

Genetic evidence for recent and incipient speciation in the evolution of Hawaiian *Metrosideros* (Myrtaceae)

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The genus *Metrosideros* Banks in Hawaii comprises five morphologically recognizable species. In the present study *M. rugosa*, *M. tremuloides*, and *M. polymorpha* were analysed for isozyme variation using starch gel electrophoresis. Fourteen populations from the island of Oahu were assessed for four enzymes encoded by six polymorphic loci. The populations exhibited high levels of variation with mean numbers of alleles per locus ranging from 2.30 to 3.30 with an overall mean of 2.97. The mean observed populational heterozygosity for different populations varied from 0.100 to 0.368 with a mean of 0.272. Gene diversity analysis indicated that 87.7 per cent of the total genetic variation resides within populations, while only 4.7 per cent was due to differentiation among taxa. The mean unbiased genetic identity for pairwise comparisons of species was 0.904, which is very high for congeneric flowering plant species. Therefore, in spite of distinct morphological divergence, the *Metrosideros* taxa studied did not show expected levels of divergence at genes coding for the enzymes studied. The data suggest that both *M. rugosa* and *M. tremuloides* have diverged recently from *M. polymorpha*. However, *M. rugosa* has diverged more ($\bar{I}=0.876$) than *M. tremuloides* ($\bar{I}=0.917$). This may therefore represent a case of recent and incipient speciation in the insular environment.

Keywords: evolution, genetic differentiation, incipient speciation, isozymes, *Metrosideros*.

Introduction

Most continental biota are so ancient that the key events of evolution that formed them have been lost (Carson, 1987). In contrast to this, island biota provide excellent opportunities for biologists to study the dynamic processes of evolution and speciation. The Hawaiian archipelago is a highly isolated group of volcanic islands in the Pacific situated 4000 km from the nearest continent. Colonization processes have populated the windward slopes of the volcanoes with forest ecosystems up to altitudes of about 2000 m. Climatic and edaphic factors vary dramatically over short distances in these islands, providing numerous niches to which plants may become adapted. This ecological diversity has been accompanied by the evolution of literally hundreds of species unique to these islands (Carson, 1987). Mueller-Dombois (1981) aptly describes the Hawaiian biota as naturally depauperated and secondarily enriched. However, the

high rate of evolution and speciation in these islands is also accompanied by an increased rate of extinction due to natural and anthropogenic habitat modification.

The genus *Metrosideros* Banks (Myrtaceae) is widely distributed in the six major islands of the Hawaiian Archipelago (i.e. Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii). This genus also occurs naturally in the other oceanic islands of the Pacific from about 52° S latitude on Auckland Island to about 25° N latitude and from Australia and Malaysia in the west to French Polynesia in the east (Smith, 1973). *Metrosideros* comprises an abundant and taxonomically confusing species complex throughout much of its range (Smith, 1973). At least five species have been described from Hawaii entirely based on morphological criteria (Rock, 1917; Skottsberg, 1944). *Metrosideros polymorpha* is the dominant tree species in Hawaii and occupies over 80 per cent of the relatively intact forests (Mueller-Dombois & Loope, 1990). The remaining four species; *M. macropus*, *M. rugosa*, *M. tremuloides* and *M.*

waialealae, are of much narrower distribution and occur sympatrically with *M. polymorpha* on the island of Oahu.

Metrosideros polymorpha has an extremely wide ecological amplitude that occurs from near sea level to the tree line at 2500 m on Mauna Loa on the island of Hawaii. It is well adapted to cloud forest conditions where it can grow to 20 m tall. On exposed, eroded ridges it occurs as a shrub or low growing tree and in bogs grows as a prostrate shrub or small tree, sometimes flowering when under 10 cm tall. *Metrosideros polymorpha* is the first tree to colonize new lava flows where it forms mature, closed stands in about 400 years in the rainforest climate (Atkinson, 1970).

Metrosideros polymorpha exhibits very high levels of morphological diversity and adaptation in the Hawaiian islands. Rock (1917) identified 11 varieties in an attempt to classify the variability within *M. polymorpha* and Porter (1972) treated all taxa of *Metrosideros* as varieties and forms of this species. Corn (1979), however, reported clinal morphological variation in Hawaiian *Metrosideros* correlated with a complex altitudinal gradient, and Stemmermann (1983) discovered a positive association of morphological variation with age of substrate. The validity of the described infraspecific taxa is therefore, questionable.

Three morphologically distinct taxa, *M. rugosa*, *M. tremuloides* and *M. polymorpha*, from the Koolau and Waianae mountains of the island of Oahu, were involved in the present study. *Metrosideros rugosa* is a shrub or small tree mainly restricted to dry, eroded ridge tops in the Koolaus, characterized by strongly rugose leaves with lower surfaces densely woolly. *Metrosideros tremuloides* is also a shrub or low growing tree with a semi-weeping habit and occurring on steep slopes of the Koolau and Waianae mountains. The leaves are distinctively small and narrow. *Metrosideros polymorpha* is highly variable in size, ranging from shrubs to tall trees, inhabiting a wide range of habitats. The leaf shape and flower colour are also highly variable.

The information on the breeding system in these taxa is lacking. However, an earlier study has shown that these taxa have different peak flushing and flowering periods but the temporal isolation is incomplete (Porter, 1972). Corn (1979) reported that there is extensive outcrossing in *Metrosideros* because the flowers are protandrous with sticky pollen that is transmitted by native birds that visit the flowers for nectar. Carpenter (1976) observed partial self-incompatibility among red-flowered types and normal seed-set occurs only with outbreeding. The presence of morphologically intermediate forms of these taxa in natural populations may also indicate that these taxa are still

connected through significant levels of gene flow. *Metrosideros* bear numerous and small seeds, but only about 15 per cent of the seeds appeared to be well filled with embryos and about 30 per cent germinate (Burton, 1982). Wind dispersal is probably the chief seed dispersal mechanism of this genus (Corn, 1972).

The extensive variability exhibited by the Hawaiian *Metrosideros* complex, involving either closely related species and/or varieties within species, offers a unique opportunity to study the evolutionary mechanisms that operate in plant populations. Morphological studies alone are often inadequate to determine evolutionary relationships in such complexes (Systma & Schaal, 1985). Hence, integrative studies involving variation for biochemical characters such as isozymes or other macromolecules, in addition to morphological comparisons, are most effective in such diverse groups.

The purpose of this study was to

- 1 assess genetic variation in the Hawaiian species complex of the genus *Metrosideros*;
- 2 determine the extent of genetic divergence among morphologically discrete taxa treated as different species; and
- 3 re-evaluate these taxa systematically in the light of genetic evidence.

Materials and methods

Three populations of *M. rugosa*, two of *M. tremuloides*, and nine of *M. polymorpha* were sampled on the island of Oahu, Hawaii (Table 1 and Fig. 1). A minimum of 30 sexually mature trees were sampled per population for fresh young leaves. The samples were transported to the laboratory on ice, stored at 4°C, and analysed within 7 days.

Electrophoresis

Leaf samples were homogenized in a buffer containing 10 per cent dimethyl sulphoxide (DMSO), 0.02 M sodium metabisulphate, and 0.005 M diethyldithiocarbamate (DIECA) in addition to the other constituents listed by Bousquet *et al.* (1987). The homogenate was absorbed onto Whatman No. 3 filter-paper wicks (3 mm × 1.0 cm) and loaded onto 12 per cent starch gels prepared with a histidine-citrate buffer at pH 6.5 (Cardy *et al.*, 1983). The gel buffer consisted of 0.016 M histidine (free base) and 0.002 M citric acid and the tray buffer of 0.065 M histidine and 0.007 M citric acid. Gels were cooled to 4°C before loading samples. Electrophoresis was conducted in a refrigerator at 4°C and at 200 V (20 V cm⁻¹) with 40 mA for 6 h. Four enzyme systems, known to be polymorphic (PGI, PGM, PER, and LAP; K.M. Aradhya,

Table 1 Populations and species of *Metrosideros* studied

Population designation	Altitude (m)	Taxon	Approximate location
1 (MR,PU-1)	550	<i>M. rugosa</i>	Off Koolau ridge summit trail, Pupukea, Koolauloa District
2 (MR,PU-2)	570	<i>M. rugosa</i>	About 0.5 km away from MR,PU-1 along ridge trail
3 (MR,P)	600	<i>M. rugosa</i>	Pauoa, above Pauoa flats, off Tantalus trail, along summit
4 (MT,P)	570	<i>M. tremuloides</i>	Pauoa, above Pauoa flats, off Tantalus trail, Honolulu District
5 (MT,PS)	570	<i>M. tremuloides</i>	Pauoa, above Pauoa flats, off Tantalus trail, along summit
6 (MP,PU)	570	<i>M. polymorpha</i>	Above Pauoa flats, along summit, Honolulu District
7 (MP,WK)	650	<i>M. polymorpha</i>	Northern ridge of Waianae Kai, Waianae District
8 (MP,PK,EWA)	750	<i>M. polymorpha</i>	Puu Kaua, Ewa side slope, Ewa District
9 (MP,PK,WS)	700	<i>M. polymorpha</i>	Puu Kaua, Waianae side slope, Waianae District
10 (MP,ML)	475	<i>M. polymorpha</i>	Mauumae ridge summit trail above Maunalani heights, Honolulu District
11 (MP,AH-1)	400	<i>M. polymorpha</i>	Aiea Heights loop trail, above Aiea Heights Park, Ewa District
12 (MP,AH-2)	425	<i>M. polymorpha</i>	Aiea Heights loop trail towards Kalauao ridge
13 (MP,AH-3)	425	<i>M. polymorpha</i>	Aiea Heights trail towards Kalauao ridge
14 (MP,AH-4)	400	<i>M. polymorpha</i>	Aiea Heights trail towards Kalauao ridge

unpublished data) were assayed following Arulsekhar & Parfitt (1986) and Shaw & Prasad (1970).

Data analysis

Genotype frequencies were inferred directly from observed isozyme phenotypes. The mean number of alleles per locus, observed and expected levels of heterozygosity and Nei's unbiased genetic identity and

distance coefficients (Nei, 1978) were computed with the BIOSYS program (Swofford & Selander, 1989).

The genetic diversity within the *Metrosideros* species complex was partitioned using the gene diversity analysis (Nei, 1973). Total gene diversity (H_T) was partitioned into gene diversity within populations (H_S) and gene diversity among populations (D_{ST}) where $H_T = H_S + D_{ST}$. H_T was calculated on the weighted average allele frequencies over all populations

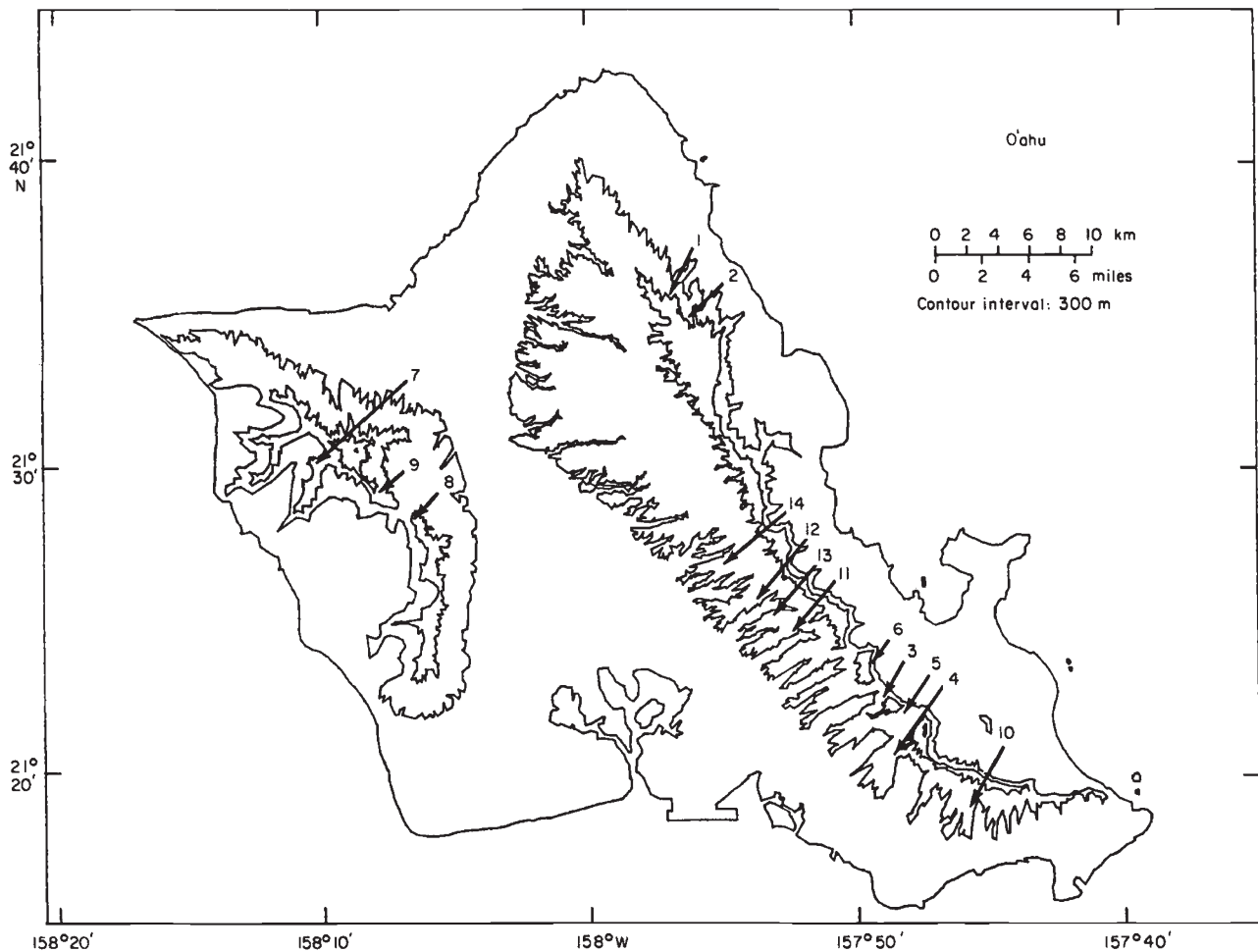


Fig. 1 Approximate location of collection sites for 14 populations of *Metrosideros*.

($H_T = 1 - \sum \bar{p}_i^2$). H_S is equal to the weighted average over all populations of the values of $1 - \sum p_i^2$ for each population. D_{ST} is obtained by subtraction ($D_{ST} = H_T - H_S$). Differentiation among populations is calculated as $G_{ST} = D_{ST}/H_T$ where G_{ST} can vary between 0 (when $H_S = H_T$) and 1 (when $H_S = 0$), i.e. populations fixed for different alleles.

Similarly specieswise pooled analysis was performed to further partition G_{ST} into gene diversity due to among populations within taxa and among taxa. It was decided not to pool the data across loci as the rate of divergence was different.

A UPGMA cluster analysis was performed on populational, pairwise genetic identity coefficients (Sneath & Sokal, 1973).

Results

Genetic variability

The six putative loci assayed were *Pgi*, *Pgm-1*, *Pgm-2*, *Per*, *Lap-1* and *Lap-2*. The allelic frequencies in 14

populations are given in Table 2. A total of 30 alleles were detected, with the number of alleles per locus ranging from three for *Lap-2* to eight for *Pgm-1*. The patterns of allelic distribution and number of alleles per locus varied across populations. For most loci, there was at least one common allele at high frequency in most populations. Two alleles (87 and 100) were equally predominant in most populations for *Per*, and allele 83 was unique to population 3 (Table 1) of *M. rugosa*. The Waianae populations of *M. polymorpha* (7–9) possessed five unique, low frequency alleles at *Pgi*, *Pgm-1* and *Pgm-2*. *Metrosideros polymorpha* populations from Aiea (11–14) did not possess allele 110 for *Lap-2*, which was expressed in all other populations. All alleles expressed in *M. tremuloides* populations (4 and 5) were a subset of those observed in *M. polymorpha* and these two species uniquely shared *Lap-1* allele 89.

The intra-populational estimates of genetic variability are presented in Table 3. The mean number of alleles per locus ranged from 2.3 to 3.3 with a mean of 3.0. The mean for *M. rugosa* populations was the

Table 2 Allelic frequencies for the six polymorphic loci in Hawaiian *Metrosideros*

Locus	Population													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Pgi</i>														
(N)	40	36	45	35	40	40	50	38	35	35	30	30	30	30
88	0.025	0.028	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
92	0.013	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.050	0.067	0.033
100	0.925	0.917	0.967	0.800	0.925	0.950	0.750	0.566	0.671	0.757	0.883	0.783	0.850	0.817
108	0.000	0.000	0.022	0.186	0.075	0.000	0.000	0.000	0.000	0.043	0.083	0.167	0.083	0.150
112	0.038	0.042	0.011	0.014	0.000	0.025	0.240	0.421	0.314	0.200	0.000	0.000	0.000	0.000
122	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.013	0.014	0.000	0.000	0.000	0.000	0.000
<i>Pgm-1</i>														
(N)	40	40	45	35	40	40	40	38	39	35	30	30	30	30
60	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.092	0.077	0.000	0.000	0.000	0.000	0.000
91	0.025	0.025	0.022	0.000	0.000	0.013	0.000	0.171	0.115	0.014	0.067	0.000	0.067	0.017
95	0.175	0.175	0.122	0.143	0.038	0.100	0.175	0.145	0.231	0.114	0.067	0.000	0.017	0.050
100	0.425	0.488	0.422	0.543	0.950	0.750	0.738	0.447	0.423	0.786	0.783	0.883	0.550	0.617
109	0.063	0.087	0.111	0.214	0.013	0.087	0.038	0.105	0.038	0.014	0.083	0.117	0.217	0.200
118	0.313	0.225	0.311	0.100	0.000	0.050	0.050	0.039	0.051	0.071	0.000	0.000	0.150	0.117
123	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000
<i>Pgm-2</i>														
(N)	40	40	45	35	40	40	40	38	37	35	30	30	30	30
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
95	0.138	0.038	0.067	0.086	0.000	0.013	0.038	0.118	0.135	0.057	0.033	0.083	0.000	0.000
100	0.700	0.762	0.600	0.829	1.000	0.938	0.863	0.789	0.703	0.943	0.967	0.917	0.917	0.900
105	0.162	0.200	0.333	0.086	0.000	0.050	0.075	0.079	0.162	0.000	0.000	0.000	0.083	0.100
109	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Per</i>														
(N)	40	40	30	34	40	40	50	38	37	39	30	30	30	30
83	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
87	0.450	0.587	0.067	0.338	0.175	0.363	0.060	0.118	0.149	0.295	0.250	0.350	0.350	0.217
100	0.538	0.412	0.917	0.662	0.825	0.637	0.510	0.882	0.851	0.705	0.750	0.633	0.617	0.783
130	0.013	0.000	0.000	0.000	0.000	0.000	0.430	0.000	0.000	0.000	0.000	0.017	0.033	0.000
<i>Lap-1</i>														
(N)	40	40	12	35	40	40	40	38	37	35	30	30	30	30
89	0.000	0.000	0.000	0.129	0.075	0.000	0.000	0.000	0.000	0.157	0.133	0.067	0.000	0.000
95	0.225	0.287	0.125	0.257	0.100	0.162	0.237	0.158	0.162	0.157	0.150	0.067	0.317	0.300
100	0.300	0.587	0.458	0.586	0.825	0.688	0.738	0.842	0.838	0.686	0.717	0.867	0.683	0.700
105	0.475	0.125	0.417	0.029	0.000	0.150	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Lap-2</i>														
(N)	40	40	12	35	40	40	40	38	37	35	30	30	30	30
97	0.000	0.150	0.000	0.000	0.075	0.050	0.000	0.000	0.000	0.000	0.033	0.133	0.383	0.250
100	0.438	0.475	0.542	0.971	0.800	0.637	0.613	0.842	0.946	0.671	0.967	0.867	0.617	0.750
110	0.563	0.375	0.458	0.029	0.125	0.313	0.387	0.158	0.054	0.329	0.000	0.000	0.000	0.000

highest (3.3) followed by the populations of *M. polymorpha* (2.9) and *M. tremuloides* (2.7). Mean observed heterozygosity ranged from 0.100 to 0.368 with an overall mean of 0.272. Mean observed heterozygosity was the highest for *M. rugosa* (0.323) followed by *M. polymorpha* (0.269) and *M. tremuloides* (0.208). The

expected heterozygosity followed the same trend with the highest recorded for *M. rugosa* (0.470) followed by *M. polymorpha* (0.360) and *M. tremuloides* (0.296).

The fixation index (F ; Wright, 1965; Jain & Workman, 1967) was calculated for all populations and averaged across loci (Table 3). The index is equal to

Table 3 Genetic variability in Hawaiian *Metrosideros* (standard errors in parentheses)

Population designation	Taxon	Mean number of alleles per locus	F^*	Mean heterozygosity	
				Direct-count	HdyWbg expected†
1.	<i>M. rugosa</i>	3.3 (0.4)	0.265‡	0.363 (0.089)	0.494 (0.079)
2.	<i>M. rugosa</i>	3.3 (0.4)	0.340‡	0.319 (0.097)	0.483 (0.077)
3.	<i>M. rugosa</i>	3.3 (0.6)	0.341‡	0.286 (0.097)	0.434 (0.106)
4.	<i>M. tremuloides</i>	3.0 (0.4)	0.198	0.316 (0.059)	0.394 (0.087)
5.	<i>M. tremuloides</i>	2.3 (0.3)	0.492‡	0.100 (0.047)	0.197 (0.056)
6.	<i>M. polymorpha</i>	3.2 (0.4)	0.438‡	0.196 (0.056)	0.349 (0.077)
7.	<i>M. polymorpha</i>	3.2 (0.3)	0.252‡	0.312 (0.050)	0.417 (0.042)
8.	<i>M. polymorpha</i>	3.2 (0.7)	0.064	0.368 (0.091)	0.393 (0.081)
9.	<i>M. polymorpha</i>	3.3 (1.0)	0.255‡	0.287 (0.087)	0.385 (0.092)
10.	<i>M. polymorpha</i>	2.8 (0.5)	0.132‡	0.322 (0.068)	0.371 (0.055)
11.	<i>M. polymorpha</i>	2.7 (0.3)	0.208‡	0.206 (0.081)	0.260 (0.069)
12.	<i>M. polymorpha</i>	2.5 (0.2)	0.429‡	0.161 (0.048)	0.282 (0.049)
13.	<i>M. polymorpha</i>	2.8 (0.5)	0.222	0.322 (0.056)	0.414 (0.071)
14.	<i>M. polymorpha</i>	2.7 (0.5)	0.326‡	0.250 (0.041)	0.371 (0.053)
Mean		3.0	0.283‡	0.272	0.371

*Fixation index (Wright, 1965).

†Unbiased estimate (see Nei, 1978).

‡ $P < 0.05$.

$(H_{\text{exp}} - H_{\text{obs}})/H_{\text{exp}}$, where H_{exp} and H_{obs} refer to expected and observed heterozygosities, respectively. There was a significant deficiency of heterozygotes as compared to Hardy-Weinberg expectations in most populations except for populations 4 (*M. tremuloides*), 8 and 13 (both *M. polymorpha*) ($P < 0.05$).

Population differentiation

The gene diversity analysis of the *Metrosideros* complex (Table 4) indicated that the total gene diversity (H_T), a measure of mean heterozygosity expected under random mating, ranged from 0.287 for *Pgm-2* to 0.578 for *Pgm-1* with an overall mean of 0.423. The

intrapopulation gene diversity summed over all loci accounted for a significant proportion of the total gene diversity (87.7 per cent), while diversity due to differentiation among populations (G_{ST}) ranged from 9.1 per cent for *Pgm-2* to 18.1 per cent for *Lap-2* with an average of 12.3 per cent. Further partitioning of the G_{ST} component into that among populations within taxa and that among taxa indicated that a greater proportion of gene diversity due to interpopulation differentiation resides among populations within taxa (7.6 per cent) compared to among taxa (4.8 per cent).

The unbiased genetic distance (D) and identity (I) measures of Nei (1978) were calculated for both pairwise comparisons among the 14 populations and

Table 4 Measures of gene diversity and population differentiation in *Metrosideros*

Locus	Differentiation among populations				Apportionment of diversity		
	H_T	H_S	D_{ST}	G_{ST}	Within populations	Among populations within taxa	Among taxa
<i>Pgi</i>	0.302	0.267	0.035	0.116	0.884	0.083	0.033
<i>Pgm-1</i>	0.578	0.522	0.056	0.097	0.903	0.057	0.040
<i>Pgm-2</i>	0.287	0.261	0.026	0.091	0.909	0.042	0.049
<i>Per</i>	0.457	0.392	0.065	0.142	0.858	0.125	0.018
<i>Lap-1</i>	0.482	0.430	0.052	0.108	0.892	0.048	0.060
<i>Lap-2</i>	0.430	0.352	0.078	0.181	0.819	0.095	0.086
Mean	0.423	0.371	0.052	0.123	0.877	0.076	0.047

H_T = total gene diversity; H_S = gene diversity within populations; D_{ST} = gene diversity among populations; G_{ST} = proportion of total gene diversity due to inter-population differentiation

		Genetic identity among populations within taxa	
		2	3
		0.938	
		0.901-0.958)	
2. <i>M. tremuloides</i>	2	0.877	0.958
		(0.831-0.917)	(0.958-0.958)
3. <i>M. polymorpha</i>	9	0.874	0.961
		(0.792-0.968)	(0.904-0.993) (0.903-0.998)

species but only the pairwise genetic identity for species comparisons are presented in Table 5. The pairwise identities among populations ranged from 0.79 to 1.00 with a mean of 0.92. Maximum divergence was observed between population 1 (*M. rugosa*) and populations 8 and 9 (both *M. polymorpha*) ($I = 0.79$ and 0.80 respectively). The genetic identities between *M. rugosa* and *M. tremuloides*, *M. rugosa* and *M. polymorpha*, and *M. tremuloides* and *M. polymorpha* were 0.88, 0.87, and 0.96, respectively.

The UPGMA cluster analysis resulted in two discrete groups of populations (Fig. 2). *Metrosideros rugosa* (1-3) populations segregated from the others at a mean genetic identity of 0.88. *Metrosideros polymorpha* populations from Waianae (7-9) were distinct from the remaining populations. The two *M. tremuloides* populations (4-5) were each more similar to *M. polymorpha* populations than they were to each other.

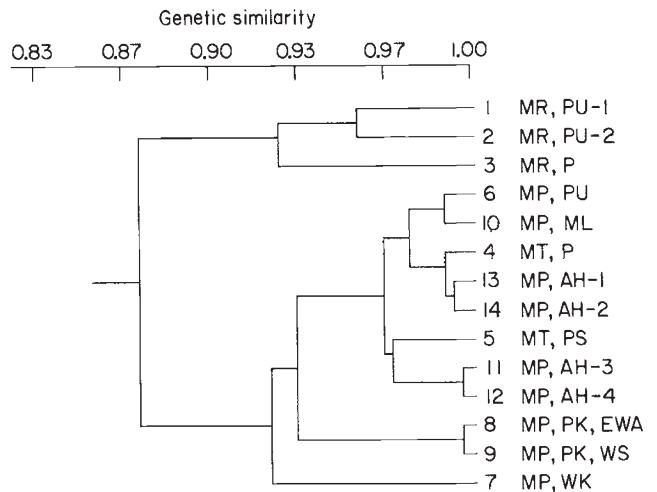


Fig. 2 Dendrogram showing genetic similarities among 14 populations of *Metrosideros*.

Discussion

Hawaiian *Metrosideros* is an excellent example of a genus that exhibits incipient speciation in an insular environment. The species in this genus may have evolved through local adaptation to various distinctive sites such as bogs, dry and eroded ridge tops and gulches, and similarly, for ecological zones such as lowland dry forests, montane rain forests, cloud forests, and sub-alpine scrub forests on high volcanoes in the islands.

Although there was evidence for differentiation of allele frequencies among the 14 *Metrosideros* populations for the six loci assessed, the extent of differentiation was marginal. Most populations shared one or two dominant alleles at high frequencies and the unique, low-frequency alleles found in several populations may have originated recently. Similar patterns were frequently observed in outcrossing long-lived trees and woody species (Gottlieb, 1981; Hamrick *et al.*, 1979). Deficiency of heterozygotes observed in some populations of *Metrosideros* could be due to some degree of self-fertilization.

Little genetic divergence among populations of *Metrosideros* complex has occurred at the molecular level with 87.7 per cent of the total genetic variation residing within populations. Similar results were obtained in conifer species which occupy large ranges (Guries & Ledig, 1981; Yeh & O'Malley, 1981). Hamrick *et al.* (1979) also demonstrated that plant species, which are widespread, long lived and primarily outcrossed, maintain high levels of intrapopulation genetic variation.

Genetic differentiation appears uniformly low across loci except for *Lap-2* and *Per*. The three taxa involved in the present study showed very little divergence for the isozyme loci studied (4.7 per cent) when compared to populations within taxa (7.6 per cent).

The foregoing gene diversity analysis of the Hawaiian *Metrosideros* complex suggests that the taxa in the present study are still in the process of speciation and that the level of isozymic differentiation is indicative of their recent origin. Furthermore, the extremely efficient seed dispersal system, coupled with high rates of outcrossing in the Hawaiian *Metrosideros*, may act as homogenizing forces to discourage rapid differentiation brought about by disruptive selection. Carlquist (1966) reported that the genetic barriers among island species, especially those which have originated on islands, will be expected to be virtually absent. Hybridization may, as Rattenbury (1962) suggests, help a species survive a 'bottleneck' caused by dwindling land area and climatic stress.

The mean pairwise genetic identity of 0.92 of the 14 populations, including three species, is comparable to values normally obtained for continental conspecific populations (Gottlieb, 1977, 1981; Crawford, 1983). The morphologically distinct *M. rugosa*, however, could be distinguished isozymically from the other two taxa, which suggests that this taxon diverged less recently from *M. polymorpha* than did *M. tremuloides*. The populations of *M. tremuloides* could not be distinguished isozymically from those of *M. polymorpha*, although the two taxa are morphologically discrete. This suggests that the latter two taxa have diverged very recently and their morphological differences for leaf characteristics may be due to small genetic differences not linked to isozyme loci. *Metrosideros polymorpha* populations from the Waianae mountains have diverged genetically from *M. polymorpha* from the Koolaus, possibly due to distinctness of these two mountains with respect to geological history, age of substrate, climate, and geographical isolation leading to limited gene flow (Fig. 2).

Narrow genetic divergence at isozyme loci among different insular taxa was also reported for *Tetramolopium* (Lowrey, 1981), *Bidens* (Ganders & Nagata, 1984; Helenurm & Ganders, 1985) and the silver-sword alliance of species belonging to the genera *Argyroxiphium*, *Dubautia*, and *Wilkesia* (Carr, 1985a,b; Witter & Carr, 1988) from the Hawaiian islands. Lowrey & Crawford (1985) found very little isozymic divergence among seven species of *Tetramolopium*, with a mean genetic identity of 0.95. Similar results were obtained for six species from two of the three recognized subgenera of the genus *Dendroseris* which is endemic to the Juan Fernandez Islands, Chile (Crawford *et al.*, 1987a). Hawaiian *Bidens* with 19 species and eight subspecies endemic to Hawaii, exhibited very little genetic differentiation for isozyme loci (mean $I=0.957$) although there is substantial morphological and ecological differentiation (Helenurm & Ganders, 1985). It has been suggested that congeneric species of insular plants may differ by more conspicuous morphological features than their counterparts on continents in spite of very little genetic divergence (Crawford *et al.*, 1987b). Similarly, Hawaiian *Drosophila* species show great variation in morphology and behaviour yet exhibit relatively little cytogenetic differentiation (Carson *et al.*, 1970).

These patterns of divergence are similar to those observed in the present study of the genus *Metrosideros* and appear to be due to the differential adaptive value of morphological versus isozymic or cytogenetic characters. Genetic differentiation at isozyme loci apparently has not occurred at the same rate as that of

adaptive morphological characters in Hawaiian *Metrosideros*.

The genetic basis for the morphological differences among different species of *Metrosideros* may be relatively simple as suggested for other taxa by Hilu (1983), Gottlieb (1984), and Crawford *et al.* (1987b). Presumably the presence of extensive morphological and genetic variation, coupled with reasonably high levels of heterozygosity, have allowed Hawaiian *M. polymorpha* to function as a tropical lowland, as well as a sub-alpine, canopy tree. Such a range of climatic adaptation is normally exhibited by different species in continental ecosystems (Mueller-Dombois, 1987).

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