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Morphological and genetic variation within *Metrosideros polymorpha* (Myrtaceae) on Hawai'i

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Abstract The highly polymorphic taxon *Metrosideros polymorpha* ('ohi'a) is the most abundant endemic tree in Hawai'i, occupying a wide but fragmented range of habitats across Federal, State, and privately managed lands. Morphological character states of 342 herbarium specimens from the island of Hawai'i distributed the five recognised varieties in ordinal space, but intermediate phenotypes were prevalent. Morphological and random amplified polymorphic DNA (RAPD) analyses were undertaken for 10 individuals at 3 sites on Hawai'i. Individuals at a high-elevation dry site and mid-elevation dry site had smaller, pubescent leaves with a higher leaf mass per area and nitrogen content than individuals at a moist mid-elevation site. While the populations were separated in ordinal space, taxonomic varieties overlapped. The high degree of overlap between the taxonomic varieties, based on genetic and morphological characteristics, does not support the current varietal subdivision of *Metrosideros polymorpha* on Hawai'i.

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INTRODUCTION

The large number of potential habitats created by variations in topography, rainfall, and other climatic and edaphic factors are thought to be contributing factors to rapid speciation in the Hawaiian Islands. *Metrosideros polymorpha* Gaud., commonly known as 'ohi'a lehua, is the dominant tree species endemic to the Hawaiian Islands. Since the initial arrival of its progenitor, most likely from New Zealand less than 1.5 m.y. ago (Wright et al. 2000), *M. polymorpha* has become a highly polymorphic taxon. Eight taxonomic varieties are currently recognised (Dawson & Stemmermann 1999). Within a particular habitat, several distinct phenotypes may be found. As such, altitudinal (Corn & Hiesey 1973), edaphic, and successional (Stemmermann 1983) ecotypes have been proposed and these largely correspond to the taxonomic varieties. On the island of Hawai'i, five varieties are currently recognised (Table 1), namely vars *glaberrima*, *incana*, *macrophylla*, *newellii*, and *polymorpha* (Dawson & Stemmermann 1999). A sixth variety, *nuda*, was recognised by Skottsberg (1944) as a glabrous form of var. *polymorpha* found above frost line on Hualalai and Mauna Loa. Variety *nuda* has vegetative characteristics of var. *glaberrima*, but with small leaves and a glabrous inflorescence (Table 1). Four specimens within the Herbarium Pacificum, Bishop Museum, Honolulu, have morphological characteristics of var. *nuda* and these were also included in our studies.

Gradients in physiological characteristics have frequently been reported for *Metrosideros polymorpha* (Cordell et al. 1998, 2000; Cordell & Goldstein 1999; Melcher et al. 2000). Under common garden conditions, Stemmermann (1983) demonstrated varietal differences in morphology and

physiology, but ecological varieties were noted to intergrade in characteristics in the field. The extent of genetic similarity for morphological and physiological differences of *M. polymorpha* varieties is largely unknown. Allozyme variation of *M. polymorpha* along altitudinal gradients on Maui and O'ahu indicated that very little differentiation has occurred, with about 90% of the total variation being found within populations (Aradhya et al. 1991, 1993). Variation in allozymes between the populations was correlated with environmental variables (Aradhya et al. 1993), but how the variation related to the current taxonomic status of the species was not discussed. Random amplified polymorphic DNA (RAPD) analysis can be used to identify variation among different individuals or populations based upon the presence or absence of amplified loci from throughout the plant genome (see Wolfe & Liston 1999). The purpose of this study was to assess the phenotypic recognition of variation expressed in the morphology of *M. polymorpha*, and to determine if this variation is supported by the genetic variability of populations on the island of Hawai'i using RAPDs.

MATERIALS AND METHODS

The distribution of the five currently recognised varieties of *Metrosideros polymorpha*, and var. *nuda*, was determined from 342 specimens deposited in the Herbarium Pacificum, Bishop Museum, Honolulu (Fig. 1). Leaf characteristics (overall shape, apex shape, basal shape, presence of hairs, and petiole length) (Table 1) were scored for each of the specimens, and the data were analysed by principal components analysis (Minitab) using the presence and absence of each characteristic.

For genetic analysis, taxonomic varieties of *Metrosideros polymorpha* from three sites with different environmental and edaphic conditions on the island of Hawai'i were sampled. Plants were

Table 1 Morphological characteristics of the *Metrosideros polymorpha* taxonomic varieties found on the island of Hawai'i as described by Dawson & Stemmermann (1999). Variety *nuda* was described by Skottsberg (1944) but not recognised by Dawson & Stemmermann (1999).

Character	*glaberrima*	*macrophylla*	*newellii*	*incana*	*polymorpha*	*nuda*
Stature	shrubs to tall trees	small to tall trees	shrubs to small trees	shrubs to tall trees	shrubs to small trees	shrubs to small trees
Leaf shape[a]	ovate or obovate to elliptic	broadly ovate, large	elliptic	ovate to suborbicular	ovate to suborbicular	ovate to suborbicular
Leaf pubescence (lower surface)[a]	glabrous	glabrous	glabrous	appressed pubescent	densely woolly or appressed pubescent	glabrous
Petiole length[a]	petiolate	petiolate	petiolate	subsessile to petiolate	subsessile	subsessile
Lamina apex[a]	rounded to acute	rounded to obtuse	obtuse to acute	rounded or retuse	rounded to retuse	rounded to retuse
Lamina base[a]	rounded or cordate to cuneate	rounded or truncate	cuneate	rounded to cuneate	cordate	cordate
Lamina margins	flat, rarely revolute	flat	flat	flat	revolute to rolled	revolute to rolled
Inflorescence	pubescent (glabrous[b])	pubescent	pubescent	pubescent	pubescent	glabrous
Elevation	mid to high	mid	low to mid, along streams	low to mid	mid to high	high

[a] Characteristics measured in morphological analysis.
[b] As described by Rock (1917).

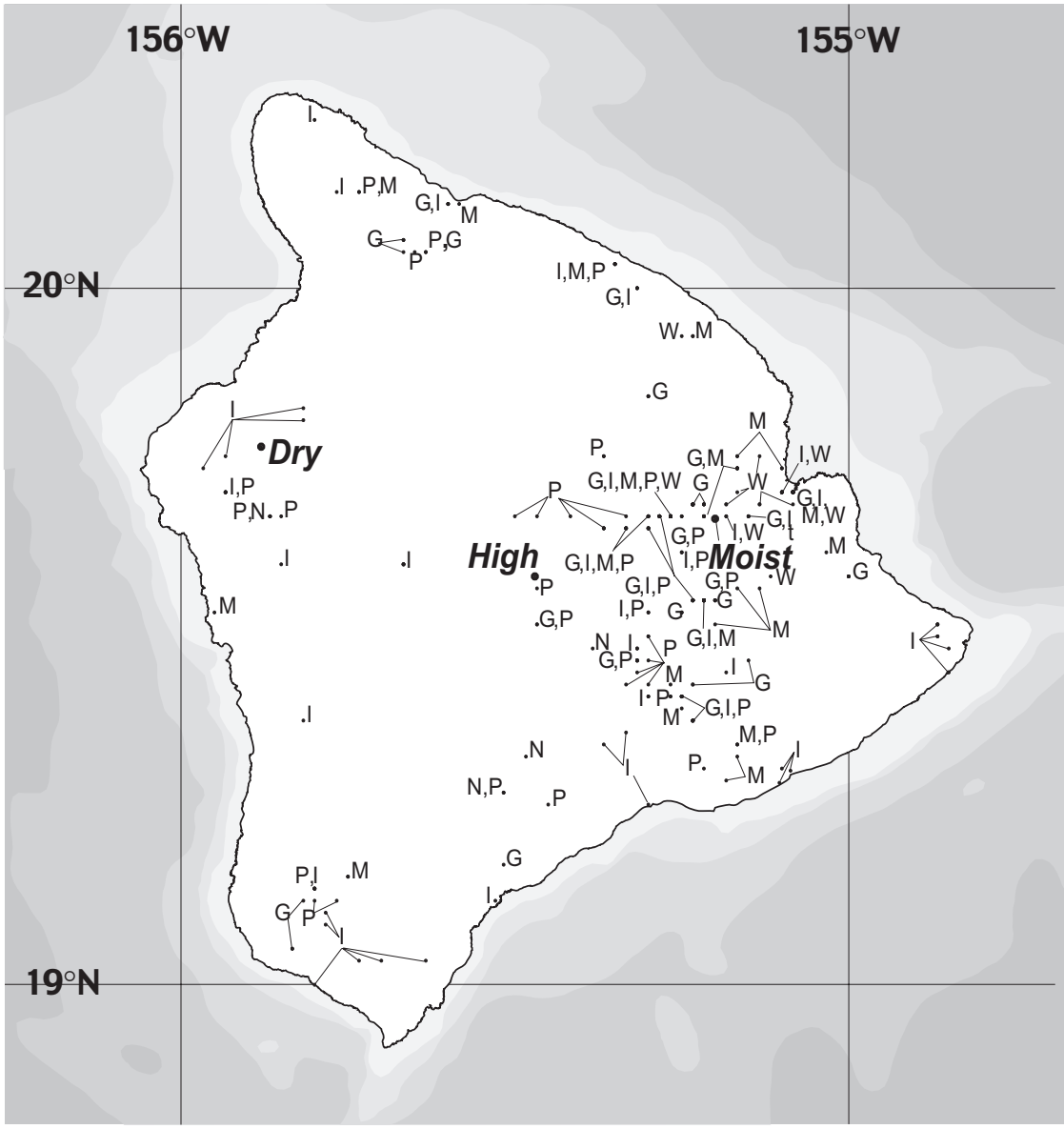

Fig. 1 Distribution map of 342 *Metrosideros polymorpha* specimens within the Herbarium Pacificum, Bishop Museum, Honolulu, collected on the island of Hawai‘i. Current taxonomic determinations have been overlaid on the data. The locations of the three field sites are indicated (Dry, High, Moist). G, var. *glaberrima*; I, var. *incana*; M, var. *macrophylla*; W, var. *newellii*; N, var. *nuda*; P, var. *polymorpha*.

collected from a moist site at mile 10.5 at an elevation of 700 m on Saddle Road (Moist site); a harsh, high-elevation site on Mauna Loa Road at 2470 m elevation (High site); and a dry site at Ka‘upulehu at 650 m elevation (Dry site) (Fig. 1; Table 2). The taxonomic variety of each individual was assessed by macroscopic morphological attributes (Table 1). Variety *polymorpha* was the only variety present at the high-elevation site, vars. *glaberrima* and *incana* were present at the moist site, and vars. *incana* and *polymorpha* were present at the dry site. Vouchers for the specimens are held in the Pacific Center for Molecular Biodiversity, Bishop Museum, Honolulu, Hawai‘i.

Assessment of foliar characteristics included leaf area, leaf mass per area (LMA), pubescence, nitrogen content, and water-use efficiency. Five pairs of young, fully expanded leaves were collected from 10 individuals at each site and leaf area was measured using a leaf area meter (LI-3100; LI-COR Inc, Lincoln, NB). Leaf pubescence was determined by removing leaf hairs with a spatula from one leaf per pair and drying the leaves at 70°C for determination of LMA. The difference between the shaved and unshaved leaves represented the amount of pubescence (g/m^2) on the leaves (Cordell et al. 1998). Unshaved leaves were finely ground and sent to Duke University for determination of leaf nitrogen on both a mass and area basis, and $\delta^{13}C$. Samples were analysed on a SIRA Series II isotope ratio mass spectrometer (Micromass, Manchester, UK).

For RAPD analyses, young leaves from 10 individuals at each site were immediately placed within activated silica gel. The dried samples were stored at –20°C. Genomic DNA was extracted from 6–10 mg dried plant material ground in 95% ethanol using the DNeasy Plant Mini Kit (QIAGEN Inc.), with the addition of 150 ng Protease (Sigma P-6911), and with the final elution in 1 m*M* Tris (pH 8.5). Fifteen 10-mer primers (University of British Columbia) were used in polymerase chain reactions (5′–3′): 153: GAGTCACGAG; 184: CAAACGGCAC; 204: TTCGGGCCGT; 218: CTCAGCCCAG; 239: CTGAAGCGGA; 244: CAGCCAACCG; 250: CGACAGTCCC; 265: CAGCTGTTCA; 338: CTGTGGCGGT; 347: TTGCTTGGCG; 375: CCGGACACGA; 389: CGCCCGCAGT; 391: GCGAACCTCG; 431: CTGCGGGTCA; 478: CGAGCTGGTC.

Polymerase chain reactions were performed in a volume of 15 µl containing 2 m*M* $MgCl_2$, 0.24 µ*M* of each dNTP, 15 ng BSA, 0.36 µ*M* primer, 0.3 ng genomic DNA, and 0.6 unit of *Taq* DNA polymerase (Promega M1861), well mixed, and the surface covered with 20 µl sterile mineral oil (Sigma M3516). DNA amplification was performed in a programmable thermal cycler (MJ Research Inc.) using a thermal cycle of 94°C (1.5 min), 38°C (2 min), 72°C (2 min) for initial strand separation, then 38 cycles of 91°C (1 min), 38°C (2 min), 72°C (2 min), and a final cycle of 91°C (1 min), 38°C (2 min), and 72°C (5 min) for final extension. Amplification products were analysed by electrophoresis (72–75 V, 60 min) in 1.5% agarose gels in 1× TBE (tris-borate-EDTA) and detected by staining with ethidium bromide. Bands were scored as present or absent. Only markers that were unambiguous, well amplified, and reproducible in replicate tests were scored.

Similarity measures were computed as presence/absence matches, divided by the maximum observed

Table 2 Environmental conditions at the moist, high-elevation, and dry sites on the island of Hawai'i.

	Elevation (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Substrate age (yr)
Moist site	700	19.3	5600	~2500
High site	2470	10.0	1500	~2500
Dry site	600	25.0	500	~1500–3000

Table 3 Foliar characteristics of *Metrosideros polymorpha* populations on the island of Hawai'i.

	Moist site	High site	Dry site
LMA (g/m^2)	130.41 ± 9.36	398.77 ± 11.0	251.69 ± 7.76
Leaf area (cm^2)	10.44 ± 0.75	3.46 ± 0.22	8.32 ± 0.86
Pubescence (g/m^2)	12.2 ± 3.8	176.3 ± 18.6	34.98 ± 5.8
N (%)	0.66 ± 0.05	0.62 ± 0.05	0.80 ± 0.05
N (g/m^2)	0.85 ± 0.04	2.48 ± 0.22	2.01
$\delta^{13}C$	–29.04 ± 0.20	–24.83 ± 0.56	–27.40 ± 0.13

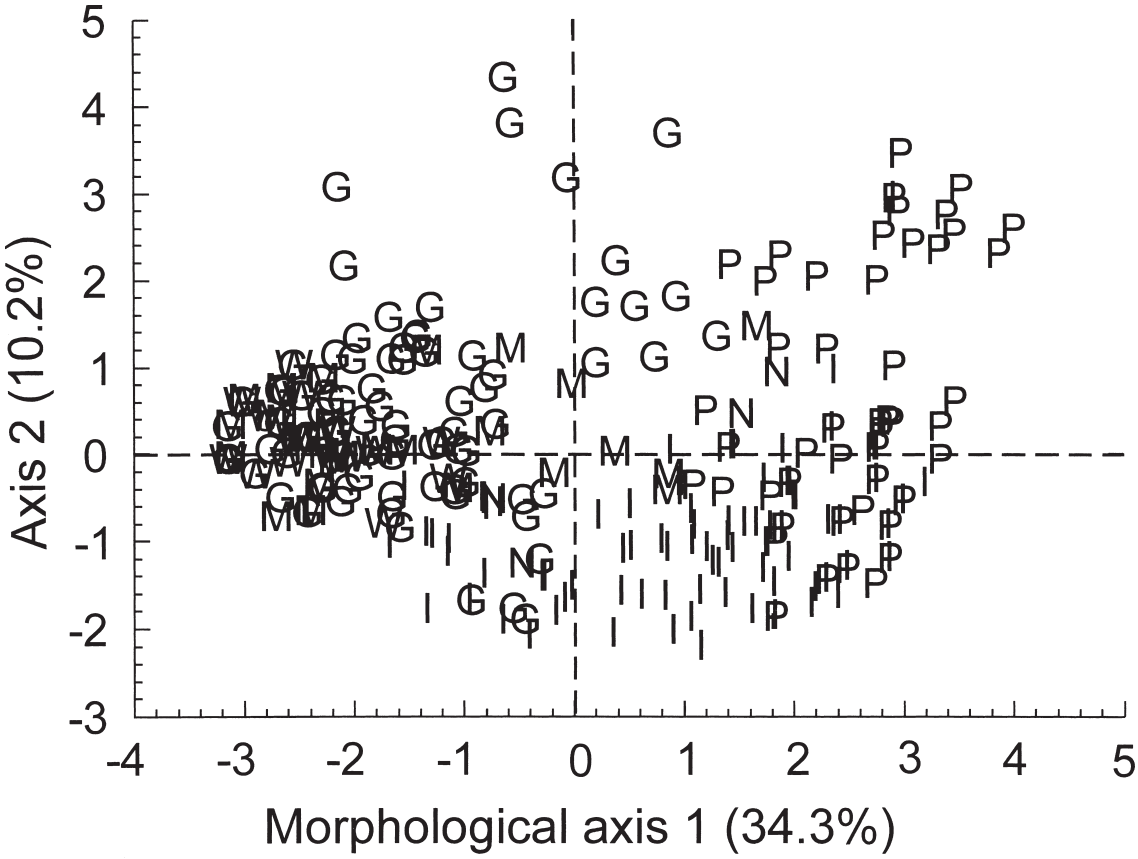

Fig. 2 Principal components analysis based on overall leaf shape, apex and base shape, petiole length, and the presence of pubescence of 342 *Metrosideros polymorpha* specimens within the Herbarium Pacificum, Bishop Museum, Honolulu. Symbols as for Fig. 1.

value for that character over all taxa. Principal coordinate analysis of the similarity matrix follows Gower (1966). The scores of each co-ordinate were statistically compared for each population using one-way analysis of variance.

RESULTS

Analysis of the morphological characteristics of 342 herbarium specimens produced no discernable clusters in the ordinate space, but when taxonomically determined variety names were overlain, vars *glaberrima* and *polymorpha* were placed at each end of the first axis (eigenvalue 34.3%), with var. *incana* bridging the two clusters (Fig. 2). Specimens with pubescent, cordate, apically retuse leaves (e.g., var. *polymorpha*) were positioned at the positive end of the first axis, and specimens having glabrous leaves with a cuneate base and acute apex were positioned at the negative end of the first axis (e.g., vars *glaberrima*, *newellii*, and *macrophylla*). Specimens with a petiole were positioned at the positive end of the second axis (eigenvalue 10.2%), with subsessile leaves at the negative end. The different taxonomic varieties overlapped throughout the ordinal space. The varieties *polymorpha* and *incana* are often difficult to separate visually, and this is reflected in the distribution of the two varieties in the ordinal space. Further, *Metrosideros polymorpha* displays heteroblastic development, and one of us (SAJ) has observed leaves on a single individual, particularly those with epicormic shoots, that correspond to several different varieties. The varieties *macrophylla* and *newellii*

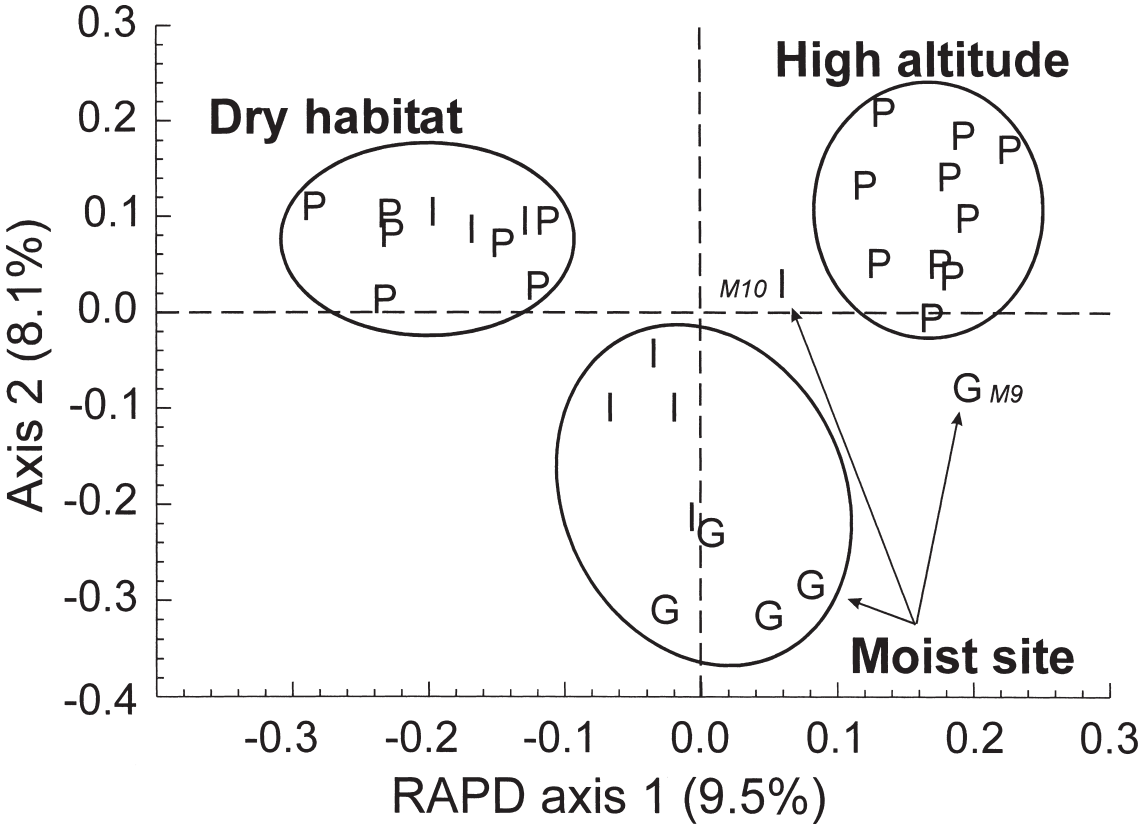

Fig. 3 Principal co-ordinates analysis based on 187 RAPDs bands for the three *Metrosideros polymorpha* populations. Individuals from the high-elevation and dry sites were separated by the second co-ordinate. The moist and high-elevation sites were grouped most closely, with some indication of interbreeding between the populations (numbered individuals). G, var. *glaberrima*; I, var. *incana*; P, var. *polymorpha*.

fell within the var. *glaberrima* ordinal space. Given that these two varieties are sympatric with var. *glaberrima* on the island of Hawai'i, but are localised in very wet areas, they may be larger-leafed phenotypes of var. *glaberrima* (Fig. 1, 2). Four specimens with morphological characteristics of var. *nuda* fell within both var. *glaberrima* and var. *polymorpha* groupings of the morphological analysis (Fig. 2). Further genetic studies are needed to determine whether the varieties of *macrophylla*, *newellii*, and *nuda* are a result of genotypic differentiation or phenotypic plasticity.

The leaf characteristic data suggest that *Metrosideros polymorpha* is morphologically and physiologically distinct at the three field sites (Table 3). Leaf area was greater and LMA and pubescence were significantly lower at the moist site than at the high or dry sites. This is most likely a result of the presence of a higher proportion of glabrous var. *glaberrima* individuals at the moist site. Leaf N, measured on a weight basis, was similar across all sites, whereas leaf N measured on an area basis was 2.4 to 2.8 times higher in the dry and high-elevation sites than in the moist site. Similarly, $\delta^{13}C$, a measure of intrinsic water-use efficiency, differed substantially from the moist site to the high-elevation site (Table 3).

Principal co-ordinates analysis (PCO) of 187 RAPD bands clustered the individuals from the three field sites separately along the first vector (eigenvalue 9.5%) (Fig. 3), but with some intergradation between the moist and high-elevation sites. Analysis of the first vector co-ordinate for each population showed that populations were significantly

different from each other ($F = 93.5$, $P < 0.001$). The second PCO RAPD vector (eigenvalue 8.1%) separated the dry habitat and high-elevation populations from the moist-site population ($F = 31.7$, $P < 0.001$) (Fig. 3). The second vector may reflect an ecological influence (e.g., aridity associated with high elevation and lack of precipitation) within the RAPD data. Third and subsequent vectors of the PCO did not yield any further significant differences between the populations and have not been shown.

Individuals of the glabrous var. *glaberrima* from the moist site clustered to the lower right of the RAPD ordination space, densely pubescent var. *polymorpha* to the upper right, and var. *incana* intermediate between vars. *glaberrima* and *polymorpha* (Fig. 3). Varietal identity was difficult to determine for plants from the dry habitat where leaves had attributes of var. *polymorpha* but had the appressed type of pubescence of var. *incana* rather than woolly pubescence of var. *polymorpha*. These specimens were scored as var. *polymorpha* but were of intermediate form. The individual M9, collected from the moist site, had genetic characteristics similar to specimens collected from the high-elevation site, but morphological characteristics of var. *glaberrima* from the moist site. Similarly, individual M10 was intermediate in morphology and genotype between the moist and high altitude sites.

DISCUSSION

Metrosideros polymorpha has been widely documented as having a broad range of morphological characteristics that overlap between varieties (e.g., Corn & Hiesey 1973; Dawson & Stemmermann 1999), and hybrids have been noted to be recognisable in the field (Porter 1973). This was evident when analysis was conducted on a range of leaf morphological characteristics of herbarium specimens collected from around the island of Hawai'i. The analysis of 187 RAPD bands for 30 individuals also showed genetic overlap between three *M. polymorpha* varieties. Such data give limited support for the current recognition of taxonomic varieties of *M. polymorpha*.

Genetic differences between the three field sites may be a result of biogeographic distance. Intergradation between the moist and high-elevation populations may be due to their closer proximity and possible interbreeding in comparison with the geographically disjunct dry-habitat population. Although the peaks of flowering by sympatric varieties such as vars *polymorpha* and *glaberrima* have been recorded as being different from each other (Porter 1973), possibly causing reproductive isolation, flowering periods were still found to largely overlap, and may be a cause of the genetic flow between varieties at the moist and high-elevation sites. *Metrosideros polymorpha* has small, wind-dispersed seeds (Corn 1972) that would also allow for distribution between the high-elevation and moist sites but more limited dispersal to the dry site, disjunct due to extensive clearing on the opposite side of Mauna Loa (4170 m).

Difference in the local environmental conditions at the three field sites may also result in adaptation. Previous work on *Metrosideros polymorpha* concluded that a small leaf size coupled with high leaf N and photosynthetic rates on an area basis, such as those leaves of var. *polymorpha* found at the high-elevation site, are potentially a consequence of trade-offs between maintenance of adequate carbon gain at the leaf level and resistance to near or below freezing temperatures. A small leaf with high LMA and dense pubescence will yield the highest area-based foliar N and photosynthetic capacity, while the morphological and anatomical implications of a small pubescent leaf, such as reduced cell size and intercellular spaces, and the decreased wettability of the leaf surface, should enhance freezing resistance properties (Cordell et al. 2000). Similarly, maximising photosynthetic capacity relative to water loss (increased water use efficiency) may selectively favour a distinct dry forest phenotype or genotype, such as that found for *M. polymorpha* at the dry field site. Such changes in leaf characteristics have been documented with elevation and aridity in natural populations and in common garden experiments (Corn & Hiesey 1973; Stemmermann 1983; Geeske et al. 1994; Cordell et al. 1998).

Corn & Hiesey (1973) noted developmental changes in leaf form from the seedling to adult form, with distinct variation in the timing of such changes. Epicormic shoots have juvenile characteristics, with juvenile leaves resembling seedling leaves and the leaves of other varieties (Porter 1973). A number of herbarium specimens had several leaf forms on a single shoot, and these were excluded from the morphological analysis. Corn (1979) reported that most trees demonstrate morphological combinations of two or three varieties, and that most plants appear intermediate between described taxa. Stemmermann (1983) similarly noted intergrading forms between vars *polymorpha* and *incana*, and between vars *glaberrima* and *macrophylla*. Despite the fact that

trees with very different morphology grow together within the same habitat, this study further supports the notion that the species is genetically labile and there is as yet little understanding of how this relates to phenotypic variation. The overlap in morphological and genetic characteristics within *M. polymorpha* indicates incomplete isolation and an overall continuum in phenotype and genotype that should be explored further to establish the varietal names.

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