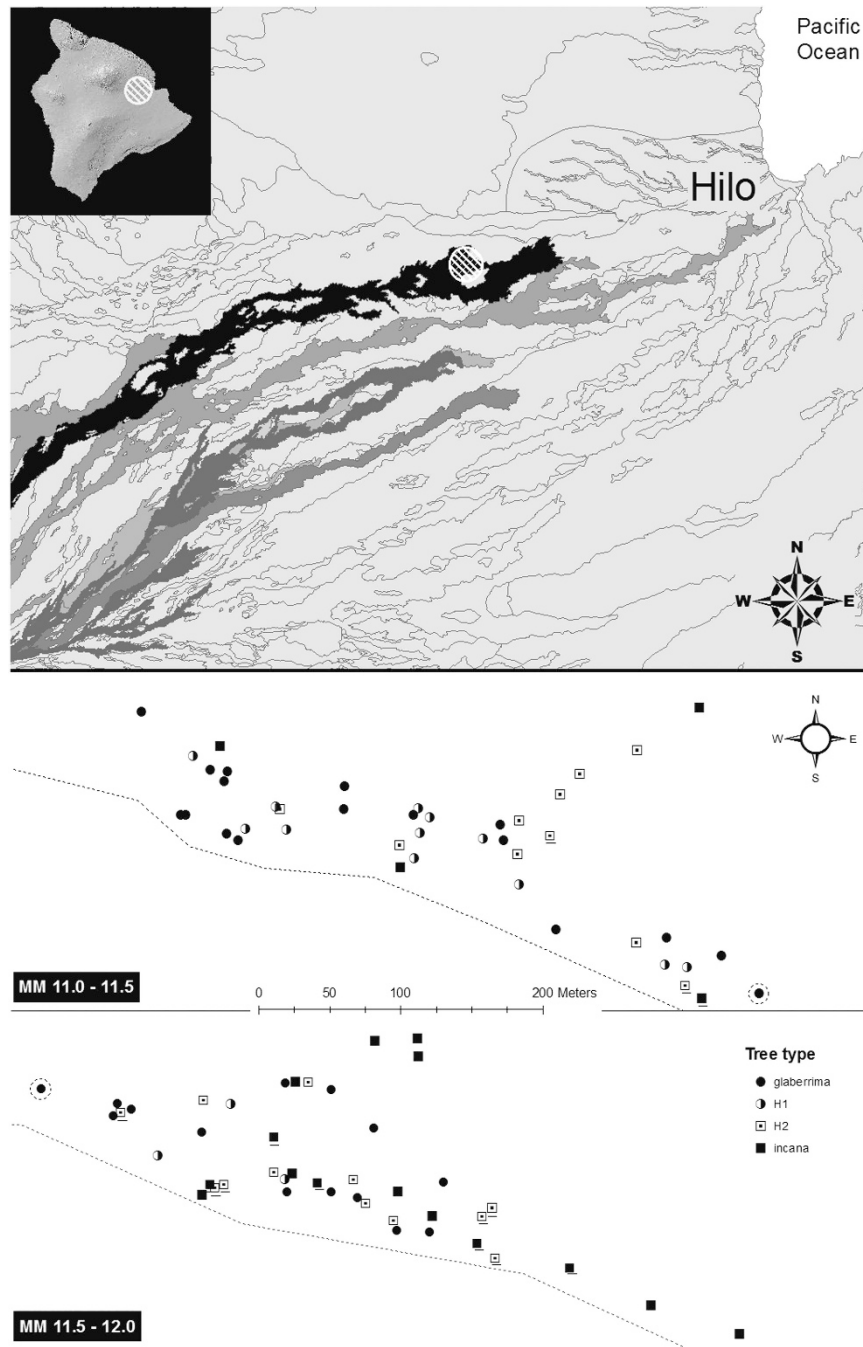
### Study site

This study centered on the population of *M. polymorpha* occurring within a ~1200 by 200 m area at ~880 m above sea level on the 1855 Mauna Loa lava flow near Saddle Road, East Hawai'i Island (Figure 1). This intermediate-aged (~150 years old at the time of the study) lava flow supports a mixed population of both vars. *incana* and *glaberrima* and morphologically intermediate trees, and the low population density at the site promotes flowering of many trees of unusually small stature (Supplementary Figure S1). These features allowed canopy access and crossing among forms that would be difficult or impossible elsewhere on the island, though restricted access to the canopies of tall (early-successional) var. *incana* trees led to fewer crosses involving this taxon. *M. polymorpha* is the sole tree species at the site.

### Morphological characterization of adults

Eighty-six trees (>1.3 m tall with reproductive structures; Figure 1) representing four tree classes (two varieties and two hybrid classes) were haphazardly chosen from among trees at the site producing ample floral buds and/or flowers during May–July 2006 and 2007. The 86 trees were well dispersed (mean  $\pm$  s.d. intertree distance =  $329 \pm 223$  m; range = 1.4–1054 m). Nine vegetative characters were measured on each tree (leaf width, leaf length, petiole length, internode length, leaf area, leaf mass, plant height, plant width and number of stems) from which three composite traits were computed (leaf shape, specific leaf area and plant shape); one index of herbivory was also recorded. Trees were sorted to type based on pubescence on the abaxial surface of mature nonsenescent leaves such that permanent leaf pubescence = var. *incana* (I); glabrous (pubescence absent) = var. *glaberrima* (G); and leaf pubescence removable by rubbing (caducous) = *incana*–*glaberrima* hybrids (H). Hybrids were further split into two groups based on the persistence of pubescence: class-1 hybrids (hereafter H1): pubescence is removed easily, and class-2 hybrids (hereafter H2): pubescence is more resistant to rubbing. Classification of all trees by this method was fully consistent across project personnel. On East Hawai'i Island, the morphotypes designated as hybrids are very rare outside of intermediate-aged lava flows (all authors, personal observation). With the exception of some adults of early-successional var. *incana*, which on average exceeded 4.5 m in height and are presumably the oldest trees on the lava flow, the canopies of trees were accessible ( $2.46 \pm 0.11$  m tall; Supplementary Figure S2). Leaf measurements were done for each of 10 well-dispersed, mature but not senescing leaves (third node from new growth) from each tree and averaged. Internode lengths were averaged across five mature internodes (second internode from growing tip) per tree, and specific leaf area was estimated using 20 leaves per tree following the formula: specific leaf area = leaf area in cm<sup>2</sup> per dry mass. Leaf area was determined with a LI-COR LI-3100 Leaf Area Meter (Lincoln, NE, USA), and dry mass calculations were taken on leaves following 48 h at 80 °C. The intensity of infestation (herbivory) by gall-forming psyllid fly larva (gall load) was also scored for each tree on a scale of 0 (no galls) to 3 (intense galling).

All response variables were examined for normality and equal variances across tree types, and were normalized as necessary using Johnson's transformation in Minitab 16 (Minitab Inc., Pennsylvania, PA, USA). The Johnson's transformation algorithm, similar to the Box–Cox algorithm, yields the function that transforms the data to best fit the normal distribution (Minitab Inc.; Johnson, 1949; Chou *et al.*, 1998). To characterize adult morphology, a principal components analysis (PCA) was done for all 86 trees on 7 traits showing pairwise Pearson's correlations <0.8 (Supplementary Table S1), and analyses of variance (ANOVAs) with Tukey's multiple comparisons ( $P < 0.05$  family error rate) were done to compare tree types for each of the three significant principal component axes. Follow-up one-way ANOVAs were done for all individual traits, followed by Tukey's *post hoc* pairwise comparisons to test for differences in vegetative morphology among tree types. The exceptions



**Figure 1** (top) Map of East Hawai'i Island showing the study site (oversized hatched circle) and (bottom) the locations of the 86 trees included in the analysis of morphology. Recent lava flows from Mauna Loa are indicated by shading, with the 1855 flow (study site) shown in black. The light gray background indicates lava flows >3000 years old. The map of the study trees is split in two halves, corresponding roughly with mile markers (MM) 11–11.5 and MM 11.5–12 on Saddle Road (dashed line); the tree circled with the dashed line is common to both halves. Adults were designated as var. *incana* (I), var. *glaberrima* (G) or an *incana*–*glaberrima* hybrid (H) based on leaf pubescence (see text). The underlined symbols indicate trees not included in the hand-crossing study ( $n=14$ ).

were internode length, number of stems and gall load that were resistant to transformation and thus analyzed using nonparametric Kruskal–Wallis tests; significant tests were followed by *post hoc* Mann–Whitney *U*-tests to identify pairwise differences between tree types.

#### Genetic structure within the hybrid zone

Two leaf buds were collected from each of the 86 trees and held at  $-80^{\circ}\text{C}$  until DNA extractions could be done. Frozen leaf material was homogenized with

Lysing Matrix A tubes in the FastPrep-24 Instrument for 40 s at  $4.0\text{ m s}^{-1}$  (MP Biomedicals, Santa Ana, CA, USA). DNA was extracted from the lysate using a QIAGEN DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol with the following modifications: extension of cell lysis incubation time to 15 min, one extra centrifuging to completely dry the membrane after washing and a decrease in the elution buffer to 75  $\mu\text{l}$ . 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). 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<sub>2</sub>, 0.5 µM each dNTP, 1.25 U Go Taq DNA polymerase (Promega, Madison, WI, USA), 0.4 µM of a dye-labeled forward primer and an unlabeled reverse primer and 1–10 ng 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 CEQ 8000 Software, version 8.0 (Beckman-Coulter).

The microsatellite data from the hybrid zone were supplemented with data from seven and four adult populations of vars. *incana* and *glaberrima*, respectively, occurring throughout Hawai'i Island (Stacy *et al.*, 2014). Allelic richness measures for the two varieties and both hybrid populations (H1 and H2) were calculated using ADZE (Szpiech *et al.*, 2008) in two ways: (1) using all populations of vars. *incana* and *glaberrima* from across the island ( $n = 138$  and 94 individuals, respectively) and (2) using just the populations of these varieties occurring within the hybrid zone (using  $n = 13$  individuals per population for rarefaction in both cases). Homozygosity by loci was also calculated analogously using Cernicalin (Aparicio *et al.*, 2006). One-way ANOVA was used to compare each of allelic richness, private allelic richness and homozygosity among island-wide var. *incana*, island-wide *glaberrima* and hybrids. MICRO-CHECKER (Oosterhout *et al.*, 2004) revealed significant null allele frequencies at three loci. The number of clusters among var. *incana*, var. *glaberrima* and their hybrids ( $K$ ) was estimated using STRUCTURE 2.3.4 with the Bayesian Markov chain Monte Carlo approach (Pritchard *et al.*, 2000), assuming admixture and correlated allele frequencies and allowing for the presence of null alleles at three loci. A burn-in period of 10 000 steps followed by 100 000 Monte Carlo replicates were simulated with  $K = 1$  through 20, and the optimal number of clusters was evaluated using an *ad hoc* statistic ( $\Delta K$ ; Evanno *et al.*, 2005). Finally, the proportion of each individual belonging to parental or hybrid classes was evaluated using NewHybrids (Anderson and Thompson, 2002), using noninformative Jeffrey priors and 100 000 iterations.

### Controlled crosses

Hand pollinations were done using 72 of the 86 hybrid zone trees, involving all possible combinations of tree types for analyses of adult fertility and cross-fertility (reported elsewhere; Stacy *et al.*, unpublished data); phenotypes of 1311, 2.5-year-old offspring from these crosses were used in the current study to estimate the genetic basis and heritabilities of the traits used to distinguish the two parental varieties. Each cross involved a single pair of trees crossed reciprocally such that each partner acted as both pollen donor and maternal tree (for example, Tree 3 → Tree 5 and Tree 5 → Tree 3). Seventy-one pairs of trees were crossed. Within each tree type, selection of individual trees for hand pollinations was haphazard from among all flowering trees of that type. Seedlings from these crosses were used in three analyses, each involving a subset of independent crosses (that is, such that each tree within the data set is used in a single cross only). First, a subset of 20 crosses involving only pure vars. *glaberrima* and *incana* adults was examined to uncover the genetic basis of the phenotypic differences between varieties, including additive vs dominant genetic elements, and to aid the identification of F<sub>1</sub> trees at the site. Second, 33 independent crosses involving all four phenotypes were used in a quantitative genetic analysis of the traits used to distinguish these varieties. Finally, 15 crosses involving only var. *glaberrima* adults were used to estimate genetic variation within varieties; inferences were also drawn from the equivalent analyses with the four pure var. *incana* crosses. Each outcross involved pollen from a single donor transferred to ≥ 20 flowers on a maternal tree, spread across two or more inflorescences. Flowers receiving pollen were emasculated at the stamen-emergence stage of anthesis and covered with mesh bags. Pollinations were done 2–3 days after emasculation when stigmas become receptive; Stacy *et al.*, unpublished data), and flowers that were asynchronous with the majority were removed. Pollen was transferred directly from anthers to stigma until the stigma was saturated, and pollinator exclusion bags were left on the experimental inflorescences for 14 days after pollination.

Upon maturation (~7.5 months later) fruits were collected and stored in open coin envelopes in an air-conditioned lab for 2 weeks to ensure capsule dehiscence. Seeds from mature experimental fruits (≤ 10 per cross) were sown (one fruit per 2, 5 × 10 cm wells) atop well-draining media covered with a thin layer of black sand and kept for 4 weeks in a misthouse under low light (~600 µmol m<sup>-2</sup> s<sup>-1</sup>) and 20 s of mist every 30 min during daylight hours) and then transferred to a greenhouse (overhead water 3 × daily with amounts adjusted as needed). At ~2 months post germination, seedlings were thinned to a maximum of six well-spaced seedlings per 5 × 10 cm well, and trays were rotated within the greenhouse monthly. At ~8 months after germination, a subset from each family (mean = 13) was transferred from communal wells to individual 10 cm pots. A six-sided dice was used to randomly select two seedlings out of the six from each well as needed for potting up. Phenotyping of seedlings was done at ~2.5 years of age when adult leaf characters had clearly emerged. Seedlings were measured or scored for eight vegetative traits (leaf length and width, petiole length, number of stems, abaxial and adaxial leaf pubescence, stem pubescence and stem color); leaf shape was then calculated as leaf length/leaf width. Length measurements were taken with dial calipers and averaged across two mature leaves, and pubescence was scored from 0 (glabrous) to 2 (permanently pubescent). Stem color was scored 1, 2 or 3 for green (light), red and purple (dark), respectively. Internode length was initially measured, but dismissed because of high variation within individual seedlings.

### Genetic basis of vegetative traits

To determine the genetic basis of traits used to distinguish vars. *incana* and *glaberrima* and to aid the identification of F<sub>1</sub> trees in the field, a subset of 20 independent within- and between-variety crosses was extracted from the full set of crosses (that is, excluding any crosses involving purported hybrid trees). One-way ANOVAs were done to compare each of the interval-scale morphological traits across the three seedling classes (within-var. *glaberrima*, within-var. *incana* and between-variety crosses; normal distributions and equal variances were confirmed), and analogous Kruskal-Wallis tests and *post hoc* pairwise Mann-Whitney *U*-tests were done for rank-order measures.

### Heritability and genetic correlation estimation through parent-offspring analysis

To estimate heritability of variety-diagnostic traits, traits were measured in adults in the field and their offspring in the greenhouse for three overlapping sets of crosses: 630 offspring from 33 independent (selected via coin toss), reciprocal crosses involving 66 randomly paired adults of all phenotypes (with seedlings from reciprocal crosses pooled within families; mean = 19.1 seedlings/family, range: 8–35); 298 offspring from 15 independent, reciprocal crosses involving 30 randomly paired adults of var. *glaberrima* (with reciprocal crosses pooled; mean = 19.9 seedlings/family, range: 16–30); and offspring from 4 independent, reciprocal crosses involving 8 randomly paired adults of var. *incana* (with reciprocal crosses pooled; mean = 14 seedlings/family, range: 9–19). The following steps were taken for each subset of crosses separately. Pearson's pairwise correlation tests were run between maternal trees and pollen donors for all interval-scale morphological measures, and Spearman's rank correlations were run for all pairwise comparisons involving either abaxial pubescence or number of stems (both rank order). For each of four leaf traits (leaf length, leaf width, leaf shape and petiole length), narrow-sense heritability was calculated using weighted (by family size) linear regression analysis of mean offspring values against mid-parent values (Falconer and Mackay, 1996). Maternal and paternal heritabilities were calculated correcting for correlations between maternal trees and pollen donors as:  $h^2 = 2b/(1+r)$ , where  $b$  is the parent-offspring correlation coefficient and  $r$  is the correlation between maternal trees and pollen donors; and maternal effect sizes were calculated by subtracting paternal  $h_n^2$  from maternal  $h_n^2$  for each leaf trait (Falconer and Mackay, 1996). The s.e. for each single-parent heritability estimate was obtained by doubling the s.e. for the regression coefficient from the weighted regression of the offspring values on the parental values. For all rank-order measures, Spearman's correlations were run to compare traits both among seedlings and between seedlings and adults. Pairwise genetic correlations among leaf length, leaf width and petiole length were calculated following:  $r = \frac{cov_{ij}}{\sqrt{(cov_{ii})(cov_{jj})}}$ , where

$cov$  is the parent–offspring covariance, and  $i$  and  $j$  are traits one and two, respectively (Falconer and Mackay, 1996).

## RESULTS

### Morphological characterization of adults

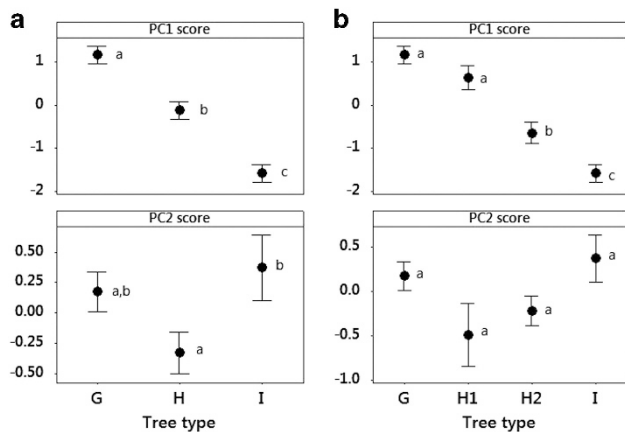
Pairwise correlations among the 12 morphological traits ranged from  $-0.359$  (number of stems and plant height) to  $0.935$  (leaf mass and leaf area; Supplementary Table S1). Analysis of adult morphology of 86 trees in the hybrid zone revealed differences among all four tree classes defined by pubescence type. PCA of adult morphological traits yielded three significant axes (eigenvalues  $>1$ ) that in combination accounted for a total of 64.4% (33.7, 16.3 and 14.4%, respectively) of the variation across the 86 trees measured (Supplementary Table S2 and Supplementary Figure S3). PC1 was loaded most heavily with leaf area, petiole length and number of stems and captured the range of

plant forms from typically large-leaved multi-stemmed var. *glaberrima* to more tree-like, smaller-leaved var. *incana* with hybrids in between. With hybrids pooled, PC1 differentiated all three tree classes ( $F_{2,83} = 33.97$ ,  $P < 0.001$ ,  $R^2 = 43.68$ ; Figure 2a). The ANOVA of PC1 scores with hybrids split into two classes showed significant differentiation between the two hybrid classes, but not between var. *glaberrima* and H1 ( $F_{3,82} = 30.11$ ,  $P < 0.001$ ,  $R^2 = 50.68$ ; Figure 2b). PC2 scores (Johnson transformed; weighted most heavily with internode length) varied significantly (with hybrids pooled:  $F_{2,83} = 4.02$ ,  $P = 0.022$ ) or nearly significantly (with hybrids separate:  $F_{3,82} = 2.72$ ,  $P = 0.05$ ) between hybrids (H1) and var. *incana* trees (Figure 2). PC2 scores of hybrids were lower than those of the two parental varieties, rather than intermediate. PC3 scores did not vary across the tree types.

Examination of individual trait variation revealed significant differences between trees of vars. *glaberrima* and *incana* at 9 of 12 vegetative traits as well as gall load (Table 1). The two varieties were largely differentiated by leaf dimensions alone (Supplementary Figure S4) in addition to leaf pubescence. The pooled hybrids were intermediate between the two varieties and differed from both varieties in leaf length, width, area and mass (Supplementary Figure S5). Divided, the two hybrid classes defined by leaf pubescence characters differed from each other at 5 of the 13 morphological traits (including gall load; Figure 3 and Supplementary Figure S6).

### Genetic structure within the hybrid zone

Allelic richness estimates per 13 individuals averaged across loci were significantly higher for var. *glaberrima* ( $6.17 \pm 0.31$  (s.d.)) than for var. *incana* ( $5.61 \pm 0.21$ ) sampled from across the island with hybrids in between (mean of pooled hybrids:  $6.12 \pm 0.086$ ;  $F_{2,12} = 9.54$ ,  $P = 0.003$ ; Supplementary Table S3). In comparison with populations of the two varieties occurring just within the hybrid zone, allelic richness of H1 ( $6.03 \pm 1.04$ ) and H2 ( $6.20 \pm 1.04$ ) were again intermediate between those of var. *incana* ( $5.68 \pm 1.01$ ) and var. *glaberrima* ( $6.52 \pm 1.01$ ); private allelic richness was lower for var. *incana* relative to the other tree classes both island wide ( $F_{2,12} = 8.70$ ,  $P = 0.005$ ) and locally (Supplementary Table S3). Homozygosity by locus did not



**Figure 2** Mean  $\pm 1$  s.e. PC1 and PC2 scores for (a) adults of var. *glaberrima* (G), var. *incana* (I) and their hybrids (H) at the study site, and (b) the same with hybrids divided into two classes (H1 and H2). Shared letters indicate no significant difference at  $\alpha = 0.05$ .

**Table 1** Comparison of 12 vegetative traits, plus gall load,<sup>a</sup> among *Metrosideros polymorpha* var. *glaberrima* (G;  $n = 30$ ), var. *incana* (I;  $n = 19$ ) and two classes of *incana*–*glaberrima* hybrids (H1;  $n = 15$  and H2;  $n = 22$ ) at ~880 m elevation on the 1855 Mauna Loa lava flow East Hawaii Island

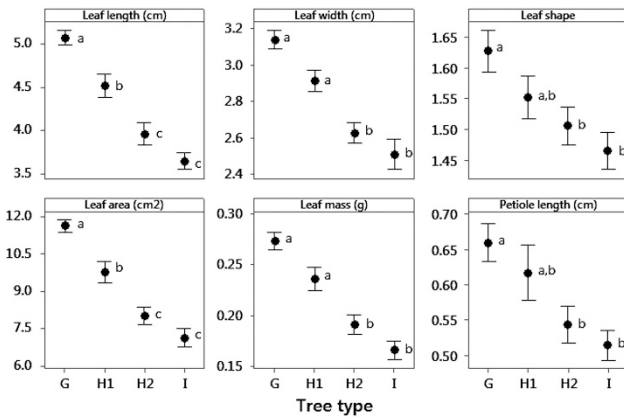
Trait	Test statistic (F or H)	d.f.	P-value	R <sup>2</sup> (adj)	Difference between varieties?
Leaf length	33.15	3,82	$<0.001$	53.16	G > I
Leaf width	23.54	3,82	$<0.001$	44.30	G > I
Leaf length/width ratio	4.93	3,82	0.003	12.18	G > I
Petiole length	5.96	3,82	0.001	14.89	G > I
Leaf area	35.06	3,82	$<0.001$	54.59	G > I
Dry leaf mass	27.08	3,82	$<0.001$	47.93	G > I
Specific leaf area	0.49	3,82	0.692	0	ND
Plant height	14.50	3,82	$<0.001$	32.27	G < I
Plant width	0.71	3,82	0.55	0	ND
Plant height/width ratio	11.19	3,82	$<0.001$	26.45	G < I
Internode length*	2.12	3	0.547	NA	ND
Number of stems*, <sup>b</sup>	25.02	3	$<0.001$	NA	G > I
Gall load*	12.44	3	0.006	NA	G > I

Abbreviations: NA, not applicable; ND, not different.

Asterisk indicates the Kruskal–Wallis test; all other tests were analyses of variance (ANOVAs). Johnson transformed variables: leaf length, petiole length, leaf area, dry leaf mass, plant height and plant width.

<sup>a</sup>A measure of herbivory.

<sup>b</sup>At 0.33 m from ground.

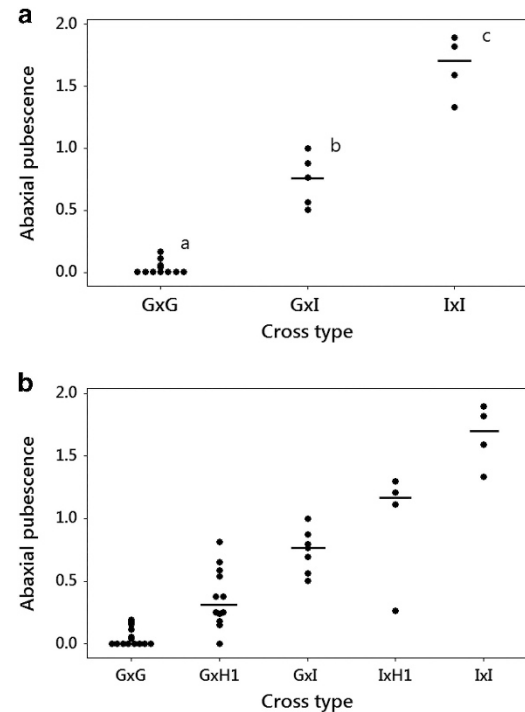


**Figure 3** Mean values  $\pm$  1 s.e. of 6 leaf traits of 86 trees in an intraspecific *M. polymorpha* hybrid zone. Tree types are as in Figure 2. Shared letters indicate no significant difference at  $\alpha=0.05$ .

differ across tree classes, ranging from  $0.36 \pm 0.01$  to  $0.39 \pm 0.04$  ( $P=0.96$ ). The STRUCTURE analysis with the H1 and H2 hybrid populations and eight and five populations of vars. *incana* and *glaberrima*, respectively, from throughout the island revealed  $k=2$  as the most likely number of clusters, but only modest differentiation between varieties and no demarcation of hybrid populations (Supplementary Figures S7 and S8). The NewHybrids analysis failed to classify parental varieties or hybrids correctly, designating a majority of trees in the  $F_2$  class; notably, however, ‘pure *incana*’ made up a significantly greater proportion of trees than did ‘pure *glaberrima*’ (Supplementary Figure S9).

#### Genetic basis of vegetative traits

The analysis of phenotypes of 2.5-year-old seedlings derived from 20 independent within- and between-variety crosses involving only pure-variety parents revealed significant differences between the varieties with  $F_1$  hybrids intermediate but slightly closer to var. *glaberrima*. Var. *glaberrima*,  $F_1$  and var. *incana* seedlings differed in abaxial leaf pubescence scores ( $H=16.11$ , d.f. = 2,  $P<0.001$ ; Figure 4a) and stem pubescence scores (Supplementary Figure S10). Adaxial leaf pubescence and stem color also differed between var. *incana* and var. *glaberrima* seedlings, with  $F_1$  seedlings intermediate but not unique (that is, not significantly different from both parental varieties), and stem number significantly lower for  $F_1$ s compared with pure-variety seedlings, although the difference was significant only in comparison with pure *incana* seedlings ( $F_{2,17}=4.99$ ,  $P=0.02$ ; Supplementary Figure S10). Examination of the key variety-diagnostic trait—abaxial pubescence—at the individual seedling level revealed variation within families and cross types. Of 298 seedlings derived from var. *glaberrima*  $\times$  var. *glaberrima* crosses, 280 were fully glabrous as expected; 18 showed nonpersistent pubescence, and none possessed the dense, permanent pubescence characteristic of var. *incana*. Of 56 var. *incana*  $\times$  var. *incana* seedlings, 45 showed dense, persistent abaxial leaf pubescence as expected, 6 showed nonpersistent pubescence and 5 were glabrous. Of 125  $F_1$  seedlings, 54 showed intermediate leaf pubescence as expected, 54 were glabrous and 17 showed heavier pubescence than expected (though none showed dense, persistent pubescence).  $F_1$  seedlings with unexpected leaf pubescence appeared in even frequencies in the two reciprocal cross types. Overall, there was a slight shift toward glabrousness in the seedling pool.



**Figure 4** Individual value plots of abaxial (bottom) leaf pubescence of parents and offspring (family-level means) from (a) 20 independent crosses involving pure-variety trees only (shared superscripts indicate no significant difference at  $\alpha=0.05$ ), and (b) the same from 42 not fully independent crosses involving pure-variety and H1 trees only (for graph only). Tree types are as in Figure 2. Solid bars indicate medians.

Expanding the analysis to include H1 adults (purported  $F_1$ s) in the pool of crosses yielded purported backcross *glaberrima* and backcross *incana* seedlings with phenotypes largely intermediate between those of  $F_1$  seedlings and vars. *glaberrima* and *incana* seedlings, respectively (Figure 4b and Supplementary Table S4). The consistency of phenotypes of the purported backcross *incana* seedlings, including leaf pubescence, with those of H2 adults at the study site suggests that the latter are backcross *incana* trees.

#### Heritability estimation through parent–offspring analysis

Mid-parent–offspring narrow-sense heritabilities were estimated for the four leaf traits that are sometimes used in addition to leaf pubescence to distinguish vars. *glaberrima* and *incana*. The analysis of 33 random independent crosses involving all tree types revealed moderate, statistically significant heritabilities for all four traits (Table 2 and Figure 5). Although not used for estimation of heritabilities, four other rank-order traits recorded for these seedlings also showed highly significant correlations with mid-parent values of abaxial leaf pubescence (Supplementary Figure S11), consistent with a heritable basis for each. Analysis of the subset of 15 independent within-var. *glaberrima* crosses revealed generally higher heritability values, though with higher s.e. values because of the lower sample size (Table 2 and Figure 6). Finally, analysis of the four independent within-var. *incana* crosses revealed high heritability estimates (leaf length:  $0.552 \pm 0.427$ ,  $P=0.325$ ; leaf width:  $1 \pm 0.048$ ,  $P=0.002$ ; leaf shape:  $>1 \pm >1$ ,  $P=0.191$ ; and petiole length:  $1 \pm 0.118$ ,  $P=0.017$ ; not shown). Maternal effect sizes ranged from 0.075 (petiole length) to 0.285 (leaf width) within var. *glaberrima* and were lower for all three traits for the 33 crosses involving all tree types (Table 2). Direct

**Table 2** Estimates of narrow-sense heritability  $\pm 1$  s.e. and maternal effect size for each of four vegetative traits used in addition to leaf pubescence to distinguish vars. *glaberrima* (G) and *incana*

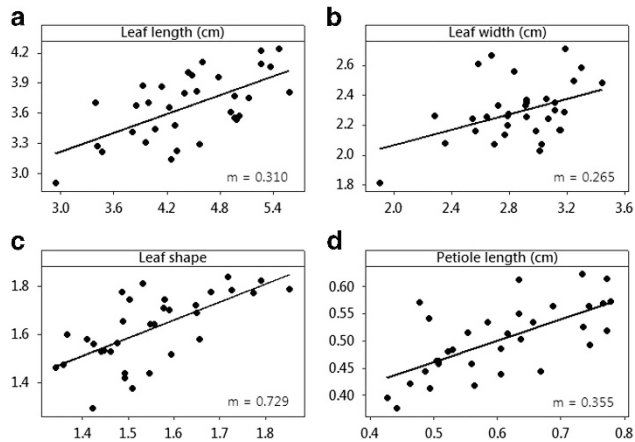
Trait	Mid-parent		Maternal		Paternal		Maternal	
	heritability		heritability <sup>a</sup>		heritability <sup>a</sup>		effect size	
	Mixed	G	Mixed	G	Mixed	G	Mixed	G
Leaf length	0.310 $\pm$ 0.071**	0.558 $\pm$ 0.238*	0.705 $\pm$ 0.111**	0.545 $\pm$ 0.278	0.614 $\pm$ 0.111**	0.354 $\pm$ 0.268	0.092	0.192
Leaf width	0.265 $\pm$ 0.111*	0.424 $\pm$ 0.234	0.509 $\pm$ 0.144*	0.501 $\pm$ 0.282	0.444 $\pm$ 0.146*	0.215 $\pm$ 0.274	0.065	0.285
Leaf shape	0.729 $\pm$ 0.159**	0.849 $\pm$ 0.202**	0.887 $\pm$ 0.187**	0.930 $\pm$ 0.244**	0.744 $\pm$ 0.244**	0.651 $\pm$ 0.260*	0.143	0.280
Petiole length	0.355 $\pm$ 0.084**	0.307 $\pm$ 0.135*	0.793 $\pm$ 0.104**	0.337 $\pm$ 0.144*	0.779 $\pm$ 0.266**	0.262 $\pm$ 0.159	0.014	0.075

Pearson's correlation coefficients and associated *P*-values for pairwise comparisons between maternal parent and pollen donor for the four traits are: Mixed: leaf length:  $r=0.31$ ,  $P=0.019$ ; leaf width:  $r=0.229$ ,  $P=0.087$ ; leaf shape:  $r=0.102$ ,  $P=0.452$ ; petiole length:  $r=0.165$ ,  $P=0.22$ ; and G: leaf length:  $r=-0.186$ ,  $P=0.326$ ; leaf width:  $r=-0.145$ ,  $P=0.445$ ; leaf shape:  $r=-0.170$ ,  $P=0.370$ ; petiole length:  $r=-0.051$ ,  $P=0.788$ .

Estimates are calculated from measurements of parents and offspring in 33 random, independent crosses involving all phenotypes at the study site (Mixed) and from measurements of parents and offspring in 15 random, independent crosses between var. *glaberrima* adults (G).

Significance at the \*0.05 and \*\*0.01 levels, respectively, of regression coefficients (mid-parent heritabilities) and correlation coefficients (single-parent heritabilities).

<sup>a</sup>The s.e. values for single-parent heritability estimates are twice the s.e. of the weighted regression coefficients.

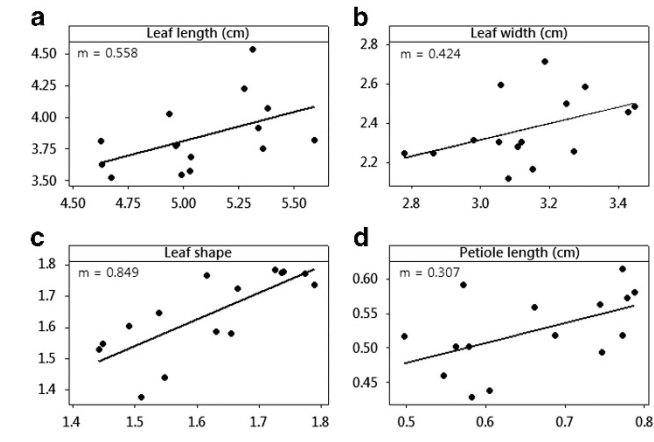


**Figure 5** Scatter plots of mid-parent (x axis) and mid-offspring (y axis) values for (a) leaf length, (b) leaf width, (c) leaf shape (leaf length/leaf width) and (d) petiole length from 33 independent crosses involving 66 randomly paired adults of all phenotypes at the study site. Weighted linear regressions: leaf length:  $F_{1,31}=18.85$ ,  $P<0.001$ ,  $R^2=35.81$ ; leaf width:  $F_{1,31}=5.68$ ,  $P=0.023$ ,  $R^2=12.76$ ; leaf shape:  $F_{1,31}=21.17$ ,  $P<0.001$ ,  $R^2=38.66$ ; petiole length:  $F_{1,31}=17.60$ ,  $P<0.001$ ,  $R^2=34.15$ .

estimates of maternal effects within var. *incana* were not possible because of the limited number of within-variety crosses.

### Genetic correlations

Pairwise genetic correlations were calculated among leaf length, leaf width and petiole length for each of the three subsets of crosses described above. Within var. *incana*, leaf length and width were very strongly, positively correlated ( $r \sim 1.0 \pm 0.004$  (s.e.)), and both traits were negatively correlated with petiole length (leaf length:  $-0.778 \pm 0.079$ ; leaf width:  $-0.627 \pm 0.028$ ). In contrast, within var. *glaberrima*, leaf length and width were decoupled ( $r = -0.104 \pm 0.152$ ), and both leaf traits were positively correlated with petiole length (leaf length:  $0.746 \pm 0.005$ ; leaf width  $0.432 \pm 0.009$ ). Genetic correlations among the three traits for the full set of 33 crosses involving all tree types were intermediate to the values calculated for the two parental varieties separately (leaf length  $\times$  leaf width:  $0.643 \pm 0.036$ ;



**Figure 6** Scatter plots of mid-parent (x axis) and mid-offspring (y axis) values for (a) leaf length, (b) leaf width, (c) leaf shape (leaf length/leaf width) and (d) petiole length from 15 independent crosses involving 30 randomly paired adults of var. *glaberrima* at the study site. Linear regressions: leaf length:  $F_{1,13}=5.49$ ,  $P=0.036$ ,  $R^2=24.26$ ; leaf width:  $F_{1,13}=3.27$ ,  $P=0.094$ ,  $R^2=13.93$ ; leaf shape:  $F_{1,13}=18.4$ ,  $P=0.001$ ,  $R^2=54.47$ ; petiole length:  $F_{1,13}=5.16$ ,  $P=0.041$ ,  $R^2=22.91$ .

leaf length  $\times$  petiole length:  $0.187 \pm 0.057$ ; leaf width  $\times$  petiole length:  $0.087 \pm 0.077$ ).

### DISCUSSION

This study verifies an intraspecific hybrid zone between two common and weakly genetically diverged varieties of the hypervariable, landscape-dominant tree, *M. polymorpha*, on Hawai'i Island. Furthermore, through comparison of phenotypes of hybrid zone adults and offspring derived from controlled crosses among them, we posit a model for hybrid zone genotypes and confirm heritabilities of the traits used to distinguish these varieties. We also show stronger genetic correlations among leaf traits in the early-successional var. *incana*, as predicted given its narrower ecological niche, and intermediate genetic correlations in the hybrid zone, consistent with hybridization.

The different plant shapes observed for successional vars. *incana* and *glaberrima* in this study can be added to a growing list of



phenotypic and ecological differences that have been observed between these varieties on Hawai'i Island, including leaf morphology, leaf nitrogen content, water retention, cuticle thickness, seedling-stage responses to light and soil nitrogen and seed germination responses to light and heat (Stemmermann, 1983; Dawson and Stemmermann, 1990; Drake, 1993; Vitousek *et al.*, 1995; Kitayama *et al.*, 1997; Morrison and Stacy, 2014). In addition to the presence-absence of leaf pubescence that distinguishes these forms, all measures of leaf size and shape, as well as plant shape, recorded for adults in the hybrid zone also differed between these forms; only specific leaf area, internode length and plant width did not differ. At the study site, pubescent, early-successional var. *incana* trees were typically single stemmed with smaller, rounder leaves, whereas glabrous, late-successional var. *glaberrima* trees were single- or multi-stemmed with larger, longer-petioled and often longer leaves.

#### Intraspecific hybrid zones on intermediate-aged lava flows

The analysis of 12 morphological characters of 86 trees at the study site indicates the presence of an *incana*–*glaberrima* hybrid zone at 880 m above sea level on the 1855 Mauna Loa lava flow on east Hawai'i Island. In fact, the abundance of morphologically intermediate trees on other intermediate-aged lava flows on east Hawai'i Island (all authors, personal observation) suggests that *incana*–*glaberrima* hybrid zones form readily on intermediate-aged substrates at low–middle elevations on the island. Aging lava flows represent an intermediate, transitional environment between fresh lava fields and mature rainforests where proximity of trees of the two varieties is increased (Howard *et al.*, 1997), the lack of a stable ecological community promotes hybrid community formation (Moore, 1977) and growth and survivorship of intervarietal hybrids may be at least as high as those of the parental varieties in their home environments (Anderson, 1948; Muller, 1952). The high rainfall at low–middle elevations (Giambelluca *et al.*, 2013) should facilitate succession and coexistence of the two varieties and their hybrids (Kitayama *et al.*, 1997). Indeed, the abundance of hybrids at the study site suggests that growth and survivorship of hybrids are high in these intermediate environments and further suggests that prezygotic barriers between the successional varieties in sympatry are weak. The high abundance of hybrids on intermediate-aged lava flows, coupled with immigrant inviability of vars. *glaberrima* and *incana* on new and old lava flows respectively (Morrison and Stacy, 2014), suggests that these ephemeral *incana*–*glaberrima* hybrid zones likely best fit the 'geographically bounded hybrid superiority model' (Moore, 1977; Hamilton and Aitken, 2013). This model would predict that growth and survivorship of hybrids in both parental environments are low, a conclusion that is supported by the low frequency of apparent hybrids outside of intermediate-aged lava flows. This observation, coupled with the paucity of pubescent trees in mature rainforests and glabrous trees on fresh lava flows below ~1200 m in elevation, suggests strong purifying selection on both varieties and their hybrids during the transition from seedlings to adulthood in parental habitats. Strong selection on juvenile stages appears to be an important determinant of adult distribution in trees (Petit and Hampe, 2006; Poorter, 2007).

With their recent common ancestry, sympatry and presumed extended history of alternating cycles of introgression and separation on the chronosequence of active volcanoes that make up the island chain, vars. *incana* and *glaberrima* form hybrid zones that likely capture elements of both primary and secondary hybrid zones (Hewitt, 1988). It is not clear from available data whether the initial divergence of these forms, which appears to have occurred on an older island (Stacy *et al.*, 2014), occurred through selection in parapatry or if

allopatry was involved. Nonetheless, a history of persistent, close association is suggested by their current sympatric or parapatric distributions on at least five main Hawaiian Islands (Lana'i, not observed), whose substrates range in age from 0 years (Hawai'i Island) to 5 million years (Kaua'i). Vars. *incana* and *glaberrima* appear to fit Endler's (1977) model of (incipient) parapatric speciation wherein a continuously distributed population diverges across a heterogeneous environment, albeit a dynamic environment of active and extinct volcanoes.

Whereas a majority of hybrid zones are thought to be long lived, resulting from climate changes such as the glacial–interglacial transition in northern temperate zones (Barton and Hewitt, 1985; Hewitt, 1988), the *incana*–*glaberrima* hybrid zones on volcanically active Hawai'i Island are ephemeral. An adult population of var. *incana* may appear on a new lava flow by roughly 27 years after cooling (Drake and Mueller-Dombois, 1993), and var. *glaberrima* adults may appear as early as 50 years after cooling (Kitayama *et al.*, 1995). Complete replacement of var. *incana* by var. *glaberrima* takes >1400 but <3000 years, depending on lava type and elevation (Drake and Mueller-Dombois, 1993; Kitayama *et al.*, 1997). Thus, pubescent and glabrous trees, including hybrids, may coexist on an aging lava flow for roughly 1400 to 2400 years. Given the fertility of hybrids (this study), and assuming a roughly 50-year generation time, this scenario would indicate very roughly 30–50 generations of introgression between varieties with each new lava flow. As conditions on a lava flow progress to the late-seral stage, replacement of trees with pubescent leaves ceases, leaving a monotypic stand of var. *glaberrima* (Drake and Mueller-Dombois, 1993) introgressed with var. *incana* alleles. This conclusion is consistent with the lower proportion of 'pure *glaberrima*' than 'pure *incana*' fractions in the NewHybrids analysis of these varieties across Hawai'i Island (this study) and the relatively greater allelic richness and private allelic richness observed for var. *glaberrima* than for var. *incana*, both in the hybrid zone and across Hawai'i Island (Stacy *et al.*, 2014; and this study).

Analysis of adult morphology at the study site suggests the presence of at least two classes of hybrids on the 1855 Mauna Loa flow on Hawai'i Island. Both hybrid classes are identifiable by their intermediate leaf pubescence, as was also observed in hybrids between glabrous and pubescent species of Asian oaks (Wei *et al.*, 2015). The relative abundances of these forms at the site and the known pattern of forest succession, coupled with the results of our parent–offspring analysis, suggest that H1 and H2 trees are F<sub>1</sub>s and backcrosses to early-successional var. *incana*, respectively. The ease with which F<sub>1</sub> seeds were produced in this study is consistent with the high abundance of purported F<sub>1</sub> trees at the study site, and the mean and median leaf pubescence scores (and means of other phenotypic traits) of F<sub>1</sub> offspring produced through reciprocal hand-crosses between the varieties were consistent with those of purported F<sub>1</sub> trees in the field. The production of backcross var. *incana* trees through frequent crossing between F<sub>1</sub>s and var. *incana* would be expected given the dominance of early-successional var. *incana* on young lava flows and the propensity of F<sub>1</sub> hybrids to mate with the more abundant parental taxon (Lepais *et al.*, 2009). The mean/median leaf pubescence scores of offspring produced through reciprocal hand-crosses between purported F<sub>1</sub>s and var. *incana* were consistent with those used to designate the purported backcross *incana* (H2) trees in the field. In fact, all 12 of the morphological traits measured on H2 adults in the hybrid zone showed values that were intermediate between those of purported F<sub>1</sub> and var. *incana*, as expected for backcross var. *incana* trees (assuming quantitative traits with an additive genetic basis). The preponderance



of  $F_1$  and backcross *incana* trees on the 1855 lava flow is fully consistent with the composition of genotypes that would be expected on a young lava flow (~150 years old) where mixing of tree varieties has occurred for only a few generations. The exceptionally weak differentiation between these varieties severely limited the utility of the SSR data for distinguishing hybrid genotypes (Nielsen *et al.*, 2003; Vaha and Primmer, 2006), and neither measures of allelic richness nor heterozygosity (inferred from homozygosity estimates) were greater for the hybrid populations than for the parental taxa sampled on either island-wide or local scales. Both of these results are consistent with a prolonged history of recurring introgression between these varieties.

### Inheritance of variety-diagnostic traits

The principal character used to distinguish vars. *incana* and *glaberrima*—the presence or complete absence of permanent abaxial leaf pubescence—is heritable (Stemmermann, 1983) and appears to have an additive genetic basis with a partial dominance of the glabrous condition. In fact, leaf morphology generally showed a slight dominance of traits from one of the parental taxa (var. *glaberrima*), as was also observed for  $F_1$  offspring between two Asian oak species (Wei *et al.*, 2015). The majority (94 and 80%) of seedlings produced through within-var. *glaberrima* and within-var. *incana* crosses were fully glabrous and permanently pubescent, respectively, as expected if pubescence is heritable. Only 43% of seedlings produced through between-variety crosses, however, showed the predicted intermediate pubescence, with another 43% of the seedlings being glabrous. Importantly, of these three cross types, seedlings with the dense, permanent pubescence diagnostic of var. *incana* were produced only through within-var. *incana* crosses (these seedlings were also produced in crosses between H2 trees and between H2 and var. *incana* trees). The additive genetic basis with a partial dominance of the glabrous condition was apparent even though the wild nature of the study trees and their long generation times prohibited diallel crosses between homogeneous lines to determine the genetic basis of leaf pubescence or any other trait (see, for example, Zaiter *et al.*, 1990; Van Dam *et al.*, 1999).

Variation in leaf pubescence and a tendency toward glabrousness also occur in open-pollinated seedlings of *M. polymorpha*. In a common garden, whereas seedlings from wild glabrous maternal trees tended to be glabrous, those from wild pubescent trees were highly variable, consistent with an overall tendency toward glabrousness, and seedling phenotypes from both classes of maternal trees on younger substrates (that is, the 1855 lava flow, the site of the current study) were highly variable (Kitayama *et al.*, 1997). The distribution of leaf pubescence scores among open-pollinated seedlings from glabrous and pubescent maternal trees in the study by Kitayama *et al.* (1997) appears fully consistent with both historical introgression and current hybridization between varieties where maternal trees are surrounded by adults of the opposite variety (that is, glabrous trees on young lava flows and pubescent trees on old). The variation in seedling pubescence characters in both studies leads to some uncertainty in our initial designations of var. *glaberrima* and hybrid individuals at the study site based on leaf pubescence alone. However, these designations are also supported by several vegetative traits of those adults and their offspring in addition to leaf pubescence. Misclassification of hybrid trees as var. *glaberrima* would only lead to underestimation of the differences between the varieties, suggesting that these estimates may be conservative.

The parent–offspring analysis of hybrid zone adults and their offspring revealed moderate to strong heritabilities for the other traits

that are also used to distinguish vars. *incana* and *glaberrima*. The range of values observed within the hybrid zone,  $h^2 = 0.27$  for leaf width to  $h^2 = 0.73$  for leaf shape, are consistent with (if slightly higher than) those generally reported for plant morphological traits (mean  $h^2 = 0.26 \pm 0.001$ ; reviewed by Geber and Griffen, 2003). Even within var. *glaberrima*, heritabilities were substantial ( $h^2 = 0.31$  to  $h^2 = 0.85$ ). This might be unexpected, given the anticipated significance of leaf traits for fitness (Nicotra *et al.*, 2011) and the contrasting directional selection imposed on juveniles of these forms in their parental habitats (Fisher, 1930). The maintenance of heritable variation within varieties may be explained by their history of recurring, prolonged episodes of introgression on intermediate-aged lava flows that may facilitate adaptation to changing environments (Grant and Grant, 1994; Seehausen, 2004). The modest heritabilities observed here for leaf traits suggest that phenotypic plasticity and possibly nonadditive genetic variation (that is, residual variation; Houle, 1992) may also be important in shaping the leaf characters of these varieties. Finally, slight maternal effects were detected in leaf traits that may help to ensure adaptation of seedlings to their local environment (Roach and Wulff, 1987).

### Genetic architecture of leaf traits

Vars. *incana* and *glaberrima* differ in the genetic architecture of simple leaf traits that may reflect their contrasting home environments (Nicotra *et al.*, 2011) as well as differences in the varieties' ecological breadths. The contrasts in leaf shape between these varieties may reflect tradeoffs between structure and function experienced in early- and late-seral forests. For example, the greater petiole length and leaf length-to-width ratio of var. *glaberrima* should permit greater light capture per unit leaf area in the wet forest understory through a larger separation of leaf area from stems (Takenaka, 1994). In contrast, the more or less fixed leaf shape of var. *incana* may be the optimal leaf shape for the high-light, water-limited conditions of new lava flows. Whereas leaf length and width were strongly genetically correlated for early-successional var. *incana*, these traits were decoupled for late-successional var. *glaberrima*. The correlation between petiole length and leaf size (length and width) also differed, being positive in var. *glaberrima* and negative in var. *incana*. The stronger genetic correlation among leaf traits in var. *incana* may reflect the narrower range of conditions under which this variety is found on Hawai'i Island (young lava flows at low to middle elevations or otherwise drier areas). Var. *glaberrima*, in contrast, is unique among Hawai'i Island varieties in its ecological breadth, ranging from low-elevation stands to beyond 1500 m in elevation, including bogs on the oldest volcano, Kohala. Moreover, because of its broader range, it co-occurs and hybridizes with each of the three other varieties of *M. polymorpha* on the island (early-successional var. *incana*, high-elevation var. *polymorpha* and riparian var. *newellii*; Stacy *et al.*, 2014). Ongoing introgression with forms of such contrasting leaf morphologies may also contribute to the weak genetic correlations within var. *glaberrima* (Grant and Grant, 1994). Finally, the lack of a genetic correlation between leaf length and width for var. *glaberrima* suggests that leaf shape in this variety should be more flexible in response to selection (Hansen, 2006). This conclusion is consistent with the purported evolution of the stenophyllous (that is, narrow)-leaved, riparian var. *newellii* from var. *glaberrima* on Hawai'i Island within the past 500 000 years (Stacy *et al.*, 2014).

### Summary

This study confirms the presence of an intraspecific hybrid zone on the 1855 lava flow at 880 m elevation on Hawai'i Island between the

common successional vars. *incana* (early) and *glaberrima* (late) of the landscape-dominant tree species, *M. polymorpha*. Results further suggest the presence of two abundant hybrid genotypes,  $F_1$  and backcross *incana* trees, as would be expected on a lava flow where mixing of varieties has occurred for only a few generations. The strong genetic basis underlying several vegetative traits that are used to distinguish vars. *incana* and *glaberrima* coupled with the weak neutral genetic differentiation between these varieties are consistent with strong disruptive selection for these traits and an extended history of recurring episodes of introgression on intermediate-aged lava flows. The contrasting heritabilities and genetic correlational structures for leaf traits in the two varieties in spite of recurring introgression suggest different genetic architectures, and thus evolutionary trajectories, of simple leaf traits in these forms. The *incana*–*glaberrima* hybrid zones should be particularly useful for studies of reproductive isolating barriers at the early stages of ecological speciation in trees and the genetic architecture of such barriers. These results highlight the value of hypervariable tree species for insights into genetic architecture at the early stages of ecological divergence in trees.

## DATA ARCHIVING

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.6f785>.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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