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Genetic structure and differentiation in *Metrosideros polymorpha* (Myrtaceae) along altitudinal gradients in Maui, Hawaii

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Summary

The level and distribution of genetic variability within and among *Metrosideros polymorpha* populations along altitudinal gradients on the island of Maui, Hawaii were examined to assess the extent of genetic differentiation. Sixteen loci encoding 11 enzymes were scored in 17 populations along the NE wet slope of Mt. Haleakala and Kipahulu Valley in East Maui and six populations along the Puu Kukui trail in West Maui. On average, 50% of the loci were polymorphic within populations with an overall mean of 2·15 alleles per locus. The observed heterozygosities for different populations were moderate (0·108–0·220) and conformed to panmixia except for one of the mid-elevation populations. The distribution of allozyme variation indicates that very little differentiation has occurred along altitudinal gradients. Approximately 90% of the total variation resides within populations in East Maui while 95% was found within West Maui populations. The mean populational pair-wise genetic identities (Nei's I) ranged from 0·909 to 0·998. The UPGMA cluster analysis on genetic identity matrices and PCA on allele frequencies revealed marginal altitudinal differentiation. Twenty one alleles out of a total 63 showed statistically significant correlations with environmental variables.

1. Introduction

Of all the oceanic islands in the world, the Hawaiian archipelago is the most geographically isolated and possesses some of the most dramatic altitudinal (up to 4200 m above MSL) and environmental gradients along the windward slopes. Latitudinal and altitudinal gradients are complex environmental gradients along which a number of environmental and edaphic factors vary jointly (Whittaker, 1975). The large numbers of potential habitats created by such variations in topography, rainfall, and other related climatic and edaphic factors are thought to be contributing factors to rapid speciation in the Hawaiian islands (Simon, 1987). As a consequence there is a high degree of endemism among flowering plants.

The remarkable geographic isolation and lack of competition coupled with relatively simple biomes have allowed certain successful species to occupy a wide range of habitats. Such widespread species are ideally suited for studies of infraspecific genetic variation and differentiation in relation to environmental factors. Although genetic structure has been described in many conspecific populations, the balance among selection, migration, and genetic drift is poorly understood (Levin, 1988). Ecological factors affecting reproduction and dispersal are likely to be particularly important in determining genetic structure (Allard, 1970; Jain, 1975).

Gene frequency clines in space and time may originate in response to ecological gradients and/or as a result of gene flow among populations of differing genetic composition (Nevo & Bar, 1976; Endler, 1977). The theory of clines assumes that the genetic structure of populations reflects both local adaptation and gene flow (Haldane, 1948; Fisher, 1950; Mayr, 1963; Dobzhansky, 1970). Adaptive genetic variation and divergence have been examined as a response to environmental gradients (Mitton *et al.* 1980; Chapin & Chapin, 1981; Anderson *et al.* 1987; Schwaegerle & Bazzaz, 1987; David *et al.* 1989; Ganders, 1990), and soil properties, such as heavy metal content, nutrient availability, and salinity (McNeilly, 1968; Wu & Antonovics, 1976; Antlfinger, 1981; Snaydon & Davies, 1982). These studies demonstrate genetic differences among populations in response to physical

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factors in the environment. However, such studies in plants are biased toward temperate species, usually annuals, short-lived perennials, or conifers (Loveless & Hamrick, 1984; Hamrick and Godt, 1990). Long-lived angiosperm trees have not been studied extensively and the population genetics of tropical insular tree species is virtually unknown.

Metrosideros polymorpha Gaud. commonly known as 'ohia lehua' is the dominant tree species endemic to the Hawaiian islands. It has an extremely wide ecological amplitude, occurring from near sea level to the tree line at 2500 m elevation, with annual rainfall and temperatures ranging from 75 to 1150 cm and 9 to 23 °C, respectively (Corn, 1979; Mueller-Dombois, 1987). As the name indicates, *M. polymorpha* is a highly polymorphic taxon with a number of varieties (Dawson & Stemmermann, 1990). Corn (1979) reported clinal morphological variation in Hawaiian *Metrosideros* associated with altitudinal gradients. Stemmermann (1983) found a positive association of morphological variation such as leaf shape, leaf pubescence, and other characters, with age of the substrates on which the plants were growing. She called these 'successional varieties'.

Ohia colonizes new lava flows as a pioneer (Eggler, 1971; Smathers & Mueller-Dombois, 1974) and also persists as one of the most abundant trees in later successional stages, similar to the climax species in continental environments. Mueller-Dombois & Loope (1990) hypothesized that *M. polymorpha*, as the dominant tree species, faced reduced competition in the early stages of its colonization on islands and thus invaded sites over an extremely broad ecological spectrum, on some of which it is not well adapted.

Metrosideros polymorpha is principally an allogamous species and pollination is accomplished by birds, insects, and wind (Aradhya, unpublished data). Carpenter (1976) observed partial self-incompatibility among red flowered types (common type), while yellow flowered types are totally self-compatible. She reported that *Metrosideros* populations seem to have differentiated along elevational gradients, with adaptations for bird pollination increasing proportionately with elevation.

A recent study on the distribution of *M. polymorpha* along an elevational gradient in East Maui has shown the existence of at least three morphologically distinct varieties restricted to low, middle and high elevations (Kitayama & Mueller-Dombois, 1992). The genetic basis for these morphological varieties is not known. A study on the level and organization of genetic variation across the species distributional range would aid in answering this question. It would also contribute to the understanding of evolution and speciation in the insular environment.

The present study was undertaken on the island of Maui which is the second largest in the Hawaiian Archipelago. The island was formed by two independent volcanoes. The West Maui mountains are geologically older (about 1·3 million years) and reach an altitude of 1764 m at the summit of Puu Kukui and Mt. Haleakala (about 0·8 million years) and reach about 3055 m (Stearns, 1985). The purpose of this study was (i) to examine the level and organization of genetic variation in *M. polymorpha* by partitioning the species-wide variation into within and between population components; (ii) to assess the genetic relationships among populations along altitudinal gradients so as to understand the extent of genetic differentiation among populations; and (iii) to examine the relationship between gene frequencies and environmental variables.

2. Materials and methods

The approximate locations of the 23 populations of *Metrosideros polymorpha* studied and corresponding elevations are shown on the map in Fig. 1.

(i) *Sampling of natural stands*

Sampling plots were established at approximately 200 m elevational intervals along the transects. The sampling sites represented a great deal of variation with respect to a number of ecological factors, including density, size structure, disturbance history, and associated vegetation. The vegetation along the windward slope of Mt. Haleakala (transect 1) has been described (Kitayama & Mueller-Dombois, 1992). Altogether 23 populations were sampled; ten from the Haleakala transect (1–10), three from Kipahulu transect (15–17), four additional populations [two along the Koolau Gap trail (11 and 12) and two from inside the crater near Paliku (13 and 14)], and six from the West Maui mountains along the Puu Kukui trail (18–23). For more details regarding climate, substrates, topography, and associated vegetation along the elevational gradients, refer to Aradhya (1992). A minimum of 40 mature trees were randomly sampled for fresh young leaves at each site. The samples were air flown the same evening to the laboratory at the University in Honolulu on ice, stored at 4 °C, and analyzed within 7 days.

(ii) *Electrophoretic analysis*

About 25 mg of leaf tissue was homogenized in 0·1 ml freshly prepared chilled extraction buffer (Aradhya, 1992). β-mercaptoethanol and polyvinylpolypyrolidone (PVPP) were added to the extraction buffer just before grinding the tissue. The resulting slurry was absorbed on Whatman No. 3 filter paper wicks (3 × 10 mm). Wicks were immediately loaded into 12% starch gels (Sigma Chemical Co.) previously prepared in histidine-citrate gel buffer and cooled to 4 °C. Among several known gel and running buffer systems tried, histidine-citrate pH 6·5 (Cardy *et al.* 1983) was found suitable for resolving enzymes in

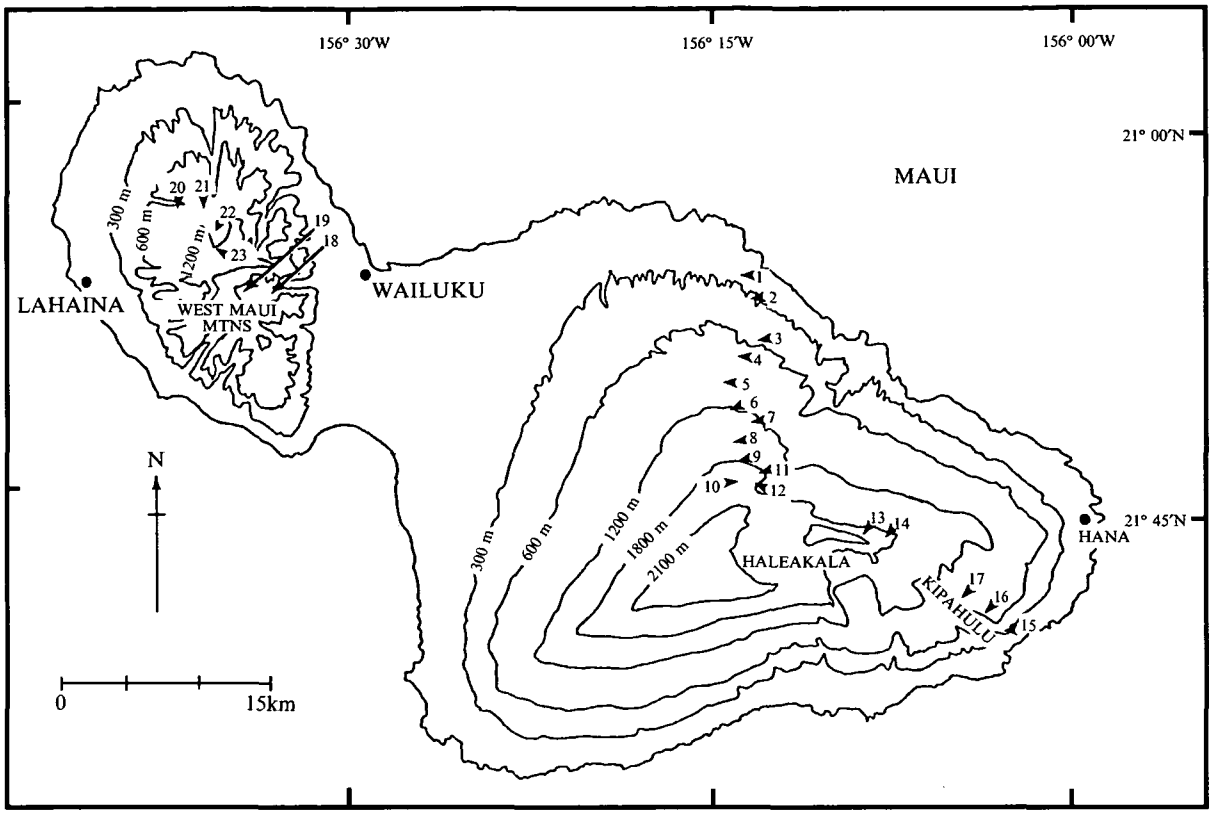

Fig. 1. Approximate location of 23 populations on the island of Maui, Hawaii. Transect 1 (Mt. Haleakala wet slope) – populations 1–10; Transect 2 (Kipahulu Valley) – populations 15–17; Transect 3 (West Maui mountains) – populations 18–23; Koolau gap trail – populations 11, 12; Crater – populations 13, 14.

Table 1. *Enzymes resolved in* Metrosideros polymorpha

Enzyme	EC number	Locus	Subunit structure
Aldolase	EC 4.1.2.13	*Ald*	Dimer
Aconitase	EC 4.2.1.3	*Aco-1*	Monomer
		Aco-2	Monomer
Leucine aminopeptidase	EC 3.4.11.1	*Lap-1*	Monomer
		Lap-2	Monomer
Malate dehydrogenase	EC 1.1.1.37	*Mdh-1*	Dimer
		Mdh-2	Dimer
Phosphoglucomutase	EC 2.7.5.1	*Pgm-1*	Monomer
		Pgm-2	Monomer
Phosphoglucoisomerase	EC 5.3.1.9	*Pgi*	Dimer
Peroxidase	EC 1.11.1.7	*Per*	Monomer
Shikimate dehydrogenase	EC 1.1.1.25	*Skdh*	Monomer
Isocitrate dehydrogenase	EC 1.1.1.42	*Idh*	Dimer
6-phosphogluconate dehydrogenase	EC 1.1.1.44	*6Pgd-1*	Dimer
		6Pgd-2	Dimer
Diaphorase	EC 1.6.4.3	*Dia*	Tetramer

Metrosideros. The gel buffer consisted of 0·016 M histidine (free base) and 0·002 M citric acid (anhydrous) and the tray buffer of 0·065 M histidine and 0·007 M citric acid.

Electrophoresis was conducted in a refrigerator at 4 °C for 6 h with 200 V (20 V/cm) and 40 mA. At the end of the electrophoresis, the gels were sliced horizontally and stained for different enzymes. Eleven enzyme systems involving 16 loci were assayed (Table 1). Staining methods and recipes were those of Arulsekar & Parfitt (1986) and Shaw & Prasad (1970) with some modifications. Although many more enzyme systems were resolved, they were inconsistent in revealing bands and hence were excluded from analyses.

Genotype frequencies were inferred directly from the isozyme phenotypes. Many enzyme systems exhibited two distinct zones of activity (Table 1) indicating the involvement of two loci in the biosynthesis of the enzymes. They were aconitase (ACO), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and 6-phosphogluconate dehydrogenase (6PGD).

(iii) *Data analysis*

Allele frequencies and genetic variability. The observed allelic frequency data were subjected to statistical analysis to compute various intrapopulational variability measures such as mean number of alleles per locus, percentage of polymorphic loci, and observed and expected levels of heterozygosity.

The fixation index (F: Wright, 1965), which is equal to $(H_{exp}-H_{obs})/H_{exp}$, where H_{exp} and H_{obs} refer to expected and observed heterozygosities respectively, was calculated for each population. F can be interpreted as the proportional increase or reduction in heterozygosity as compared to panmictic expectations. The value of F ranges from $-1{\cdot}0$ to $1{\cdot}0$; positive

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values indicate a deficit of heterozygotes and negative values an excess.

Population differentiation. Interpopulational relationships were examined by computing the unbiased genetic identity and distance coefficient (Nei, 1978) for all possible pair-wise comparisons. All computations were performed using the computer program BIOSYS (Swofford & Selander, 1989). A cluster analysis was performed on the genetic identity matrix with the UPGMA algorithm included in the BIOSYS program.

The gene diversity analysis was performed on the allele frequency data from the two mountains, Mt. Haleakala (transects 1, 2) and West Maui (transect 3) by the method suggested by Nei (1973). Hierarchical gene diversity analysis was performed on the groups generated by UPGMA cluster analysis from East Maui (transect 1) according to Nei's (1973) method, as extended by Chakraborthy (1980) where the components of gene diversity were partitioned according to hierarchy.

Principal components analysis. The multivariate relationships among populations of *Metrosideros* along the gradients were analyzed separately for East and West Maui by the principal components analysis (PCA) (Gauch, 1982). The input data matrix consisted of frequencies of all alleles scored across 16 loci for 17 populations from East Maui and 6 populations from West Maui. Two separate variance–covariance matrices were generated and from each the eigenvalues and vectors were extracted. The first three orthogonal vectors were multiplied with the original allele frequency matrices and the resultant product vectors were plotted in two-dimensional space to establish the clusters of populations.

Correlation and regression analyses. Pearson's product–moment correlation between allele frequencies and environmental variables, such as mean annual temperature, annual rainfall and an environmental index associated with different elevations, were computed with PROC CORR procedure (SAS Institute, 1990). The environmental index was calculated by following Eberhart & Russell (1966):

$$\mathbf{I}_j = (\Sigma_i \mathbf{E}_{ij}/n) - (\Sigma_i \Sigma_j \mathbf{E}_{ij}/pn), \quad \Sigma_j \mathbf{I}_j = 0,$$

where i is the number of populations (1 to p) and j is the number of environmental parameters (1 to n). Simple linear regressions were computed with PROC GLM procedure with allele frequencies as dependent variable to quantify the relationships for those alleles which showed significant correlations with selected environmental variables. All computations were performed on the pooled data from both mountains.

3. Results

(i) *Intrapopulational genetic variability*

The observed allelic frequencies for different populations are given in Aradhya (1992). All the 16 loci that could be resolved in *Metrosideros* were polymorphic in at least one population. The number of alleles per locus varied from 2 in *6Pgd-1* to 6 in *Pgm-1*, with a total of 60 alleles recognized for East Maui, as compared to 55 for West Maui with a range from 2 for *Idh* and *Mdh-1* to 7 for *Pgm-1*. With the exception of *Ald, Lap-1, Pgm-1, Pgi,* and *Per*, where two or more alleles occurred equally predominantly, the same allele was most common in all populations at each locus.

The low frequency alleles at some loci were found restricted to a few populations, although no trend in their distributional pattern was observed. West Maui populations were unique in possessing one extra allele in *Pgm-1* (*124*), *Pgi* (*118*) and *6Pgd-1* (*109*), while the East Maui populations were unique in possessing an extra allele in *Aco-2* (*94*), *Lap-2* (*114*), *Mdh-1* (*105*) *and Dia* (*124*) and two extra alleles in *Per* (*83* and *136*) and *Idh* (*82* and *91*).

Measures of genetic variability within populations of *M. polymorpha* from East and West Maui are summarized in Table 2. The mean number of alleles per locus for East Maui populations varied from 1·8 to 2·6, with an overall mean of 2·1, and it varied from 1·9 to 2·4, with an overall mean of 2·2, for West Maui populations.

The proportion of polymorphic loci in East Maui averaged 68·8%, with a range from 56·3% for two mid-elevation populations (6 and 7) and a high elevation population (10) to 81·3% for two populations, one each from transect I (1) and Kipahulu (20). The West Maui populations averaged 75·0%, with a range from 62·5% for population 23 to 81·3% for populations 20, 21 and 22.

The mean observed heterozygosity for 17 polymorphic populations from East Maui ranged from 0·094 for population 10 from transect I to 0·220 for population 11 (Koolau gap), with an overall mean of 0·143. The mean heterozygosity based on Hardy–Weinberg expectations ranged from 0·124 for population 8 (transect I) to 0·252 for population 11, with an overall mean of 0·170. The observed heterozygosity for West Maui populations averaged 0·144, with a range from 0·112 to 0·170, whereas it ranged from 0·146 to 0·183, with an overall mean of 0·162, based on Hardy-Weinberg expectations. There was deficiency of heterozygotes in most populations, but the F values were not statistically significant except for population 5 (transect I).

(ii) *Population differentiation along altitudinal gradients*

The genetic identity (I) and distance (D) coefficients for pair-wise comparisons among populations of East and West Maui and groups from East Maui were computed but only the matrix of pair-wise comparison for groups is presented in Table 3.

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Table 2. *Genetic variability at 16 loci in 23 populations of* Metrosideros polymorpha *from East and West Maui*
(Standard error in parentheses)

Population[a]		*N*	*A*	P	*F*	H_{obs}	H_{exp}
East Maui							
1. 200 m	H	44	2·6 (0·3)	81·3	0·119	0·178 (0·054)	0·202 (0·049)
2. 400 m	H	46	2·4 (0·2)	75·0	0·123	0·171 (0·059)	0·195 (0·049)
3. 600 m	H	44	2·1 (0·2)	68·8	0·216	0·134 (0·039)	0·171 (0·047)
4. 800 m	H	40	2·1 (0·3)	68·8	0·184	0·142 (0·046)	0·174 (0·053)
5. 1000 m	H	45	2·2 (0·2)	75·0	0·347*	0·094 (0·027)	0·144 (0·045)
6. 1200 m	H	45	1·9 (0·2)	56·3	0·214	0·125 (0·046)	0·159 (0·054)
7. 1400 m	H	45	1·9 (0·2)	56·3	0·133	0·124 (0·038)	0·143 (0·047)
8. 1600 m	H	45	2·1 (0·4)	62·5	0·129	0·108 (0·044)	0·124 (0·051)
9. 1800 m	H	45	2·3 (0·3)	75·0	0·204	0·113 (0·042)	0·142 (0·053)
10. 2000 m	H	38	1·8 (0·2)	56·3	0·129	0·115 (0·038)	0·132 (0·044)
11. 1950 m	H	40	2·3 (0·3)	75·0	0·127	0·220 (0·055)	0·252 (0·054)
12. 1950 m	H	36	2·1 (0·3)	68·8	−0·004	0·217 (0·060)	0·216 (0·057)
13. 1800 m	CR	45	2·0 (0·2)	68·8	0·125	0·126 (0·040)	0·144 (0·048)
14. 1850 m	CR	45	1·8 (0·2)	62·5	0·084	0·131 (0·043)	0·143 (0·050)
15. 400 m	K	30	1·9 (0·2)	68·8	0·206	0·146 (0·036)	0·184 (0·046)
16. 600 m	K	30	2·1 (0·2)	68·8	0·158	0·160 (0·043)	0·190 (0·047)
17. 800 m	K	25	1·9 (0·1)	81·3	0·246	0·132 (0·037)	0·175 (0·048)
Mean			2·1	68·8	0·161	0·143	0·170
West Maui							
18. 400 m		38	2·0 (0·2)	68·8	0·152	0·128 (0·035)	0·151 (0·049)
19. 600 m		40	2·3 (0·3)	75·0	0·116	0·153 (0·040)	0·173 (0·049)
20. 800 m		40	2·3 (0·3)	81·3	0·089	0·133 (0·040)	0·146 (0·045)
21. 1000 m		40	2·1 (0·2)	81·3	0·018	0·167 (0·049)	0·170 (0·046)
22. 1200 m		40	2·4 (0·4)	81·3	0·071	0·170 (0·051)	0·183 (0·045)
23. 1400 m		40	1·9 (0·3)	62·5	0·238	0·112 (0·051)	0·147 (0·051)
Mean		40	2·2	75·0	0·114	0·144	0·162

N, no. of trees sampled; *A*, no. of alleles/locus; P, proportion of polymorphic loci (frequency of most common allele < 0·99), H, heterozygosity (observed and expected); *F* = fixation index (Wright, 1965).
* $P < 0·05$.
[a] H, Haleakala; CR, Crater; K, Kipahulu valley.

The genetic identities varied from 0·909 between population 16 (Kipahulu) and population 12 (Koolau gap) to 0·998 between populations 13 and 14 (both from crater), 1 and 2 (both from transect I), and 8 (transect I) and 13 (crater) for East Maui. The average pair-wise identity among groups was 0·957, with a range from 0·932 to 0·977. For West Maui populations, it varied from 0·974 between populations 18 (400 m)

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Table 3. *Genetic identity and distance coefficients among* Metrosideros polymorpha *population groups based on UPGMA cluster analysis*

(Below diagonal: Nei (1978), unbiased genetic identity; above diagonal: Nei (1978), unbiased genetic distance)

	No. of pops	1	2	3	4
1 Group I	5	—	0·036	0·038	0·071
2 Group II	9	0·965	—	0·024	0·048
3 Group III	1	0·963	0·977	—	0·050
4 Group IV	2	0·932	0·953	0·951	—

Group I, 200 m, 400 m populations from Haleakala, and 400 m, 600 m and 800 m populations from Kipahulu.
Group II, 600–1800 m populations, and the two from crater of Haleakala.
Group III, 2000 m population from Haleakala.
Group IV, two Koolau populations from Haleakala.

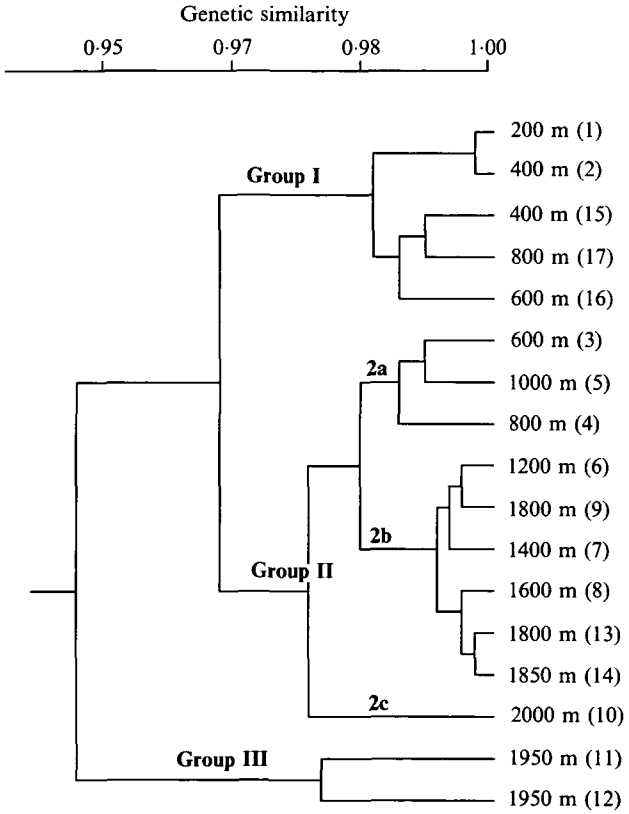

Fig. 2. Dendrogram showing genetic similarities among 17 populations from East Maui, Hawaii (figures in parentheses indicate population number as shown in Fig. 1).

and 22 (1200 m) to 0·998 between populations 20 (800 m) and 21 (1000 m).

The cluster analysis for East Maui populations resulted in three groups at about 97% level of genetic similarity (Fig. 2). Group I represents those populations coming from lower elevations on Haleakala and all three populations from Kipahulu. However, populations from Haleakala and Kipahulu valley

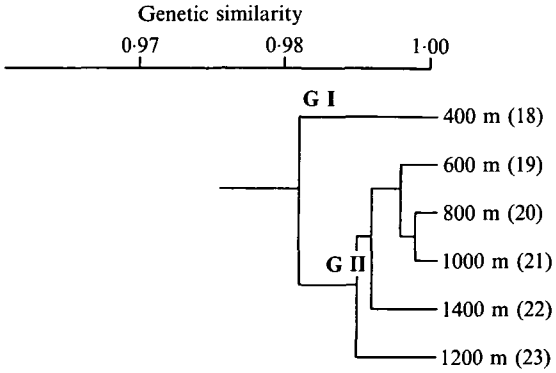

Fig. 3. Dendrogram showing genetic similarities among 6 populations from West Maui, Hawaii (figures in parentheses indicate population number as shown in Fig. 1).

showed greater affinity within themselves as compared to between them. Group II comprises all populations from middle and high elevations on Haleakala (3–10, and 13 and 14 from the crater). A most striking feature of this group is that it contains three subdivisions roughly corresponding to mid-elevation from populations 3–5 (2a), high elevation from 6 to 9, and 13 and 14 from the crater (2b), and a single population (10) from the 2000 m which is relatively distinct from other populations (2c). A third group which includes the two populations from the Koolau gap area (11 and 12) can be recognized as the most distinct group.

West Maui populations clustered into two groups at about 98% level of similarity (Fig. 3). Group I possess one population from the lowest elevation (18) and Group II contains the remaining populations (19–23) representing middle to high elevation. However, in group II, the populations 22 and 23 appeared relatively distinct from the rest of the group.

(iii) *Distribution of genetic variation within and between populations*

The results of gene diversity analysis are summarized for East and West Maui populations in Tables 4 and 5 respectively. The gene diversity components are a function of the allele frequencies in the subpopulations and provide estimates of population subdivision (Nei, 1973). Total gene diversity (H_T) varied considerably in magnitude among loci, ranging from 0·013 for *6Pgd-1* to 0·616 for *Pgm-1* with an average of 0·192 for East Maui and from 0·004 for *Idh* to 0·583 for *Pgm-1* with a mean of 0·168 for West Maui.

Summing over all loci, approximately 90% of the total variation resides within populations in East Maui, while it is 95% for West Maui. The remaining 10% was due to among population variation for East Maui. This was further partitioned hierarchically into variation due to genetic differences among populations within groups, which amounted to about 4%, and variation due to genetic differences among groups

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Table 4. *Measures of gene diversity for 17 populations of* Metrosideros polymorpha *from East Maui and apportionment of diversity into among populations within cluster and among clusters*

	Differentiation among populations				Apportionment of diversity			
Locus	H_T	H_S	D_{ST}	G_{ST}	Within pops	Among pops within cluster	Among clusters	*Nm*
Ald	0·500	0·418	0·082	0·164	0·836	0·062	0·102	1·27
Aco-1	0·036	0·034	0·002	0·064	0·936	0·028	0·028	3·66
Aco-2	0·185	0·165	0·020	0·110	0·891	0·032	0·076	2·02
Lap-1	0·509	0·419	0·090	0·176	0·824	0·077	0·100	1·17
Lap-2	0·226	0·153	0·073	0·324	0·676	0·035	0·288	0·52
Mdh-1	0·034	0·033	0·001	0·036	0·964	0·009	0·029	6·69
Mdh-2	0·048	0·046	0·002	0·050	0·950	0·029	0·021	4·75
Pgm-1	0·616	0·562	0·054	0·087	0·913	0·031	0·057	2·62
Pgm-2	0·183	0·168	0·015	0·083	0·917	0·038	0·044	2·76
Pgi	0·181	0·171	0·010	0·057	0·943	0·044	0·011	4·14
Per	0·229	0·213	0·016	0·070	0·930	0·031	0·039	3·32
Skdh	0·190	0·154	0·036	0·189	0·811	0·105	0·084	1·07
Idh	0·024	0·023	0·000	0·001	0·993	0·008	0·000	36·50
6Pgd-1	0·013	0·012	0·000	0·000	0·969	0·031	0·000	7·81
6Pgd-2	0·023	0·022	0·001	0·029	0·972	0·000	0·033	8·37
Dia	0·070	0·065	0·005	0·072	0·928	0·030	0·043	3·22
Mean	0·192	0·166	0·026	0·097	0·903	0·037	0·058	5·60

H_T, total genetic diversity; H_S, genetic diversity within populations; D_{ST}, genetic diversity among populations; $G_{ST} = D_{ST}/H_T$, and *Nm* = gene flow.

Table 5. *Measures of gene diversity for 6 populations of* Metrosideros polymorpha *from West Maui*

	Differentiation among populations				
Locus	H_T	H_S	D_{ST}	G_{ST}	*Nm*
Ald	0·484	0·474	0·010	0·022	11·3
Aco-1	0·049	0·048	0·001	0·010	22·5
Aco-2	0·149	0·145	0·004	0·024	10·2
Lap-1	0·223	0·211	0·012	0·055	4·3
Lap-2	0·407	0·374	0·033	0·081	2·8
Mdh-1	0·037	0·036	0·001	0·028	8·8
Mdh-2	0·053	0·051	0·002	0·043	5·5
Pgm-1	0·583	0·559	0·024	0·041	5·9
Pgm-2	0·118	0·111	0·008	0·065	3·6
Pgi	0·181	0·174	0·008	0·041	5·8
Per	0·106	0·104	0·003	0·028	8·7
Skdh	0·081	0·073	0·008	0·100	2·3
Idh	0·04	0·003	0·001	0·010	22·0
6Pgd-1	0·013	0·012	0·001	0·025	9·9
6Pgd-2	0·096	0·085	0·010	0·114	1·9
Dia	0·096	0·090	0·006	0·067	3·5
Mean	0·168	0·159	0·008	0·047	8·1

H_T, total genetic diversity; H_S, genetic diversity within populations; G_{ST}, proportion of total genetic diversity due to population differentiation; Nm, gene flow.

amounting to 6% (Table 4). However, the enzyme loci differed with respect to the distribution of variation within and among populations and within and among clusters of populations.

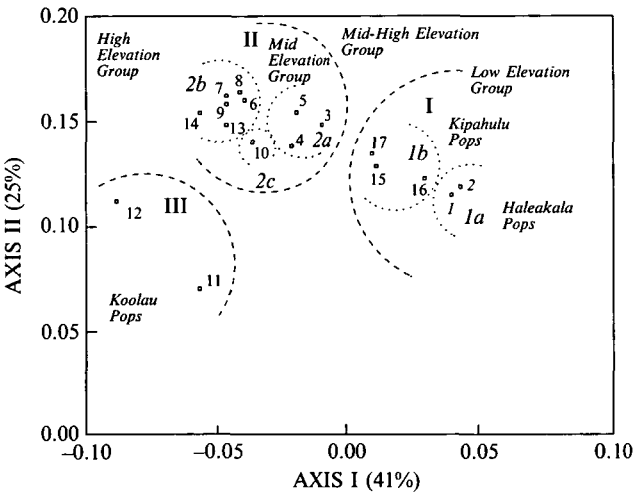

Fig. 4. Principal components analysis of 17 populations from East Maui, Hawaii.

(iv) *Principal components analysis*

The overall relationship among populations of *M. polymorpha*, represented as a multivariate structure of allele frequencies along the altitudinal gradients can be illustrated by principal components analysis (PCA). The first two principal components for East Maui populations accounted for 66% of total variation.

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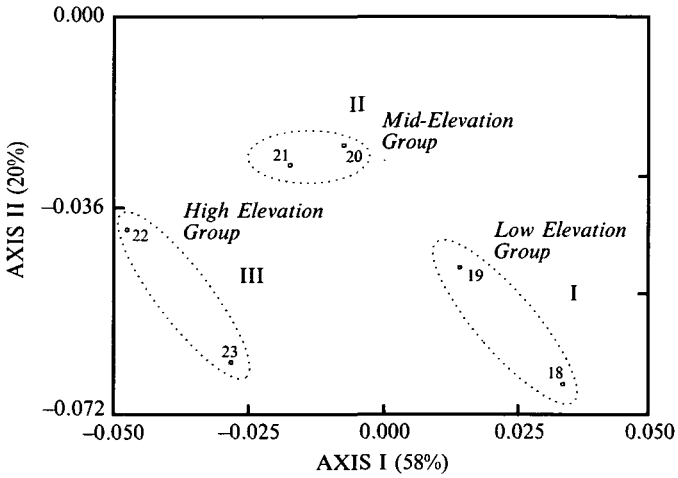

Fig. 5. Principal components analysis of 6 populations from West Maui, Hawaii.

The populations were projected onto a plane defined by the first two PCA axes to visualize the relationship among the populations (Fig. 4). The number of groups and their compositions are similar to that of the one obtained by the UPGMA cluster analysis.

The PCA of West Maui populations revealed the existence of three clusters corresponding to low, middle and high elevations as compared to two from the UPGMA cluster analysis results (Fig. 5). The first two orthogonal axes in this case accounted for 77% of the total variation.

(v) *Relationship between allele frequencies and environmental variables*

Pearson's correlation coefficients were computed between allele frequencies for different populations from East and West Maui and associated mean annual temperature, annual rainfall, and the environmental index (Table 6). The temperature and rainfall data were obtained by extrapolating the data from isotherms and isohytes for Maui (Giambelluc *et al.* 1986). Out of the 63 alleles 21 showed statistically significant correlations. It was evident from the linear regression analysis that the regression coefficients (b), though small, were significant in most cases, indicating a gradual clinal response of these alleles to the environmental variables (Table 6). However, sharp gradients of allele frequencies were seen locally across narrow altitudinal belts.

(vi) *Gene flow between populations*

According to Crow and Aoki (1984) assuming island model, the commonly used measure of gene differentiation $\mathbf{G}_{ST}$ is approximately equal to $[4Nm\alpha+1]^{-1}$ where $\alpha=[n/(n\text{-}1)]^2$, and Nm is the number of migrants per generation. As suggested by the above authors, the locus-wise gene flow estimates computed assuming island model of population structure was multiplied by 2 to get a better approximation of *Nm*

Table 6. *Correlation and regression coefficients between allele frequencies and environmental variables*

		Temperature		Rainfall		Index	
Locus	Allele	*r*	*b*	*r*	*b*	*r*	*b*
Ald	100	0·75**	0·043**			0·53**	0·073**
Ald	115	−0·74**	−0·043**			−0·51**	−0·072**
Aco-1	90	0·57**	0·003**				
Aco-2	100	−0·45*	−0·013*				
Lap-1	95	−0·41*	−0·018*				
Mdh-1	96	−0·49*	−0·004*	−0·62**	−0·011**	−0·70**	−0·014**
Mdh-1	100	0·52**	0·004**	0·61**	0·010**	0·71**	0·015**
Mdh-2	100	−0·64**	−0·008**				
Mdh-2	109	0·65**	0·008**				
Pgm-1	95	−0·52**	−0·023**			−0·44*	−0·047*
Pgm-1	109	0·57**	0·023**			0·57**	0·056**
Pgm-1	95	0·45**	0·005*				
Pgm-2	105	−0·56**	−0015**			−0·50**	−0·30**
Pgi	100	−0·51**	−0·012**			−0·43*	−0·024*
Per	87	−0·54**	−0·014**	−0·58**	−0·031**	−0·71**	−0·046
Per	100			0·52**	0·028**	0·50**	0·032*
Per	130	0·54**	0·006**			0·48*	0·012*
Skdh	91			0·49**	0·012*		
Skdh	100	−0·49*	−0·019*			−0·53**	−0·051**
Skdh	109	0·50**	0·019**			0·46*	0·042*
Dia	87	−0·50**	−0·008*	−0·39*	−0013*	−0·56**	−0·023**
Dia	100	0·51**	0·009**			0·54**	0·023**

r, correlation; *b*, regression.
** $P < 0{\cdot}01$; * $P < 0{\cdot}05$.
Blanks indicate both *r* and *b* are insignificant.

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for a population structure closer to a stepping-stone model in *Metrosideros* along the elevational gradients. Migration (gene flow) on an average was higher among West Maui populations (8·1) as compared to East Maui (5·6). The migration rate estimates varied for different loci among both East and West Maui populations (Tables 4, 5).

4. DISCUSSION

(i) *Genetic variability*

Although it was not possible to provide an absolute estimate of genetic variability in the entire species range, it was evident from the study along climatic gradients based on the number of variable loci observed and the mean heterozygosity per locus that genetic diversity is comparable, if not higher, than that found in other allogamous tree species (Brown, 1979; Hamrick, 1983; Loveless & Hamrick, 1984; Hamrick and Godt, 1990).

The allelic composition and distribution among populations along altitudinal gradients did not show definite trend in both East and West Maui. However, clinal variation in allele frequencies was apparent for many loci across narrower altitudinal belts. This indicates that the gene flow in the neighbourhood is more prevalent than among populations located farther apart along gradients.

A contingency χ^2 analysis for heterogeneity of gene frequencies indicated significant differences between populations. Twenty one alleles out of a total of 63 observed were significantly correlated with environmental factors, such as temperature, rainfall and the combined environmental index. This suggests that natural selection for these environmentally important adaptations could cause considerable differences at a few loci, with others that are neutral or only weakly selected being relatively uniform throughout a species range.

Among plants, tree species seem to possess higher levels of variability than do those of short-lived species (Hamrick *et al.* 1979). Late successional and climax tree species tend to maintain higher levels of within-population genetic variation and are less prone to random microdifferentiation than those of woody pioneer species, because the former are more stable in time and gene flow therein tends to be over greater distances (Levin & Kerster, 1974; Venable & Levin, 1983).

The higher level of genetic variation observed in *M. polymorpha* is probably due to its allogamous breeding system, resulting in frequent recombination of alleles from genetically diverse populations. This tends to maintain a higher level of genetic polymorphism in the form of higher average number of alleles coupled with higher levels of heterozygosity within populations. Another possible explanation for such higher levels of genetic variation would be that genetically diverse forms might have been introduced on more than one occasion to the islands. Such multiple introductions of genetically diverse founders would help species to overcome the genetic bottlenecks in the initial stages of colonization. However, most colonial populations experience bottlenecks during their initial colonization and establishment. Small heterozygote deficiency when compared to panmixia observed in populations of *M. polymorpha* could be due to spatial and temporal genetic structure within a sampling area.

(ii) *Population differentiation*

The broad ecological amplitude exhibited by *M. polymorpha* indicates that the species displays either a wide range of adaptation or a number of local adaptations to different habitats along altitudinal gradients and in areas differing appreciably in rainfall, temperature, and soil characteristics. Earlier studies of this species showed the existence of local adaptations in the form of altitudinal and successional ecotypes, varieties, and clinal variation for many morphological traits (Corn, 1979; Stemmermann, 1983).

In most continental biota which are so ancient and genetically more variable, the genetic differentiation normally originates as a result of reshuffling of already existing genetic variation within and between their populations due to the interplay of evolutionary forces such as selection, gene flow, and drift. On the other hand, genetic divergence in island taxa depends on the build up of genetic variation after initial colonization solely through the origin, distribution, and fixation of new mutations in their distributional range. Genetic divergence in sexually reproducing organisms may involve the development of intrinsic barriers to gene flow following shifts in the adaptive peaks leading to the establishment of multiple stable equilibria (Wright, 1977). However, congeneric species on oceanic islands may lack such genetic barriers after initial adaptive divergence, but in certain instances (as with Hawaiian *Bidens*, *Tetramolopium*, the silverswords) geographic and ecological separation prevent hybridization (Lowrey and Crawford, 1985; Ganders & Nagata, 1984; Carr, 1985). Nevertheless, genetic divergence can occur within populations, overriding the homogenizing effects of gene flow, if selection is strong enough to bring about specific ecological adaptations (McNeilly, 1968; Wu & Antonovics, 1976; Snaydon & Davies, 1982).

The level of genetic differentiation observed for *Metrosideros* is comparable to that of continental populations of conifers and other allogamous flowering plant species (Gottlieb, 1975; Weeler & Guries, 1982; Wendel & Parks, 1985; Bousquet *et al.* 1987; Yeh, 1988). Hawaiian examples of studies of genetic differentiation are found in the Hawaiian silversword alliance (Compositae: Madiinae), con-

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sisting of 28 species in three endemic genera, *Argyroxiphium*, *Dubautia*, and *Wilkesia*, where interspecific and intergeneric gene flow are still possible (Carr, 1985; Witter & Carr, 1988); Hawaiian *Tetramolopium*, involving 11 species with wide ecological amplitudes which exhibit very little interspecific divergence (Lowrey & Crawford, 1985); and Hawaiian *Bidens*, involving 27 congeneric taxa exhibiting little interspecific divergence (Helenurm & Ganders, 1985).

Nei's populational pair-wise unbiased genetic identities and distances provided little indication of genetic divergence. The genetic distances observed are typical of the values observed for conspecific populations in a wide variety of plants including conifers (Yeh & Layton, 1979; Guries & Ledig, 1982; Dancik & Yeh, 1983). Although genetic distances did not increase linearly with altitudinal separation, the phenetic and PCA discovered a relationship between the distribution of genetic variability and altitude. Substantial amounts of within-population variation ($H_S = 90\%$) coupled with high populational pair-wise genetic identities, however, indicate that relatively little genetic differentiation has occurred along the altitudinal gradients. Nevertheless, three broad groups could be recognized at 97·5% similarity level among East Maui populations and two at 98% similarity level among West Maui populations (Figs 2, 3). Populations in these groups may reflect altitudinal adaptations approximately corresponding to the different vegetation zones. Kitayama & Mueller-Dombois (1992) recognized three morphological forms of *Metrosideros* roughly corresponding to the altitudinal groups recognized in the present study.

The populations from lowland dry and mesic forests including some from lowland bog forests showed greater genetic affinity. Apparently in this group, the Kipahulu populations are in the process of diverging from the Haleakala populations. The second group represents populations from lowland wet and montane mesic and wet forests which include populations from the cloud forest zone where trade winds cause very heavy rainfall. Since selective climatic factors vary gradually along the altitudinal gradient, the divergence of populations may not be very apparent in this broad altitudinal zone. However, marginal divergence showing specific local adaptations to lowland wet forest and montane wet forest environments was evident.

The two populations sampled along the Koolau gap trail were distinct from the rest. These populations also possessed high levels of heterozygosity as compared to most other populations. The increased heterozygosity is due to concomitant increase at most loci except for *Mdh-2, Skdh* and *6Pgd-1* and *6Pgd-2* for which the populations were monomorphic. Since these populations are located just above the inversion zone, they are subjected to frequent cyclical environmental fluctuations such as sudden discontinuities in temperature and humidity and may show special adaptations.

Altitudinal segregation of populations was also evident on West Maui, however, the level of differentiation was less marked than that on East Maui. The higher within-population genetic variation as compared to East Maui suggests the prevalence of extensive gene flow.

Since evolutionary forces operate on multilocus structures, rather than on individual loci, adaptive multilocus responses may be complex and simultaneously involve composite changes at many loci (Koehn, 1969; Johanson & Powell, 1974; Brown *et al.* 1976). Hence, methods like the PCA is useful to describe the multivariate relationships among populations (Gauch & Whittaker, 1972; Gauch, 1982). The altitudinal segregation of populations was evident in the PCA performed on both East and West Maui data and it confirms the results obtained by the UPGMA cluster analyses. The altitudinal segregation of populations was more evident in the PCA than in the UPGMA cluster analysis for West Maui populations.

The study indicates that species-wide gene flow is relatively high and is comparable to those of many allogamous species (Hamrick, 1987). In general, insular species may not develop genetic barriers following initial differentiation and may circulate the genetic variation within the species complex. This is a feature commonly found among insular taxa.

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