

GENECOLOGY OF HAWAIIAN *METROSIDEROS*

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ABSTRACT

Metrosideros polymorpha is the dominant and a highly polymorphic species endemic to Hawaii. It has a wide ecological amplitude ranging from near sea level to treeline at 2500 m on the high volcanic mountains. *Metrosideros macropus*, *M. rugosa*, *M. tremuloides* and *M. waialealae*, are of much narrower distribution.

Twenty three populations of *Metrosideros polymorpha*, along altitudinal gradients on the island of Maui, Hawaii, were analyzed for allelic variation in 11 enzymes encoded by 16 loci. On the average 50 per cent of loci were polymorphic, the number of alleles per locus was 2.15, and the expected and observed heterozygosities were 0.166 and 0.144, respectively. Gene diversity analysis indicated that nearly 90 % of the total variation resides within populations, while the rest was due to differentiation among populations along altitudinal gradients. The between mountains component of variation was less than 1 % of the total as compared to the within mountains (9 %). The populational pair-wise genetic identities ranged from 0.909 to 0.998 for East Maui and 0.974 to 0.998 for West Maui. The UPGMA cluster analysis of the genetic identity matrix and the principal components analysis of the allele frequencies indicated marginal altitudinal differentiation. Twenty one

alleles out of a total of 63 observed exhibited significant correlations with rainfall and temperature.

Genetic differentiation was more pronounced along altitudinal gradients on the successional younger Mt. Haleakala than on the older West Maui mountains. High genetic similarities among populations across broad altitudinal ranges indicate that gene flow is an important factor in the evolution of *Metrosideros*.

A study of genetic differentiation among the three species, *M. polymorpha*, *M. rugosa* and *M. tremuloides* on Oahu, indicated that nearly 88 per cent of the total variation resides within populations, while only about 5 per cent was due to differences among taxa. The mean unbiased genetic identity for pair-wise comparisons of species was 0.904, which is very high for congeneric flowering plant species. The results suggest that both *M. rugosa* and *M. tremuloides* have diverged recently from *M. polymorpha*, but *M. rugosa* has diverged more than *M. tremuloides*. Hence, this may represent a case of recent and incipient speciation in the insular environment.

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PREFACE

Studies in genecology, or ecological genetics, involve both field observations and laboratory analyses. The genetic structure of populations, i.e., the amount and organization of genetic variation in space and time, play a dominant role in the evolution of natural populations. Population genetic approaches to studies of genecology allow biologists to partition the genetic variation in natural populations into "within" and "between" components to assess the level of genetic differentiation among populations.

Most continental biota are very ancient, and hence the key events of evolutionary processes that formed them are lost in the shadows of the past. But on isolated volcanic islands, the dynamic processes of evolution are still in progress and are far more accessible for investigations. The Hawaiian archipelago is the most isolated chain of islands, situated at least 4000km from the nearest continental land mass. Colonization events through long-distance dispersal have populated the windward slopes of the volcanoes up to altitudes of about 2500m with rich forest ecosystems. Features such as dramatic climatic gradients along these slopes, topographic heterogeneity due to erosion, occurrence of different-aged substrates, and rain-shadowed dry leeward

slopes have provided large numbers of ecologically diverse habitats and opportunities for biota to evolve into hundreds of species unique to these islands.

Evolution of the outcrossing mode of reproduction results in lack of reproductive isolation among conspecifics after initial genetic differentiation in island biota. This in turn results in circulation of genetic variability within species complexes. Hence, the population genetic approach to study the dynamic processes of evolution should be the main focus in evolutionary biology on islands.

In this dissertation, one such attempt has been made to study the level and organization of genetic variability in the dominant tree species, *Metrosideros polymorpha* commonly known as Ohia lehua. The results of the study of the genetic structure along altitudinal gradients and comparison of two successional stages conducted on the island of Maui, Hawaii, are reported in this dissertation in Chapters 2 and 3, respectively.

The genus *Metrosideros* has five endemic species described mainly by morphology. Two species with a narrow range of distribution, *M. rugosa* and *M. tremuloides*, and the dominant *M. polymorpha* were studied for the level of genetic differentiation among them by assessing genetic variation within and between species. The results are reported in Chapter 4.

Starch gel electrophoresis was employed to assess genetic variation using enzyme markers. Eleven enzyme systems encoded by 16 loci were successfully resolved to assess genetic variability. This basic study may be of use to ecologists, population geneticists, evolutionary biologists, and in general for biologists who are interested in island biogeography and evolution.

I would like to thank Prof. Dieter Mueller-Dombois and Prof. H. L. Carson for the initial encouragement to pursue my dream of doing evolutionary biology research.

PART I

BACKGROUND

CHAPTER I

LITERATURE REVIEW AND WORKING HYPOTHESES

A. INTRODUCTION

Biogeographically isolated insular ecosystems are ideal testing grounds for evolutionary hypotheses. The flora and fauna of islands have several unique features that distinguish them from the flora and fauna of continents. Many of these features are related to difficulties of dispersal. Once on an island, a species may evolve so as to lose its ability to disperse far, with many interesting effects on both morphology and reproduction (Williamson, 1981). Extreme isolation resulting in lack of competition and extraordinary opportunities for species to radiate on isolated oceanic islands has made them appropriate sites for studies of the kinetics of evolutionary processes of adaptation and speciation (Carson, 1983).

The Hawaiian archipelago is the most isolated group of islands in the world, lying at least 4000 km from the nearest continental land mass (Simon, 1987). The Hawaiian islands, being volcanic in origin, are uniquely suited to study the dynamic processes of evolution in a biogeographically isolated insular ecosystem. The linear

arrangement of the islands in known chronological sequence and the presence of a large number of endemic plants have added value to these islands as excellent natural evolutionary laboratory. The vegetation on these islands is supported along the slopes of high volcanic mountains. These provide some of the extraordinarily sharp topographic and climatic gradients to which the species populations exhibit adaptation. The Hawaiian vegetation has been extensively studied from the viewpoint of altitudinal distribution, forest dynamics, succession and dieback (Mueller-Dombois et al., 1981; Mueller-Dombois, 1983, 1985, 1987, 1988a,b, 1990). Description of the vegetation zones in Hawaii has emphasized plant community formation arranged in altitudinal belts with only a few species. The low number of canopy species is an important characteristic differentiating the Hawaiian mountains from other tropical mountains (Mueller-Dombois et al., 1981).

Metrosideros polymorpha is the most important endemic canopy tree species on all the high islands of the Hawaiian archipelago. Its range includes a wide spectrum of habitats from near sea level up to about 2500m elevation along the wet windward slopes on these high islands, with an annual average rainfall ranging from 750 to 11,570mm. As the name indicates, it is a highly polymorphic taxon, which has led taxonomists to classify the variation into several varieties (Dawson and

Stemmermann, 1990). At least four other species have been described in the genus *Metrosideros* Banks from Hawaii entirely based on morphological criteria (Rock, 1917; Skottsberg, 1944; Dawson and Stemmermann, 1990). However, their distributional ranges are much narrower and occur sympatrically with *M. polymorpha* on some older islands. Porter (1972) treated all taxa of *Metrosideros* as varieties and forms of the species *M. polymorpha*. Corn (1979) reported clinal morphological variation in Hawaiian *Metrosideros* correlated with complex altitudinal gradients. Stemmermann (1983) described a correlation of morphological variation, such as leaf shape and pubescence, with age of substrate and identified these variations as successional varieties of *M. polymorpha*. The genetic basis for these variations was indicated through a common garden study.

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 and Schaal, 1985). Hence, integrative studies involving variation in macromolecules, in addition to morphological comparisons, are most beneficial.

B. GENEKOLOGY: EARLY STUDIES OF INFRASPECIFIC VARIATION IN PLANTS

Genecology or ecological genetics represents a union between population genetics and population ecology, combining certain aspects of each discipline, but also differing in certain respects from both (Merrell, 1981). Although genecology had its beginnings along with Darwin's theory of "the origin of species through natural selection", it was formally recognized only when Turesson for the first time published his series of papers on ecological genetics in the 1920s (Turesson, 1922a,b, 1925 and 1930). Turesson's contribution to our understanding of the patterns of variation within species is of great importance. He demonstrated clearly the widespread occurrence of infraspecific habitat-correlated genetic variation. He coined a number of terms such as "ecotype", "ecospecies" and "ecophene". He wrote in 1922, " The species problem, as it appears so far, seems to be in large measure an ecological problem " and in 1925, "It seems appropriate for several reasons to denote this study of species ecology by the term genecology (from the Greek 'genos', race, and 'ecology') as distinct from the ecology of the individual species, for which study the old term autecology seems to be the adequate expression. "

Turesson (1922, 1925) also demonstrated that the ability of organisms to occupy a wide range of habitats depends not only on their ecological tolerance of a wide range of conditions, but also on genetic modifications of the type proposed by Darwin, namely natural selection. The "ecotype" was originally envisioned by Turesson (1922) as the "ecological unit to cover the product arising as a result of the genotypical response of an ecospecies to a particular habitat". Until the late 1950s, the term ecotype had primarily been used to refer to a within species grouping of populations in relation to a type of habitat or climate (Quinn, 1978). Although Turesson's definition is literally equivalent to a local population, he made his intentions clear by proposing a system designating ecotypes by the habitat-type they occupied. The genecology research of the Carnegie group of Clausen, Keck and Hiesey reinforced the ecotype concept with the grouping of local populations of *Potentilla glandulosa* into climatic races or ecotypes corresponding to the subspecies *typica*, *reflexa*, *hanseni*, and *nevadensis* (Clausen et al., 1940; Clausen and Hiesey, 1958). *Achillea* was also studied along the central California seacoast-to-High Sierra transect and reported to exhibit a sequence of climatic races or ecotypes (Clausen et al., 1948).

Quinn (1987) argued that the use of the term "ecotype" creates both practical and conceptual problems

in the identification and characterization of ecological and evolutionary units. He emphasized that the Turesson's ecotype concept assumes lack of ecologically significant genetic variability among the genotypes and populations of an ecotype.

Darwin's theory was that the adaptation of populations to their environments resulted from natural selection and that if this process continued long enough, it would ultimately lead to the origin of local races or varieties and that these finally differentiate into subspecies and species. Ecological genetics focuses on the processes of evolution and speciation through adaptation in natural populations to their physical and biological environments, and on the mechanisms of population responses to environmental changes (Merrell, 1981).

The classical review of genecology by Heslop-Harrison (1964) vividly describes progress made in the field from the 1920s to the early 1960s. He states that three basic propositions governed earlier works:

1. Plant species with wide ecological amplitudes exhibit spatial and temporal variations in morphological and physiological characters.
2. Much of the infraspecific variation can be correlated with habitat differences.
3. To the extent that ecologically correlated variation is not simply due to plastic response to environment, it is

attributable to the action of natural selection in molding locally adapted populations from the pool of genetical variation available to the species as a whole.

Genetic differentiation within species in response to habitat differences has now been repeatedly observed and has become an established phenomenon; it is no longer assumed that members of a species from one habitat are identical to those from another. Langlet (1934) pointed out that the most important habitat factors, such as temperature and rainfall, commonly vary in a continuous fashion, and thus one would expect graded variation in many widespread species rather than discontinuous variation. In 1938, Huxley, after surveying the literature, coined the useful term "cline" for character variations in relation to environmental gradients. Clinal variation has been described in a large number of species, and the list being very long, it will not be presented here.

Genecologists have recognized that if natural selection is to be studied, it is appropriate to investigate it by studying infraspecific variation in grossly dissimilar environments differing in exposure, temperature, light, moisture, topography, aspect, rainfall etc.

In earlier studies, genetic polymorphism for visible traits, such as plant pigmentation in different parts, and

even some easily analyzable chemical traits, such as cynogeneic glycosides, etc., were extensively utilized to study the role of natural selection in speciation. With the development of isozyme techniques, botanists were provided with a most valuable tool for investigating variation in natural populations. Smithies (1955 a & b) introduced the starch gel for zone electrophoresis and reviewed its theoretical advantages. Hubby and Lewontin (1966) felt that this technique detected a substantial fraction of the variation in multiple forms of enzymes in a species. The multiple forms of enzymes, separable by electrophoretic procedures, occurring within the same organism and having similar or identical function, are generally termed "isozymes or isoenzymes". The term "isozyme" was first introduced by Markert and Moller (1959), but the alternative form "isoenzyme" is also widely accepted. Since the polypeptide constituents of a protein may be coded by more than one genetic locus, the term isozyme has been restricted to mean various proteins produced by combinations of several polypeptides specified by distinct genetic loci. A new term "allozyme" has been coined to designate the variant proteins produced by different alleles at the same locus (Prakash et al., 1969).

C. ENZYME ELECTROPHORESIS: AN APPROACH TO THE STUDY OF INFRASPECIFIC GENETIC VARIATION AND EVOLUTION

The electrophoretic study of protein variation in natural populations initiated a new era in the understanding of the genetic basis of evolutionary processes (Bullini, 1982). The initial applications of gel electrophoresis of enzymes to studies of genetic variation in natural populations of plants and animals has had a remarkable impact on research in population genetics, evolution, and systematics (Harris, 1966; Hubby and Lewontin, 1966). It proved to be a particularly powerful tool in population genetics, because only small samples of material are required from single individual, and large numbers of individuals can be screened precisely and relatively rapidly, as compared to any other existing method. Hence, it has facilitated geneticists in uncovering large stores of enzyme variation within populations.

The first comprehensive investigations demonstrating multiple molecular forms was reported by Gomori et al. (1959) and Markert and Moller (1959). One of the earliest papers on electrophoretic investigations on higher plants was by Jermyn and Thomas (1954). More recently, a variety of isoenzyme systems have been reported in higher plants.

The presence of isoenzymes detected by the zymogram method quickly led researchers to extend the techniques to investigation of infraspecific enzyme variability. Hall et al. (1969) made comparative investigations of several enzyme systems using eleven plant genera and various tissues within genera. They demonstrated that wide diversity between genera is a general rule of all enzymes studied and that tissue specific enzymes were common.

The initial applications of gel electrophoresis of enzymes to studies of genetic variation in natural populations of plants and animals has had a remarkable impact on research in population genetics, evolution, and systematics, as it has permitted estimation of levels of genetic variation and its organization among conspecific populations (Harries, 1966; Hubby and Lewontin, 1966) and among related species (Choudhary and Singh, 1987). This technique makes it possible to study genetic variation, and the similarities and differences among organisms, at the level of enzymes, or other proteins, and DNA. Hence, electrophoretic variation can be directly equated with genetic differences in many cases (Gottlieb, 1971). This permits a characterization at the molecular level, of the amounts and types of genetic variability in populations of practically any organism, and an estimate of the extent of genetic diversity within and among conspecific populations and related species. Studies of enzyme loci have been

used to discover or confirm the existence of cryptic or dubious species in a variety of organisms (Carter and Thorpe, 1981; Hedgecock, 1979; Smith and Robertson, 1981) and also have encouraged a more realistic view on the species concept from the genetic point of view (Graves and Rosenblatt, 1980; Moyse et al., 1982). Previously, the study of genetic variation in natural populations was unsatisfactory for the reason that it depended on the identification and enumeration of rare recessive mutants that, when homozygous, yielded visible morphological changes, or on infrequent morphological polymorphisms which are quite plastic in their expression. Most other characters are polygenic in nature and hence are affected by environment. As easily detectable morphological polymorphisms are infrequent, they constitute only a very small proportion of genetic variation in natural populations.

An essential prerequisite for analyzing the genetic diversity and structure of populations is the ability to discriminate between individual genotypes as accurately as possible. Hubby and Lewontin (1966) suggested four criteria for choice of techniques and the kind of genetic markers that are useful for population genetic studies: 1) allelic expression should be distinguishable in populations, 2) the effect of each allelic substitution should be locus specific, and distinguishable from

substitution at other loci, 3) all changes at the molecular level should be detectable, and 4) loci should be sampled at random, irrespective of their function or likely level of polymorphism.

The isozyme technique meets these criteria more closely than any preexisting method. At present, protein electrophoresis is the most widely used technique for obtaining genetic data from natural populations. The application of electrophoretic technique depends on correct genetic interpretation of the enzyme variability observed on the gel. Inheritance studies for the isozyme loci under consideration are advisable before using them for population surveys.

D. GENETIC BASIS OF ELECTROPHORETIC PATTERNS

The pattern or zymogram of enzyme bands on a gel following electrophoresis depends on the particular enzyme assayed, its mode of inheritance, and the genotype and ploidy level of the individual examined. The structure and number of polypeptides involved in the enzyme determines the number of bands displayed in a heterozygous individual. For example, a plant heterozygous at a locus specifying a monomeric enzyme will have only two allozymes. If the enzyme is dimeric, the zymogram pattern will comprise three bands: two homodimers and a

heterodimer usually with intermediate mobility and more intense staining. Tetrameric enzymes show five bands when the coding gene is heterozygous (Figure 1.1)

The expected numbers of bands and their relative intensities for individuals heterozygous for protein coding loci can be predicted from a binomial expansion of the two categories of allelic subunits (a and a'). For a dimeric protein the expression would be

$$(a + a')^2 = a^2 + 2aa' + a'^2$$

In reference to the left-hand side of the binomial formula, the a and a' represent the actual protein subunits and the exponent (2) represents the number of subunits in the protein. On the expanded right-hand side of the formula, the three terms represent the number of bands, and their respective coefficients (1:2:1) represent their relative intensities. For a tetramer, the exponent becomes 4 and the relative intensities of the bands would be 1:4:6:4:1. More complicated electrophoretic patterns can occur when the same type of protein is encoded by two or more loci. These complications include additional protein bands arising from combinations of subunits, encoded by different loci (interlocus heterodimers) with different electrophoretic mobilities. Occasionally, different gene loci are encountered that specify the subunits of isozymes which "overlap" on the gel, because

they have the same mobility in the particular electrophoretic conditions (Weeden and Gottlieb, 1979). Partial degradation of enzymes can occur when plant tissues are not properly stored before they are used in isozyme analysis. In such cases, there may be ghost bands appearing along with the regular banding thus making it difficult to visualize the banding patterns. The storage conditions can vary from species to species, but generally tissues stored at less than 4° C can retain enzyme activity for up to a week in many species.

The important determinant of electrophoretic patterns in population surveys is the amount of genetic variability within the sampled populations: the proportion of the isozyme gene loci that are polymorphic, the number and relative frequencies of alleles at these loci, and the proportion of gene loci heterozygous per individual.

E. FACTORS AND PROCESSES AFFECTING DIVERSITY AND POPULATION GENETIC STRUCTURE

Plant populations are not random assemblages of individuals but are structured in space and time (Allard, 1975; Brown et al., 1978; Linhart et al., 1981; Schaal, 1975; Hamrick, 1982). A spatial genetic structure may exist with certain alleles or genotypes being patchily distributed within the population. Such nonrandom

distribution of genetic variation is often referred to as the genetic structure of a population (Loveless and Hamrick, 1984). Genetic structure results from the joint actions of mutation, migration, selection, and drift, which in turn operate within the historical and biological context of each plant species (Loveless and Hamrick, 1984). The rate at which mutation creates new alleles at a locus is quite low, at least when are not strongly detrimental. Further increase and spread of such beneficial alleles depends on the nature of selection, gene flow, and population size. The genetic structure of a population, i.e., the amount and organization of genetic variation in space, plays a dominant role in its evolution by influencing the consequences of interactions among conspecifics, which include levels of selection, the amount of genetic variation which is maintained, and the ability to exploit ecological opportunity (Wade, 1978, 1980; Wilson, 1979; Wright, 1980). Micro-habitat adaptation due to spatially variable selection and limited pollen and seed dispersal are the major factors causing spatial genetic structure. Although genetic structure has been described in a few species populations, the balance among selection, genetic drift, and migrations is poorly understood (Levin, 1988). Ecological factors affecting growth, reproduction, dispersal, and establishment are probably important in determining the genetic structure of

populations (Allard, 1975; Jain, 1975; Clegg, 1980). The most critical factors determining patterns in plant populations are those affecting seed viability, dispersal, seedling establishment and survival (Whittaker, 1969; Harper and White, 1974). Variation in edaphic and microclimatic conditions are especially important at these stages. Hence, the patterns observed in space and time may coincide with those of the environment as a result of differential selection favoring different genotypes in different environments (Levin, 1988). It is suggested that the amount of genetic variation may be regarded as an adaptive strategy for increasing population fitness in a spatio-temporally heterogeneous and uncertain environment (Nevo, 1978).

The habitat patterns are produced by the interaction of various climatic and edaphic factors on a spatial and temporal scale. Specific patterns of physiological response often can be associated with specific environmental variables. A variety of attributes or adaptive responses of populations develop within the selective regimes associated with the habitat heterogeneity (Wiens, 1976). Selection is viewed as incessantly trimming all but the best adapted or optimal genotypes from a population, and competition is usually presumed to be the major driving force in this selection process (Wiens, 1976). Based on theoretical treatments,

Levene (1953) and Levins (1965, 1968) predicted that genetic heterogeneity can increase with spatial and/or temporal environmental variation. In particular, when species, which occupy a patchy environment (heterogeneous) are exposed to different intensities and directions of selection in the different patch types, and where they occupy small patches their gene frequencies are subjected to founder effects or drift. Further, the patch structure and selection pressures are likely to change through time (Dickinson and Antonovics, 1973). Under such conditions a wide array of theoretical and observational studies indicate that genetic polymorphism should be great (Maynard Smith, 1970; Antonovics, 1971; Christiansen, 1974; Gillespie, 1974). Species populations which are subjected to selection in a fine-grained environment experience a variety of environmental conditions during different stages of their development, but different individuals within a population probably encounter roughly the same average set of conditions. Thus, individual flexibility in responsiveness to several short spans of environmental fluctuations within the same generation may result in phenotypic plasticity which should be favoured by selection in such conditions.

The trophic resources model correlates low genetic variation with instability in food resource supplies, and high genetic variation with trophically stable

environments such as the tropics and the deep sea (Valentine, 1976). This contradicts the body of ecological-genetic theory which predicts a positive correlation of genetic variation with environmental instability. Questioning the tropic resources model, Hedrick et al. (1976) suggested that "the high heterozygosity in the tropics may be due to a large spatial biotic heterogeneity as seen in the species measures". Models with spatial heterogeneity and limited migration or habitat selection are very effective at maintaining heterozygosity. Loci whose enzymes utilize substrates originating from the external environment are far more polymorphic than loci whose enzymes utilize internal metabolites (Gillespie and Kojima, 1968; Johnson, 1974). Loci encoding enzymes for variable substrates are in general relatively more polymorphic and heterozygous than their more specific counterparts (Nevo, 1978).

F. SELECTION, GENE FLOW AND GENETIC DRIFT

Selection is a wide spread feature of natural populations which alters gene and genotype frequencies and acts in close association with other processes of adaptation and evolution to generate genetic structure (Slatkin, 1973; Felsenstein, 1976; Karlin, 1976; Endler, 1979). As a result of natural selection those characters

in which stability is vital for the organism are likely to show greater stability than those characters in which some plasticity is not a disadvantage (Bradshaw, 1965).

Natural selection can be much more effective than genetic drift in either preventing or establishing local differences. Selection in favour of the same alleles or the same traits would produce geographic uniformity regardless of any gene flow. Selection favouring different alleles in different locations will succeed in producing local differences reflecting genetic adaptedness to local conditions. Roughly speaking, the fitness differences measured by selection " s ", exceed the fraction of immigration, " m " (Haldane, 1930; Nagylaki, 1975).

Although genetic drift affects all loci in the same way, natural selection does not. Natural selection for locally important adaptations could cause substantial differences at a few loci, with other loci that are neutral or only weakly selected being relatively uniform throughout a species range. Natural selection should differ for different genes, so that alleles involved in one ecologically adaptive function may vary independently of those involved with other functions (Bryant, 1974).

Hence, natural selection may alter the genetic structure of populations by differentially selecting the alleles belonging to several genes responsible for ecological adaptation. Such multi-dimensional selection may further

vary spatially and temporally leading to the maintenance of high levels of polymorphism in highly dynamic environments. In terms of the relative strengths of evolutionary forces, gene flow might be weaker than selection at some loci yet much stronger than genetic drift at other loci (Slatkin, 1987). A basic "rule of thumb" is that very little genetic exchange between populations will prevent divergence in the absence of natural selection (Allendorf and Phelps, 1981). In nature, widespread species populations constitute a system of comparatively small and partially isolated populations which tend to inbreed more than interbreed. As far as plants are concerned, even quite a narrow strip of unsuitable habitat separating interfertile colonies may be sufficient barrier to check the interflow of genes (Gregor, 1944). Partial isolation of intraspecific populations tends to increase the local effectiveness and rate of ecological selection. Differentiation into species is usually assumed to be impossible without barriers because gene flow is supposed to "swamp out" any differences evolved in response to local environmental factors (Wright, 1931; Mayr, 1940; Haldane, 1948; Fisher, 1950; Dobzhansky, 1970). It is accepted that "migration" at the rate of one migrant individual per local population per generation is generally sufficient to obscure any disruptive effects of drift (Spieth, 1974). But recent

studies both theoretical and experimental have suggested that gene flow may not have as great an effect as has been postulated (Thoday, 1958; Jain and Bradshaw, 1966; Endler, 1973; Slatkin, 1973). It is argued that selection is both the primary cohesive and disruptive forces in evolution, and that the selection regime itself determines what influence gene flow will have. Some form of selection (balancing, directional, disruptive, frequency-dependent, etc.) may be the major determinant of population genetic structure and differentiation (Nevo, 1978). Several studies have shown the strength of selection in overcoming the homogenizing effects of gene flow and allowing local differentiation (Jain and Bradshaw, 1966; Ehrlich and Raven, 1969; Brussard and Ehrlich, 1970; Gibson, 1973; Endler, 1977). Since selection operates on multilocus structures, rather than on single genes, adaptive multilocus associations must be scrutinized in addition to single-locus adaptive patterns (Johnson and Powell, 1974; Brown *et al.*, 1976). Gene flow, including all movement of genetic units from one population to another of the same species, largely determines the extent to which local populations (demes) can be independent during their differentiation in diverse environments or under geographic separation of more uniform environments that permit random genetic drift.

The importance of gene flow as a creative and a disruptive force in microevolutionary processes has been studied by various workers (Wright, 1931; Mayr, 1963; Ehrlich and Raven, 1969; Slatkin, 1985, 1987 for reviews). Ehrlich and Raven (1969) summarized their views on selection and gene flow: (1) gene flow in nature is much more restricted than commonly thought; (2) populations that have been completely isolated for long periods often show little differentiation; and (3) populations freely exchanging genes, but under different selection regimes, may show marked differentiations. Levin and Kerster (1974) have reviewed information on gene flow coming from various lines of study. They consider that even in species with seeds and fruits apparently well adapted with wings or plumes may travel relatively short distances from the parent plants. Wind dispersal of pollen is also observed to be leptokurtic. Although pollen can be dispersed great distances at times, the chances of it falling on a receptive stigma as well as competitiveness in fertilization at any great distances are slight (Ehrlich and Raven, 1969). Similarly, propagules that are dispersed over longer distances may not be successful in establishment and, interbreeding with members of an alien population. Hence, the realized gene flow rapidly and substantially diminishes as the distance from the source increases. The consequences of gene flow depend, not only

on the frequency and pattern of immigration, but also on the fitness of immigrants relative to locals (Levin, 1984). The greater the relative fitness of the immigrants, the greater their impact. This remains true even in the case of insect- and bird-pollinated species. The amount of effective gene flow through pollen dispersal is influenced by the spatial separation of populations, relative sizes and densities, their phenologies, and intervening vegetation (Levin and Kerster, 1974). In a dense, homogeneous population, animal pollen vectors may conform to optimum foraging models (Levin, 1978; Pyke, 1978; Waddington and Heinrich, 1981). Indirect evidence in support of the differences among wind- and insect-pollinated species comes from the analyses of Brown (1979) and Hamrick et al. (1979), who have demonstrated that wind-pollinated species maintain more variation within their populations and less between populations than insect- or self-pollinated species. However, there is no satisfactory method of estimating the gene flow in natural populations to accurately predict its possible role in population differentiation. Indirect estimates based on allele frequency distributions produce higher estimates of gene flow than direct measures based on following dispersal units or marker genes (Hamrick, 1987). Wright (1931) showed that for selectively neutral alleles, genetic differentiation among populations, F_{ST} , is

inversely related to the number of migrants exchanged per generation, Nm . On the other hand, Slatkin (1981) proposed a qualitative method to estimate levels of gene flow from allozyme data, in which the average frequency of rare alleles is used to estimate gene flow; the frequency distribution of rare alleles depends strongly on the overall levels of gene flow among populations, but is nearly independent of both mutation rates and selection intensity. Nm is approximately linearly related to the logarithm of the average frequency of "private" alleles (Slatkin, 1985a). The relationship between gene flow and selection in shaping local variation patterns in a heterogeneous environment, and the spatial scale of such variations, are best considered in terms of a one-dimensional gene flow scale (l) the square root of the mean squared dispersal distance (Fisher, 1950; Slatkin, 1973; Endler, 1977). The gene flow distance (l) is related to the neighborhood radius by $r = l^2$. The smaller the l , the greater the isolation by distance between two subpopulations, and the more rapidly and fully these aggregates may respond to disruptive selection. However, the influence of gene flow on the structure and evolution of natural plant populations remains controversial.

Genetic drift is defined in Li (1955) as the variation of gene frequencies due to random deviations as

a result of sampling accidents from one generation to the next. If the effective population is large, all gene frequencies remain at a stable equilibrium point, which is determined by the counteracting, but systematic, processes of selection, mutation, and migration; provided the environment remains constant, no genetic change takes place. If the effective population size is small, Wright (1931) demonstrated mathematically that gene frequencies would diverge at random from their equilibrium points, and most genes will eventually become fixed or lost by chance. For this to happen, random drift effects must override those of selection; though some cases of random drift may fortuitously be advantageous, some unfavorable genes will also be fixed against selective pressure. Wright (1948) theorized that if the population is sufficiently small all loci will become homozygous even though the result will be disadvantageous; thus the organism could not be fully adapted and would be genetically inflexible; it will therefore become extinct in due course, particularly if there are fluctuations in the environment. Such genetically depauperate populations have the best chance of survival in "vacant" environments where competition is minimal and rapid spread will eventually lead to return of heterozygosity (Mayr, 1954). Colonization episodes on isolated islands often involve a small nucleus of dispersing individuals may experience severe genetic

drift. Similar situations occur in the Hawaiian rain forest environment during primary succession following massive lava flows, when *Metrosideros* rain forests were reduced to small patches (kipukas). The trees in kipukas become the main seed source reaching the new lava surface upon cooling. Kipukas being isolated and with small population size may experience a genetic bottleneck. As a result of inbreeding (genetic drift), the progenies which get established on the new lava surface may show reduced heterozygosity (founder effect), but still get established due to lack of competition and rapidly regain heterozygosity in a few generations of turnover. Wright (1940) suggested that an ideal situations for rapid evolution exist in small partially isolated populations. This has been demonstrated experimentally by Dobzhansky and Pavlovsky (1953) in *Drosophila*. There are also reports that a single event of population bottleneck may release hidden genetic variability, especially for polygenic traits, thus favouring evolution (Mayr, 1954; Lewis and Roberts, 1956; Carson, 1971 and 1990).

G. SPATIAL AND TEMPORAL GRADIENTS AND POPULATION RESPONSE

Environmental changes are often gradual. Temperature, moisture, exposure, and altitude commonly vary gradually on a geographic scale (Pickett, 1976).

Another important consideration is that environmental factors seldom vary independently and must always be considered jointly as complex gradients (Whittaker, 1970). Spatial variation in the intensity of natural selection caused by these environmental factors can play an important role in determining the genetic structure of natural populations (Slatkin, 1973). The environment of a plant species may not only change in time, it may also change in space. If spatial changes in environmental factors occur over longer distances, plant species usually adapt by the formation of localized races or ecotypes (Bradshaw, 1965). The distances for such differentiation are now known to be as little as 10 meters in herbaceous species (Bradshaw, 1963). The gradational interpretation of the environment allows for varying degree of discontinuity and consequent discontinuity of population response (Beals, 1969). It is highly unlikely that all genotypes will have exactly equal mean survival and reproductive rates for more than a short span of time and also distance (Endler, 1977). Under conditions of random drift and gene flow, what happens in a few generations may determine the general pattern of gene frequencies for the next few hundred generations. An environmental factor which is geographically variable within a species range but acts only once or twice in a long time may be sufficient to maintain regional differentiation of gene

frequencies, provided the decay rate of the cline is not faster than the time between selective periods. The geographically environmental factors would have to act more continuously to maintain stepped clines (Endler, 1977). The basic models, namely stepping stone and continuous models of population structure, explain the role of dispersal (gene flow) and selection against hybrids along a cline. The former consists of a set of discrete demes, each exchanging members or genes only with nearest neighbors, and having discrete, non-overlapping generations. The latter assumes a continuous dense distribution of organisms, each of which has a certain probability of leaving offspring at any given distance away per unit time. The breadth of phenological adaptation of any one population on a gradient is limited. The optimization of populations on a particular range of spatial gradients is wide spread. Some species are known to exhibit gradual variation along gradients (clinal variation) (Haldane, 1948; Fisher, 1950; Mayr, 1963; Heslop-Harrison, 1964; Dobzhansky, 1970; Endler, 1977). This phenomenon can be explained in the simplest way as the product of selection for optimal genotypes that vary continuously along geographic gradients. Clinal patterns over space and time at one or more loci are commonly found in plants, animals, and humans. They often correlate with gradually changing physical parameters such as

temperature, rainfall, and edaphic factors (Nevo, 1978). Most of the "ecoclines" (clines developed and maintained by natural selection) extending over large geographic areas supposedly result from a systematic change in gene frequencies caused by variable selection values of alleles of genes along parallel ecological gradients (Stern and Roche, 1974). The theory of clines assumes that the genetic structure of populations reflects both local adaptation combined with the influence of gene flow (Haldane, 1948; Mayr, 1963; Dobzhansky, 1970). Barton and Hewitt (1981, 1985) claim that the dynamic equilibrium between dispersal and selection along environmental gradients results in a cline. Such clines in allele frequencies may be temporarily stable or changing with time, resulting in either a stable cline or a transient cline, respectively. Further other clines may be stepped, with a large change in frequencies over a short distance or gradual, where the allele frequency change in the cline forms a gentle gradient (Endler, 1977). In other words, the pattern of cline verses ecotype is a reflection of the gradual verses abrupt changes in allele frequencies. The width of clines diminishes as the selection intensity increases. Under high selection intensities, pollen flow will generate wider clines than by seed dispersal, because nearly all of the immigrants would be killed prior to

reproduction (Antonovics, 1968). At low selection intensities, the opposite will be true.

H. GENETIC CHANGES DURING SUCCESSION

Succession is the change, with time, of species composition and community structure on a site. The basic selective forces responsible for changes in the vegetation also modifies genetic composition of species involved in the vegetation communities (Gray, 1987). Such changes are due to the complex interplay of ecological, edaphic and environmental factors which act as evolutionary forces over space and time. A major trend in succession is the amelioration of environmental extremes and hence it is a complex gradient of decreasing physical stress through time on vegetation (Pickett, 1976). However, it is certain that perturbations affecting environmental and ecological factors across a species range will act as a selective force on the species populations. Apparently there is a limit to the width of a habitat that a population can exploit, beyond which it is maladapted (Pickett, 1976). Mueller-Dombois (1988) argues that on volcanic islands like Hawaii primary successional events represent a temporal gradient of decreasing stress for plants in the younger stages up to about 1000 to 2000 years. During that period habitats development through

weathering and secondary enrichment of soils provide favorable growing conditions and then primary succession enters a regressive phase. During the regressive phase soils become increasingly acidic resulting in nutrient imbalances which result in decreased biomass and forest stature. Hence, Mueller-Dombois argues that primary succession is not only a temporal gradient of decreasing stress for plants ending in maximum biomass and community development (climax) on volcanic islands but is followed by a regressive phase associated to soil and geomorphological aging of ecosystems.

Succession provides a complex temporal gradient of physical and biotic environments, analogous to spatial gradients, to which species populations respond in both ecological and evolutionary time. Selection adjusts the positions of populations on successional gradients in the same way they are adjusted on spatial gradients (Pickett, 1976). Further evidence from natural populations occupying early stages of succession indicates that pioneer plants tend to allocate relatively early more of their resources to sexual reproduction (Gadgil and Solbrig, 1972). Such changes in reproductive behavior of early successional plants may be due to the need for higher levels of genetic recombination to maximize fitness in the heterogeneous environment. Adequate information on genetic variation and differentiation along secondary

successional gradients in herbaceous plants is available (Solbrig and Simpson, 1974 in *Taraxacum officinale*; Law et al., 1977 in *Poa annua*; Hancock and Wilson, 1976 in *Erigeron annuus*; Reinartz, 1984 in *Verbascum thapsus*).

A major difference between primary and secondary succession is that many colonizers in secondary succession are dispersed through time rather than through space (Grubb, 1987). As opposed to "conventional" type of primary succession where the pioneers are shorter-lived than the later-invading species (Grubb, 1987), on the Hawaiian islands lava flows many herbaceous species arrive only after the initial colonization and pre-conditioning of habitat by *M. polymorpha* (Smathers and Mueller-Dombois, 1974). Hamrick et al. (1979) demonstrated that species which are widespread, long lived, and primarily outcrossed by wind pollination had high life-time fecundities, and were characteristic of the later stages of succession. These taxa also maintained higher levels of intrapopulation genetic variation than species with other combinations of these characteristics. However, information on genetic changes during primary succession in long-lived perennials and trees is inadequate at present.

Assuming that a successional gradient occupied by a high amplitude species in which early, middle, and late stages are characterized by populations with high

frequencies of different genotypes, the genetic changes could be modeled to explain the process of differentiation in populations (Gray, 1987). The following four basic genetic models explain the ways in which differentiation may occur due to selection by successional change:

Model 1: Differential selection of genotypes occurs at each successional stage from a more or less homogeneous seed population which promotes micro-evolutionary changes in these populations.

Model 2: Differences in genotype frequency have arisen from differences in individual rates of development among the founder populations. Analogous to the initial floristic composition model of vegetation succession (Egler, 1954), such differentiation might stem from genetic differences in phenology or from germination polymorphisms of the type known to exist in some populations.

Model 3: This applies to mid successional stages. A population disperses offspring to nearby areas, which are in different successional stages along the chronosequential gradient. Here the new environment will select different genotypes.

Model 4: This also applies to mid-successional stages. But here discrete populations occupy areas which happen to be on a successional gradient.

These models represent cases which are not mutually exclusive. Elements of all four models could operate simultaneously over any set of adjacent successional stages. Although succession as a process succeeds perturbations in the species habitat which generate the requisite environmental heterogeneity for selection to operate in models 3 and 4. However, succession is not by itself an agent of selection. Genetic drift and similar non-selective forces during different stages of succession may possibly cause the observed non-random distribution of genotypes. Although a large body of information is available on the genetic changes during secondary succession, all of this is not relevant in the current context, which deals with genetic changes along primary successional gradients.

I. GENETIC DIVERSITY AND DIFFERENTIATION PATTERNS IN INSULAR SPECIES

Isolated oceanic islands have offered a great deal to biologists in understanding evolutionary processes since the 19th century (Carson, 1987). These isolated land masses were created by volcanic action in the open ocean. Because living organisms arrived by long distance dispersal, evolution virtually begins anew from a few colonists. Most continental biota are so ancient that the

key events of evolution that formed them are lost in the remote shadows of the past, whereas these events on oceanic islands are more recent and far more accessible for investigation (Carson, 1987).

The properties of the populations of plants and animals on islands depend to a large extent on the nature and history of the islands on which they are found. The flora and fauna of islands have several features that distinguish them from the flora and fauna of continents. Organisms that can disperse well are more likely to be found on islands than those which cannot. Once on an island, particularly an oceanic island, a species may evolve so as to lose its ability to disperse far, with many interesting effects on both morphology and reproduction (Williamson, 1981). The variation in dispersal ability and the subsequent evolution on islands produces a disharmonic biota. In many cases gigantism and other bizarre life forms are found on islands. Island biota are vulnerable to extinction and composed of interesting relict and endemic forms of species. J.D. Hooker, in his famous lecture to the British Association in 1866 pointed out that in general there were fewer species on oceanic islands than in continental areas of the same size, and that on islands genera were represented by few species and families by few genera as a consequence both of small area and impoverishment. The disharmony is

very evident in the fact that there are many evergreen woody angiosperms but no gymnosperms on remote oceanic islands, an over-representation of ferns and few if any indigenous annuals. Alpine and sub-alpine plants are rare from island mountains. Many members of the island biota are endemic and more distantly related to continental forms. Those endemic species that are so distinct that they have no known continental relatives are often abundant on the oceanic islands on which they have evolved. Lorence (1978) noted that the pteridophytes which are very efficient dispersers have fewer endemic species on oceanic islands. Perennial plants are known to possess an advantage over annuals because their longevity increases the likelihood of securing sufficient pollination to produce enough seeds to establish and maintain a species (Wallace, 1958). Baker (1955) claimed that hermaphroditism and self-pollination are advantageous for colonizers on islands, but species with long-term tenure on islands seem to develop outcrossing as a means of maximizing their fitness.

The most important evolutionary event on islands, both from an ecological and from a genetical point of view, is the formation of new species (Williamson, 1981). Long-distance dispersal to an island and successful establishment is a major event in the history of a species, an event which begins a train of consequences

(Carlquist, 1965). On volcanic islands, when colonists become established from long-distance dispersal, evolution virtually begins anew from a few founders (Carson, 1987). The survival and further evolution of these colonizing species involves acquisition of characters which can compensate for the broken contact from their continental counterparts which had accumulated very high levels of genetic variability. Dioecism, gynodioecism, monoecism, and various other dichotomous floral conditions tend to increase genetic variability and promote evolution on islands. Wind pollination and self-sterility are also very important for generation of variability through genetic recombination. However, in general, all those mechanisms through which species can maximize genetic variability are very important for survival and expansion on islands.

The habitat diversity, the effectiveness of inter-island isolation, and the size of the land areas available for colonization and spread of a particular species are other important dimensions which would influence the genetic diversity (Carlquist, 1965). Small population size and narrow distributional area are obvious reasons for the vulnerability of island species to extinction (Mueller-Dombois and Loope, 1990). Lack of competition on islands often allows aggressive species to indiscriminately colonize a wide spectrum of habitats.

Subsequently, such species may be subjected to differential selection pressure in different habitats leading to genetic divergence with incomplete genetic barriers. Hybrid swarms appear occasionally in such species along their peripheries. Rattenbury (1962) has stressed the value of hybridization as a means of retaining genetic vigor and variability.

Carlquist (1965) suggested that the relative paucity of successful immigrants in the Hawaiian angiosperm flora, coupled with great ecological opportunities, has resulted in abundant radiation. The products of such radiations are interfertile species or sub-species or races. The development of prolonged flowering and hybridization among the insular taxa may be expected to contribute to the high degree of heterozygosity and polymorphism among the waif floras. Hence hybrid swarms among recently diverged insular taxa are so frequent that they cause taxonomic problems. Reports of hybridization in insular floras depend on the opinion of systematists to recognize the presence of hybrids or to neglect them (Carlquist, 1965). In the Hawaiian islands, hybrids have been reported in several plant families even to the extent of inter-generic hybrids (Carr and Kyhos, 1981; Carr, 1985a)

The genetic significance of a high degree of hybridism in insular groups is like that of outcrossing: both tend to maintain a high level of genetic

heterozygosity, which permits evolutionary flexibility despite 1) loss of genetic contact with mainland relatives, and 2) small land area. Hybridization may, as Rattenbury (1962) suggests, help a species to overcome a bottleneck of dwindling land area and climatic stress.

A species successful on islands of reasonable size and ecological diversity would be expected to be polymorphic. Polymorphism in the Hawaiian flora has been emphasized by Hillebrand (1888) and Fosberg (1948). Patterns of almost continuous variability, such as occurs in Hawaiian *Metrosideros* are evidence of extensive outbreeding (Baker, 1953). According to Williamson (1981), well isolated, reasonably large, and environmentally heterogeneous islands are those best suited to speciation. Wagner et al. (1990) estimated that 956 species of higher plants occur in the Hawaiian islands, of which 850 are endemic derived from only about 272 or so ancestral stocks (Fosberg, 1948). Many groups of plants have undergone spectacular evolution in Hawaii. The flora is notably disharmonic and secondarily enriched.

J. PLANT EVOLUTION AND SPECIATION ON OCEANIC ISLANDS

An understanding of the evolutionary biology of insular flora must be based on sound principles of mode of dispersal and biogeography of islands (Carlquist, 1966,

1974). Congeneric species of plants on oceanic islands may differ conspicuously for more morphological characters than distinguish congeners on continents (Crawford et al., 1987). In some instances early taxonomic treatments recognized several genera to accommodate the variability (Stuessy et al., 1984). The eleven species of the genus *Tetramolopium* (Compositae) in Hawaii include such diverse life forms as succulent-leaved, cushion-forming plants, and caespitose alpine shrubs but show extremely low or no genetic divergence among the different species (Lowrey and Crawford, 1985). Helenurm and Ganders (1984) reported that the 19 species and eight subspecies of the genus *Bidens* (Compositae) endemic to the Hawaiian islands evolved from a single ancestral species. Morphologically they are more diverse than the 200 or so species of *Bidens* occurring on five continents, but all Hawaiian species are interfertile. Most striking of all is the morphological diversity in the Hawaiian silversword alliance (Compositae-Madiinae) comprising three genera namely *Dubautia*, *Argyroxiphium*, and *Wilkesia*, in the family Compositae (Carr and Kyhos, 1981; Carr, 1985a,b; Witter and Carr, 1988). This natural, doubtlessly monophyletic assemblage includes life forms such as cushion plants, semi-shrubs, trees, monocarpic rosette plants, and lianas (Carr, 1985b). The habitats occupied by these plants range from near sea level to 3750m including very recent lava

flows, cinder cones, alpine deserts, dry scrub, dry forests, mesic forests, rain forests, and bogs (Carr, 1987). Despite the remarkable diversity of the silversword alliance, they exhibit very high levels of interspecific and also intergeneric fertility (Carr, 1987). Natural interspecific and intergeneric hybrids were also noticed.

The wide ecological amplitude of congeners on islands has led to greater morphological variation and is often paralleled by the diversity of habitats in which they grow. The eleven species of *Tetramolopium* occur in a wide range of habitats from sea level to above 3000m (Lowrey and Crawford, 1985). Hawaiian *Bidens*, with nineteen species, occupy a great diversity of habitats from sea level to about 2200m including coastal dunes, rain forests, cliffs, dry cinder cones, montane ridges, rain forests, and bog margins with annual rainfall ranging from 0.3m to 7.0m (Ganders, 1989).

Similarly, Hawaiian *Metrosideros* exhibits a wide range of morphological diversity, which taxonomists classified into five species, based on morphological criteria, including several varieties within the major endemic species, *Metrosideros polymorpha*, (Rock, 1917; Skottsberg, 1944; Dawson and Stemmermann, 1990). *Metrosideros polymorpha* occurs over an extremely wide ecological amplitude ranging from near sea level to tree

line at 2500m on Mauna Loa on the island of Hawaii and even into subalpine scrub. *Metrosideros polymorpha* is well adapted to cloud forest conditions where it can grow about 20m tall, on exposed eroded ridges it occurs as a shrub or low growing tree and, in bogs, it grows as a prostrate shrub or small tree, sometimes flowering when under 10 cm tall.

K. ECOLOGICAL DIVERSITY AND DIFFERENTIATION PATTERNS ON ISLANDS

Habitats on oceanic islands

A person attempting to study the diversity of congeneric species on islands should first of all consider the diversity of habitats available for species to colonize and spread on islands. Most oceanic islands developed over volcanic hotspots. On a geological time scale, volcanic islands are relatively short lived features (Carlquist, 1974). Therefore oceanic islands exhibit the early and mid-stages in the phylaxis of a waif biota. Volcanic islands may disappear due to erosion even before evolutionary products are well advanced to the levels of distinct families in the phylogeny.

Evolution started on older islands could continue on younger islands, which are formed later as the tectonic

plate moves over the hotspot, through colonization of species belonging to advanced evolutionary stages. Hence it can be speculated that geologically younger islands may harbor evolutionarily more advanced members of an endemic lineage.

Factors influential for an island's biotic richness which could promote genetic differentiation and speciation are habitat diversity, degree of isolation, and the number of barriers within an island that can bring about geographic isolation (Carlquist, 1974). Other factors assisting rapid genetic differentiation and evolution on oceanic islands include lack of competition and predation, and presence of a wide spectrum of ecological opportunities for colonization and the presence of ridges and gulches that isolate small and rapidly changing populations where stochastic processes (genetic drift) can quickly advance the process of evolution. Oceanic islands may offer a diverse mosaic of habitats composed of different aged lava flows differing in structure, composition, and topography with tremendous diversity in microenvironments for the newly arriving propagules to establish and adapt to the conditions prevailing.

Habitats on oceanic islands are present for shorter periods on a geological time scale than on continents, thus little or no habitat pre-conditioning by other plants or animals takes place before the invasion by new plant

species (Crawford et al., 1987). In addition, the mountains formed by volcanoes on most oceanic islands can provide extraordinarily diverse habitats within short distances where environmental factors such as orographic rainfall, temperature, relative humidity, and solar radiation, and edaphic factors, can vary significantly. Further, erosion of older land surfaces can also create diverse habitats by altering the existing habitats and species population sizes thus providing new, open habitats for recolonization in the form of primary and secondary succession. Populations of late successional or climax species are less prone to random microdifferentiation than those of pioneer species, because the former are more stable in time, and gene flow therein tends to be over greater distances (Venable and Levin, 1983)

Adaptive radiation on insular archipelagos

The term "adaptive radiation" often used to describe the origin of adaptation and differentiation as result of diversifying selection which operate on an endemic taxon originally exhibiting a wide ecological amplitude. This may result in a number of sub-populations now narrowly adapted to habitats within the original amplitude or dispersed to newer habitats where populations enjoyed better fitness. In other words, recent differentiation due to local adaptation in an endemic taxon proliferated

from a single event of introduction. On the other hand, adaptive evolution can be defined simply as the process by which populations through natural selection increase their fitness in a given environment (Loeschcke, 1987). Thus the study of adaptive radiation in a particular group on an island or archipelago assumes that the group is monophyletic, as a result of a single introduction or invasion (Crawford et al., 1987).

Adaptive radiation is one of the more intriguing concepts in evolution, because of its manifold overtones (Carlquist, 1974). The process of adaptive radiation has been invoked to explain the diversity of species on oceanic islands. Carlquist (1974) applied the term "adaptive radiation" to describe evolution and speciation on islands. Helenurm and Ganders (1985) defined the term "adaptive radiation" as the evolution of many species adapted to a variety of different ecological niches from a single ancestor.

Once colonized on oceanic islands, opportunities for speciation may be greater than on continents because of the availability of habitats not yet occupied by other species. Rapid cladogenetic evolution to exploit vacant habitats results in adaptive radiation (Helenurm and Ganders, 1985). One implication of adaptive radiation is that the basic process of diversification has occurred quite recently. Classical examples of adaptive radiation

on islands are Darwin's finches in the Galapagos (Lack, 1947) and honeycreepers in Hawaii (Carlquist, 1980). The Hawaiian archipelago is rich with examples of adaptive radiation, including *Lipochaeta* (Gardner, 1976), *Bidens*, *Cyrtandra*, *Euphorbia*, *Pelea*, *Scaevola*, seven genera of *lobeliads* (Carlquist, 1980), and three genera of the silversword alliance (Carr, 1987).

L. SUMMARY AND HYPOTHESES

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. *Metrosideros polymorpha* is the dominant species among the five endemic species in the genus *Metrosideros* in Hawaii, described entirely by morphological criteria. *Metrosideros polymorpha* exhibits very high morphological diversity and adaptation in Hawaiian islands. In a recent taxonomic treatment, eight varieties of *M. polymorpha* have been described to accommodate this species morphological variation. There is still insufficient information to determine the genetic basis for the observed variation.

The species occupies wide spectrum of habitats from near sea level to the tree line at 2500m on Mauna Loa on

the island of Hawaii. Earlier studies to understand the nature of local adaptation and ecotypic differentiation were mainly on morphological traits and were limited to common garden experiments. In one study, all taxa of *Metrosideros* were treated as varieties and forms of *M. polymorpha*.

The genetic basis for the observed variation and its association with climatic and edaphic factors are essential to understand the evolutionary relationships in such complex taxa. Morphological criteria alone are inadequate and sometimes misleading in delineating the taxonomic relationships in such diverse groups.

The genecological approach to understand the level and organization of variation within and between populations of such complex taxa offers an excellent solution to the problem. Enzyme markers permit characterization at the molecular level of the amounts and organization of genetic variability to answer questions related to ecological adaptation, ecotypic differentiation, evolution, and biosystematics in diverse groups of organisms. Enzyme electrophoresis allows for the estimation of the number of alleles and their frequencies at gene loci in populations and taxa of plants. These allelic data may then be employed to estimate the genetic variation within and between populations.

In the present dissertation research I use genecological approach by employing enzyme electrophoresis to study the level and organization of genetic variability in the Hawaiian *Metrosideros*. The proposed research will test the following hypotheses:

1. *Metrosideros polymorpha* has genetically differentiated along the windward slopes of both East and West Maui mountains.
2. Genetic structure and differentiation patterns in *M. polymorpha* vary between the younger East and older West Maui mountains.
3. *Metrosideros rugosa* and *M. tremuloides* represent a case of recent and incipient speciation in the insular ecosystem

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<i>Subunit structure</i>	<i>AA</i>	<i>Aa</i>	<i>aa</i>	<i>Subunit combinations</i>
<i>Monomer</i>	—	— —		<i>A</i> <i>a</i>
<i>Dimer</i>	—	— — —	—	<i>AA</i> <i>Aa</i> <i>aa</i>
<i>Tetramer</i>	—	— — — — —	—	<i>AAAA</i> <i>AAAa</i> <i>AAaa</i> <i>Aaaa</i> <i>aaaa</i>

Figure 1.1. Electrophoretic phenotypes for monomeric, dimeric and tetrameric enzymes

PART II

ORIGINAL RESEARCH

CHAPTER 2

GENETIC STRUCTURE AND DIFFERENTIATION IN *METROSIDEROS* *POLYMORPHA* ALONG ALTITUDINAL GRADIENTS ON THE ISLAND OF MAUI, HAWAII

A. ABSTRACT

Metrosideros polymorpha is the dominant and a highly polymorphic tree species endemic to Hawaii. It occurs from near sea level up to 2500m along the windward slopes of volcanic mountains. Earlier attempts by taxonomists to classify the variation within this taxon resulted in the recognition of a number of varieties. In the present study starch-gel electrophoresis was used to examine the level and organization of genetic variability along complex altitudinal gradients on the island of Maui, Hawaii. Seventeen populations were sampled at approximately 200m elevational intervals along the NE wet slope of Mt. Haleakala and Kipahulu Valley in East Maui and six along Puu Kukui trail in West Maui. These were assessed for allelic variation in 11 enzyme systems encoded by 16 loci. On the average, 50 per cent of the loci studied were polymorphic per population with an overall mean of 2.15 alleles per locus. The mean observed and expected heterozygosities were 0.143 and 0.170 for

East Maui populations and 0.144 and 0.162 for West Maui respectively. The observed heterozygosities for different populations conform to panmixia except the 1000m population from Haleakala. The levels of heterozygosity observed for different loci among the populations of *M. polymorpha* are comparable with that of continental allogamous taxa including conifers.

Gene diversity analysis of East and West Maui populations indicated that 90 per cent of the total genetic variation was found within populations in East Maui while 95 per cent was found within West Maui populations. The populational pair-wise genetic identity (Nei's I) varied from 0.909 to 0.998 for East Maui populations and from 0.974 to 0.998 for West Maui. The UPGMA cluster analysis of the genetic identity matrix revealed three altitudinal groups at 97 per cent similarity level for East Maui populations, with more groups at the 98 per cent level of similarity. The West Maui populations fell into two groups, with the 400m population alone forming a distinct group. The level of altitudinal differentiation was moderate in both East and West Maui. Principal components analysis showed similar patterns for both East and West Maui populations. The partitioning of variation indicated that 6 per cent of the total variation was due to variation between groups while 4 per cent was due to differences among populations within

groups in East Maui. Twenty one alleles, out of a total of 63 observed for the 16 loci, exhibited statistically significant correlations with environmental variables. The regression coefficients indicated gradual allele frequency clines in response to environmental factors.

B. INTRODUCTION

Of all the oceanic volcanic islands in the world, the Hawaiian archipelago is the most geographically isolated and possesses some of the most dramatic altitudinal (up to 4205m above MSL) and environmental gradients along the windward slopes. Latitudinal and altitudinal gradients are environmental gradients along which a number of environmental and edaphic factors vary jointly as complex gradients (Whittaker, 1975). The large numbers of potential habitats created by such topographical variations, steep rainfall gradients, 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 climatically benign environments coupled with relatively simple biomes have allowed certain successful species to occupy a wide range of habitats. Species populations along altitudinal

and environmental gradients may consist of many narrowly adapted local subpopulations (demes) or of a broadly adapted widespread population. Such widespread species are ideally suited for studies of infraspecific genetic variation and differentiation in relation to the environmental factors forming the gradients. Information about population response patterns can be applied to evolutionary predictions: the extent to which ecological response breadth is divided among separate populations strongly influences whether or not selective divergence will occur between these entities (Bazzaz and Sultan, 1987).

The genetic structure in natural populations is assumed to have resulted from the interplay of mutation, migration, selection, and drift (Loveless and Hamrick, 1984). Although genetic structure has been described in many conspecific populations, the balance among selection, migration, and genetic drift are poorly understood (Levin, 1988). Rapid establishment of spatial patterns in gene frequencies may be possible, provided that gene flow is restricted, resulting in small neighborhoods as per Wright's neighborhood model (Wright, 1943a, 1946 and 1978). Ecological factors affecting reproduction and dispersal are likely to be particularly important in determining the genetic structure (Allard, 1970; Jain, 1975).

Gene frequency clines or character gradients 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 and 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). The genetic structure of a population, i.e. the amount and organization of genetic variation in space, plays a very important role in evolution by influencing the consequences of interactions among conspecifics, levels of selection, the amount of variation which is maintained, and the ability to exploit ecological opportunities (Wilson, 1979; Wright, 1969 and 1980; Wade, 1985). Adaptive genetic variation and divergence have been examined as a response to complex altitudinal and latitudinal environmental gradients (Mitten et al., 1980; Chapin and Chapin, 1981; ; Anderson et al., 1987; Schwaegerle and Bazzaz, 1987; David et al., 1989; Ganders, 1990), and soil properties, such as heavy metal content, nutrient availability, and salinity (Jain and Bradshaw, 1966; McNeilly, 1968; Wu and Antonovics, 1976; Antlefinger, 1981; Snaydon and Davies, 1982). These studies demonstrate genetic differences among populations in response to physical factors in the environment. However, such studies in plants are biased toward

temperate species, usually annuals, short-lived perennials, or confers (Loveless and Hamrick, 1984). Long-lived angiosperm trees and other herbaceous perennials have not been studied extensively, and the population genetics of tropical insular tree species is virtually unknown. The paucity of studies on allozyme variation in perennial angiosperms may be due partially to high phenol to protein ratios in this group which pose difficulties in resolving the enzymes (Soltis et al., 1980).

Ohia lehua, *Metrosideros polymorpha* Gaud. 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 2500m elevation, with annual rainfall and temperatures ranging from 75 to 1150cm and 9 to 23°C, respectively (Doty and Mueller-Dombois, 1966; Corn, 1979; Mueller-Dombois, 1981, 1987). As the name indicates, *M. polymorpha* is a highly polymorphic taxon with a number of varieties (Dawson and 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 and 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, where it can reach 25 - 30m tall (Baldwin, 1953). Mueller-Dombois and Loope (1990) stated 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. Carpenter (1976) observed partial self-incompatibility among red flowered types (common type), while yellow flowered types are totally self-compatible. She reported that the native birds and insects are essential for good seed set. She also 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, mid- and high elevations (Kitayama, 1992). The genetic basis for these morphological

varieties is not known. A study on the level of genetic variation and its organization across the species distributional range would aid in answering this question and would also be important for understanding 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 purpose of this study was (1) to examine the level and organization of genetic variation in *M. polymorpha* by partitioning the species-wide variation into within and between population components; (2) to assess the genetic relationships among populations along altitudinal gradients so as to understand the extent of genetic differentiation among populations; and (3) to examine the possible linear association of gene frequencies with environmental variables.

C. DESCRIPTION OF THE STUDY AREA

Three altitudinal transects were established in 1987: two on East Maui, one along the NE wet slope (windward) of Mt. Haleakala and a second in the Kipahulu valley on the outer ESE slopes of Haleakala; and the third transect was along the Puu Kukui trail in the West Maui Mountains (Figure 2.1).

The NE wet slope transect of Mt. Haleakala (Transect 1)

This transect approximately overlaps Transect No. 3 of the Fish and Wildlife Service, US Department of Interior. It extends from near sea level at PaPa'a'ea reservoir, beside Kahului - Hana highway near Kailua, roughly parallel to the Waikamoi stream to slightly above the timber-line at 2000m above MSL. The upper part of the transect area is under the administrative control of the Haleakala National Park.

The northern slope of Mt. Haleakala lies directly in the path of the NE trade winds, which bring abundant rainfall except in the winter (Leopold, 1949; Blumenstock, 1961). Under trade wind conditions there is frequently a pronounced moisture discontinuity between 1200-2200m above MSL. Below these heights the air is moist and above it is dry. The heaviest rainfall (6000-9000mm/year) is received in the altitudinal zone around 1000m elevation. Rainfall drops off rapidly west of Kailua stream and east of the Ko'olau - Hana district boundary. The temperature decreases slightly with increasing elevation at the rate of 0.5° C per 100m in an uniform manner up to the inversion layer. At the inversion the temperature increases, sometimes quite suddenly. The higher temperature may extend upward for a few hundred meters

before it begins once more to decrease upward in the usual manner.

The entire transect area belongs to the Kula volcanic series. This consists chiefly of thick alkali 'a'a flows, which were deposited in a viscous state and contain many interstratified, thin ash-soil layers with intermittent cinder cones embedded. Some olivine basalts and picrite occur in the Kula series (Sterns, 1985). Kula lava series flowed in the early and middle Pleistocene.

The lower section of the transect has an average slope of 10 per cent which increases to about 20 per cent in the mid-elevations and to 50 to 60 per cent in higher elevations. As compared to higher elevations, valleys are shallower in the lower elevations due to erosion and silting of streams.

The soils of the lower section of the transect, between 300 and 1000m elevations, belong to the " Honomanu - Amalu Association " (USDA, 1972). Honomanu soils are well drained and ash derived, occupying the slopes of gulches. Amalu soils are poorly drained, having developed from organic matter and materials weathered from basic igneous rocks. These occupy the flatter ridge tops. The remaining part of the transect, above 900m elevation, belongs to the " Hydrandepts - Tropaquods Association ". Hydrandepts occupy about 60 per cent of this association and are well- to moderately well-drained, much like the

Honomanu series. They are associated with steeper slopes. Tropaquods are poorly drained and similar to the Amalu series, occur on less sloping terrain. This association is derived from volcanic ash and material weathered from cinder and basic igneous rocks.

The Kipahulu transect of Mt. Haleakala (Transect 2)

This transect extends from 400m to 800m in the Kipahulu valley on the eastern slopes of Haleakala, along the ridge between Palikea stream and Koukouai stream (Figure 2.1). The valley extends in a northwesterly direction from about 300m above MSL at 'Ohe'o to Pohakupalaha and Haleakala crater at about 2300m.

There are two major topographical aspects in the valley; 1) the extended cinder cone, Palikea, at about 600m; and, 2) the valley, which is divided along its length with an upper shelf on the southern side 200m above the northern side. A major gorge, Koukouai gulch, formed by the intermittent Koukouai stream, borders the southern end of the valley. The valley is an erosional feature formed during a quiescent period following the kula volcanic series. Later volcanic activity during the Hana series partially filled in the valley with 'a'a flows.

There are two major soil types found within the Kipahulu valley transect. They are a " Hydrandepts-Tropaquods Association " and a silty clay of the Maka'alae

series. The Hydrandepts-Tropaquods Association is confined to the forest areas above Pu'u 'Ahu'ula from Koukouai gulch to Palikea stream and the ridge top above Waimoku falls where the entire transect is located.

The average maximum temperature in coastal areas varies from 28° C in December and January to 30° C between July and September. The average annual rainfall ranges from about 2500mm at the lowest point on the transect to about 5000mm at 1200m elevation.

Kipahulu Valley below the transect has been converted into grassland for cattle. All dominant species in the lower grassland are alien, principally grasses and sedges. Below 400m, several distinct alien forest communities occur, including bamboo thickets, stands of Java plum, Christmasberry, and common guava. These alien plants persist all through the transect along with ohia, strawberry guava, and rose apple. Strawberry guava and rose apple are major competitors with the native species.

In addition to these two transects, which accounted for 13 populations of *M. polymorpha* (10 along the NE Wet slope and 3 along SE Kipahulu transect), four more populations were sampled: two from along the trail to Koolau gap (11 and 12), one of which from the border along the gap; and two from inside the crater at the eastern end, near Paliku (13 and 14). At Paliku, one came from the floor of the crater in the wet NE corner where there

is a small grove of *Metrosideros*, and another was on the slope of the wall of the crater where there is a notch through which clouds drift into the crater, resulting in a mean annual rainfall of 4500mm. Trees in these areas are dominated by pubescent forms. The leaves are thick and covered with dense wooly pubescence on both sides.

The West Maui Puu Kukui transect (Transect 3)

The third transect was located in West Maui. The lower aspect of which includes 400 and 600m populations. This transect extends from above the Iao Valley State Park visitors' lookout along a trail on the ridge between Kinihapai stream and Nakalaloa stream (Figure 2.1). Iao Valley is an old caldera tapped by the Wailuku River and enlarged by erosion. Extrusion of basalts, which constitutes the mass of the mountain was followed by a rest period during which a few cm to a few meters of soil formed. These basaltic masses form the Wailuku Volcanic Series. The soils along this transect are described as a Hydrandepts-Tropaquods Association, including well to poorly drained soils. They developed in material weathered from volcanic ash, cinders, and basic igneous rocks (USDA, 1972).

The upper section of the transect extends upwards from above the Kaulalewelewe cabin located at about 700m elevation along the Puu Kukui trail NW of West Maui

mountains. Sampling points corresponding to 800, 1000, 1200 and 1400m elevations were located along this transect. Puu Kukui is the second wettest Hawaiian mountain with a mean annual rainfall of 952cm at its summit bogs (Carlquist, 1980). Puu Kukui possess a series of channel-like valleys which tend to funnel tradewinds towards the summit and hasten condensation over the summit. The major volcanic mass forming the Wailuku Volcanic Series erupted in late Pleistocene (Sterns, 1985). Soils along the upper transect are included in the Hydrandepts-Tropaquods Association. The foot of the mountain was converted into agricultural land by the Maui Pineapple Co. for pineapple production. The transect where the study populations were located is relatively well protected with abundant native vegetation.

D. MATERIALS AND METHODS

The approximate locations of the 23 populations of *Metrosideros polymorpha* sampled and their corresponding elevations are shown on the map in Figure 2.1.

Sampling of natural stands

Sampling plots were established at 200m elevational intervals along the transects. A minimum of 40 mature trees were randomly sampled for fresh young leaves at each

site. 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 associated vegetation at each sampling site along transect 1 was described by following the releve method (Kitayama, 1992). Altogether 23 populations were sampled; ten from the Haleakala transect (1 to 10), three from Kipahulu transect (15 to 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 to 23). 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.

Electrophoretic analysis

Several grinding buffers were tried to select a suitable buffer to extract enzymes in *Metrosideros* (Appendix A). None of them gave satisfactory results. Addition of 10 per cent dimethyl sulfoxide (DMSO). 0.02 M sodium metabisulphate, and 0.005 M diethyldithiocarbamate (DIECA) in addition to the other constituents listed by Bousquet et al. (1987) yielded satisfactory results. About 25 mg of leaf tissues were homogenized in 0.1 ml freshly prepared chilled extraction buffer. B-

mercaptoethanol and polyvinylpolypyrrolidone (PVPP) were added to the extraction buffer just before grinding the tissue. The grinding was accomplished quickly on ice. The resulting slurry was absorbed on to Whatman #3 filter paper wicks (4 x 10mm). Wicks were immediately loaded into 12 per cent 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 (Appendix B), histidine-citrate pH 6.5 (Cardy et al., 1983) was found suitable for resolving enzymes in *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 and at 200 volts (20 v/cm) with 40 milliamperes for 6 hours. At the end of the electrophoresis, the gels were sliced horizontally into six slices and stained for different enzymes.

Eleven enzyme systems were assayed (Table 2.1). Staining methods and recipes were those of Arulsekhar and Parfitt (1966) and Shaw and Prasad (1970) with some modifications (Appendix C). Although many more enzyme systems were resolved, they were inconsistent in revealing bands and hence were excluded from the analysis.

Genotype frequencies were inferred directly from the isozyme phenotypes. Genetic studies utilizing segregating progenies were not possible because of the perenniality of the species and very poor seed set upon selfing. Many enzyme systems exhibited at least two distinct zones of activity (Table 2.1, Appendix F) indicating the involvement of two loci in the biosynthesis of the enzymes. They were aconitase (ACO), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucosmutase (PGM), and 6-phosphoglucosnate dehydrogenase (6PGD). Altogether, the populations were screened for 16 loci, coding for 11 enzymes.

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 percentage of polymorphic loci and the number alleles/locus were calculated using only those alleles present at a minimum frequency of 0.05.

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 -1.0 to 1.0; positive values indicate a deficit of heterozygotes and negative values an excess.

Population differentiation

Interpopulational relationships were established by computing the unbiased genetic identity and distance coefficients (Nei, 1978) for all possible pair-wise comparisons. All computations were performed using the computer program BIOSYS (Swofford and 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 (transect 1 and 2) and West Maui mountains (transect 3) by the method suggested by Nei (1973). The total genetic diversity (H_T) was apportioned into gene diversity within populations (H_S) and gene diversity among populations (D_{ST}) where $H_T = H_S + D_{ST}$. H_T is defined as the Hardy-Weinberg expectation of heterozygosity obtained with the weighted average of allele frequencies over all populations,

i.e.
$$p_k = \sum_i w_i p_{ik} / n \quad (1)$$

where w_i is the number of individuals from the i th subpopulation, p_{ik} is the frequency of the k th allele in the i th subpopulation and n is the total number of individuals over all populations

and
$$H_T = 1 - \sum_k p_k \quad (2)$$

H_T can be interpreted as the probability of nonidentity of two alleles sampled from the total population.

H_S is equal to the weighted average of the Hardy-Weinberg expectation of heterozygosity (H_i) over all populations

i.e.
$$H_i = 1 - \sum_k p_{ik} \quad (3)$$

and
$$H_S = \sum_i w_i H_i / n \quad (4)$$

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. population fixed for different alleles.

Hierarchical gene diversity analysis was performed on populations sampled in East Maui according to Nei's (1973) method, as extended by Chakraborty (1980) where the components of genetic diversity are partitioned according to hierarchy. Considering the groups based on the UPGMA cluster analysis, H_T can be further partitioned as:

$$H_T = H_S + D_{SG} + D_{GT} \quad (5)$$

where $D_{SG} = H_G - H_S$ and $D_{GT} = H_T - H_G$ and H_G is the average gene diversity of groups, defined as the average over groups of H_{Gj} , where H_{Gj} is the Hardy-Weinberg expectation of heterozygosity obtained from the average frequencies in group (j). For consistency with the assumptions of equal weights to subpopulations, the average over groups should be made with weights proportional to the number of subpopulations in the group (Chakraborty, 1980; Chakraborty et al., 1982). Dividing equation (5) with H_T ,

$$1 = H_S/H_T + G_{SG(T)} + G_{GT} \quad (6)$$

and $G_{ST} = G_{SG(T)} + G_{GT}$ has been split into two components due to variation between subpopulations within groups and between groups within total. Equations (5) and (6)

describe population structure by an additive partitioning into components.

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 63 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 eigen values and vectors were extracted. The first three orthogonal vectors were multiplied with the original allele frequency matrices. 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 and Russell (1966):

$$I_j = (\sum_i E_{ij} / n) - (\sum_i \sum_j E_{ij} / pn), \sum_j I_j = 0 \quad (8)$$

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 (SAS Institute, 1990) 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.

E. RESULTS

Intrapopulation genetic variability

The observed allelic frequencies are presented in Tables 2.2 and 2.3. 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 observed 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.

The frequency of the most common allele (100) in different loci along the altitudinal gradient in both East

(Figures 2.2, 2.3) and West Maui (Figures 2.4, 2.5) did not follow any definite trend. However, in some loci a local trend in the gene frequencies was evident. 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.

The low frequency alleles at some loci were found to be 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 Tables 2.4 and 2.5 respectively. 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, while 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 47.8 per cent, with a range from 25.0 per cent for the 1600m population to 68.8 per cent for the 1950m population located closer to Koolau gap. The West Maui populations averaged 51.1 per cent, with a range of 31.3

per cent for the 1400m population to 62.5 for the 600 and the 1200m populations.

The mean observed heterozygosity for 17 polymorphic populations from East Maui ranged from 0.094 for the 1000m population from transect 1 to 0.220 for the 1950m population at Koolau gap, with an overall mean of 0.143. The mean heterozygosity based upon Hardy-Weinberg expectations ranged from 0.124 for the 1600m population to 0.252 for the 1950m Koolau gap population, both from transect 1, 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.

All populations exhibited a deficiency of heterozygotes, except the one sampled at 1950m along the Koolau gap trail (Koolau-2) which recorded a slight excess of heterozygotes. However, F values were not statistically significant in most cases except for the 1000m population.

Population differentiation along gradients

Genetic differentiation along the altitudinal gradients were analyzed separately for East and West Maui by computing pair-wise unbiased genetic identity (I) and distance (D) measures among populations (Nei, 1972). The

coefficients for East and West Maui populations are presented in Tables 2.6 and 2.7, respectively. The genetic identity among different populations from East Maui varied from 0.909 between the 600m population from transect 2 and the 1950m population from Koolau gap area to 0.998 between a population sampled inside the crater near Paliku and the 1600m population from transect 1. For West Maui populations, it varied from 0.974 between the 400m and the 1200m population to 0.998 between the 800 and the 1000m population.

The genetic identity matrices among all pair-wise comparisons for East and West Maui were subjected to a cluster analysis with the UPGMA algorithm (Figures 2.6, 2.7) The cluster analysis for East Maui populations resulted in three groups at 95 per cent level of genetic similarity. Group I represents those populations coming from lower elevations, which includes 200m, and 400m populations from Haleakala and all three populations from Kipahulu. However, populations from Haleakala and Kipahulu valley showed greater affinity within themselves as compared to between them. Group II is comprised of all populations from mid- and high elevations on Haleakala (600m to 2000m and the two from crater). A most striking feature of this group is that it contains three sub-groups roughly representing mid-elevation from 600m to 1000m (2a), high elevation from 1200m to 1800m and the two from

the crater (2b), and a single population from 2000m which is relatively distinct from other populations (2c). A third group which includes the two populations from the Koolau gap area (1950m) can be recognized as the most distinct group.

West Maui populations tend to cluster into two groups at about 98 per cent level of similarity. Group I possessed one population from low elevation (400m) and Group II had the remaining populations (600m to 1400m) representing mid to high elevation. However, in group II, the 1200m and the 1400m populations appeared relatively distinct from rest of the group.

Nei's unbiased genetic identity and distance coefficients were computed among the groups (Table 2.8). The average pair-wise identity among groups was 0.957, with a range from 0.932 to 0.977.

Distribution of genetic variation within and between populations

The results of gene diversity analyses are summarized for East and West Maui populations in Tables 2.9 and 2.10 respectively. The gene diversity components are a function of the allele frequencies in the subpopulations and provide estimates of population subdivision (Nei, 1973). The gene diversity analysis can be extended to include many hierarchical subdivisions (Nei, 1973;

Chakraborty, 1974). Total gene diversity (H_T), a measure of mean heterozygosity expected under random mating varied considerably in magnitude among loci, ranging from 0.013 for *6Pgd-1* to 0.616 for *Pgm-1* with an averaged of 0.192 for East Maui and it is from 0.012 for *6Pgd-1* to 0.582 for *Pgm-1* with a mean of 0.168 for West Maui.

Summing over all loci, approximately 90 per cent of the total variation resides within populations in East Maui, while it is 95 per cent for West Maui. The remaining 10 per cent was due to between population variation for East Maui. It was further partitioned hierarchically into variation due to genetic differences among populations within groups, which amounted to about 4 per cent, and variation due to genetic differences among groups which amounted to 6 per cent (Table 2.9). However, the enzyme loci differed with respect to the distribution of variation within and between populations and within and between subdivisions of populations.

Principal components analysis

The overall relationship among populations of *M. polymorpha*, represented as a multivariate structure of allele frequencies of 16 loci along the altitudinal gradients in East Maui, can be better illustrated by principal components analysis (PCA). The first two principal components accounted for 66 per cent of the

total variation. The populations were projected onto a plane defined by the first two PCA axes to visualize the relationship among the populations (Figure 2.8). The number of groups and their compositions are similar to that of the one obtained by the UPGMA cluster analysis with the genetic identity matrix. It appears that the alleles correlated with altitudinally related environmental variables make up the first PCA axis. This is indicated by the near linear trend in arrangement of clusters from right to left of the first PCA axis in the order of increasing elevation.

Similarly, the PCA of West Maui populations revealed the existence of two clusters, confirming the UPGMA cluster analysis results (Figure 2.9). The first two orthogonal axes in this case accounted for 77 per cent of the total variation. The elevational trend along the first axis was similar to that observed for the East Maui populations.

Relationship between allele frequencies and environmental variables (clinal variation)

The heterogeneity observed in the pattern of distribution of alleles among populations for different loci stimulated further examination of the nature of the relationship of individual alleles with some important environmental variables. These environmental factors may

act as potential selective forces in the maintenance and pattern of distribution of alleles and genotypes along the gradients. Pearson's correlation coefficients were computed between allele frequencies in different populations from East and West Maui and associated mean annual temperature, annual rainfall, and the environmental index for different elevations (Table 2.11). The temperature and rainfall data were obtained by extrapolating the data from isotherms and isohyets for Maui (DLNR, 1986). Out of the 63 alleles that were involved in the computation of correlations with environmental parameters, 21 showed statistically significant correlations. The simple linear regression analysis was performed with environmental factors as the independent variables and allele frequencies as the dependent variable. The results are presented in Table 2.11. It was evident from the regression analysis that the rates of variation (b), though small, were significant in most cases, indicating a gradual clinal response of these alleles to the environmental variables.

Gene flow between populations

Wright (1951) has shown that the commonly used measure of genetic differentiation, F_{ST} , is approximately equal to $1/(4Nm + 1)$, assuming an island model, in which a subpopulation receives immigrants at a rate m chosen at

random from other subpopulations. As a result, genetic differentiation will be greatly reduced, even with low levels of migration or gene flow. Using the above relationships, locuswise estimate of average number of immigrants per generation each subpopulation would receive from the population as a whole (N_m) was estimated for both East and West Maui (Tables 2.9, 2.10). For species with a population structure closer to the stepping-stone model, wherein immigrants are from adjacent subdivisions, Crow and Aoki (1984) showed that the better approximation of N_m is obtained if the island model estimate is multiplied by 2. 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 among different loci both among East and West Maui populations.

F. DISCUSSION

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 and Hamrick, 1984).

The allelic composition and its pattern of distribution among populations along altitudinal gradients did not show definite trends in both East and West Maui. Similar results have been reported for many allogamous tree species with wide ranges of distribution (Gottlieb, 1981). However, contingency chi-square analysis for heterogeneity of gene frequencies between populations indicated significant differences between populations. Although gene frequencies for the most common alleles in different loci did not show any global trends across the entire altitudinal range, the local trends were very obvious in many loci across narrower altitudinal belts. This indicates that the gene flow across populations in the neighborhood (demes) is more prevalent than among populations located farther away along the elevation. 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 indicates that alleles involved in one ecologically adaptive function may vary independently of those involved with other functions in response to natural selection.

Among plants, tree species seem to maintain higher levels of variability than do those of short-lived species

(Hamrick et al., 1979). Populations of late successional or 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 and Kerster, 1974; Venable and Levin, 1983)

The higher levels of genetic variation observed in *M. polymorpha* when compared to other insular taxa is probably due to its allogamous breeding system, resulting in frequent recombination of alleles from genetically diverse and geographically distant 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 the existence of higher levels of genetic variation would be that probably more genetically diverse forms might have been introduced on more than one occasion to the islands. Such multiple introductions of genetically diverse founders help species to overcome the genetic bottlenecks in the initial stages of colonization and spread. However, most colonial populations experience bottlenecks during their initial colonization and establishment. Rattenbury (1962) suggests that hybridization among related insular taxa helps them to survive a "bottleneck" of dwindling land

area and climatic stress. Small heterozygote deficiency when compared to panmixia observed in some populations of *M. polymorpha* were statistically not significant. This appears to be true of most wind-pollinated species (O'Malley et al., 1979; Guries and Ledig, 1981)

Population differentiation

The broad ecological amplitude exhibited by *Metrosideros polymorpha* indicates that the species displays either a wide range of adaptation or a number of local adaptations to different habitats along the 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, 1973; Stemmermann, 1986). However, inferring genetic differences associated with such morphological differences and ecological preferences displayed by conspecific populations of insular species is difficult (Crawford et al., 1987).

Most continental biota are so ancient and are rich in genetic variability that 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 processes

such as selection, gene flow and drift. However, the genetic divergence in island taxa depends on the build up of genetic variation after initial colonization solely through origin, distribution and fixation of new mutations in their distributional range. Genetic divergence in sexually reproducing organisms involves the development of intrinsic barriers to gene flow following shifts in the adaptive peaks on an adaptive landscape leading to the establishment of multiple stable equilibria. 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, 1981; Ganders and Nagata, 1984; Carr, 1985b).

The information on the level and distribution of genetic variation is important to understand the role of complex ecological variables operating on the species populations along complex environmental gradients such as altitudinal gradients. Partitioning of genetic variation in *M. polymorpha* indicated marginal differentiation among populations along the altitudinal gradients. Substantial amounts of variation reside within the populations ($H_S = 90\%$). The pattern of distribution of the remaining 10 per cent between population variation appeared to be somewhat altitudinally structured. Considerably higher

levels interpopulational gene flow observed in both the mountains may counteract genetic differentiation between populations due to disruptive selection along the gradients. The homogenizing effect of gene flow is more marked among populations in successional advanced West Maui mountains.

Geologically younger Hawaiian mountains with their gentle slopes do exhibit distinct altitudinal zonations of vegetation when defined by total florestic composition (Kitayama and Mueller-Dombois, 1992). However, island tree species range over wider altitudinal segments than tree species on older, continental mountains. The gradually varying selective environmental and edaphic factors coupled with reasonably high levels of gene flow between populations along altitudinal gradients can reduce the magnitude of genetic differentiation considerably and increase within population genetic variation. However, it is known that genetic divergence can occur within populations, overriding the homogenizing effects of gene flow, if the selection is strong enough to bring about specific ecological adaptations (McNeilly, 1968; Wu et al., 1975; Wu and Antonovics, 1976; Snaydon and Davies, 1982).

The level of genetic differentiation observed for *Metrosideros* is comparable with that of continental populations of conifers and other outcrossing flowering

plant species (Gottlieb, 1975; Weeler and Guries, 1982; Wendel and Parks, 1985; Bousquit et al., 1986 Yeh, 1988; Yeh et al., 1986). Hawaiian examples of study of genetic differentiation are found in the Hawaiian silversword alliance (Compositae: Madiinae), consisting of 28 species in three endemic genera, *Argyroxiphium*, *Dubautia*, and *Wilkesia*, where interspecific and intergeneric gene flow are still possible (Carr, 1985; Witter and Carr, 1988); Hawaiian *Tetramolopium*, involving 11 species with wide ecological amplitudes exhibits very little interspecific divergence (Lowrey and Crawford, 1985); and Hawaiian *Bidens*, involving 27 congeneric taxa exhibits narrow interspecific divergence (Helenurm and Ganders, 1985).

Nei's populational pair-wise unbiased genetic identities and distances based on the adaptive multilocus association did not give any indication of genetic divergence. The genetic distances observed are typical of the values observed among conspecific populations in a wide variety of plants including conifers (Yeh and Layton, 1979; Guries and Ledig, 1982; Dancik and Yeh, 1983). Genetic distances did not increase linearly with altitudinal separation and altitudinal patterns of differentiation were not apparent. The low G_{ST} values coupled with very high populational pair-wise genetic identities indicate that relatively little genetic

differentiation has occurred along the altitudinal gradients.

Despite high genetic identities between populations three broad groups could be recognized at the level of 97.5 per cent similarity among East Maui populations and two at a 98 per cent similarity level among West Maui populations (Figures 2.6 and 2.7). Populations in these groups reflected altitudinal adaptations approximately corresponding to the different vegetation zones. Kitayama (1992) while studying vegetation along the same gradient on Haleakala 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 bog forming dieback 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 forest where trade winds cause very heavy rainfall and includes populations from the cloud forest zone. Since the vegetation zones are overlapping along the altitudinal gradient, the divergence of populations is not very apparent in this broad altitudinal zone. However, marginal divergence showing specific local adaptations to lowland wet forest and montane wet forest environments

were evident. The two populations sampled along the Koolau gap trail exhibit very unique adaptation by being relatively distinct from other populations. These populations also possessed very high levels of heterozygosity as compared to the other populations. These two populations, located just above the inversion zone where they are subjected to frequent cyclical environmental fluctuations which include sudden discontinuities in temperature and humidity, may show special adaptations. Another distinctive feature of the Koolau gap habitat is that it allows for a distinctive microclimate such as heavy clouds brought by tradewinds. These pass through and around the gap instead of being lifted above the inversion layer, as happens towards the west side. Genetic identities between these groups are of a magnitude comparable to differences between *Metrosideros* populations occupying extreme environments along the altitudinal gradient.

Altitudinal segregation of populations was also evident on West Maui. However, the level of differentiation was marginally lower than that on East Maui. The within populations genetic variation was also relatively higher than that on East Maui indicating 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 and Powell, 1974; Brown et al., 1976; Marinkovic and Ayala, 1975). The multivariate ordination technique, such as the principal components analysis (PCA), considers composite variations at many loci simultaneously and hence it is very useful in describing the multivariate relationships among populations (Gauch, 1977; Gauch and Whittaker, 1972). 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 pronounced in the PCA of West Maui than in the UPGMA cluster analysis.

The migration rate (gene flow) estimates, following Wright's Infinite Island Model (Wright, 1951), indicated relatively high levels of species-wide gene flow for different loci. The average gene flow estimates for *M. polymorpha* are comparable with those from many allogamous species (Hamrick, 1987). Since endemic Hawaiian birds and some native insects are the major pollinators of *Metrosideros*, the levels of gene flow depend on pollinator behavior. Temporal fluctuations in pollinator populations and the inability of these pollinators to respond instantly to changes in the intensity of bloom have

important consequences for pollination, outbreeding, and, consequently, gene flow (Carpenter, 1976).

G. SUMMARY

The level and organization of genetic variability in *Metrosideros polymorpha* has a number of outstanding features. First, populations contain heterozygosity levels which are much higher than the majority of plant species that have been electrophoretically examined. The genetic variability is relatively uniform throughout the species range. Generally the observed and expected levels of heterozygosity in different populations suggest that populations conform to panmixia.

Secondly, there was a differential response of alleles indicating the complex multidimensional nature of response to environmental factors which are potential selective forces acting along the complex altitudinal gradients. Twenty-one alleles out of 63 exhibited clinal patterns of variation and the others did not show any significant relationships with environmental variables. Nearly 90 per cent of the total variation resided within populations and the remaining 10 per cent was highly structured along the environmental gradients. In insular species, after initial differentiation, the populations generally lack genetic barriers and may circulate the

genetic variation within the species complex. This results in higher levels of polymorphism, a feature commonly characteristic of island taxa.

Finally, the UPGMA cluster analysis of genetic identity matrices suggested that *M. polymorpha* populations are somewhat genetically structured along the gradients in both East and West Maui. The PCA ordinations of allele frequencies from different populations further support the above findings. *Metrosideros polymorpha*, being allogamous, the gene flow between populations in the neighborhood was extensive thus increasing the neighborhood size.

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Table 2.1. Enzymes resolved in *Metrosideros polymorpha*

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

Table 2.2. Allele frequencies for 16 polymorphic loci among 17 populations of *Metrosideros polymorpha* (1 thru 9) from East Maui

Locus	Population								
	1	2	3	4	5	6	7	8	9
<i>Ald</i>									
(N)	44	46	44	40	45	45	45	45	45
85		0.011			0.011				
100	0.955	0.837	0.682	0.650	0.589	0.367	0.289	0.411	0.256
115	0.045	0.152	0.318	0.350	0.400	0.633	0.711	0.589	0.744
<i>Aco-1</i>									
90	0.023	0.033			0.011				0.022
100	0.955	0.946	1.000	0.988	0.989	1.000	1.000	1.000	0.978
112	0.023	0.022		0.013					
<i>Aco-2</i>									
94									
100	0.705	0.739	0.943	0.900	1.000	1.000	0.989	1.000	0.956
114	0.295	0.239	0.057	0.087			0.011		0.044
126		0.022		0.013					
<i>Lap-1</i>									
89			0.045	0.338	0.033	0.011	0.022		0.033
95	0.011		0.386	0.013	0.067	0.411	0.200	0.189	0.333
100	0.625	0.554	0.568	0.650	0.900	0.578	0.778	0.811	0.611
105	0.364	0.446							
112									0.022
<i>Lap-2</i>									
97	0.068								
100	0.898	0.870	0.864	0.762	0.867	1.000	1.000	1.000	1.000
110	0.034	0.087	0.080	0.237	0.067				
114		0.043	0.057		0.067				
<i>Mdh-1</i>									
96									0.022
100	1.000	1.000	1.000	1.000	1.000	0.989	1.000	1.000	0.978
105						0.011			
<i>Mdh-2</i>									
92									
100	0.920	0.924	0.955	1.000	0.989	1.000	1.000	0.978	0.967
109	0.080	0.076	0.045		0.011			0.022	0.033
<i>Pgm-1</i>									
86								0.011	
91	0.057	0.065			0.022	0.011	0.022	0.022	0.078
95	0.125		0.045	0.213	0.122	0.289	0.389	0.289	0.189
100	0.523	0.565	0.614	0.575	0.556	0.411	0.511	0.444	0.433
109	0.295	0.370	0.341	0.213	0.300	0.289	0.078	0.189	0.233
118								0.044	0.067

Table 2.2. (Continued)

Locus	Population								
	1	2	3	4	5	6	7	8	9
<i>Pgm-2</i>									
95	0.023	0.011	0.045	0.013	0.011	0.011	0.011	0.022	0.011
100	0.966	0.989	0.920	0.938	0.911	0.900	0.933	0.978	0.978
105	0.011		0.034	0.050	0.078	0.089	0.056		0.011
<i>Pgi</i>									
88				0.038		0.033		0.022	0.022
92		0.022		0.013	0.078			0.011	
100	0.875	0.902	0.943	0.725	0.889	0.922	0.922	0.900	0.944
108	0.023	0.043	0.045	0.100	0.033			0.056	0.022
112	0.102	0.033	0.011	0.125		0.044	0.078	0.011	0.011
<i>Per</i>									
83									
87	0.045	0.022	0.034	0.013		0.089	0.100	0.044	0.044
100	0.864	0.935	0.864	0.988	0.933	0.889	0.856	0.956	0.956
130	0.068	0.043	0.102		0.067	0.022	0.044		
136	0.023								
<i>Skdh</i>									
91	0.023	0.022	0.034						
100	0.659	0.554	0.841	1.000	0.889	0.822	0.811	1.000	1.000
109	0.318	0.424	0.125		0.111	0.178	0.189		
<i>Idh</i>									
82	0.023								
91	0.023	0.011							
100	0.932	0.967	1.000	1.000	0.989	1.000	1.000	0.989	0.989
114	0.023	0.022			0.011			0.011	0.011
<i>6Pgd-1</i>									
86				0.038					
100	1.000	1.000	1.000	0.962	1.000	1.000	1.000	1.000	1.000
<i>6Pgd-2</i>									
89						0.022	0.011	0.044	
100	1.000	1.000	0.989	0.988	0.989	0.978	0.967	0.956	1.000
111			0.011	0.013	0.011		0.022		
<i>Dia</i>									
80								0.011	
87	0.023								0.056
100	0.977	1.000	1.000	1.000	1.000	1.000	1.000	0.989	0.922
124									0.022

Table 2.2. (Continued)

Locus	Population							
	10	11	12	13	14	15	16	17
<i>Ald</i>								
(N)	38	40	36	45	45	30	30	25
85	0.013	0.013	0.028				0.033	
100	0.605	0.525	0.375	0.422	0.400	0.683	0.833	0.660
115	0.382	0.463	0.597	0.578	0.600	0.317	0.133	0.340
<i>Aco-1</i>								
90						0.017		0.020
100	1.000	0.875	1.000	1.000	1.000	0.983	1.000	0.980
112		0.125						
<i>Aco-2</i>								
94							0.033	
100	0.895	0.750	0.972	0.889	0.989	0.800	0.850	0.780
114	0.105	0.250	0.028	0.111	0.011	0.200	0.117	0.220
126								
<i>Lap-1</i>								
89	0.013	0.050	0.069	0.033				
95	0.250	0.013	0.361	0.100	0.144	0.017	0.017	
100	0.737	0.450	0.569	0.867	0.856	0.567	0.400	0.680
105		0.488				0.417	0.583	0.320
112								
<i>Lap-2</i>								
97	0.026			0.011				
100	0.855	0.387	0.361	0.978	1.000	1.000	1.000	0.940
110	0.118	0.613	0.639	0.011				0.060
114								
<i>Mdh-1</i>								
96	0.066	0.100	0.014	0.033	0.022		0.017	
100	0.934	0.900	0.986	0.956	0.978	1.000	0.983	1.000
105				0.011				
<i>Mdh-2</i>								
92	0.013							
100	0.987	1.000	1.000	1.000	1.000	0.867	1.000	0.980
109						0.133		0.020
<i>Pgm-1</i>								
86		0.038						
91			0.014	0.022				
95		0.425	0.389	0.300	0.267	0.017	0.017	0.040
100	0.961	0.450	0.542	0.456	0.522	0.750	0.450	0.460
109	0.039	0.075	0.056	0.222	0.211	0.233	0.533	0.500
118		0.013						

Table 2.2 (Continued)

Locus	Population							
	10	11	12	13	14	15	16	17
<i>Pgm-2</i>								
95			0.014			0.117	0.133	0.060
100	1.000	0.800	0.681	0.856	0.800	0.850	0.800	0.940
105		0.200	0.306	0.144	0.200	0.033	0.067	
<i>Pgi</i>								
88	0.026	0.013	0.056					
92					0.022			
100	0.947	0.975	0.875	0.989	0.978	0.900	0.800	0.760
108			0.056	0.011		0.100	0.200	0.240
112	0.026	0.013	0.014					
<i>Per</i>								
83							0.050	
87	0.329	0.175	0.250	0.056	0.222	0.133	0.067	0.060
100	0.671	0.825	0.750	0.944	0.778	0.767	0.867	0.920
130						0.100	0.017	0.020
136								
<i>Skdh</i>								
91								
100	1.000	1.000	1.000	1.000	1.000	0.950	0.783	0.980
109						0.050	0.217	0.020
<i>Idh</i>								
82								
91		0.050	0.014					
100	1.000	0.950	0.986	1.000	1.000	1.000	1.000	1.000
114								
<i>6Pgd-1</i>								
86						0.033	0.050	0.020
100	1.000	1.000	1.000	1.000	1.000	0.967	0.950	0.980
<i>6Pgd-2</i>								
89					0.011			
100	1.000	1.000	1.000	0.989	0.967	1.000	1.000	1.000
111				0.011	0.022			
<i>Dia</i>								
80		0.013						
87		0.175	0.111	0.100	0.044		0.033	
100	1.000	0.813	0.889	0.900	0.956	1.000	0.967	0.960
124								0.040

Table 2.3. Allele frequencies for 16 polymorphic loci among 6 populations of *Metrosideros polymorpha* from West Maui

Locus	Population					
	1	2	3	4	5	6
<i>Ald</i>						
(N)	38	40	40	40	40	40
85	0.039					
100	0.697	0.663	0.525	0.550	0.538	0.637
115	0.263	0.338	0.475	0.450	0.463	0.363
<i>Aco-1</i>						
90	0.026	0.050	0.025	0.025	0.013	
100	0.974	0.950	0.962	0.975	0.988	1.000
112			0.013			
<i>Aco-2</i>						
100	0.829	0.913	0.925	0.925	0.950	0.975
114	0.079	0.087	0.075	0.075	0.050	0.025
126	0.092					
<i>Lap-1</i>						
89	0.145					0.013
95	0.039	0.125	0.038	0.063	0.038	0.025
100	0.816	0.850	0.962	0.938	0.913	0.788
105					0.050	0.175
110		0.025				
<i>Lap-2</i>						
97	0.053					
100	0.947	0.800	0.712	0.663	0.600	0.613
110		0.200	0.287	0.338	0.400	0.387
<i>Mdh-1</i>						
96	0.013		0.050	0.050		
100	0.987	1.000	0.950	0.950	1.000	1.000
<i>Mdh-2</i>						
92		0.013				
100	0.921	0.925	0.988	1.000	1.000	1.000
109	0.079	0.063	0.013			
<i>Pgm-1</i>						
86					0.013	0.087
91					0.038	
95	0.276	0.162	0.087	0.213	0.038	0.038
100	0.474	0.475	0.650	0.625	0.750	0.625
109	0.171	0.250	0.250	0.150	0.087	0.225
118	0.079	0.112	0.013	0.013	0.050	
124					0.025	0.025

Table 2.3. (Continued)

Locus	Population					
	1	2	3	4	5	6
<i>Pgm-2</i>						
95		0.025			0.013	
100	1.000	0.925	1.000	0.863	0.850	0.988
105		0.050		0.138	0.138	0.013
<i>Pgi</i>						
88		0.013	0.038			
92		0.013	0.038	0.063		
100	0.921	0.813	0.887	0.837	0.988	0.975
108		0.087	0.025	0.087		0.025
112	0.079	0.075	0.013		0.013	
118				0.013		
<i>Per</i>						
87	0.092	0.063	0.100	0.013	0.063	
100	0.908	0.938	0.887	0.988	0.938	1.000
130			0.013			
<i>Skdh</i>						
91					0.050	0.175
100	0.987	1.000	1.000	0.988	0.950	0.825
109	0.013			0.013		
<i>Idh</i>						
100	1.000	1.000	0.988	1.000	1.000	1.000
114			0.013			
<i>6Pgd-1</i>						
86					0.013	
100	1.000	1.000	1.000	1.000	0.962	1.000
109					0.025	
<i>6Pgd-2</i>						
89			0.013		0.038	
100	1.000	0.988	0.988	0.962	0.775	0.988
111		0.013		0.038	0.188	0.013
<i>Dia</i>						
80				0.013	0.013	0.025
87		0.013	0.013	0.075	0.150	
100	1.000	0.988	0.988	0.913	0.837	0.975

Table 2.4. Genetic variability at 16 loci in 17 populations of *Metrosideros polymorpha* from East Maui (Standard error in parantheses)

Popula- tion ^d	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic ^b	F ^c	Mean heterozygosity	
					Direct- count	HdyWbg expected ^a
1. 200m H	44	2.6 (0.3)	56.3	0.119	0.178 (0.054)	0.202 (0.049)
2. 400m H	46	2.4 (0.2)	62.5	0.123	0.171 (0.059)	0.195 (0.049)
3. 600m H	44	2.1 (0.2)	56.3	0.216	0.134 (0.039)	0.171 (0.047)
4. 800m H	40	2.1 (0.3)	43.8	0.184	0.142 (0.046)	0.174 (0.053)
5. 1000m H	45	2.2 (0.2)	50.0	0.347*	0.094 (0.027)	0.144 (0.045)
6. 1200m H	45	1.9 (0.2)	43.8	0.214	0.125 (0.046)	0.159 (0.054)
7. 1400m H	45	1.9 (0.2)	43.8	0.133	0.124 (0.038)	0.143 (0.047)
8. 1600m H	45	2.1 (0.4)	25.0	0.129	0.108 (0.044)	0.124 (0.051)
9. 1800m H	45	2.3 (0.3)	31.3	0.204	0.113 (0.042)	0.142 (0.053)
10. 2000m H	38	1.8 (0.2)	43.8	0.129	0.115 (0.038)	0.132 (0.044)
11. 1950m H	40	2.3 (0.3)	68.8	0.127	0.220 (0.055)	0.252 (0.054)
12. 1950m H	36	2.1 (0.3)	50.0	-0.004	0.217 (0.060)	0.216 (0.057)
13. 1800m CR	45	2.0 (0.2)	43.8	0.125	0.126 (0.040)	0.144 (0.048)
14. 1850m CR	45	1.8 (0.2)	31.3	0.084	0.131 (0.043)	0.143 (0.050)
15. 400m K	30	1.9 (0.2)	56.3	0.206	0.146 (0.036)	0.184 (0.046)
16. 600m K	30	2.1 (0.2)	56.3	0.158	0.160 (0.043)	0.190 (0.047)
17. 800m K	25	1.9 (0.1)	50.0	0.246	0.132 (0.037)	0.175 (0.048)
Mean		2.1	47.8	0.161	0.143	0.170

^b A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

^a Unbiased estimate (see Nei, 1978)

^c Fixation index (Wright, 1965)

* P < 0.05

^d H= Haleakala, CR= Crater, and K=Kipahulu

Table 2.5. Genetic variability at 16 loci in 6 populations of *Metrosideros polymorpha* from West Maui (standard errors in parentheses)

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic ^a	F ^c	Mean heterozygosity	
					Direct- count	HdyWbg expected ^b
1. 400m West Maui	38.0 (0.0)	2.0 (0.2)	50.0	0.152	0.128 (0.035)	0.151 (0.049)
2. 600m West Maui	40.0 (0.0)	2.3 (0.3)	62.5	0.116	0.153 (0.040)	.173 (0.049)
3. 800m West Maui	40.0 (0.0)	2.3 (0.3)	43.8	0.089	0.133 (0.040)	0.146 (0.045)
4. 1000m West Maui	40.0 (0.0)	2.1 (0.2)	56.3	0.018	0.167 (0.049)	0.170 (0.046)
5. 1200m West Maui	40.0 (0.0)	2.4 (0.4)	62.5	0.071	0.170 (0.051)	0.183 (0.045)
6. 1400m West Maui	40.0 (0.0)	1.9 (0.3)	31.3	0.238	0.112 (0.051)	0.147 (0.051)
Mean		2.2	51.1	0.114	0.144	0.162

- ^a A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
^b Unbiased estimate (see Nei, 1978)
^c Fixation index (Wright, 1965)
* P < 0.05

Table 2.6. Populational pair-wise genetic identity and distance coefficients among 17 populations of *Metrosideros polymorpha* from East Maui
Below diagonal: Nei (1978) unbiased genetic identity,
Above diagonal: Nei (1978) unbiased genetic distance

Population	1	2	3	4	5	6	7	8
1 200m Haleakala		0.002	0.023	0.032	0.028	0.048	0.053	0.046
2 400m Haleakala	0.998		0.024	0.037	0.029	0.047	0.053	0.049
3 600m Haleakala	0.977	0.976		0.015	0.008	0.012	0.022	0.015
4 800m Haleakala	0.968	0.964	0.985		0.010	0.026	0.026	0.016
5 1000m Haleakala	0.973	0.971	0.992	0.990		0.014	0.013	0.006
6 1200m Haleakala	0.953	0.954	0.989	0.975	0.986		0.004	0.006
7 1400m Haleakala	0.948	0.948	0.978	0.975	0.987	0.996		0.004
8 1600m Haleakala	0.955	0.952	0.985	0.984	0.994	0.994	0.996	
9 1800m Haleakala	0.941	0.943	0.982	0.975	0.983	0.996	0.993	0.996
10 2000m Haleakala	0.959	0.955	0.985	0.976	0.981	0.970	0.973	0.977
11 1950m Koolau-1	0.940	0.939	0.944	0.961	0.947	0.940	0.942	0.945
12 1950m Koolau-2	0.911	0.910	0.956	0.963	0.955	0.960	0.959	0.957
13 1800m Crater-1	0.957	0.953	0.982	0.983	0.993	0.990	0.993	0.998
14 1850m Crater-2	0.950	0.946	0.981	0.978	0.991	0.991	0.994	0.996
15 400m Kipahula	0.984	0.983	0.985	0.977	0.981	0.968	0.966	0.974
16 600m Kipahulu	0.987	0.989	0.974	0.963	0.966	0.955	0.942	0.953
17 800m Kipahulu	0.983	0.982	0.984	0.985	0.987	0.972	0.966	0.981

Table 2.6. (Continued)

	9	10	11	12	13	14	15	16	17
1	0.061	0.042	0.062	0.093	0.044	0.051	0.016	0.013	0.017
2	0.059	0.046	0.063	0.094	0.048	0.055	0.017	0.011	0.018
3	0.018	0.015	0.057	0.045	0.018	0.019	0.015	0.027	0.016
4	0.026	0.024	0.040	0.037	0.017	0.022	0.023	0.038	0.015
5	0.017	0.019	0.055	0.046	0.007	0.009	0.019	0.034	0.013
6	0.004	0.030	0.061	0.041	0.010	0.009	0.032	0.046	0.028
7	0.007	0.028	0.060	0.042	0.007	0.006	0.034	0.060	0.034
8	0.004	0.024	0.057	0.044	0.002	0.004	0.026	0.048	0.019
9		0.030	0.060	0.044	0.007	0.009	0.033	0.057	0.027
10	0.971		0.057	0.044	0.025	0.019	0.018	0.054	0.031
11	0.942	0.945		0.022	0.048	0.053	0.050	0.065	0.050
12	0.957	0.957	0.978		0.041	0.037	0.067	0.095	0.067
13	0.993	0.975	0.954	0.960		0.002	0.025	0.048	0.019
14	0.991	0.981	0.948	0.964	0.998		0.025	0.051	0.025
15	0.967	0.983	0.951	0.935	0.975	0.975		0.013	0.008
16	0.945	0.947	0.937	0.909	0.953	0.950	0.987		0.010
17	0.973	0.969	0.951	0.936	0.981	0.975	0.992	0.990	

Table 2.7. Genetic identity and distance coefficients among 6 populations
of *Metrosideros polymorpha* from West Maui
Below diagonal: Nei (1978) unbiased genetic identity
Above diagonal: Nei (1978) unbiased genetic distance

Population	1	2	3	4	5	6
1 400m West Maui		0.004	0.012	0.014	0.026	0.018
2 600m West Maui	0.996		0.004	0.004	0.014	0.009
3 800m West Maui	0.988	0.997		0.002	0.007	0.006
4 1000m West Maui	0.986	0.996	0.998		0.004	0.007
5 1200m West Maui	0.974	0.986	0.993	0.996		0.008
6 1400m West Maui	0.982	0.991	0.994	0.993	0.992	

Table 2.8. Genetic identity 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

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

Table 2.9. 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	Nm
<i>Ald</i>	0.500	0.418	0.082	0.164	0.836	0.062	1.27
<i>Aco-1</i>	0.036	0.034	0.002	0.064	0.936	0.028	3.66
<i>Aco-2</i>	0.185	0.165	0.020	0.110	0.891	0.032	2.02
<i>Lap-1</i>	0.509	0.419	0.090	0.176	0.824	0.077	1.17
<i>Lap-2</i>	0.226	0.153	0.073	0.324	0.676	0.035	0.52
<i>Mdh-1</i>	0.034	0.033	0.001	0.036	0.964	0.009	6.69
<i>Mdh-2</i>	0.048	0.046	0.002	0.050	0.950	0.029	4.75
<i>Pgm-1</i>	0.616	0.562	0.054	0.087	0.913	0.031	2.62
<i>Pgm-2</i>	0.183	0.168	0.015	0.083	0.917	0.038	2.76
<i>Pgi</i>	0.181	0.171	0.010	0.057	0.943	0.044	4.14
<i>Per</i>	0.229	0.213	0.016	0.070	0.930	0.031	3.32
<i>Skdh</i>	0.190	0.154	0.036	0.189	0.811	0.105	1.07
<i>Idh</i>	0.024	0.023	0.000	0.001	0.993	0.008	36.50
<i>6Pgd-1</i>	0.013	0.012	0.000	0.000	0.969	0.031	7.81
<i>6Pgd-2</i>	0.023	0.022	0.001	0.029	0.972	0.000	8.37
<i>Dia</i>	0.070	0.065	0.005	0.072	0.928	0.030	3.22
Mean	0.192	0.166	0.026	0.097	0.903	0.037	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; Nm = gene flow

Table 2.10. Measures of gene diversity for 6 populations of *Metrosideros polymorpha* from West Maui

Differentiation among populations					Nm
Locus	H _T	H _S	D _{ST}	G _{ST}	
<i>Ald</i>	0.484	0.474	0.010	0.022	11.3
<i>Aco-1</i>	0.049	0.048	0.001	0.010	22.5
<i>Aco-2</i>	0.149	0.145	0.004	0.024	10.2
<i>Lap-1</i>	0.223	0.211	0.012	0.055	4.3
<i>Lap-2</i>	0.407	0.374	0.033	0.081	2.8
<i>Mdh-1</i>	0.037	0.036	0.001	0.028	8.8
<i>Mdh-2</i>	0.053	0.051	0.002	0.043	5.5
<i>Pgm-1</i>	0.583	0.559	0.024	0.041	5.9
<i>Pgm-2</i>	0.118	0.111	0.008	0.065	3.6
<i>Pgi</i>	0.181	0.174	0.008	0.041	5.8
<i>Per</i>	0.106	0.104	0.003	0.028	8.7
<i>Skdh</i>	0.081	0.073	0.008	0.100	2.3
<i>Idh</i>	0.004	0.003	0.001	0.010	22.0
<i>6Pgd-1</i>	0.013	0.012	0.001	0.025	9.9
<i>6Pgd-2</i>	0.096	0.085	0.010	0.114	1.9
<i>Dia</i>	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.

Table 2.11. Correlation and regression coefficients between allele frequencies and environmental variables.

Allele	Temperature		Rainfall		Index	
	Correlation	Regression	Correlation	Regression	Correlation	Regression
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**	-0.015**			-0.50**	-0.030**
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*	-0.013*	-0.56**	-0.023**
Dia 100	0.51**	0.009**			0.54**	0.023**

** Significant at 1 per cent level and * at 5 per cent level

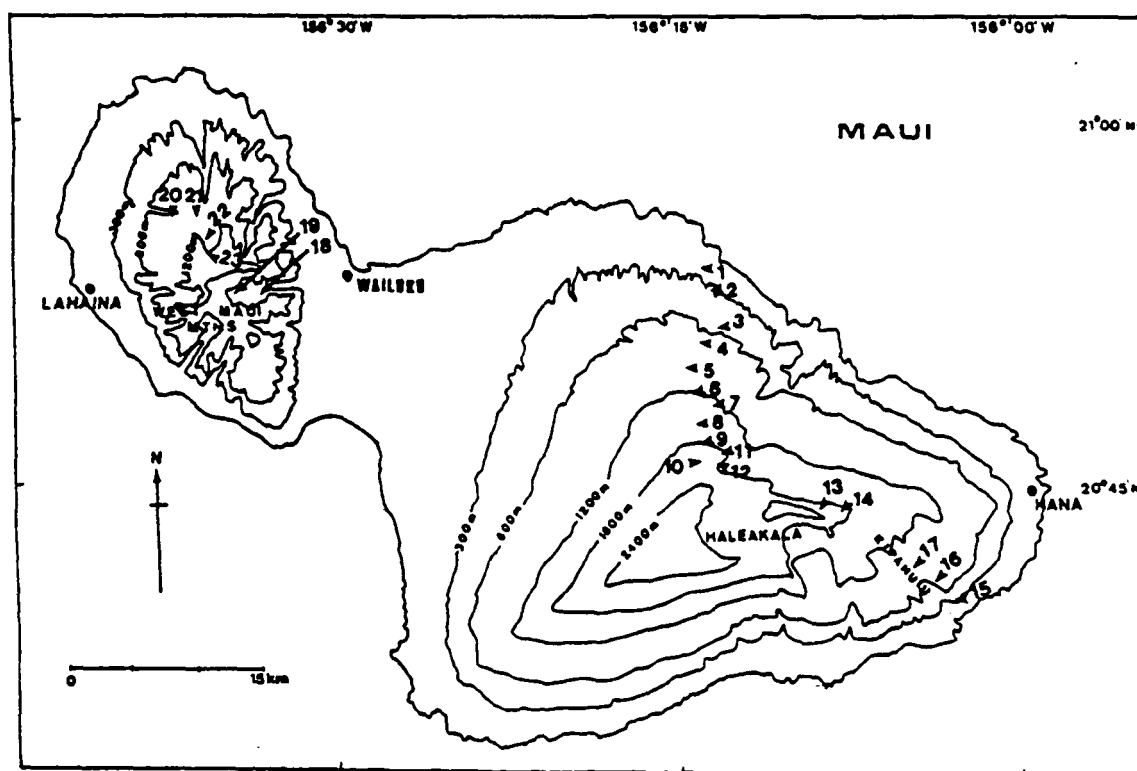
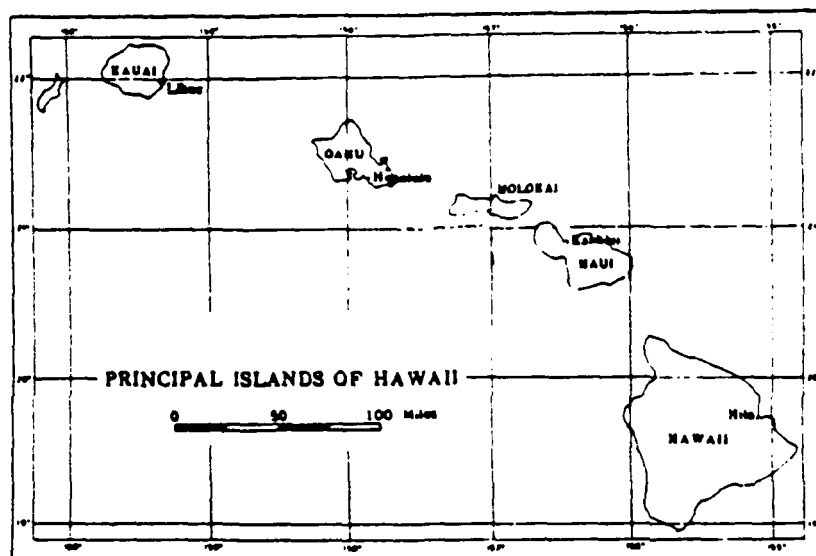


Figure 2.1. Approximate location of 23 populations of *Metrosideros polymorpha* on the island of Maui, Hawaii
 Note: Populations 1 to 12 - Transect 1 (NE slope of Mt. Haleakala), populations 15 to 17 - Transect 2 (SE slope of Haleakala, Kipahulu), populations 18 to 23 - Transect 3 (West Maui mountains), and populations 13 and 14 (Haleakala crater)

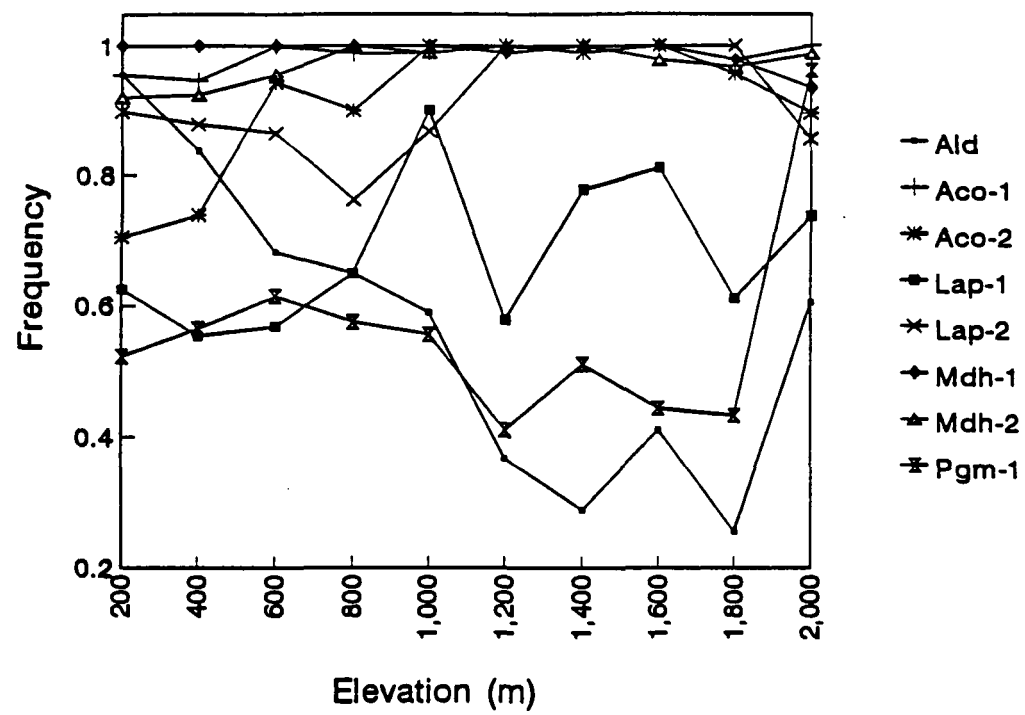


Figure 2.2. Frequencies of the most common allele (100) for different loci along the altitudinal gradient in East Maui (Mt. Haleakala)

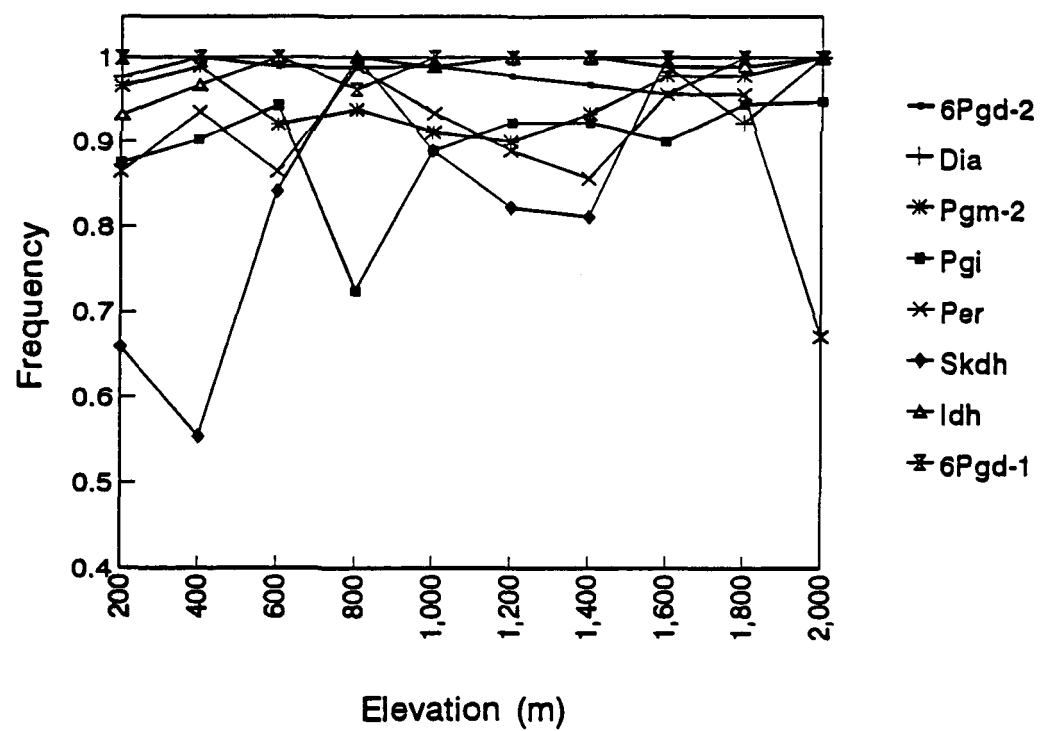


Figure 2.3. Frequencies of the most common allele (100) for different loci along the altitudinal gradient in East Maui (Mt. Haleakala)

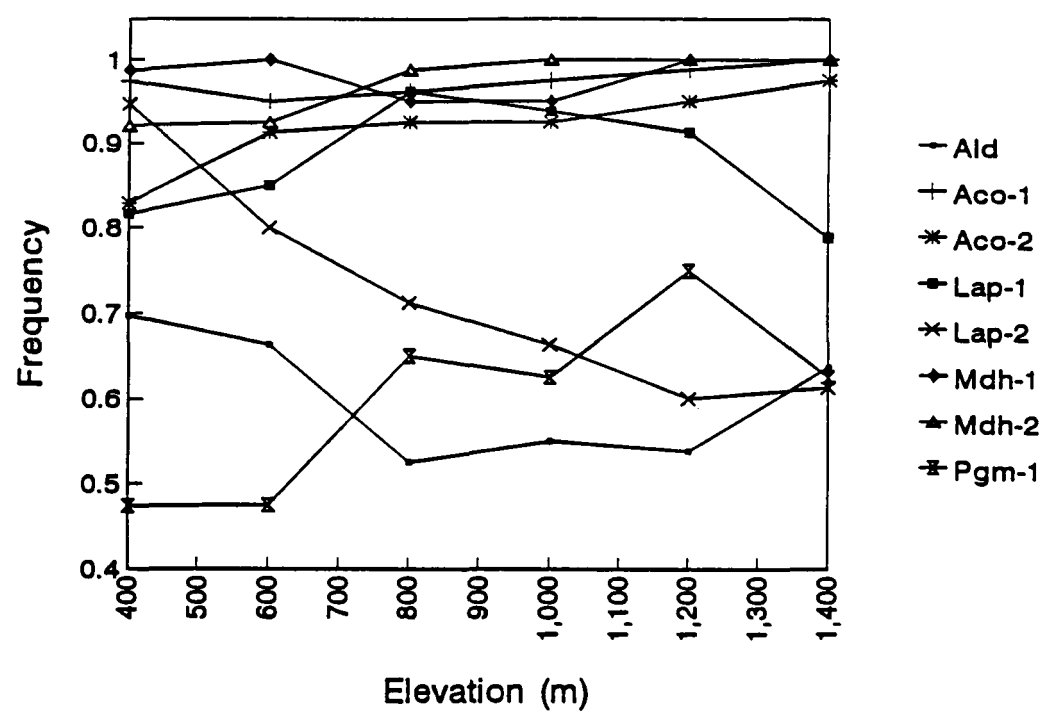


Figure 2.4. Frequencies of the most common allele (100) for different loci along the altitudinal gradient in West Maui

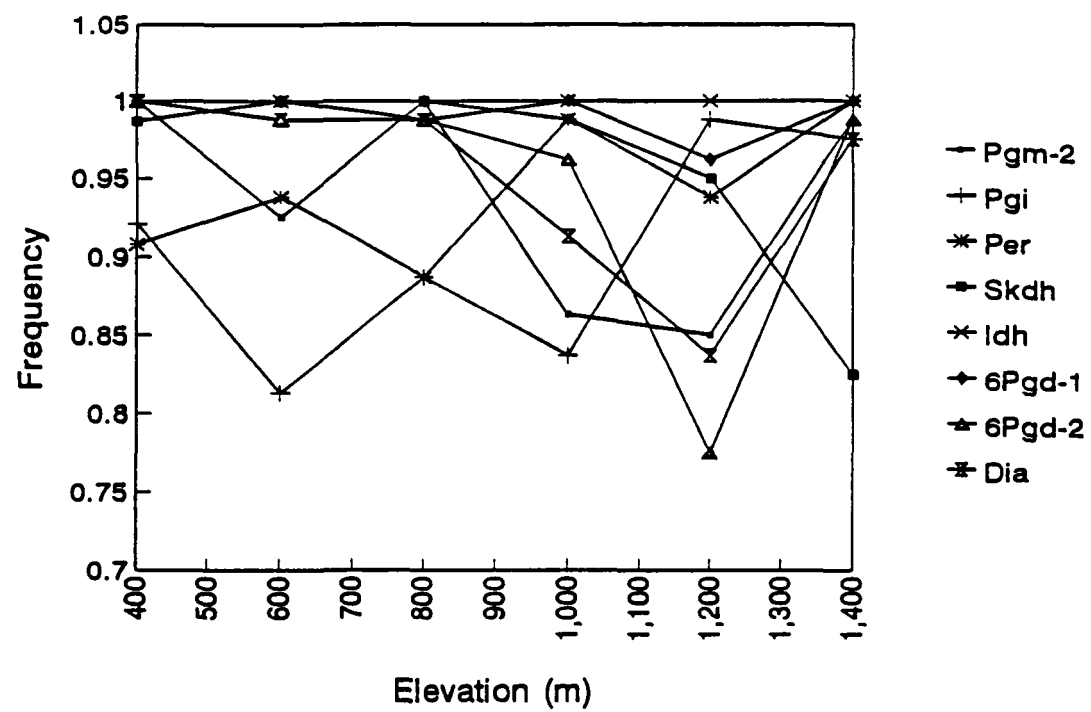


Figure 2.5. Frequencies of the most common allele (100) for different loci along the altitudinal gradient in West Maui

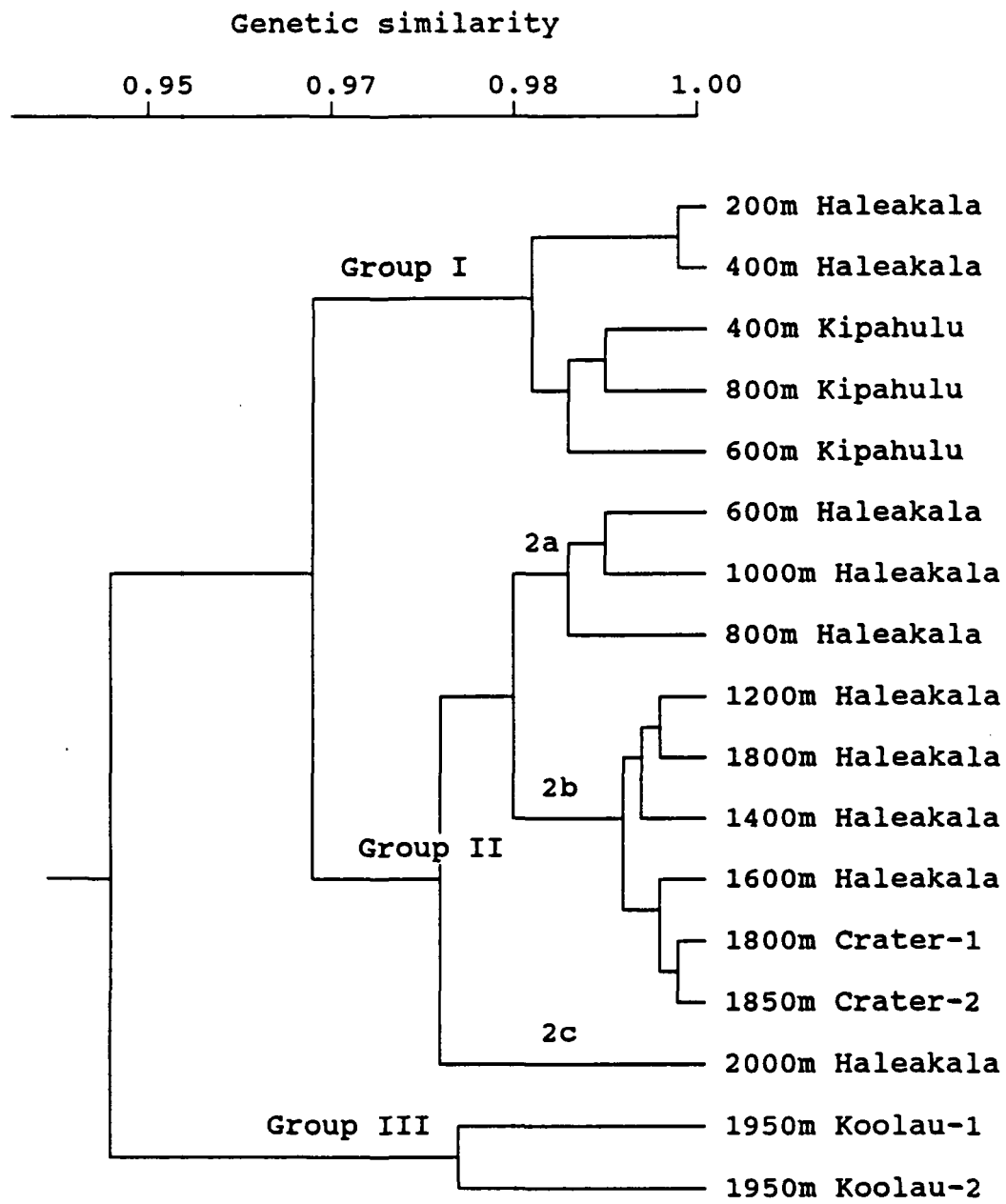


Figure 2.6. Dendrogram showing genetic similarities among 17 populations of *Metrosideros polymorpha* from East Maui

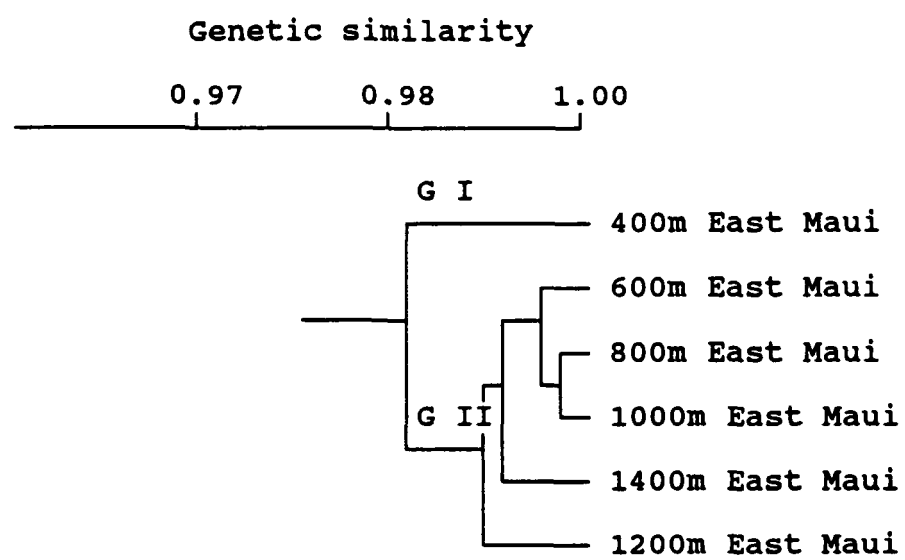


Figure 2.7. Dendrogram showing genetic similarities among 6 populations of *Metrosideros polymorpha* from West Maui
 Note: G-I= Group 1, G-II= Group 2

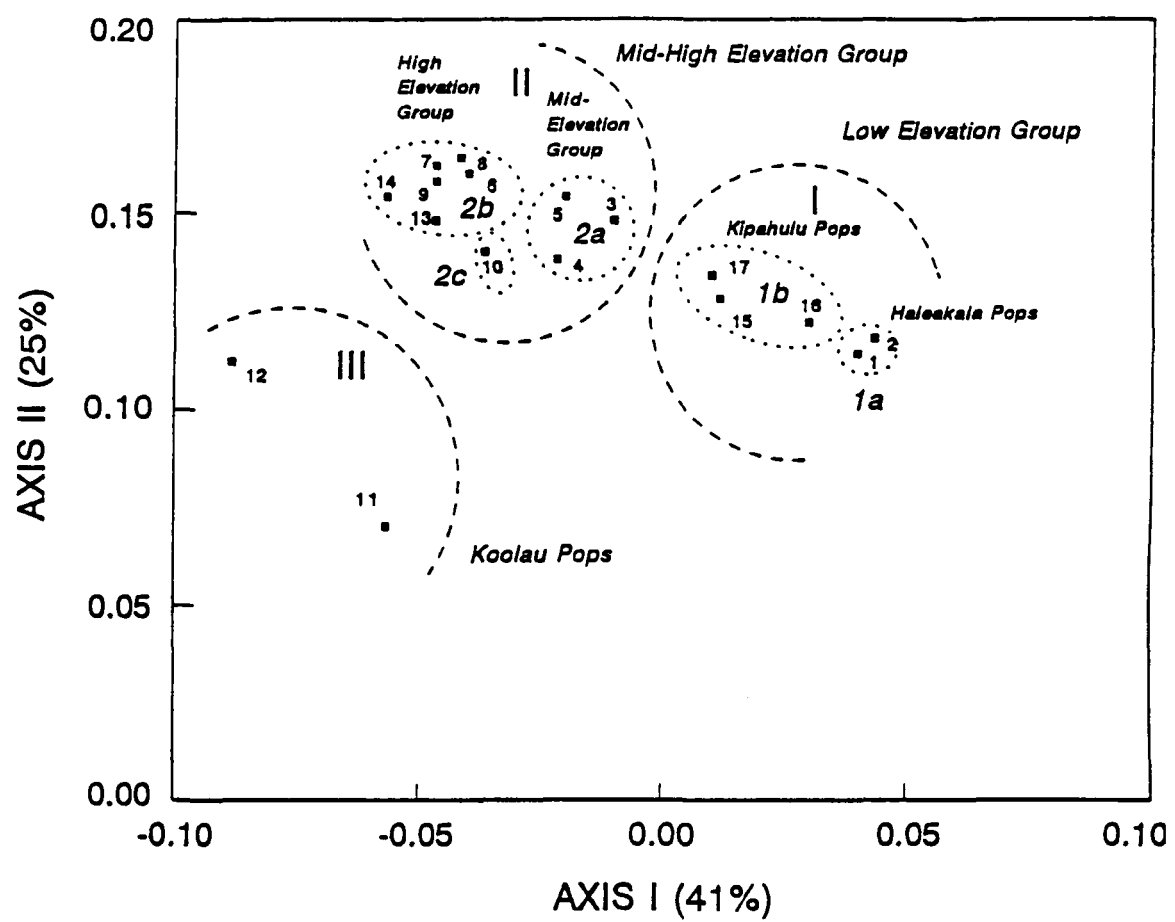


Figure 2.8. PCA ordination of 17 populations of *Metrosiders polymorpha* from East Maui

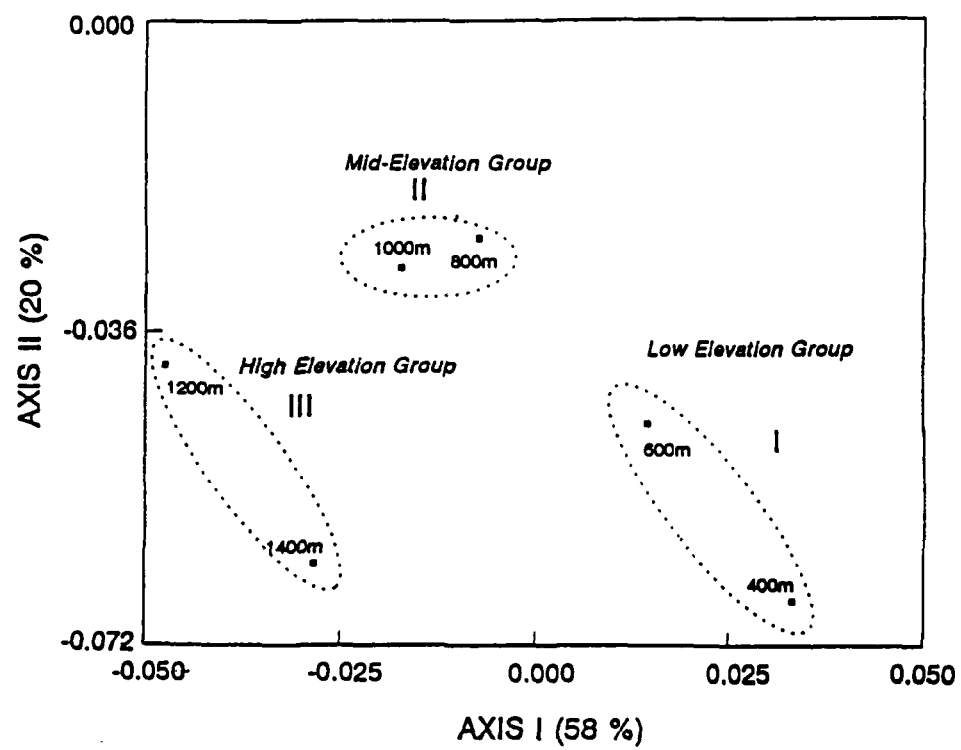


Figure 2.9. PCA ordination of 6 populations of *Metrosideros polymorpha* from West Maui

CHAPTER III

GENETIC DIVERSITY AND PATTERNS OF DIFFERENTIATION IN *METROSIDEROS POLYMORPHA* BETWEEN DIFFERENT SUCCESSIONAL STAGES ON THE ISLAND OF MAUI, HAWAII.

A. ABSTRACT

Metrosideros polymorpha occupies successional habitats ranging from recent lava flows, as a pioneer to very late successional environments on older islands where it forms a climax forest. A comparison of two volcanic mountains representing two consecutive stages in the successional gradient on the island of Maui suggested that *Metrosideros* harbors relatively more genetic variation (95 per cent) within its populations in the later successional stage (West Maui) than in the younger stage (90 per cent) (Mt. Haleakala). Population differentiation along altitudinal gradients was more pronounced on the successional younger Mt. Haleakala than on the West Maui mountains which represent a relatively advanced stage in succession. The proportion of variation due to genetic differences between populations on East and West Maui was less than 1 per cent as compared to genetic differences among populations within mountains (9 per cent). The UPGMA cluster analyses of the pair-wise genetic identity

matrix of all 23 populations for 16 loci resulted in clusters which indicated that altitudinally associated environmental and edaphic factors are the major selective forces in determining the level and organization of genetic variation in this species rather than successional related factors. The principal components analysis indicated similar trends with population clusters based on altitude irrespective of the successional stages from which they were sampled. However, each mountain possessed several unique low frequency alleles, which must have originated via recent mutational events.

B. INTRODUCTION

The Hawaiian archipelago is an ideal natural laboratory for studying evolution in an isolated insular environment. These islands have originated sequentially as a result of movement of the Pacific Tectonic Plate north-westward over a volcanic hot spot. The linear chronological order of islands of known ages adds enormously to the value of these islands as an ideal natural testing ground of evolutionary hypotheses in an isolated environment and in the absence of competition (Simon, 1987; Carson and Kaneshiro, 1976).

The terrestrial biota descended from waifs which originally dispersed across the ocean by long-distance

dispersal (Carlquist, 1970). Chance events played a key role not only on the first arrivals, but also on subsequent arrivals as each new island emerged to the southeast of Kauai (Carson and Kaneshiro, 1976). There were new opportunities for the repeated establishment of populations by stochastic colonization.

The sequential formation of islands of different ages coupled with subsequent colonization have produced an excellent successional series in the Hawaiian archipelago. This natural linear arrangement allows biologists to compare evolutionary patterns that follow colonization along the successional gradient. Evolution can begin in a species on the older islands and can continue on subsequently formed islands as propagules are dispersed from older islands to newly formed ones at various stages of evolution and speciation. Continued volcanic activities may alter the population size through fragmentation and thus changing the genetic structure of species populations. In fact the biota on Hawaiian islands have evolved along with volcanism (Mueller-Dombois and Loope, 1990). Communities in various stages of primary succession are found on islands where there have been relatively recent lava flows. Distinguishing between climax communities and successional communities can be a major problem on oceanic islands. Indeed, one might argue that all of the vegetation is successional in Hawaii. The

island chain from Kauai up to Hawaii forms a neat successional series to study vegetation dynamics, evolution and speciation in endemic taxa in relation to their age.

Successional changes in plant communities may be a factor maintaining genetic diversity in some populations (Gray, 1987). Many species with narrow genetic bases are vulnerable to local extinction during early successional stages and may undergo genetic bottlenecks leading to a loss of genetic variation due to random fixation of alleles. Some others with relatively high genetic variation may recover and rebuild their lost genetic variation by recombining and expanding their population.

Successional changes in the present study refers to the change, with time, of the genetic composition of populations along a primary successional gradient. The random and selective effects of succession as such may be inseparable in practice from the genetic consequences of changes in plant distribution and density imposed by such factors as changes in pollination range and neighborhood relationships (Antonovics and Levin, 1980). In general, early-successional environments vary greatly within one growing season and hence, early successional species must deal with such variation and their response may vary ontogenetically (Bazzaz, 1987). However, a major trend in succession is the amelioration of environmental extremes

over time. In other words, there is a complex gradient of decreasing physical stress through time (Pickett, 1976). Hence, populations extending across such gradients are subjected to differential selection pressures leading to local adaptation and genetic differentiation. Mueller-Dombois (1988) suggested that primary successional stages, up to between 1000 and 3000 years, may be considered as a temporal gradient of decreasing stress. After that succession enters a regressive phase when site deterioration may cause decreases in forest stature and biomass associated with landscape and soil aging in the Hawaiian Islands.

Founder effects during colonization and early successional stages can lead to pronounced divergence among populations mainly because the populations are transient, long-distance dispersal is less effective in homogenizing populations, and strong within-population subdivision is less likely (Loveless and Hamrick, 1984). The same species in late successional stages may possess larger effective population sizes and reduced interpopulational differentiation. There is a surprising lack of empirical evidence demonstrating that habitat characteristics during successional change are major factors maintaining genetic diversity in populations. However, the outcome of complex interaction of ecological variables on populations dictate the genetic structures at

various stages during succession. Hence, it is necessary to consider the conditions under which successional change may act as a selective force and how species may respond to it. Apparently, it is difficult to delineate those ecological variables that could act as selective forces in determining the nature of genetic differentiation. Although information on the population genetic structure of herbaceous and weedy species along secondary successional gradients is available (Solbrig and Simpson, 1974; Law et al., 1977; Roos and Quinn, 1977); Scheiner and Goodnight, 1984), the same has not been documented for tree species.

The present study focuses on the comparison of genetic diversity and the pattern of distribution of genetic variation in the dominant tree species *Metrosideros polymorpha* along altitudinal gradients within and between two volcanic mountains, Mt. Haleakala (East Maui) and the West Maui Mountains. These two mountains originated as independent volcanoes and later coalesced to form the island of Maui as it exists today. The West Maui Mountains reach an altitude of 1764m at the summit of Puu Kukui. They originated about 1.3 million years ago and were probably first colonized both by long distance dispersal from non-Hawaiian sources as well as propagules from older Hawaiian islands (Stearns, 1985). This mountain range represents a successional more advanced

stage in the development of the island vegetation as compared to East Maui. The East Maui mountain, which reaches an altitude of 3055 m, originated about 0.8 million years ago (Stearns, 1985). These circumstances have resulted in a successional gradient representing two consecutive stages of succession in the Hawaiian archipelago on what is presently the same land mass. *Metrosideros polymorpha* Gaud. is the major component of the forests on both mountains and it is therefore possible to compare its populations for genetic similarities and differences. Altitudinal gradients from different successional environments form combined ecological gradients offering unique opportunities to study the composite effect the evolution of *M. polymorpha* in a biogeographically isolated island ecosystem.

The purpose of this study is (1) the comparison of populations of *M. polymorpha* for their genetic similarities and differences between mountains representing different successional stages and (2) the comparison of levels of genetic differentiation along altitudinal gradients as influenced by successional environments.

C. MATERIALS AND METHODS

Details regarding the establishment of elevational transects, sampling sites, and methods for sampling populations of *M. polymorpha* are explained in detail in chapter 2 of this dissertation.

Twelve *M. polymorpha* populations along the NE wet slope of Mt. Haleakala, two from inside the crater near Paliku and three from Kipahulu Valley were sampled in East Maui. These populations represent complex altitudinal gradients on the successional younger Mt. Haleakala.

Six populations located along the altitudinal gradient on West Maui, as explained in chapter 2, form the study populations representing a successional more advanced stage.

The populations were scored for eleven enzyme systems. The electrophoretic procedures and the method and recipes for staining of enzymes are presented in chapter 2.

Data analysis

Allele frequencies and genetic variability

The genotype frequencies for different loci were recorded directly from the gels by interpreting the isozyme phenotypes. Allele frequencies for different loci

were calculated from the genotype frequencies. Single locus genetic variability parameters were computed separately for the two mountains. They included the average number of alleles per locus, number of polymorphic populations expressed in percentage, average observed heterozygosity for each locus, and locus-wise proportion of heterozygosity within and between populations. All possible pair-wise Nei's unbiased genetic identity and distance coefficients were computed on the pooled data from both mountains with the computer program BIOSYS (Swofford and Selander, 1989). Hierarchical gene diversity analysis (Nei, 1973) was performed on the pooled data. The total genetic diversity (H_T) was partitioned into genetic variation within populations (H_S), between populations within mountains (D_{SM}) and between mountains (D_{MT}). Therefore,

$$H_T = H_S + D_{SM} + D_{MT} \quad (1)$$

where $D_{SM} = H_M - H_S$ and $D_{MT} = H_T - H_S - D_{SM}$. H_S is the within populations variation which is equivalent to the average probability of nonidentity of two alleles sampled from the same subpopulation, H_M is the average gene diversity of the mountains and D_{SM} is the component of gene diversity due to variation between mountains within the total.

H_T was calculated on the weighted average allele frequencies over all populations,

$$\text{i.e.} \quad H_T = 1 - \sum_k P_k \quad (2)$$

where $P_k = \sum_i w_i p_{ik} / n$; w_i is the number of individuals in the i th subpopulation, k = number of alleles, i = number of populations and n is the total number of individuals overall populations.

H_S is equal to the weighted average of the Hardy-Weinberg expectation of heterozygosity ($H_i = 1 - \sum_k p_{ik}$) over all populations

$$\text{i.e.} \quad H_S = \sum_i w_i H_i / n \quad (3)$$

H_M is equal to the weighted average of the Hardy-Weinberg expectation of heterozygosity over the two mountains.

Therefore,

$$H_M = 1 - \sum_k P_{mk} \quad (4)$$

where k = number of alleles and m = number of subgroups (mountains)

Cluster analysis

Nei's populational pair-wise unbiased genetic identity matrix was subjected to a UPGMA cluster analysis (Sneath and Sokal, 1973) which included all populations of *Metrosideros* sampled on the island.

As an alternative approach to elucidate the multivariate pattern of allele frequency distribution among populations, the allele frequency data from all the 16 loci for 23 populations was subjected to principal components analysis (PCA) (Gauch, 1982). A variance-covariance matrix of allele frequencies (63 x 63) was employed to extract the eigen values and vectors. The first and the second PCA axes were plotted to reveal the genetic relationships among the populations.

The distribution of heterozygosity among the populations was analyzed for the two mountains separately by computing diversity indices for different loci across populations (Shannon and Weaver, 1949). This index combines measures of evenness of distribution of heterozygosity and the extent of its distribution.

D. RESULTS

Genetic variation within populations

Eleven enzyme systems encoded by 16 putative loci were successfully resolved. A total of 63 alleles were

observed across 23 populations. Because of the large size of the data set, only the frequencies of the most common alleles (100) at the polymorphic loci are presented here for each population (Table 3.1). Please see chapter 2 of this dissertation for the complete data set.

Single-locus estimates of genetic variability are presented in table 3.2. The two mountains differed with respect to the total number of alleles encountered. Populations from East Maui possessed 60 alleles whereas 55 alleles were found in West Maui. East Maui populations possessed unique low frequency alleles for *Aco-2*, *Lap-2*, *Mdh-1*, *Per*, *Idh*, and *Dia* which were not found in West Maui populations. Similarly, West Maui populations exhibited low frequency alleles for *Pgm-1*, *Pgi*, and *6Pgd-1* which were missing among East Maui populations.

The overall average of percentage of polymorphic loci across populations on East Maui was 68.8 % with a range from 24 % for *6Pgd-1* to 100 % for a few loci such as *Ald*, *Lap-1*, *Pgm-1*, and *Per*. The overall average was 74 % for West Maui with a range from 17 % for *Idh* and *6Pgd-1* to 100 % for several loci (Table 3.2).

The diversity indices computed on heterozygosity values for different genetic loci indicated great variation in the symmetry of distribution of heterozygosity across different populations for both East and West Maui populations (Table 3.3). The Shannon

diversity index for East Maui varied from 0.582 for *6Pgd-1* to 1.213 for *Lap-1* while it varied from 0.000 for *Idh* and *6Pgd-1* to 0.778 for *Ald* for West Maui populations.

The observed heterozygosity for East Maui populations varied from 0.034 for *Aco-1* to 0.405 for *Lap-1* with an overall mean of 0.190 while it varied from 0.036 for *Mdh-1* to 0.560 for *Pgm-1* with an overall average of 0.160 for West Maui. Patterns of distribution of heterozygosity was more even across loci among East Maui populations than among West Maui populations.

Gene diversity and partitioning of variation

Nei's gene diversity analysis indicated that 90 per cent of the total variation resides within populations on East Maui as compared to 95.3 per cent on West Maui (Table 3.2). However, the loci varied greatly with respect to the proportions of within and between population components of variation in both East and West Maui. The pooled gene diversity analysis revealed that nearly 91 per cent of the total variation was found within populations (Table 3.4). The proportion of variation due to genetic differences between East and West Maui populations was extremely low (about 1.0 per cent of total) as compared to variation among populations within mountains (8.6 per cent of total).

Genetic similarities and dissimilarities among populations

Genetic similarity among *M. polymorpha* populations was computed for all possible pair-wise combinations among the populations from both East and West Maui by the distance statistics proposed by Nei (1973) (Table 3.5). The unbiased genetic identity for pair-wise comparisons of populations across the 16 loci ranged from 0.910 between a population sampled at 400m and another at 1950m on Haleakala to 0.998 between three pairs of populations (the 200m and 400m Haleakala populations, the two populations sampled inside the crater, and the 800m and 1000m populations of West Maui). There was no evidence for intermountain genetic differentiation of *Metrosideros*.

The UPGMA cluster analysis of the pooled genetic identity matrix revealed three major clusters at the 97 per cent level of similarity among populations (Figure 3.1). Cluster 1 was composed of two lower elevation populations from the Haleakala transect (200 and 400m) and the three Kipahulu transect populations (400, 600, and 800m). Cluster 2 consisted of the majority of the remaining populations except for the two populations from 1950m sampled along Koolau gap trail. At the 98 per cent similarity level, cluster 2 splits into three subclusters. Subcluster 2a consisted of three populations from the Haleakala transect (600, 800, and 1000m) and the six populations of West Maui transect (400, 600, 800, 1000,

1200, and 1400m). Subcluster 2b contained the populations from 1200, 1400, 1600 and 1800m on the Haleakala transect and the two populations collected from inside the crater. Subcluster 2c contained a single population from 2000m on the Haleakala transect.

The genetic variability residing in the complex multivariate data structure consisting of 63 alleles scored across the 16 putative loci was subjected to the PCA to visualize the multivariate relationships among the populations. The first two orthogonal vectors extracted from the variance-covariance matrix of the allele frequencies accounted for 70 per cent of the total variation. The plotting of these vectors resulted in the formation of three major clusters similar to the those obtained through UPGMA cluster analysis (Figure 3.2). The composition within clusters also matched that of the UPGMA. Cluster 2 was slightly different, however, having only two subclusters representing groups from mid- and high elevation populations respectively.

E. DISCUSSION

The vegetation communities on the Hawaiian islands essentially represent different successional stages. It is possible to distinguish among different successional communities on the younger islands as they are of

comparatively recent origin when compared to older islands. This is so because the age of the substrates or lava flows on which they are established is known in most instances. *Metrosideros polymorpha* occurs as a dominant forest tree species on all the major islands over a wide range of successional habitats. The *Metrosideros* forests on the older islands can be considered as successional advanced stages as compared to the forests on the more recently formed islands. On some islands, depending on the age and extent of continued volcanic activity, a relatively large number of successional stages can be recognized.

Genetic changes along successional gradients in species populations have not been understood clearly. The selective effects at different stages of succession as such may be practically difficult to separate from genetic consequences resulting from the combination of various evolutionary forces that come into operation due to the heterogeneity in the environment (Antonovics and Levin, 1980). However, a major trend in succession is the amelioration of environmental extremes. Succession is thus a complex gradient of decreasing physical stress on populations through time (Pickett, 1976).

Mueller-Dombois (1988) argues that on volcanic islands like Hawaii primary successional events represent a temporal gradient of decreasing stress on plants in the

younger stages up to about 1500 to 2000 years. During this period habitats development through weathering and secondary enrichment of soils provide favorable growing conditions and then primary succession enters a regressive phase. During the regressive phase site deterioration results in increasing stress on plants leading to reduced stature of trees and biomass. Mueller-Dombois further differentiates this whole process between soils derived from Pahoehoe and volcanic ash deposits in Hawaii. However, the genetic composition of populations will change continually with long-term climatic and geological changes associated with succession. The degree of recombination allowed by the genetic system is the reflection of the species' position on spatial and successional gradients. Genetic systems favouring reduced recombination are selected for in relatively severe, early successional habitats (Pickett, 1976).

Since almost all empirical studies on population differentiation during succession involve observation and measurement at one point in time, inferences are drawn about the processes which may have led to the observed pattern of differences. Ideally the population should be observed before, during, and after selection and the relative fitness of all individuals measured (longitudinal study). However, such studies are infrequent.

It has been generally assumed that early primary successional habitats are more variable and unpredictable than are late successional habitats and hence are highly fragmented and heterogeneous and composed of numerous microhabitats to which the colonizing species populations have to respond. Colonization of primary successional habitats without long distance dispersal may occur without significant genetic change (Carson, 1987). In the presence of preadaptation and efficient dispersal, the entire gene pool of a species may be transported without alteration to the new site. However, the heterogeneous habitat may impose differential selection leading to minor fluctuations in gene frequencies and minor fine-grain adaptation to microsites (Carson, 1987). In early primary successional situations selection by the physical environment is strong and may override the homogenizing force of continued gene flow. In later successional habitats, which will be increasingly long lasting, genetic systems allowing gradual release of recombination will produce some offspring capable of meeting slow, directional change in the environment, as well as some capable of exploiting the present environment (Stebbins, 1958; Grant, 1958).

Genetic changes during succession are likely to occur in populations which are reasonably persistent, yet within which there is a turnover of generations. In addition the

genetic system, and particularly the breeding system, would need to be capable of recombining and circulating the genetic variability on which selection acts (Gray, 1978). As a corollary, the rates of change in the surrounding vegetation, soil properties, and other environmental factors which characterize successional stages must be sufficiently rapid in relation to the species' temporal niche to act as a selective force.

Despite high levels of genetic diversity in natural plant populations, despite clear patterns of differences between early- and late-successional species and even congeners, and despite the fact that "most plants occupy stages in successions" (Harper, 1977) there is a surprising lack of empirical evidence that successional change is a major factor maintaining genetic diversity in species populations (Gray, 1978). Theory predicts that spatial heterogeneity in the environment will be more important in maintaining genetic polymorphisms than temporal heterogeneity and that the conditions under which temporal variation in selection might operate effectively to maintain diversity are rather restricted (Hedrick, 1976; Hedrick, Ginevan and Ewing, 1976; Ennos, 1983).

Metrosideros polymorpha is the first tree species to appear on new lava flows where it forms a forest ecosystem in about 400 years in a tropical rain forest climate in Hawaii (Atkinson, 1970). Hence, its populations are

subjected to the differential selection pressure of evolutionary forces over space and time while establishing itself and through various stages of succession before reaching the climax stage. Species-wide genetic variation undergoes several reorganizations during these dynamic phases of establishment. Mueller-Dombois (1980) hypothesized that there are ecotypes in *M. polymorpha* replacing one another in a successional sequence. Further, Stemmermann (1983) attempted to find the genetic basis for the existence of such successional ecotypes or varieties through common garden experiments. She concluded that pubescent forms of *M. polymorpha* are favoured on young lava flows while older forests are dominated by glabrous types. She also showed that these characters bred true.

It was evident from the measures of genetic variability that *M. polymorpha* populations from the successional older mountain, West Maui, harbor relatively more variation within populations as compared to between populations. On the contrary, East Maui which is successional younger than West Maui, supported *Metrosideros* which had relatively more variability due to differences between populations. The unique low frequency alleles found restricted to one or the other mountain must have arisen as recent mutational events.

Although the two mountains compared in the present study support *Metrosideros* which are geographically isolated by a zone of low land scrubby vegetation devoid of *Metrosideros* and by extensive agricultural development, *M. polymorpha* on these mountains seems to have been evolving under more or less similar evolutionary constraints, except for a few minor differences. Altitudinal differentiation among populations was more marked in the successional younger mountain (East Maui) as compared to older West Maui mountains as indicated by the pattern of distribution of genetic variation within and between populations. The altitudinal divergence of populations was more striking than intermountain differentiation. Clear patterns of altitudinal divergence could be seen from the results of the UPGMA cluster analyses and the PCA. All the populations from West Maui have exhibited greater affinity with the lowland wet and montane mesic group of Haleakala. The populations from lowland dry and mesic forests of Haleakala have again shown greater affinity among themselves. These striking affinities observed in the cluster analysis indicate that factors related to altitudinal gradients are responsible for population divergence. It is evident from the broad altitudinal grouping of populations that gene flow among local populations within these groups may be an important role in genetic differentiation.

In conclusion, *M. polymorpha* has diverged more along the altitudinal gradients than successional gradients, although this needs further verification with broader sampling across a number of successional stages. While the species harbors more genetic variation within populations than between populations, the latter portion of variation exhibited discernable trends in the nature of its distribution along altitudinal gradients and associated successional stages. Secondly, succession provides a complex gradient of physical and biotic environmental factors to which species respond in both ecological and evolutionary time. Hence, the cause and effect of successional and altitudinal gradients are inseparable. Thirdly, the results suggest that as species enter advanced stages in succession, genetic variation within populations tends to increase at the cost of variation between populations, and hence the populations show less differentiation. Finally, the prevailing environmental and edaphic factors along the altitudinal gradients are the major selective forces for genetic differentiation in *M. polymorpha* on the island of Maui.

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Table 3.1. Frequency of allele 100 at 16 polymorphic loci in 23 populations of *Metrosideros polymorpha* from the island of Maui

Allele	Population											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Ald</i>	0.96	0.84	0.68	0.65	0.59	0.37	0.29	0.41	0.26	0.61	0.53	0.38
<i>ACO-1</i>	0.96	0.95	1.00	0.99	0.99	1.00	1.00	1.00	0.98	1.00	0.88	1.00
<i>ACO-2</i>	0.71	0.74	0.94	0.90	1.00	1.00	0.99	1.00	0.96	0.90	0.75	0.97
<i>Lap-1</i>	0.63	0.55	0.57	0.65	0.90	0.58	0.78	0.81	0.61	0.74	0.45	0.57
<i>Lap-2</i>	0.90	0.87	0.86	0.76	0.87	1.00	1.00	1.00	1.00	0.86	0.39	0.36
<i>Mdh-1</i>	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.98	0.93	0.90	0.99
<i>Mdh-2</i>	0.92	0.92	0.96	1.00	0.99	1.00	1.00	0.98	0.97	0.99	1.00	1.00
<i>Pgm-1</i>	0.52	0.57	0.61	0.58	0.56	0.41	0.51	0.44	0.43	0.96	0.45	0.54
<i>Pgm-2</i>	0.97	0.99	0.92	0.94	0.91	0.90	0.93	0.98	0.98	1.00	0.80	0.68
<i>Pgi</i>	0.88	0.90	0.94	0.73	0.89	0.92	0.92	0.90	0.94	0.95	0.98	0.88
<i>Per</i>	0.86	0.94	0.86	0.99	0.93	0.89	0.86	0.96	0.96	0.67	0.83	0.75
<i>Skdh</i>	0.66	0.55	0.84	1.00	0.89	0.82	0.81	1.00	1.00	1.00	1.00	1.00
<i>Idh</i>	0.93	0.97	1.00	1.00	0.99	1.00	1.00	0.99	0.99	1.00	0.95	0.99
<i>6Pgd-1</i>	1.00	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>6Pgd-2</i>	1.00	1.00	0.99	0.99	0.99	0.98	0.97	0.96	1.00	1.00	1.00	1.00
<i>Dia</i>	0.98	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.92	1.00	0.81	0.89

Table 3.1. (Continued)

Allele	Population											
	13	14	15	16	17	18	19	20	21	22	23	
<i>Ald</i>	0.42	0.40	0.68	0.83	0.66	0.70	0.66	0.53	0.55	0.54	0.64	
<i>Aco-1</i>	1.00	1.00	0.98	1.00	0.98	0.98	0.95	0.96	0.98	0.99	1.00	
<i>Aco-2</i>	0.89	0.99	0.80	0.85	0.78	0.83	0.91	0.93	0.93	0.95	0.98	
<i>Lap-1</i>	0.87	0.86	0.57	0.40	0.68	0.82	0.85	0.96	0.94	0.91	0.79	
<i>Lap-2</i>	0.98	1.00	1.00	1.00	0.94	0.95	0.80	0.71	0.66	0.60	0.61	
<i>Mdh-1</i>	0.96	0.98	1.00	0.98	1.00	0.99	1.00	0.95	0.95	1.00	1.00	
<i>Mdh-2</i>	1.00	1.00	0.87	1.00	0.98	0.92	0.93	0.99	1.00	1.00	1.00	
<i>Pgm-1</i>	0.46	0.52	0.75	0.45	0.46	0.47	0.48	0.65	0.63	0.75	0.63	
<i>Pgm-2</i>	0.86	0.80	0.85	0.80	0.94	1.00	0.93	1.00	0.86	0.85	0.99	
<i>Pgi</i>	0.99	0.98	0.90	0.80	0.76	0.92	0.81	0.89	0.84	0.99	0.98	
<i>Per</i>	0.94	0.78	0.77	0.87	0.92	0.91	0.94	0.89	0.99	0.94	1.00	
<i>Skdh</i>	1.00	1.00	0.95	0.78	0.98	0.99	1.00	1.00	0.99	0.95	0.83	
<i>Idh</i>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	
<i>6Pgd-1</i>	1.00	1.00	0.97	0.95	0.98	1.00	1.00	1.00	1.00	0.96	1.00	
<i>6Pgd-2</i>	0.99	0.97	1.00	1.00	1.00	1.00	0.99	0.99	0.96	0.78	0.99	
<i>Dia</i>	0.90	0.96	1.00	0.97	0.96	1.00	0.99	0.99	0.91	0.84	0.98	

Table 3.2. Single locus estimates of genetic variability for 16 polymorphic loci in *Metrosideros polymorpha*

Locus	No. of alleles		Percentage of polymorphic population		Observed heterozygosity		Proportion of heterozygosity			
							Within pops.		Between pops.	
	EM	WM	EM	WM	EM	WM	EM	WM	EM	WM
<i>Ald</i>	3	3	100	100	0.419	0.473	83.6	97.8	16.4	2.2
<i>ACO-1</i>	3	3	47	83	0.034	0.049	93.6	98.9	6.4	1.1
<i>ACO-2</i>	4	3	82	100	0.175	0.147	89.1	97.6	10.9	2.4
<i>Lap-1</i>	5	5	100	100	0.405	0.212	82.4	94.6	17.6	5.4
<i>Lap-2</i>	4	3	59	100	0.129	0.372	67.6	91.9	32.4	8.1
<i>Mdh-1</i>	3	2	47	50	0.062	0.036	96.4	97.3	3.6	2.7
<i>Mdh-2</i>	3	3	53	50	0.055	0.052	95.0	95.7	5.0	4.3
<i>Pgm-1</i>	6	7	100	100	0.371	0.560	91.3	95.9	8.7	4.1
<i>Pgm-2</i>	3	3	94	66	0.213	0.110	91.7	95.5	8.3	6.5
<i>Pgi</i>	5	6	100	100	0.152	0.174	94.3	95.9	5.7	4.1
<i>Per</i>	5	3	100	83	0.290	0.150	93.0	97.2	7.0	2.8
<i>Skdh</i>	3	3	53	66	0.213	0.073	91.2	90.1	18.8	9.9
<i>Idh</i>	4	2	41	17	0.101	0.004	99.2	98.9	0.8	1.1
<i>6Pgδ-1</i>	2	3	24	17	0.108	0.012	96.9	97.5	3.1	2.5
<i>6Pgδ-2</i>	3	3	47	80	0.145	0.085	97.1	88.6	2.9	11.4
<i>Dia</i>	4	3	53	80	0.160	0.090	93.0	93.3	7.0	6.7
Mean	3.8	3.4	69	74	0.190	0.160	90.3	95.3	9.7	4.7

EM = East Maui and WM = West Maui

Table 3.3. Locus-wise diversity estimates for the observed heterozygosities among populations of *Metrosideros polymorpha* from East and West Maui

Locus	East Maui			West Maui		
	I	E	N	I	E	N
<i>Ald</i>	1.212	0.985	17	0.778	0.999	6
<i>Aco-1</i>	0.768	0.850	8	0.662	0.947	5
<i>Aco-2</i>	1.043	0.910	14	0.721	0.927	6
<i>Lap-1</i>	1.213	0.986	17	0.725	0.932	6
<i>Lap-2</i>	0.941	0.941	10	0.743	0.955	6
<i>Mdh-1</i>	0.792	0.877	8	0.424	0.890	3
<i>Mdh-2</i>	0.840	0.880	9	0.398	0.835	3
<i>Pgm-1</i>	1.211	0.984	17	0.773	0.993	6
<i>Pgm-2</i>	1.108	0.920	16	0.516	0.857	4
<i>Pgi</i>	1.147	0.932	17	0.673	0.865	6
<i>Per</i>	1.163	0.945	17	0.639	0.914	5
<i>Skdh</i>	0.886	0.929	9	0.407	0.676	4
<i>Idh</i>	0.735	0.870	7	0.000	0.000	1
<i>6Pgd-1</i>	0.582	0.967	4	0.000	0.000	1
<i>6Pgd-2</i>	0.842	0.933	8	0.418	0.598	5
<i>Dia</i>	0.853	0.894	9	0.524	0.750	5

I = Shannon diversity index, E = Evenness of distribution of allele across populations and N = Number of populations possessing at least one heterozygote for the locus.

Table 3.4. Gene diversity estimates for 23 populations of *Metrosideros polymorpha* from the island of Maui and partitioning of total diversity into among populations with mountains and between mountains

Locus	Differentiation among populations				Apportionment of diversity	
	H _T	H _S	D _{ST}	G _{ST}	Within pops. among mountains	Among pops. within mountains
<i>Ald</i>	0.497	0.433	0.064	0.130	0.870	0.128
<i>Aco-1</i>	0.039	0.037	0.002	0.043	0.957	0.041
<i>Aco-2</i>	0.176	0.160	0.016	0.091	0.909	0.089
<i>Lap-1</i>	0.448	0.366	0.083	0.184	0.816	0.157
<i>Lap-2</i>	0.282	0.210	0.072	0.256	0.744	0.223
<i>Mdh-1</i>	0.035	0.034	0.001	0.039	0.961	0.039
<i>Mdh-2</i>	0.049	0.047	0.002	0.049	0.951	0.049
<i>Pgm-1</i>	0.609	0.561	0.048	0.079	0.921	0.075
<i>Pgm-2</i>	0.167	0.153	0.014	0.081	0.919	0.079
<i>Pgi</i>	0.181	0.171	0.010	0.053	0.947	0.053
<i>Per</i>	0.199	0.185	0.014	0.072	0.928	0.065
<i>Skdh</i>	0.164	0.133	0.031	0.190	0.810	0.173
<i>Idh</i>	0.019	0.018	0.000	0.027	0.973	0.026
<i>6Pgd-1</i>	0.013	0.012	0.000	0.031	0.969	0.031
<i>6Pgd-2</i>	0.042	0.038	0.004	0.087	0.913	0.074
<i>Dia</i>	0.077	0.071	0.006	0.073	0.927	0.072
Mean	0.187	0.164	0.023	0.093	0.907	0.086

H_T = total genetic diversity; H_S = genetic diversity within populations;
D_{ST} = genetic diversity among populations; G_{ST} = D_{ST}/H_T.

Table 3.5. Genetic identity and distance coefficients for 23 populations of *Metrosideros polymorpha* from the island of Maui
Below diagonal: Nei (1978) unbiased genetic identity
Above diagonal: Nei (1978) unbiased genetic distance

Population	1	2	3	4	5	6	7	8
1 200m Haleakala		0.002	0.023	0.032	0.028	0.048	0.053	0.046
2 400m Haleakala	0.998		0.024	0.037	0.029	0.047	0.053	0.049
3 600m Haleakala	0.977	0.976		0.015	0.008	0.012	0.022	0.015
4 800m Haleakala	0.968	0.964	0.985		0.010	0.026	0.026	0.016
5 1000m Haleakala	0.973	0.971	0.992	0.990		0.014	0.013	0.006
6 1200m Haleakala	0.953	0.954	0.989	0.975	0.986		0.004	0.006
7 1400m Haleakala	0.948	0.948	0.978	0.975	0.987	0.996		0.004
8 1600m Haleakala	0.955	0.952	0.985	0.984	0.994	0.994	0.996	
9 1800m Haleakala	0.941	0.943	0.982	0.975	0.983	0.996	0.993	0.996
10 2000m Haleakala	0.959	0.955	0.985	0.976	0.981	0.970	0.973	0.977
11 1950m Koolau-1	0.940	0.939	0.944	0.961	0.947	0.940	0.942	0.945
12 1950m Koolau-2	0.911	0.910	0.956	0.963	0.955	0.960	0.959	0.957
13 1800m Crater-1	0.957	0.953	0.982	0.983	0.993	0.990	0.993	0.998
14 1850m Crater-2	0.950	0.946	0.981	0.978	0.991	0.991	0.994	0.996
15 400m Kipahulu	0.984	0.983	0.985	0.977	0.981	0.968	0.966	0.974
16 600m Kipahulu	0.987	0.989	0.974	0.963	0.966	0.955	0.942	0.953
17 800m Kipahulu	0.983	0.982	0.984	0.985	0.987	0.972	0.966	0.981
18 400m West Maui	0.978	0.969	0.987	0.992	0.993	0.979	0.981	0.991
19 600m West Maui	0.973	0.968	0.990	0.994	0.997	0.981	0.980	0.992
20 800m West Maui	0.962	0.960	0.984	0.989	0.995	0.976	0.981	0.989
21 1000m West Maui	0.958	0.956	0.981	0.991	0.994	0.974	0.980	0.987
22 1200m West Maui	0.950	0.949	0.974	0.980	0.985	0.963	0.970	0.976
23 1400m West Maui	0.970	0.972	0.985	0.987	0.990	0.969	0.970	0.979

Table 3.5. (Continued)

Population	9	10	11	12	13	14	15	16
1 200m Haleakala	0.061	0.042	0.062	0.093	0.044	0.051	0.016	0.013
2 400m Haleakala	0.059	0.046	0.063	0.094	0.048	0.055	0.017	0.011
3 600m Haleakala	0.018	0.015	0.057	0.045	0.018	0.019	0.015	0.027
4 800m Haleakala	0.026	0.024	0.040	0.037	0.017	0.022	0.023	0.038
5 1000m Haleakala	0.017	0.019	0.055	0.046	0.007	0.009	0.019	0.034
6 1200m Haleakala	0.004	0.030	0.061	0.041	0.010	0.009	0.032	0.046
7 1400m Haleakala	0.007	0.028	0.060	0.042	0.007	0.006	0.034	0.060
8 1600m Haleakala	0.004	0.024	0.057	0.044	0.002	0.004	0.026	0.048
9 1800m Haleakala		0.030	0.060	0.044	0.007	0.009	0.033	0.057
10 2000m Haleakala	0.971		0.057	0.044	0.025	0.019	0.018	0.054
11 1950m Koolau-1	0.942	0.945		0.022	0.048	0.053	0.050	0.065
12 1950m Koolau-2	0.957	0.957	0.978		0.041	0.037	0.067	0.095
13 1800m Crater-1	0.993	0.975	0.954	0.960		0.002	0.025	0.048
14 1850m Crater-2	0.991	0.981	0.948	0.964	0.998		0.025	0.051
15 400m Kipahulu	0.967	0.983	0.951	0.935	0.975	0.975		0.013
16 600m Kipahulu	0.945	0.947	0.937	0.909	0.953	0.950	0.987	
17 800m Kipahulu	0.973	0.969	0.951	0.936	0.981	0.975	0.992	0.990
18 400m West Maui	0.978	0.980	0.948	0.946	0.991	0.986	0.983	0.965
19 600m West Maui	0.981	0.982	0.958	0.964	0.991	0.987	0.981	0.965
20 800m West Maui	0.980	0.987	0.960	0.967	0.989	0.987	0.977	0.951
21 1000m West Maui	0.976	0.980	0.968	0.976	0.990	0.986	0.971	0.949
22 1200m West Maui	0.967	0.979	0.964	0.972	0.980	0.978	0.967	0.939
23 1400m West Maui	0.969	0.977	0.968	0.965	0.978	0.974	0.976	0.962

Table 3.5. (Continued)

Population	17	18	19	20	21	22	23
1 200m Haleakala	0.017	0.022	0.027	0.039	0.042	0.052	0.031
2 400m Haleakala	0.018	0.031	0.033	0.041	0.045	0.052	0.029
3 600m Haleakala	0.016	0.013	0.010	0.016	0.019	0.026	0.016
4 800m Haleakala	0.015	0.008	0.006	0.011	0.009	0.020	0.013
5 1000m Haleakala	0.013	0.007	0.003	0.005	0.006	0.015	0.010
6 1200m Haleakala	0.028	0.021	0.019	0.024	0.026	0.038	0.031
7 1400m Haleakala	0.034	0.019	0.020	0.020	0.021	0.031	0.031
8 1600m Haleakala	0.019	0.009	0.008	0.011	0.013	0.024	0.022
9 1800m Haleakala	0.027	0.022	0.019	0.021	0.024	0.034	0.032
10 2000m Haleakala	0.031	0.020	0.018	0.013	0.021	0.021	0.023
11 1950m Koolau-1	0.050	0.053	0.043	0.040	0.033	0.036	0.032
12 1950m Koolau-2	0.067	0.055	0.037	0.033	0.024	0.028	0.036
13 1800m Crater-1	0.019	0.009	0.009	0.011	0.010	0.020	0.022
14 1850m Crater-2	0.025	0.014	0.013	0.013	0.014	0.022	0.027
15 400m Kipahulu	0.008	0.017	0.019	0.023	0.029	0.034	0.024
16 600m Kipahulu	0.010	0.036	0.035	0.050	0.053	0.063	0.039
17 800m Kipahulu		0.014	0.010	0.017	0.022	0.034	0.019
18 400m West Maui	0.986		0.004	0.012	0.014	0.026	0.018
19 600m West Maui	0.990	0.996		0.004	0.004	0.014	0.009
20 800m West Maui	0.983	0.988	0.997		0.002	0.007	0.006
21 1000m West Maui	0.978	0.986	0.996	0.998		0.004	0.007
22 1200m West Maui	0.966	0.974	0.986	0.993	0.996		0.008
23 1400m West Maui	0.981	0.982	0.991	0.994	0.993	0.992	

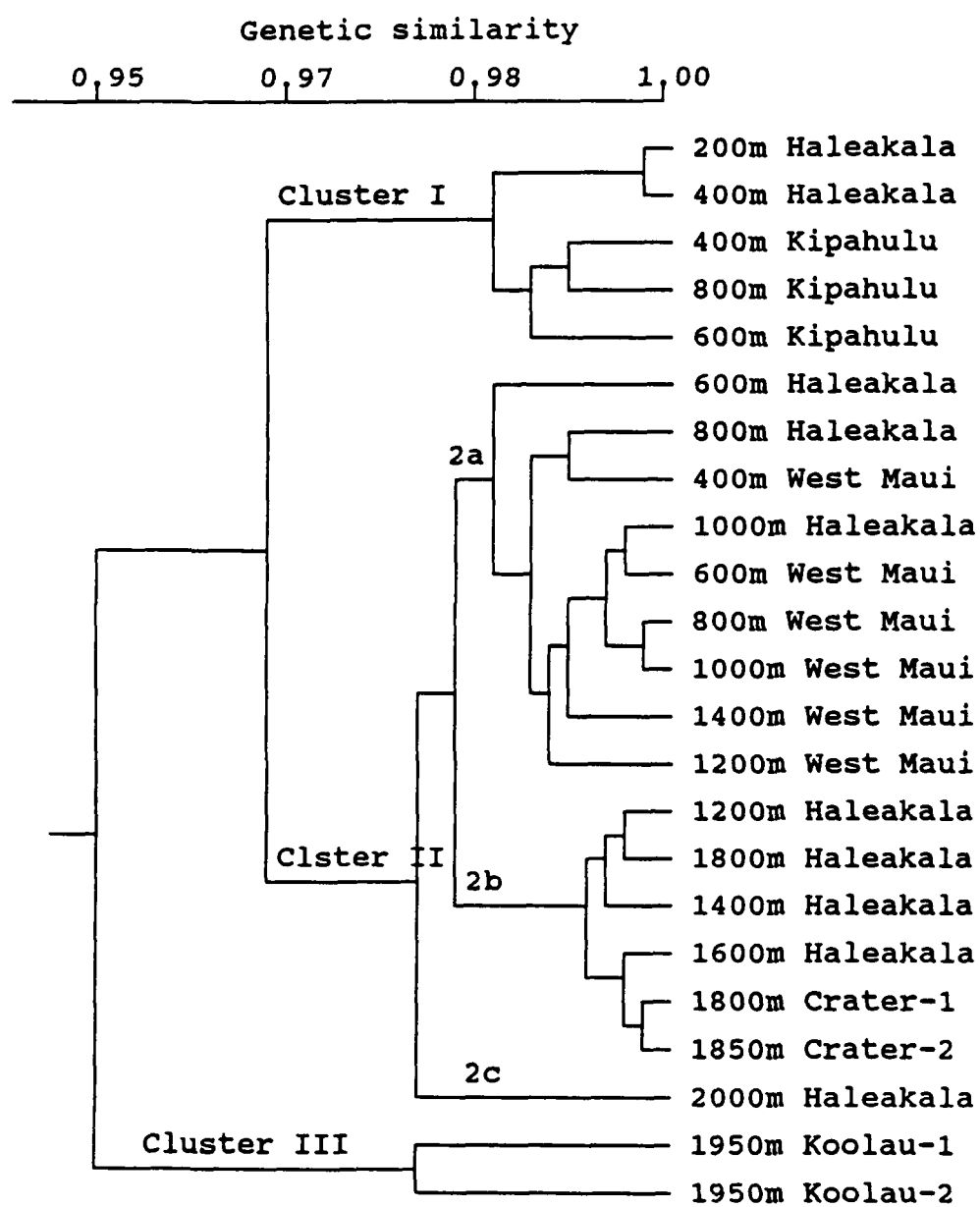


Figure 3.1. Dendrogram showing the similarities among 23 populations of *Metrosideros polymorpha* on the island of Maui

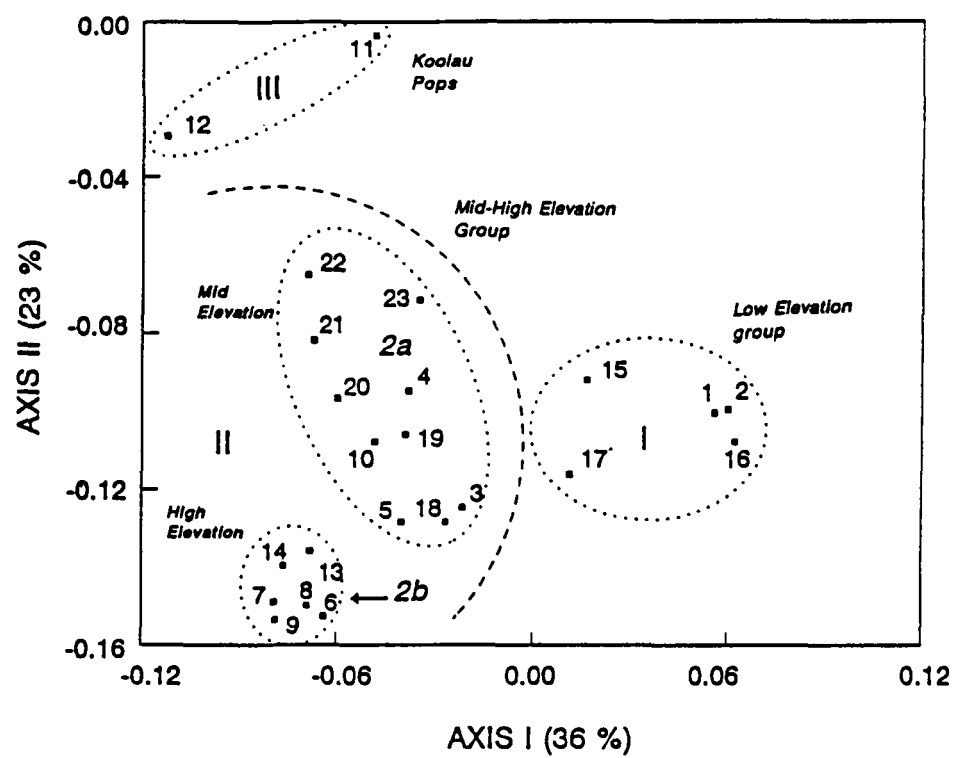


Figure 3.2. PCA ordination of 23 populations of *Metrosideros polymorpha* from the island of Maui, Hawaii

CHAPTER IV

GENETIC EVIDENCE FOR RECENT AND INCIPIENT SPECIATION IN THE EVOLUTION OF HAWAIIAN *METROSIDEROS* (MYRTACEAE)

A. ABSTRACT

The genus *Metrosideros* Banks in Hawaii comprises five morphologically recognizable species. In the present study *M. rugosa*, *M. tremuloides*, and *M. polymorpha* were analyzed 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 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 was residing 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, inspite 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* diverged recently from *M. polymorpha*. However, *M. rugosa* has diverged more ($I = 0.876$) than *M. tremuloides* ($I = 0.917$). Hence, this may represent a case of recent and incipient speciation in the insular environment.

B. INTRODUCTION

Most continental biota are so ancient that the key events of evolution that formed them are lost in the remote shadows of the past (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 kilometers from the nearest continent. Colonization processes have populated the windward slopes of the volcanoes with forest ecosystems up to altitudes of about 2000 meters. 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, occupying over 80 per cent of the relatively intact forests (Mueller-Dombois and 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 occurring 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 extremely 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 the subject of this investigation.

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 color 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* since the flowers are protandrous with sticky pollen that is transmitted by native birds visiting 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 well filled with embryos and about 30 per cent germinate (Burton, 1982). Wind dispersal is 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 an 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 and 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.

C. 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 4.1 and Figure 4.1). 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 analyzed within 7 days.

Electrophoresis

Leaf samples were homogenized in a buffer containing 10 per cent dimethyl sulfoxide (DMSO), 0.02 M sodium

metabisulfate, 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 x 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 volts (20v/cm) with 40 milliamps for 6 hours. Four enzyme systems, known to be polymorphic (PGI, PGM, PER, and LAP; Aradhya, unpublished data) were assayed following Arulsekhar and Parfitt (1986) and Shaw and Prasad (1970).

Data analysis

Genotype frequencies were inferred directly from observed isozyme phenotypes. 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 computer program BIOSYS (Swofford and 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 ($H_T = 1 - \sum_i \bar{p}_i^2$ where p_i = mean frequency of the i th allele). H_S is equal to the weighted average over all populations of the values of $1 - \sum_i p_i^2$ 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 were different.

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

The multivariate relationships among the species populations was examined by employing the principal components analysis, PCA (Gauch, 1982). The input data matrix consisted of allele frequencies for 30 alleles in 14 populations. From this matrix, a variance-covariance matrix was computed from which principal components were extracted which represented orthogonal vectors through the multidimensional space defined by 30 alleles. Species relationships were visualized by projecting the species

populations onto a plane defined by the first two principal axes.

D. 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 4.2. A total of 30 alleles were detected, with the number of alleles per locus ranging from 3 for *Lap-2* to 8 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. For *Per*, two alleles (87 and 100) were equally predominant in most populations and allele 83 was unique to population 3 (Table 4.1) 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-population estimates of genetic variability are presented in table 4.3. 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 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 and Workman, 1967) was calculated for all populations and averaged across loci (Table 4.3). The index is equal to $(H_{exp} - H_{obs})/H_{exp}$, 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 4.4) 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 intrapopulational gene diversity

summed over all loci amounted for a significant proportion of the total gene diversity (87.7 %), 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 G_{ST} component into among populations within taxa and among taxa indicated that greater proportion of gene diversity due to interpopulational differentiation was residing among populations within taxa (7.6 %) compared to among taxa (4.8 %).

The unbiased genetic distance (D) and identity (I) measures of Nei (1978) were calculated for both pairwise comparisons among the 14 populations and species, but only the pairwise genetic identity for species comparisons are presented in table 4.5. 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 (Figure 4.2). *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.

The principal component analysis has effectively reduced the complex multidimension variation among the allele frequencies from 14 species populations to simple and meaningful dimensions in the form of a set of orthogonal vectors. The first two of them accounted for about 53 per cent of the total variation. The plotting of these two vectors (Figure 4.3) has resulted in the formation of two major clusters similar to that observed in the UPGMA analysis. Cluster 1 contained all the three populations of *M. rugosa* and the cluster 2 included all the populations of *M. polymorpha* and *M. tremuloides*. However, *M. polymorpha* populations from Waianae mountains showed greater affinity among themselves (cluster 2a) as compared to that from Koolaus.

E. DISCUSSION

Hawaiian *Metrosideros* is an excellent example of a genus exhibiting 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 6 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 sort of assortative mating resulting in inbreeding leading to population substructuring within species.

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

Genetic differentiation appears uniformly low across loci except for *Lap-2* and *Per* which may indicate selective

advantage of certain alleles of these loci in some populations. The three taxa involved in the present study showed very little divergence for the isozyme loci studied (4.7 %) when compared to populations within taxa (7.6 %).

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. Further, the extremely efficient seed dispersal system coupled with high rates of outcrossing in the Hawaiian *Metrosideros* may act as homogenizing forces discouraging rapid differentiation brought about by divisive forces of 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 surviving a "bottleneck" of 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 which are 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 geographic isolation leading to limited gene flow (Figure 4.2).

Similar conclusions can be drawn from the results of the principal component analysis (Figure 4.3) where *M. rugosa* populations clustered together distinctly when compared to the rest of the populations indicating that it is relatively genetically more diverged than *M. tremuloides* which has clustered along with *M. polymorpha*. However, there is an indication of genetic distinctness of *M. polymorpha* populations from Waianae mountains (cluster 2a) as compared to *M. polymorpha* from Koolaus.

Narrow genetic divergence at isozyme loci among different insular taxa was also reported for *Tetramolopium* (Lowrey, 1981), *Bidens* (Ganders and Nagata, 1984; Helenurm and Ganders, 1985) and the silversword alliance of species belonging to the genera *Argyroxiphium*, *Dubautia*, and

Wilkesia (Carr, 1985a,b; Witter and Carr, 1988) from the Hawaiian islands. Lowrey and 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 8 subspecies endemic to Hawaii, exhibited very little genetic differentiation for isozyme loci (mean $\bar{I}$ = 0.957) although there is substantial morphological and ecological differentiation (Helenurm and 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 behavior 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 vs. 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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Table 4.1. 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

Table 4.2. Allelic frequencies for the six polymorphic loci in Hawaiian *Metrosideros*

Locus	Population						
	1	2	3	4	5	6	7
<i>Pgi</i>							
(N)	40	36	45	35	40	40	50
88	0.025	0.028				0.025	
92	0.013	0.014					
100	0.925	0.917	0.967	0.800	0.925	0.950	0.750
108			0.022	0.186	0.075		
112	0.038	0.042	0.011	0.014		0.025	0.240
122							0.010
<i>Pgm-1</i>							
(N)	40	40	45	35	40	40	40
60							
86							
91	0.025	0.025	0.022			0.013	
95	0.175	0.175	0.122	0.143	0.038	0.100	0.175
100	0.425	0.488	0.422	0.543	0.950	0.750	0.738
109	0.063	0.087	0.111	0.214	0.013	0.087	0.038
118	0.313	0.225	0.311	0.100		0.050	0.050
123			0.011				
<i>Pgm-2</i>							
(N)	40	40	45	35	40	40	40
80							
95	0.138	0.038	0.067	0.086		0.013	0.038
100	0.700	0.762	0.600	0.829	1.000	0.938	0.863
105	0.162	0.200	0.333	0.086		0.050	0.075
109							0.025
<i>Per</i>							
(N)	40	40	30	34	40	40	50
83			0.017				
87	0.450	0.587	0.067	0.338	0.175	0.363	0.060
100	0.538	0.412	0.917	0.662	0.825	0.637	0.510
130	0.013						0.430
<i>Lap-1</i>							
(N)	40	40	12	35	40	40	40
89				0.129	0.075		
95	0.225	0.287	0.125	0.257	0.100	0.162	0.237
100	0.300	0.587	0.458	0.586	0.825	0.688	0.738
105	0.475	0.125	0.417	0.029		0.150	0.025
<i>Lap-2</i>							
(N)	40	40	12	35	40	40	40
97		0.150			0.075	0.050	
100	0.438	0.475	0.542	0.971	0.800	0.637	0.613
110	0.563	0.375	0.458	0.029	0.125	0.313	0.387

Table 4.2. (Continued)

Locus	Population						
	8	9	10	11	12	13	14
<i>Pgi</i>							
(N)	38	35	35	30	30	30	30
88							
92				0.033	0.050	0.067	0.033
100	0.566	0.671	0.757	0.883	0.783	0.850	0.817
108			0.043	0.083	0.167	0.083	0.150
112	0.421	0.314	0.200				
122	0.013	0.014					
<i>Pgm-1</i>							
(N)	38	39	35	30	30	30	30
60		0.026					
86	0.092	0.077					
91	0.171	0.115	0.014	0.067		0.067	0.017
95	0.145	0.231	0.114	0.067		0.017	0.050
100	0.447	0.423	0.786	0.783	0.883	0.550	0.617
109	0.105	0.038	0.014	0.083	0.117	0.217	0.200
118	0.039	0.051	0.071			0.150	0.117
123		0.038					
<i>Pgm-2</i>							
(N)	38	37	35	30	30	30	30
80	0.013						
95	0.118	0.135	0.057	0.033	0.083		
100	0.789	0.703	0.943	0.967	0.917	0.917	0.900
105	0.079	0.162				0.083	0.100
109							
<i>Per</i>							
(N)	38	37	39	30	30	30	30
83							
87	0.118	0.149	0.295	0.250	0.350	0.350	0.217
100	0.882	0.851	0.705	0.750	0.633	0.617	0.783
130					0.017	0.033	
<i>Lap-1</i>							
(N)	38	37	35	30	30	30	30
89			0.157	0.133	0.067		
95	0.158	0.162	0.157	0.150	0.067	0.317	0.300
100	0.842	0.838	0.686	0.717	0.867	0.683	0.700
105							
<i>Lap-2</i>							
(N)	38	37	35	30	30	30	30
97				0.033	0.133	0.383	0.250
100	0.842	0.946	0.671	0.967	0.867	0.617	0.750
110	0.158	0.054	0.329				

Table 4.3. Genetic variability in Hawaiian *Metrosideros*
(standard errors in parentheses)

Popula- tion	Taxon	Mean no. of alleles per locus	F ¹	Mean heterozygosity	
				Direct- count	HdyWbg expected ²
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

1 Fixation index (Wright, 1965)

2 Unbiased estimate (see Nei, 1978)

* P < 0.05

Table 4.4. Measures of gene diversity and population differentiation
in *Metrosideros*

Differentiation among populations				Apportionment of diversity	
Locus	H _T	H _S	D _{ST}	G _{ST}	Within pops. Among pops. within taxa
<u>Pgi</u>	0.302	0.267	0.035	0.116	0.884
<u>Pgm-1</u>	0.578	0.522	0.056	0.097	0.903
<u>Pgm-2</u>	0.287	0.261	0.026	0.091	0.909
<u>Per</u>	0.457	0.392	0.065	0.142	0.858
<u>Lap-1</u>	0.482	0.430	0.052	0.108	0.892
<u>Lap-2</u>	0.430	0.352	0.078	0.181	0.819
Mean	0.423	0.371	0.052	0.123	0.877

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 interpopulational differentiation

Table 4.5. Estimates of mean genetic identity among populations within taxa (diagonal) and between taxa of *Metrosideros* (below diagonal) (range within parentheses)

SPECIES	No. of pops.	1	2	3
1. <i>M. rugosa</i>	3	0.938 (0.901-0.958)		
2. <i>M. tremuloides</i>	2	0.877 (0.831-0.917)	0.958 (0.958-0.958)	
3. <i>M. polymorpha</i>	9	0.874 (0.792-0.968)	0.961 (0.904-0.993)	0.948 (0.903-0.998)

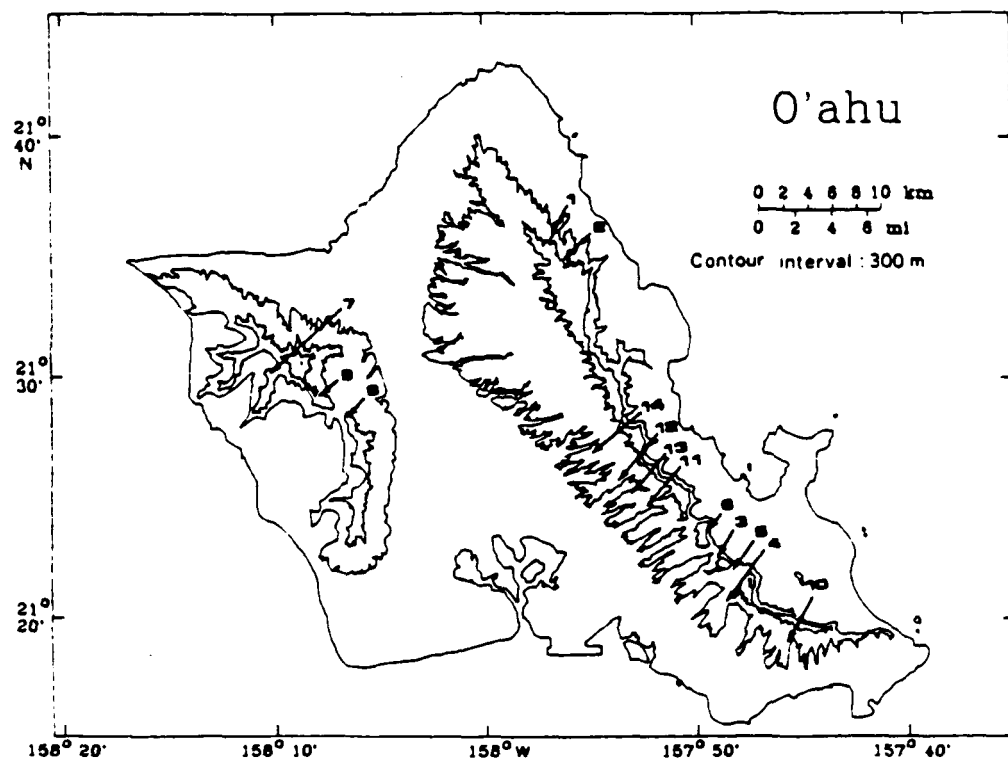
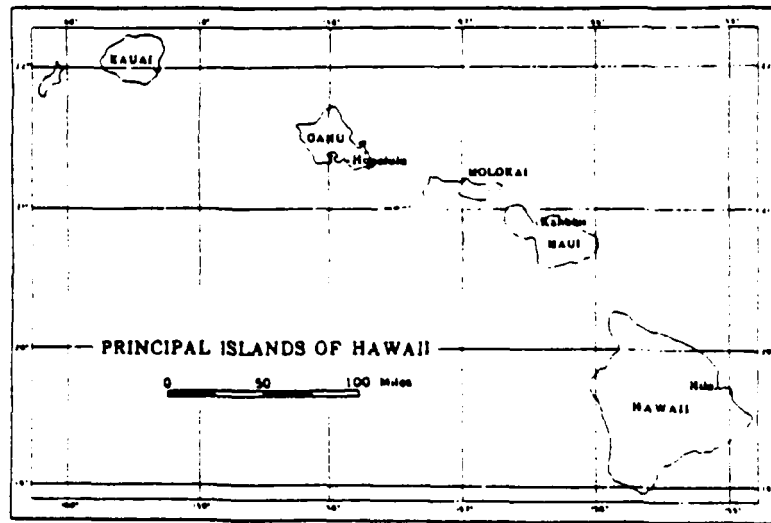


Figure 4.1. Approximate location of 14 populations of *Metrosideros* species on the island of Oahu, Hawaii

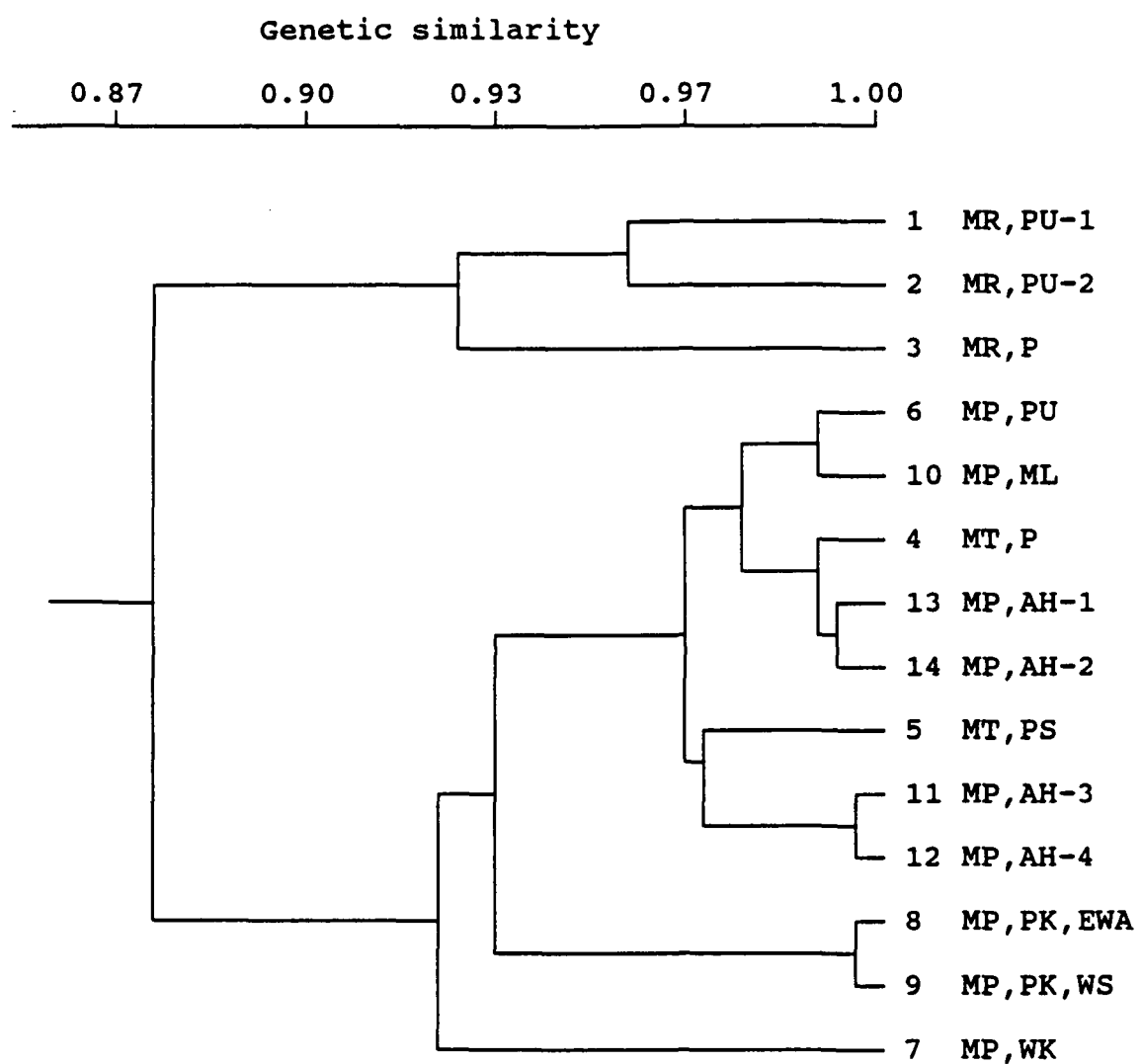


Figure 4.2. Dendrogram showing genetic similarities among 14 populations of *Metrosideros*

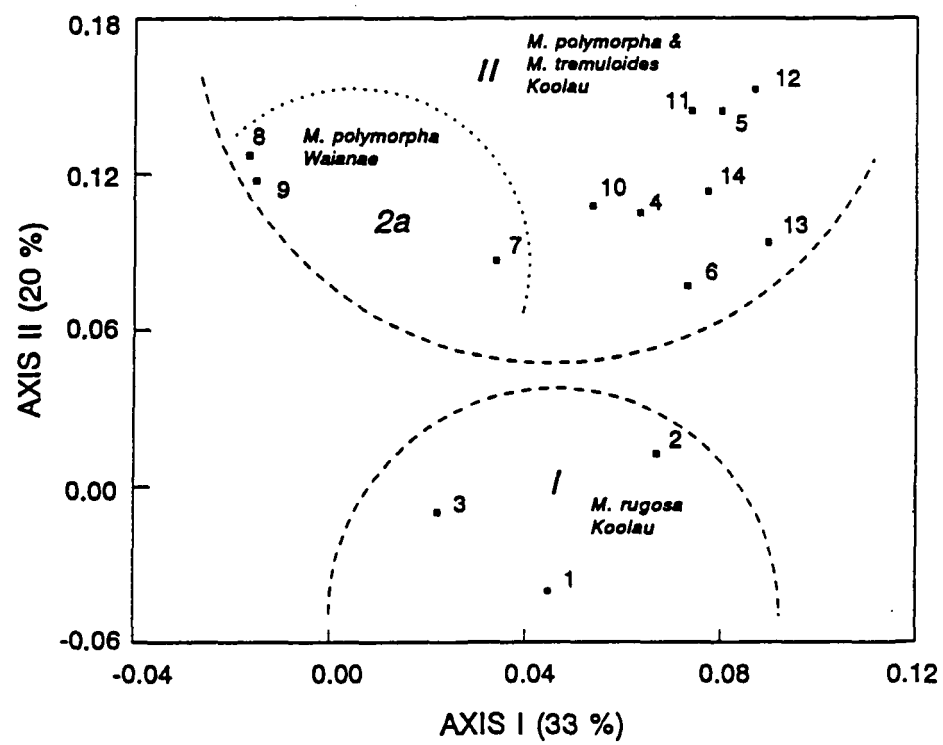


Figure 4.3. PCA ordination of 14 populations of *Metrosideros* species from the island of Oahu, Hawaii

APPENDIX A

EXTRACTION BUFFERS

Interference of plant phenolic compounds during extraction of plant enzymes

Plants produce a variety of phenolic compounds which often interfere seriously in the extraction of plant enzymes (Loomis, 1969). These wide variety of phenolic compounds are known to complex with enzymes following cellular destruction (Dirr et al., 1973; Loomis, 1974; Loomis and Battalie, 1966). The paucity of electrophoretic investigations of perennial angiosperms and gymnosperms may be due partially to high phenol to protein ratio in these groups (Kelly and Adams, 1977). Phenols combine with proteins in two ways: reversibly by hydrogen bonding, and irreversibly by oxidation to quinones following covalent condensation of the quinones with reaction groups of proteins. The quinones may also oxidize essential groups of proteins. Thus little oxidation of phenolic compounds occurs in intact plant cells because the phenolics are spatially separated from O-diphenoloxidases by the tonoplast. However, in ageing cells, breakdown of the tonoplast occurs (Shaw and Manocha, 1965) and oxidation of the vacuolar phenolics by the cytoplasmic enzymes ensues; it may be significant that this is concomitant with the death

of the cell (Spencer, 1965). Most plant tissues contain a wide range of phenolic compounds which are oxidized by copper-containing enzyme broadly grouped as phenol oxidases (Bonner, 1957). The most active of these enzymes in plant tissues is O-diphenol: O₂ oxidoreductase (E.C. 1.10.3.1 trivial name O-diphenoloxidase) which oxidizes O-diphenols to the corresponding quinones.

Techniques for extracting enzymes or organelles from plants that contain phenolic compounds should not only separate the phenols from proteins, but also prevent oxidation of the phenols. In the living plant cell, compartmentalization protects the protoplasm components from secondary products. Therefore, living tissues can usually be stored for sometime if kept moist and cold-but not frozen. Freezing destroys cell compartmentalization, and oxidative changes may occur in ordinary frozen storage.

Improved extraction techniques

Methods to sequester the phenolic interference in enzyme extraction has been outlined in papers by Loomis and Battalie (1966); Loomis (1974), and Anderson (1968).

Use of phenol complexing agent

At neutral or acid pH the only effective means of removing bound phenolics from plant proteins is to supply a phenol-complexing agent that can compete with the peptide

linkages of the plant proteins. To date, the most satisfactory and widely used agent appears to be various grades of polyvinylpyrrolidone (PVP). Foreign proteins and synthetic poly-amides (Nylons) have also been used successfully. Poly ethyleneglycols (PEG) of high molecular weight 20,000 have proved useful in certain methods (Badran and Jones, 1965; Dilley, 1966). Hydroxyl groups of phenolic compounds act as very strong proton donors in hydrogen bonding. The -Co - N group of PVP is a very strong protein acceptor and cannot act as a proton donor. For this reason PVP appears to be an excellent and more specific phenol-binding agent than any other available material. A cross-linked, insoluble grade of PVP called polyvinylpolypyrrolidone (PVPP) is very effective in isolating soluble plant enzymes. Polyethylene glycol (PEG, 20,000) is very useful in extracting soluble enzymes especially from phenol-rich plant species (Young, 1965; Badran and Jones, 1965). It is desirable to maintain the pH of the extraction buffer at 7.00 especially when PVPP is used in the extraction buffer.

Bovine Serum Albumin

BSA is one of the most widely used and effective additives in plant enzyme extraction (Raison and Leyons, 1970; Hobson, 1970). It protects plant enzymes during isolation by reacting with phenolic compounds in all of the

principle ways that proteins combine with phenols. It is also known for its capacity to bind lipids by hydrophobic forces, and to bind anions (Foster, 1960) consistent with its high content of hydrophobic amino acids and lysine. BSA appears also to be an effective quinone scavenger (Wehr, 1973) by virtue of its high lysine and cysteine content.

Antioxidants and phenoloxidase inhibitors

Several Antioxidants and phenoloxidase inhibitors have been recommended for use in extraction of plant enzymes. These agents either prevent oxidation of phenols or reduce quinones as soon as they are formed. Thiols such as cysteine, mercaptoethanol, thioglycollate, dithiothreitol (DTT, Cleland's reagent), diethyldithiocarbamate (DIECA) are known to inhibit O-diphenoloxidase activity (Pierpoint, 1966; Palmer and Roberts, 1967) but the greater enzyme activity of extracts prepared with thiols has been correlated with preventing accumulation of O-diphenoloxidase products in only a few cases (Slack, 1966; Anderson and Rowan, 1967). Therefore the crucial part of the effectiveness of thiols depends on removal of any quinones as it is formed. The thiols can be broadly grouped in to two classes, depending on the type of reaction between thiols and quinones. 1. Thiols which reduce quinones as they are formed back to the O-diphenols; such thiols (thioglycollate and mercaptobenzothiazole) tend to be

powerful inhibitors of O-diphenoloxidase. 2. Thiols which combine with quinones to form a product which is not further oxidized and does not inhibit enzymes, such thiols (cysteins, DIECA and ethyl xanthate) tend to be less powerful inhibitors of O-diphenoloxidase than those of class (1). Glutathione and thiourea also combine with quinones (Henze, 1956). In practice, both classes of thiols provide the same degree of protection of enzymes during extraction.

Many workers have used ascorbate in attempts to increase the efficiency of enzyme extraction (Anderson and Rowan, 1967; Abukharma and Woolhouse, 1966). Ascorbate readily reduces quinones with the regeneration of the phenol and is widely used to measure spectrophotometrically O-diphenoloxidase activity.

Metabisulphate is yet another antioxidant as efficient as thiols for extracting some enzyme by inhibiting O-diphenoloxidase. Borate and germinate are also known for their ability to form complexes with polyols, including O-diphenols (King, 1971) and to inhibit O-diphenoloxidases

Rapid extraction procedures

Several workers have emphasized the importance of minimizing the time period during which the homogenate is in contact with air. Use of low temperature is another (between 0 - 4° C). Rapid grinding in a prechilled grinding buffer has been effective. Grinding of plant tissue

preserved in liquid nitrogen should be done by transferring the tissue in to the cold extraction buffer before it thaws.

Extraction buffers surveyed for selecting a suitable extraction buffer for *Metrosideros polymorpha*

1. Grinding buffer for Ohia lehua - modified from Bousquet et al., 1987

0.10 M Tris(hydroxymethyl)aminomethane
0.20 M Sucrose
0.30 mM Nicotinamide adenine dinucleotide phosphate (NADP)
0.40 mM Nicotinamide adenine dinucleotide (NAD)
0.50 mM Ethylenediaminetetraacetic acid (EDTA, disodium)
0.005 M Dithiothreitol (DTT)
0.012 M Cysteine-HCL
0.025 M Ascorbic acid
0.02 M Sodium metabisulfate
0.005 M Diethyldithiocarbamate (DIECA)
1 % Polyethylene glycol (PEG)
10 % Dimethyl sulfoxide (DMSO)
0.1 % Bovine serum albumin (BSA)
10 % β mercaptoethanol
2 % Tween 80
10 % Polyvinyl polypyrrolidone (PVPP)

pH of the buffer is adjusted to 7.5. β mercaptoethanol and PVPP are added just before use.

2. Phosphate grinding buffer-PVP solution (Soltis et al., 1983)

0.100 M phosphate buffer pH 7.5

0.029 M sodium tetraborate

0.017 M sodium metabisulfate

0.200 M L-ascorbic acid sodium salt

0.016 M diethyldithiocarbamate (DIECA)

4 % (w/v) PVP (mol. wt. 40,000, Sigma PVP 40T)

1 % 2-mercaptoethanol

(to make 100 ml of phosphate buffer dissolve 1.36 g KH_2PO_4 in dH_2O , add 9.0 ml 1 M NaOH, and bring volume to 100 ml with dH_2O)

3. Tris-maleate grinding buffer-PVP solution (Soltis et al., 1983)

0.200 M sodium tetraborate

0.020 M sodium metabisulfate

0.250 M L-ascorbic acid sodium salt

0.026 M diethyldithiocarbamate (DIECA)

0.100 M maleic acid

0.100 M Tris(hydroxymethyl)aminomethane (TRIS)

0.1 % 2-mercaptoethanol

4 % (w/v) PVP (mol. wt. 10,000)
(to make 25 ml of buffer, dissolve amounts indicated in 19
ml dH₂O; mix thoroughly and adjust pH to 7.5 with 1.0 M HCl,
finally adjust the volume with dH₂O)

**4. Tris-HCl grinding buffer-PVP solution (Soltis et al.,
1983)**

25 ml Tris-HCl buffer
0.001 M EDTA (tetrasodium salt)
0.010 M Potassium chloride
0.010 M magnesium chloride hexahydrate
0.1 % 2-mercaptoethanol
10 % (w/v) PVP (mol. wt. 40,000)
(adjust the pH to 7.5 before adding PVP and 2-
mercaptoethanol)

**5. Extraction buffer used for fruit tissues such as Almond,
Walnut, Pistachio, and Fig (Arulsekhar and Parfitt, 1986)**

0.05 M tris
0.007 M citric acid (monohydrate)
0.1 % (w/v) cysteine hydrochloride
0.1 % (w/v) Ascorbic acid (sodium salt)
1.0 % Polyethylene glycol
1 mM 2-mercaptoethanol
10 % Polyvinyl pyrrolidone (PVPP)
(final pH is adjusted to 8.0, before adding PVPP)

6. Extraction buffer used for juniper (Kelley and Adams, 1977), slightly modified.

0.10 M Tris-Maleate buffer pH 7.0

0.20 M sodium tetraborate

0.25 M sodium ascorbate

0.02 M sodium metabisulfate

0.02 M diethyldithiocarbamate (DIECA)

0.01 M germanium dioxide

10 % (v/v) dimethyl sulfoxide (DMSO)

10 % (w/v) PVP (mol. wt. 40,000)

(final pH is 7.0)

7. Extraction buffer used for species of Astereae (Gottlieb, 1981)

0.10 M Tris-HCl buffer pH 7.5

0.014 M 2-mercaptoethanol

1 mM EDTA (tetra sodium)

10 mM Magnesium chloride

10 mM Potassium chloride

5 - 10 % (w/v) PVPP

8. Extraction buffer used for Tea (Wendel and Parks, 1982)

0.04 M phosphate buffer pH 7.3

0.005 M ascorbic acid (sodium salt)

0.003 M sodium metabisulfate

0.003 M dithiothreitol (DTT)
0.006 M DIECA
0.001 M EDTA
0.2 M sucrose
5 % (w/v) PVP-40
0.1 % 2-mercaptoethanol
(Final pH is adjusted to 7.5)

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APPENDIX B

GEL AND TRAY BUFFERS

The following gel and tray buffers were tried in the electrophoretic analysis of *Metrosideros polymorpha*:

1. Histidine - citrate pH 6.5 (Cardy et al., 1981)

Tray : 0.065 M histidine (free base)
0.007 M citric acid (anhydrous)
Gel : 0.016 M histidine (free base)
0.002 M citric acid (anhydrous)

2. Morpholine - citrate pH 6.1 and 8.0 (Clayton and Tretiak, 1972)

Tray : 0.04 M citric acid (anhydrous)
(adjust the pH of required quantity to 6.1 or
8.0 with N-(3-aminopropyl)-morpholine
Gel : a 1:20 dilution of tray buffer

3. Histidine - citrate pH 7.0 (Fildes and Harris, 1966)

Tray : 0.410 M citric acid, trisodium salt
(adjust the pH to 7.0 with 1 N HCl)
Gel : 0.005 M histidine-HCl
(adjust the pH to 7.0 with 1 M NaOH)

4. LiOH/borate - Tris/citrate pH 8.1/8.4 (Selander et al., 1971)

Tray : 0.03 lithium hydroxide
0.19 boric acid
(adjust the pH to 8.1 with LiOH)

Gel : 0.05 M tris
0.008 M citric acid
(adjust the pH to 8.4 with 4 M citric acid)

Note: Gel buffer is 1 part tray buffer +
9 parts gel buffer

5. Sodium/borate - Tris/citrate pH 8.2/8.7 (Selander et al., 1971)

Tray : 0.07 M sodium hydroxide
0.3 M boric acid
(pH 8.2)

Gel : 0.076 M tris
0.005 M citric acid
(pH 8.7)

6. Tris-EDTA-borate pH 8.6 Gottlieb (pers. comm. to Soltis)

Tray : 0.18 M tris
0.004 M EDTA (tetra-sodium)
0.1 M boric acid
(adjust the pH to 8.6 with boric acid)

Gel : 1:3 dilution of tray buffer

7. Tris - citrate pH 7.5 (Shaw and Prasad, 1970)

Tray : 0.223 M tris
0.086 M citric acid
(adjust pH to 7.5 with 1 M NaOH)

Gel : 0.008 M tris
0.003 M citric acid
(adjust pH to 7.5)

8. Tris - citrate pH 7.2 (Shaw and Prasad, 1970)

Tray : 0.223 M tris
0.069 M citric acid
(adjust pH to 7.2)

Gel : dilute 35 ml tray buffer to 1 liter

9. Tris - maleate pH 7.4 (Selander et al., 1971)

Tray : 0.1 M tris
0.1 M maleic acid
0.01 M EDTA (disodium)
0.01 M magnesium chloride
(adjust pH to 7.4)

Gel : 1:9 dilution of tray buffer

Many of the above mentioned buffers are slight
modifications of the original formulations of the authors.

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APPENDIX C

ACTIVITY STAINING OF ENZYMES

During the course of electrophoretic investigations of *Metrosideros*, I tried many staining protocols and recipes presented in the literature. Many of the staining schedules are slight modifications of each others procedures and recipes. Generally most staining protocols prescribe much larger quantities of ingredients than required for normal staining procedures. I have cut down the quantity (up to 30 to 40 per cent) of some of these ingredients such as NAD, NADP, linking enzymes, MTT, PMS and also substrates for most enzyme systems. Linking enzymes, dependent on the inexpensive cofactors such as NAD in place of NADP would be considerable saving. I have used 20 ml of staining mixture as against 50 ml recommended by most protocols without sacrificing the quality of staining.

Stock solutions of MTT, PMS, NAD and NADP can be made in advance and stored in dark bottles in the refrigerator for up to 1 month without deterioration. They are dispensed using 1 ml pipettes with plastic bulbs attached. The quantity of substrates were meant only as a general guide. The analytical spatula is used to estimate the quantities of most substrates, unless they are very expensive or proportions are crucial. The gels were incubated with

staining solutions in an oven at 37°C until bands appear.
Some systems can be stained at the room temperature.

The following are some stock solutions regularly used in day
to day staining procedures:

Chemical	concentration	quantity/100 ml
NAD	10 mg/ml	1.0 g
NADP	5 mg/ml	0.5 g
MTT	5 mg/ml	0.5 g
NBT	5 mg/ml	0.5 g
PMS	1 mg/ml	0.1 g
MgCl ₂	10 mg/ml	1.0 g

(refrigerate all the above solutions except MgCl₂)

Staining buffers

1. 0.2 M Tris HCl, pH 8.0 (for 10 liters)

Tris 242.2 g

(adjust the pH to 8.0 with concentrated HCl)

2. 0.2 M Acetate buffer, pH 5.0 (for 2 liters)

Sodium acetate 54.4 g

(adjust the pH to 5.0 with concentrated glacial
acetic acid)

3. Tris-Maleate buffer, pH 7.4 (for 1 liter)

Tris 24.2 g

Maleic acid 23.2 g

4. Phosphate buffer pH 6.4 (for 1 liter)

Sodium phosphate (monobasic) 13.9 g

Sodium phosphate (dibasic) 5.4 g

(pH is approximately 6.4)

**The recipe for preparing glucose-6-phosphate
dehydrogenase solution**

0.0005 M glycine

0.1 per cent albumin

add 80 ml dH₂O, then add

MgCl₂ 1.0 ml

NAD 2.0 ml

G6PDH 5000 NADP units

(adjust pH to 8.0)

dH₂O to final volume of 100 ml

(refrigerate the solution)

Staining recipes and protocol

1. Aconitase (EC 4.2.1.3.)

0.1 M Tris-HCl buffer pH 8.0 20 ml

cis-aconitic acid 30 mg

Isocitrate dehydrogenase 3 - 4 units

1.0 M MgCl ₂	0.5 ml
NADP	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

2. Aldolase (EC 4.1.2.13)

0.1 M Tris-HCl buffer pH 8.0	20 ml
Fructose-1,6-diphosphate (trisodium)	60 mg
1.0 M arsenic acid, sodium salt	0.5 ml
Glyceraldehyde-3-phosphate dehydrogenase	50 units
NAD	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

3. Diaphorase (EC 1.6.4.3)

0.1 M Tris-HCl buffer pH 8.0	20 ml
2,6-dichlorophenolindophenol	few grains
NADH	10 mg
MTT	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

4. Isocitrate dehydrogenase (EC 1.1.1.42)

0.1 M Tris-HCl buffer pH 8.0	20 ml
Isocitric acid, trisodium	40 mg
1.0 M MgCl ₂	1.0 ml
NADP	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

5. Leucine aminopeptidase (EC 3.4.11.1)

0.2 M Tris-Maleate buffer	20.0 ml
0.2 M NaOH	8.0 ml
dH ₂ O	12.0 ml
L-leucine-β-naphthylamide (free base)	10 mg
Black K salt	10 mg

Stain at room temperature in dark; a modification of Arulsekhar and Parfitt, 1986

6. Malate dehydrogenase (EC 1.1.1.37)

0.1 M Tris-HCl buffer pH 8.0	20 ml
2.0 M Malate solution	5 ml
NAD	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

7. Peroxidase (EC 1.11.1.7)

0.05 M Sodium acetate buffer pH 5.0	40 ml
3-amino-9-ethyl carbazole (dissolved in dimethyl formamide)	30 mg
0.1 M CaCl ₂	1.0 ml
3 % H ₂ O ₂	1.0 ml

Stain at room temperature; a modification of Shaw and Prasad, 1970

8. Phosphoglucisomerase (EC 5.3.1.9)

0.1 M Tris-HCl buffer pH 8.0	20 ml
Fructose-6-phosphate, disodium salt	20 mg
1.0 M MgCl ₂	1.0 ml
Glucose-6-phosphate dehydrogenase	10 units
NAD/NADP (depends on G6PDH)	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

9. Phosphoglucomutase (EC 2.7.5.1)

0.1 M Tris-HCl buffer pH 8.0	20 ml
Glucose-1-phosphate, disodium	20 mg

1.0 M MgCl ₂	1.0 ml
Glucose-6-phosphate dehydrogenase	10 units
NAD/NADP (depends on G6PDH)	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

10. 6-Phosphogluconate dehydrogenase (EC 1.1.1.44)

0.1 M Tris-HCl buffer pH 8.0	20 ml
6-phosphogluconic acid, barium salt	20 mg
1.0 M MgCl ₂	1.0 ml
NADP	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

11. Shikimate dehydrogenase (EC 1.1.1.25)

0.1 M Tris-HCl buffer pH 8.0	20 ml
Shikimic acid	20 mg
NADP	1.0 ml
MTT/NBT	1.0 ml
PMS	1.0 ml

Stain at 37°C in dark; a modification of Shaw and Prasad, 1970

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APPENDIX D

LOCUSWISE GENOTYPE DATA FOR DIFFERENT POPULATIONS OF *METROSIDEROS POLYMORPHA*

The raw data consisted of genotypes of individual trees scored for eleven enzyme systems encoded by 16 loci. A total of 23 populations from the island of Maui were included in the present study.

Each row represents the genotypes (P = pubescent, G = glabrous) of individual trees scored for 16 loci.

The data is arranged to analyze for genetic diversity and differentiation among populations.

GENETIC STRUCTURE ANALYSIS OF OHIA LEHUA ON MAUI

NOTU=23 , NLOC=16 , NALL=10 , CRT;

(10(1X,A5)/6(1X,A5))

ALD-1 ACO-1 ACO-2 LAP-1 LAP-2 MDH-1 MDH-2 PGM-1 PGM-2 PGI-1

PER-1 SDH-1 IDH-1 PGD-1 PGD-2 DIA-1

STEP DATA:

DATYP=1,ALPHA;

(A4,16(1X,A1,A1))

MP1	200M	HALEAKALA	1	1
01	BB	BB	BB	CC
02	BB	BB	BB	DD
03	BB	BB	BB	CC
04	BB	BB	BB	CD
05	BB	BB	CC	CC
06	BB	BB	BB	CC
07	BB	BB	BB	CC
08	BB	BB	BB	CC
09	BB	BB	BB	CC
10	BB	BB	BB	CC
11	BB	BB	BB	CC
12	BB	BB	BB	CC
13	BB	BB	BB	CC
14	BB	BB	BB	CC
15	BB	BB	BB	CC
16	BB	BB	BB	CC
17	BB	BB	BB	CC
18	BB	BB	BB	CC
19P	BB	BB	BB	CC
20	BB	BB	BB	CC
21	BB	BB	BB	CC
22	BB	BB	BB	CC
23	BB	BB	BB	CC
24	BB	BB	BB	CC
25	BB	BB	BB	CC

26	BB	BB	BB	CD	BB	BB	BB	CE	BB	CC	CC	BC	CC	BB	BB	CC	
27	BB	AB	BB	CC	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC	
28	BB	BB	CC	CD	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
29	BB	BB	CC	CD	BB	BB	BC	EE	BB	CC	CD	BC	CC	BB	BB	CC	
30	BB	BB	CC	DD	BC	BB	BB	DD	AB	CC	CC	BC	CC	BB	BB	CC	
31	BB	BB	CC	DD	BB	BB	BC	DE	BB	CC	CC	BB	CC	BB	BB	CC	
32	BB	BB	CC	CC	AA	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
33P	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
34	BB	BB	BB	DD	BB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC	
35	BB	BB	BB	CC	BB	BB	BC	DD	BB	CE	CC	BC	CC	BB	BB	CC	
36	BB	BB	BB	CD	BB	BB	BB	DE	BC	CC	BC	BC	CC	BB	BB	BC	
37P	BB	BB	CC	CD	BB	BB	BC	DE	BB	CE	CC	BC	CC	BB	BB	CC	
38	BB	BB	CC	DD	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
39	BB	BB	BB	CD	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC	
40	BC	BB	CC	CC	BC	BB	BB	CE	BB	CC	CD	BC	CC	BB	BB	CC	
41	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
42	BB	BB	BC	CD	BB	BB	BB	BE	BB	CE	CE	BC	CC	BB	BB	CC	
43	BB	BB	CC	CC	BB	BB	BB	CC	BB	CC	CE	BC	CC	BB	BB	CC	
44P	BB	AB	BB	CD	BB	BB	BB	EE	BB	CE	CC	BC	CC	BB	BB	CC	
NEXT																	
MP2	400M HALEAKALA								1	1							
01P	BB	BB	CC	DD	BB	BB	BB	EE	BB	CD	CD	BC	CC	BB	BB	CC	
02	BB	AB	BC	CD	BB	BB	BB	BD	BB	CC	CC	BC	BC	BB	BB	CC	
03	BB	BB	BB	CD	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
04	BB	BB	BC	DD	BB	BB	BC	BD	BB	CC	CC	BC	CC	BB	BB	CC	
05	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
06	BB	AB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BC	CC	BB	BB	CC	
07	BB	BB	CC	CC	BB	BB	BB	EE	BB	CC	CD	BB	CC	BB	BB	CC	
08	BB	BB	BB	DD	BC	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
09	BB	BB	BB	DD	BB	BB	BC	BD	BB	CE	CC	BC	CC	BB	BB	CC	
10P	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
11	CC	BB	DD	DD	BB	BB	CC	DE	BB	CC	CC	BC	DD	BB	BB	CC	
12	BB	BB	BB	CD	BB	BB	BB	DE	BB	CC	CD	BC	CC	BB	BB	CC	
13	BB	BB	CC	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
14	BC	BB	BC	DD	BB	BB	BB	BD	BB	BC	CC	BC	CC	BB	BB	CC	
15	BB	BB	BB	DD	BD	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
16	BB	BB	BB	CC	BB	BB	BB	DE	BB	CD	CC	BC	CC	BB	BB	CC	
17	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
18	BB	BB	BB	DD	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
19	BB	BB	BB	CC	BB	BB	BC	DE	BB	CC	CC	BC	CC	BB	BB	CC	
20P	AB	BB	BC	CD	BB	BB	BB	DE	BB	CC	BC	AB	CC	BB	BB	CC	
21	CC	BB	BB	CD	BB	BB	BC	EE	BB	CC	CC	BC	CC	BB	BB	CC	
22P	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
23	BC	BB	BB	CC	BC	BB	BB	DE	BB	BC	CC	BB	CC	BB	BB	CC	
24	BB	BB	BC	DD	BD	BB	BB	DE	AB	CC	CC	BC	CC	BB	BB	CC	
25	BB	BB	CC	DD	BC	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
26	BC	BB	BB	CC	BC	BB	BC	DE	BB	CC	CC	BC	CC	BB	BB	CC	
27	BC	BB	BB	CD	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
28	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
29	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
30	BB	BB	BB	DD	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
31	CC	BB	BB	CC	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	

32	BB	BB	CC	CD	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
33	BB	CC	BC	CC	BC	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
34	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
35	BB	AB	BB	CC	BB	BB	BB	DE	BB	CD	CC	BC	CC	BB	BB	CC
36	BC	BB	BB	CD	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
37P	CC	BB	BB	DD	BB	BB	BB	DD	BB	CE	CC	AB	CC	BB	BB	CC
38P	BB	BB	CC	CC	BB	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
39	BB	BB	BB	DD	BB	BB	BB	BE	BB	CC	CC	BC	CC	BB	BB	CC
40	BB	BB	BB	CD	BB	BB	BB	BE	BB	CC	CD	BC	CC	BB	BB	CC
41	BB	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
42	BC	BB	BB	DD	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
43	BB	BB	BC	CD	BB	BB	BB	DD	BB	CE	CC	BC	CC	BB	BB	CC
44	BB	BB	CC	CD	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
45P	BB	BB	BB	CC	DD	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
46	BB	BB	BB	DD	BC	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC

NEXT

MP3	600M	HALEAKALA		1	1											
01	CC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
02	BC	BB	BB	BC	BB	BB	BB	EE	BC	CC	CC	BB	CC	BB	BB	CC
03	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
04	CC	BB	BC	CC	BC	BB	BB	DD	BC	CC	CC	AB	CC	BB	BB	CC
05	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
06P	CC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
07P	CC	BB	BB	BC	BB	BB	BB	DD	BB	CD	CD	BB	CC	BB	BB	CC
08	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
10	BC	BB	BB	AC	BB	BB	BB	DE	BB	CC	BC	BC	CC	BB	BB	CC
11	BB	BB	BB	AC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
12	BB	BB	BB	BC	BB	BB	BB	DE	AB	CC	CC	BB	CC	BB	BB	CC
13P	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CD	BC	CC	BB	BB	CC
14P	BB	BB	BB	BC	BB	BB	CC	DE	BB	DD	CC	BB	CC	BB	BB	CC
15P	BB	BB	BB	BB	BB	BB	BB	DE	AB	CC	CC	BC	CC	BB	BB	CC
16	BC	BB	BB	BB	BB	BB	BB	CD	BB	CC	CD	BB	CC	BB	BB	CC
17	BC	BB	BB	BC	BB	BB	BB	DE	BB	CE	CC	BB	CC	BB	BB	CC
18	BC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
19	CC	BB	BB	BB	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
20	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	BB	BB	BB	BB	BB	BB	BB	DE	BB	CC	CD	BC	CC	BB	BB	CC
22	BB	BB	BB	CC	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	BB	BB	AC	BB	BB	BB	DE	AB	CC	CC	BB	CC	BB	BB	CC
24	CC	BB	BB	BB	BC	BB	BB	CD	BB	CC	CD	BB	CC	BB	BC	CC
25P	CC	BB	BB	BB	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
26P	BB	BB	BB	CC	BD	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
27	CC	BB	BB	BB	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC
28	BB	BB	BB	BC	BB	BB	BC	DD	BB	CD	CD	BC	CC	BB	BB	CC
29	BB	BB	BB	BB	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
30	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
31	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CD	BB	CC	BB	BB	CC
32	BC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CD	BC	CC	BB	BB	CC
33P	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
34	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
35	BB	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC

36	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
37	BB	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
38	BB	BB	CC	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
39	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	AB	CC	BB	BB	CC
40	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
41	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
42	BB	BB	BB	AC	BB	BB	BB	DE	AB	CC	BC	BB	CC	BB	BB	CC
43	BC	BB	CC	CC	DD	BB	BB	DD	BB	CC	CD	AB	CC	BB	BB	CC
44	BB	BB	BB	CC	DD	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC

NEXT

MP4	800M	HALEAKALA		1	1											
01	BB	BB	BB	BC	BB	BB	BB	CC	BB	AC	CC	BB	CC	BB	BB	CC
02	BB	BB	BB	AA	BC	BB	BB	DE	BB	CC	CC	BB	CC	AB	BB	CC
03	BC	BB	BC	CC	BB	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	CC
04	BC	BB	BB	CC	BC	BB	BB	DD	BB	EE	CC	BB	CC	BB	BB	CC
05	BC	BB	BB	CC	BC	BB	BB	DD	BB	EE	CC	BB	CC	BB	BB	CC
06	BC	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
07	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
08	BB	BB	BB	AA	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	CC	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
10	BB	BB	BB	AC	BB	BB	BB	CC	BB	CD	BC	BB	CC	BB	BB	CC
11	BC	BB	BB	AA	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
12	BC	BB	BB	CC	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
13	BB	BB	BB	AC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
14P	BC	BB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
15	BB	BB	BB	AA	BC	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
16	BB	BB	BB	AA	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
17	BB	BB	BB	CC	BB	BB	BB	DD	BB	AA	CC	BB	CC	BB	BB	CC
18	BC	BB	BB	AA	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
19	BC	BC	BC	CC	BC	BB	BB	CE	BB	CE	CC	BB	CC	BB	BB	CC
20	BC	BB	BB	CC	BB	BB	BB	CE	CC	CE	CC	BB	CC	BB	BB	CC
21	BC	BB	BB	AA	BC	BB	BB	DD	BB	CE	CC	BB	CC	BB	BB	CC
22	BB	BB	BB	AC	BB	BB	BB	CC	BB	DD	CC	BB	CC	BB	BB	CC
23	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	AB	BB	CC
24	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
25	BC	BB	BB	CC	BC	BB	BB	CE	AB	CC	CC	BB	CC	BB	BB	CC
26	CC	BB	BB	AA	CC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
27	BC	BB	BB	CC	BC	BB	BB	DD	BB	CE	CC	BB	CC	BB	BB	CC
28	BC	BB	BB	AC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
29	CC	BB	BC	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
30	BC	BB	BB	CC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
31	BC	BB	BB	CC	BB	BB	BB	CE	BB	CD	CC	BB	CC	BB	BB	CC
32	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
33	BB	BB	BB	AA	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
34	BB	BB	BB	AA	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
35	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
36	BC	BB	BB	CC	BB	BB	BB	CD	BB	BC	CC	BB	CC	BB	BB	CC
37	BC	BB	BC	AA	BC	BB	BB	CD	BC	CE	CC	BB	CC	BB	BC	CC
38	BC	BB	BC	CC	BC	BB	BB	DE	BB	CE	CC	BB	CC	BB	BB	CC
39	BC	BB	BD	AC	BB	BB	BB	DE	BB	CC	CC	BB	CC	AB	BB	CC
40	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC

NEXT

MP5	1000M	HALEAKALA	1	1													
01	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
02	BB	AB	BB	CC	BB	BB	BB	CE	BB	CC	CD	BB	CC	BB	BB	CC	
03	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
04	CC	BB	BB	CC	BB	BB	BB	DD	BB	BC	CD	BB	CC	BB	BB	CC	
05	CC	BB	BB	CC	BB	BB	BB	DE	BC	CD	CC	BB	CC	BB	BB	CC	
06	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
07	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC	
08	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
09P	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
10P	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
11	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
12	BB	BB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BC	CC	
13	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
14	BB	BB	BB	CC	BB	BB	BB	DE	BC	CC	CC	BC	CC	BB	BB	CC	
15	CC	BB	BB	AC	BB	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC	
16	BB	BB	BB	BC	BB	BB	BB	CD	BB	BC	CC	BB	CC	BB	BB	CC	
17	BB	BB	BB	CC	CC	BB	BC	DE	BB	BC	CC	BC	CC	BB	BB	CC	
18	BB	BB	BB	BC	BB	BB	BB	DE	BC	CC	CC	BC	CC	BB	BB	CC	
19P	CC	BB	BB	CC	BD	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
20	AB	BB	BB	CC	BB	BB	BB	DE	BB	BC	CC	BB	CC	BB	BB	CC	
21	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	CC	CC	BB	BB	CC	
22	CC	BB	BB	CC	BB	BB	BB	CC	BC	CC	CC	BB	CC	BB	BB	CC	
23	CC	BB	BB	AC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
24P	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
25	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
26	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
27	CC	BB	BB	CC	DD	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
28	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
29	BB	BB	BB	CC	CC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
30	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
31	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
32	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
33	BB	BB	BB	CC	BB	BB	BB	CC	BB	BD	CC	BB	CC	BB	BB	CC	
34	BB	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC	
35	BB	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BC	CC	BB	BB	CC	
36	CC	BB	BB	AC	BB	BB	BB	EE	BB	CC	CD	BB	CD	BB	BB	CC	
37	BB	BB	BB	CC	BD	BB	BB	DD	BB	BC	CC	BB	CC	BB	BB	CC	
38P	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
39	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CD	BB	CC	BB	BB	CC	
40	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
41	BB	BB	BB	CC	BB	BB	BB	DD	AB	CC	CD	BB	CC	BB	BB	CC	
42	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
43P	BB	BB	BB	CC	BB	BB	BB	DD	BB	BC	CD	BB	CC	BB	BB	CC	
44P	BC	BB	BB	BB	BD	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
45P	BB	BB	BB	CC	BD	BB	BB	BB	BB	CC	CC	BB	CC	BB	BB	CC	
NEXT																	
MP6	1200M	HALEAKALA	1	1													
01	BC	BB	BB	BB	BB	BB	BB	DE	BB	CE	CC	BC	CC	BB	BB	CC	
02	CC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
03	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
04	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC	

05	CC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
06	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	AA	CC
07	CC	BB	BB	BC	BB	BB	BB	DD	BC	AC	BB	BB	CC	BB	BB	CC
08	CC	BB	BB	BC	BB	BB	BB	CC	CC	CC	BC	BB	CC	BB	BB	CC
09	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
10	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
11	CC	BB	BB	CC	BB	BB	BB	DD	BC	AC	CC	BB	CC	BB	BB	CC
12	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
13	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
14	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
15	CC	BB	BB	BC	BB	BB	BB	CC	BC	CC	CD	BC	CC	BB	BB	CC
16P	BB	BB	BB	BB	BB	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
17	CC	BB	BB	BB	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
18	BC	BB	BB	BB	BB	BB	BB	EE	BB	CC	CC	BC	CC	BB	BB	CC
19P	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CD	BC	CC	BB	BB	CC
20	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	BB	BB	BB	BC	BB	BB	BB	BD	BB	CC	CC	BB	CC	BB	BB	CC
22	BC	BB	BB	BC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
23	BC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	BC	BB	BB	BB	DD	AB	CC	BC	BB	CC	BB	BB	CC
25	BB	BB	BB	BC	BB	BB	BB	CD	BC	CC	BC	BB	CC	BB	BB	CC
26	BC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC
27	CC	BB	BB	BC	BB	BB	BB	CD	BC	CC	CC	BC	CC	BB	BB	CC
28	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC
29P	CC	BB	BB	BC	BB	BC	BB	DD	BB	CC	BC	BC	CC	BB	BB	CC
30	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC
31	BC	BB	BB	BC	BB	BB	BB	DE	BB	CE	CC	BB	CC	BB	BB	CC
32	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
33	CC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
34	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
35	CC	BB	BB	BC	BB	BB	BB	EE	BC	CC	CC	BB	CC	BB	BB	CC
36P	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
37P	CC	BB	BB	CC	BB	BB	BB	EE	BB	CE	CC	BB	CC	BB	BB	CC
38	BC	BB	BB	AB	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
39	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
40	BC	BB	BB	BC	BB	BB	BB	EE	BB	AE	CC	BB	CC	BB	BB	CC
41	CC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
42P	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC
43P	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
44	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
45	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC

NEXT

MP7 1400M HALEAKALA

1 1

01P	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CD	BC	CC	BB	BC	CC
02	CC	BB	BB	AC	BB	BB	BB	CC	BC	CC	CD	BC	CC	BB	BB	CC
03	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	BB	BB	CC	BB	BB	CC
04	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
05	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
06	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
07	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
08	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
09	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	BC	BB	CC	BB	AB	CC

10	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BC	CC	BB	BB	CC
11	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
12	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
13	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BC	CC	BB	BB	CC
14	CC	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
15	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
16	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CD	BC	CC	BB	BB	CC
17P	CC	BB	BB	BC	BB	BB	BB	CD	BB	EE	CD	BC	CC	BB	BB	CC
18	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BC	CC
19	BC	BB	BB	CC	BB	BB	BB	BB	BC	CC	BC	BC	CC	BB	BB	CC
20	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	BC	BC	CC	BB	BB	CC
21P	CC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
22P	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
23	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
25	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BC	CC	BB	BB	CC
26	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
27	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
28	BC	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
29	CC	BB	BB	BC	BB	BB	BB	DD	AB	CE	CC	BB	CC	BB	BB	CC
30	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BC	CC	BB	BB	CC
31	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
32	CC	BB	BB	AC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
33	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	BC	BC	CC	BB	BB	CC
34	CC	BB	BB	BC	BB	BB	BB	CD	BC	CE	CC	BB	CC	BB	BB	CC
35	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
36P	CC	BB	BB	CC	BB	BB	BB	CC	BB	CE	CC	BB	CC	BB	BB	CC
37P	BB	BB	BB	BB	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
38P	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
39	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
40	CC	BB	BB	CC	BB	BB	BB	CD	BB	CE	CC	BB	CC	BB	BB	CC
41P	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BC	CC	BB	BB	CC
42P	CC	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
43	CC	BB	BB	CC	BB	BB	BB	DD	BB	CE	CC	BC	CC	BB	BB	CC
44	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
45	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC

NEXT

MP8	1600M	HALEAKALA			1	1										
01	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
02	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
03	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
04P	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
05	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
06	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
07	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
08	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	AC
09	BC	BB	BB	CC	BB	BB	BB	DF	BB	CC	CC	BB	CC	BB	BB	CC
10	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
11	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
12	BB	BB	BB	BC	BB	BB	BB	EF	BB	CC	CC	BB	CC	BB	BB	CC
13	BB	BB	BB	BC	BB	BB	BB	BD	BB	CC	CC	BB	CC	BB	BB	CC
14	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC

15P	CC	BB	BB	CC	BB	BB	BC	CD	BB	CD	CC	BB	CC	BB	BB	CC
16	CC	BB	BB	CC	BB	BB	BB	CD	BB	AC	CC	BB	CC	BB	BB	CC
17P	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
18P	CC	BB	BB	BC	BB	BB	BB	CD	BB	CD	CC	BB	CC	BB	BB	CC
19	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
20	BB	BB	BB	CC	BB	BB	BC	EE	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
22	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
23	BC	BB	BB	CC	BB	BB	BB	CD	BB	BC	CC	BB	CC	BB	BB	CC
24	CC	BB	BB	CC	BB	BB	BB	DF	BB	CC	CC	BB	CC	BB	BB	CC
25	BC	BB	BB	BC	BB	BB	BB	CD	BB	CD	CC	BB	CC	BB	BB	CC
26	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CD	BB	AB	CC
27P	BC	BB	BB	CC	BB	BB	BB	AE	BB	CC	CC	BB	CC	BB	BB	CC
28	BB	BB	BB	CC	BB	BB	BB	DF	AB	CC	CC	BB	CC	BB	BB	CC
29	BC	BB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
30	CC	BB	BB	BC	BB	BB	BB	BC	BB	CC	CC	BB	CC	BB	BB	CC
31	BC	BB	BB	CC	BB	BB	BB	CD	BB	CE	CC	BB	CC	BB	BB	CC
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34	BC	BB	BB	CC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
35	BC	BB	BB	BC	BB	BB	BB	CD	BB	AC	BC	BB	CC	BB	BB	CC
36	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
37	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	AA	CC
38P	BC	BB	BB	CC	BB	BB	BB	CE	AB	CC	CC	BB	CC	BB	BB	CC
39P	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	AB	CC
40	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
41	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
42	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
43	BB	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
44P	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
45	CC	BB	BB	CC	BB	BB	BB	CD	BB	CD	CC	BB	CC	BB	BB	CC
NEXT																
MP9	1800M	HALEAKALA							1		1					
01	CC	BB	BB	AA	BB	BB	BC	BD	BB	CC	CC	BB	CC	BB	BB	CC
02	BC	BB	BB	BC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
03	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
04	BC	BB	BC	BC	BB	AB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
05	CC	BB	BB	EB	BB	BB	BB	DD	BB	AC	BC	BB	CC	BB	BB	CC
06P	BB	BB	BB	EB	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
07	BC	BB	BC	BC	BB	BB	BB	BE	AB	CC	CC	BB	CC	BB	BB	CC
08	BC	AA	BB	BC	BB	BB	BB	DE	BB	CD	CC	BB	CC	BB	BB	BC
09	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
10	BB	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
11	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
12	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
13	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	BC
14	CC	BB	BB	CC	BB	BB	BC	BC	BB	CC	CC	BB	CC	BB	BB	CC
15	CC	BB	BC	BB	BB	BB	BC	CD	BB	CC	CC	BB	CC	BB	BB	CC
16	BC	BB	BB	BB	BB	BB	BB	CD	BB	CE	CC	BB	CC	BB	BB	CC
17	CC	BB	BB	BB	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
18	CC	BB	BB	BB	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	BC
19	BB	BB	BB	BB	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC

20	CC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CD
21	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CD	BB	BB	CC
22	BB	BB	BC	CC	BB	AB	BB	CF	BB	CC	BB	BB	CC	BB	BB	CC
23	CC	BB	BB	CC	BB	BB	BB	BD	BB	CC	CC	BB	CC	BB	BB	BC
24	BB	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
25	CC	BB	BB	CC	BB	BB	BB	EF	BB	CC	CC	BB	CC	BB	BB	CC
26	CC	BB	BB	BC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
27	CC	BB	BB	BC	BB	BB	BB	BE	BB	CC	CC	BB	CC	BB	BB	CC
28	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
29	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
30P	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
31	BC	BB	BB	CC	BB	BB	BB	DD	BB	AC	CC	BB	CC	BB	BB	CC
32	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
33	CC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	BC
34	BB	BB	BB	CC	BB	BB	BB	FF	BC	CC	CC	BB	CC	BB	BB	CC
35	BC	BB	BB	AB	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
36	CC	BB	BB	CC	BB	BB	BB	FF	BB	CC	CC	BB	CC	BB	BB	CC
37P	CC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CD
38	BB	BB	BB	CC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
39	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
40	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
41P	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
42	BC	BB	BB	BC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
43	CC	BB	BB	CC	BB	BB	BB	BC	BB	CC	CC	BB	CC	BB	BB	CC
44	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
45P	CC	BB	BB	CC	BB	BB	BB	BD	BB	CC	BC	BB	CC	BB	BB	CC

NEXT

MP10	2000M	HALEAKALA		1	1											
01	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
02	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
03	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
04	BB	BB	BB	CC	BC	BB	BB	DD	BB	CE	BC	BB	CC	BB	BB	CC
05	BC	BB	BB	CC	BC	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
06	CC	BB	BB	BB	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
07	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
08G	BB	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09G	AC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
10	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
11	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
12	BB	BB	BB	CC	BB	BB	BB	DD	BB	CE	BC	BB	CC	BB	BB	CC
13G	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
14G	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
15	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
16	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
17	BB	BB	BB	CC	BC	BB	AB	DE	BB	CC	BB	BB	CC	BB	BB	CC
18	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
19	BC	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
20	BB	BB	BB	CC	BB	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BC	BC	BB	BB	BB	DD	BB	AC	BC	BB	CC	BB	BB	CC
22	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
23	BC	BB	BC	CC	BC	AB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
24	CC	BB	BB	CC	AB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC

25	BC	BB	BB	BB	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
26	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
27	BB	BB	CC	BB	BC	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
28	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
29	BB	BB	BB	CC	BC	AB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
30G	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
31G	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
32G	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
33	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
34	CC	BB	BB	BC	BB	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
35	BB	BB	CC	CC	AB	BB	BB	DD	BB	AC	CC	BB	CC	BB	BB	CC
36	BC	BB	BB	AC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
37	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
38G	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
NEXT																
MP11	1950M	KOOLAU	GAP						1		1					
01	BB	CC	BB	DD	BC	BB	BB	CD	BC	CC	BC	BB	BB	BB	BB	BC
02	BC	BC	BC	DD	BC	AB	BB	EE	BC	CC	BC	BB	CC	BB	BB	BC
03	BC	BB	BC	DD	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
04	BB	BB	BB	DD	BC	BB	BB	DD	BC	CC	CC	BB	BB	BB	BB	BC
05	BC	BB	BB	DD	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
06	BB	BB	BB	DD	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
07	BB	BB	BB	DD	BC	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
08	CC	BB	BB	AA	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
09	BB	BC	BC	CC	BC	AB	BB	CC	BB	CC	CC	BB	CC	BB	BB	BC
10	CC	BB	BC	DD	BC	BB	BB	CC	BC	CC	CC	BB	CC	BB	BB	AC
11	BC	BB	BB	CC	BC	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
12	BC	BB	BB	BD	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
13	CC	BB	CC	AC	BB	BB	BB	CE	BB	CC	BC	BB	CC	BB	BB	CC
14	BC	BB	BB	DD	CC	AB	BB	CD	BC	CC	BC	BB	CC	BB	BB	BC
15	AB	BB	CC	DD	CC	BB	BB	DD	BB	AC	BC	BB	CC	BB	BB	CC
16	BC	BB	BB	DD	CC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
17	BB	BC	CC	CC	BC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
18	CC	BB	BB	DD	CC	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	BC
19	BC	BB	BB	CC	BC	BB	BB	CD	BB	CC	BB	BB	CC	BB	BB	CC
20	BC	BC	BC	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BC	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
22	BC	BB	BC	DD	CC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	BC	BC	CC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	BC
24	BC	BB	BC	CC	CC	AB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
25	BB	BC	BB	CC	CC	AB	BB	CD	BC	CC	CC	BB	CC	BB	BB	BC
26	BB	BC	BB	CC	CC	AB	BB	CD	BB	CC	BB	BB	CC	BB	BB	CC
27	BC	BB	BB	DD	CC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
28	BB	BB	BB	DD	CC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
29	CC	BB	BB	DD	CC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	BC
30	BB	BB	BC	CC	BC	BB	BB	CC	BC	CC	CC	BB	CC	BB	BB	BC
31P	CC	BB	BB	CC	BC	BB	BB	CF	BC	CC	CC	BB	CC	BB	BB	BC
32W	BC	BB	BB	DD	CC	AB	BB	CD	CC	CC	BC	BB	CC	BB	BB	CC
33P	CC	BB	BC	DD	BC	BB	BB	AD	BB	CC	CC	BB	CC	BB	BB	CC
34P	BC	BB	BC	DD	BC	AB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
35W	BC	BC	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
36W	CC	BB	BC	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	BB

37W	BC	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
38W	CC	BB	BB	CC	BB	BB	BB	AD	BC	CC	CC	BB	CC	BB	BB	BC
39	BC	BB	BB	AC	BC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
40	BC	BB	BC	CC	BC	BB	BB	AD	BB	CE	BC	BB	CC	BB	BB	CC
NEXT																
MP12	1950M	EXTRA							1	1						
01	BC	BB	BB	AA	CC	BB	BB	CD	BB	CC	BC	BB	CC	BB	BB	CC
02	CC	BB	BB	BB	CC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
03	BC	BB	BB	BC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
04	CC	BB	BB	CC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
05	BC	BB	BB	BB	CC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
06	BC	BB	BB	BC	BC	BB	BB	CC	BB	CC	BC	BB	CC	BB	BB	CC
07	CC	BB	BB	BC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
08	BC	BB	BB	AC	BC	BB	BB	CC	BC	AC	CC	BB	CC	BB	BB	CC
09	AB	BB	BC	BC	BC	BB	BB	CD	BC	CE	BB	BB	CC	BB	BB	BC
10	CC	BB	BC	BC	BC	BB	BB	DD	AB	CC	CC	BB	CC	BB	BB	CC
11	BC	BB	BB	BC	BC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
12	CC	BB	BB	BC	BC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
13	BC	BB	BB	BC	BC	BB	BB	CC	BC	DD	BC	BB	CC	BB	BB	CC
14	BB	BB	BB	BC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
15	CC	BB	BB	AC	BC	BB	BB	DD	CC	AC	BB	BB	CC	BB	BB	BC
16	BC	BB	BB	BC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
17	BC	BB	BB	CC	BC	BB	BB	DD	BC	CC	BC	BB	CC	BB	BB	CC
18	BC	BB	BB	BB	BC	BB	BB	CD	BB	CD	BB	BB	CC	BB	BB	CC
19P	AC	BB	BB	CC	CC	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	CC
20P	CC	BB	BB	CC	CC	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
21P	BB	BB	BB	CC	CC	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	BC
22P	BB	BB	BB	BC	CC	BB	BB	CD	BC	CC	BC	BB	CC	BB	BB	BC
23P	CC	BB	BB	BC	CC	BB	BB	DE	BC	CC	BC	BB	CC	BB	BB	BC
24P	BB	BB	BB	BC	CC	BB	BB	CD	BC	CC	BC	BB	CC	BB	BB	BC
25P	CC	BB	BB	BC	CC	BB	BB	DD	BB	CD	CC	BB	BC	BB	BB	CC
26	BB	BB	BB	CC	CC	BB	BB	BE	BB	CC	BC	BB	CC	BB	BB	CC
27P	BC	BB	BB	CC	CC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
28P	CC	BB	BB	CC	BC	BB	BB	DD	BC	CC	BC	BB	CC	BB	BB	CC
29P	CC	BB	BB	CC	BC	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
30P	BC	BB	BB	BC	BB	BB	BB	DD	BC	AC	CC	BB	CC	BB	BB	CC
31P	CC	BB	BB	BC	BB	BB	BB	CD	BC	AC	CC	BB	CC	BB	BB	CC
32P	CC	BB	BB	BC	BC	AB	BB	CD	BC	CC	BB	BB	CC	BB	BB	CC
33P	BC	BB	BB	BC	BC	BB	BB	DD	CC	CC	BC	BB	CC	BB	BB	BC
34P	CC	BB	BB	AC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
35P	BC	BB	BB	BC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
36P	BB	BB	BB	BC	BC	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
NEXT																
MP13	1800M	CRATER-1							2	1						
01	BC	BB	CC	CC	BB	AB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
02	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
03	CC	BB	CC	CC	BB	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
04	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
05	CC	BB	BB	CC	AB	BB	BB	DD	BC	CC	BC	BB	CC	BB	BB	CC
06	CC	BB	CC	BC	BB	BB	BB	BC	BB	CC	CC	BB	CC	BB	BB	CC
07	CC	BB	BB	CC	BB	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	BC
08	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC

09	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
10	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
11	BC	BB	BB	AC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
12	BC	BB	BC	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
13	BB	BB	BC	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
14	CC	BB	BB	CC	BB	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
15	BC	BB	BB	CC	BB	BB	BB	BC	BB	CC	CC	BB	CC	BB	BB	BB
16	CC	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	BC
17	BC	BB	BB	BC	BB	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC
18	CC	BB	BB	CC	BB	BB	BB	CC	BC	CD	CC	BB	CC	BB	BB	CC
19	BB	BB	BB	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
20	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
22	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
23	BC	BB	BB	CC	BB	AB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
25	BB	BB	BB	CC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
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27	BC	BB	BC	CC	BB	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
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29	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
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34	BC	BB	BB	CC	BB	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC
35	BC	BB	BB	BC	BB	BB	BB	CE	BB	CC	BC	BB	CC	BB	BB	CC
36	BC	BB	BB	CC	BB	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	CC
37	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
38	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
39	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
40	CC	BB	BB	CC	BB	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	CC
41	CC	BB	BB	CC	BB	BC	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
42G	BB	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
43G	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	BC
44G	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
45G	BC	BB	BB	AC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
NEXT																
MP14	1850M	CRATER-2							2		1					
01	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	BC
02	BB	BB	BB	BC	BB	BB	BB	CE	BB	CC	BC	BB	CC	BB	BB	BC
03	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC
04	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
05	BB	BB	BB	BC	BB	BB	BB	DD	BB	BC	CC	BB	CC	BB	BB	CC
06	BC	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
07	CC	BB	BB	CC	BB	BB	BB	CD	BB	CC	BC	BB	CC	BB	BB	CC
08	BC	BB	BB	BC	BB	BB	BB	DD	BC	CC	BC	BB	CC	BB	BB	CC
09	BC	BB	BB	CC	BB	AB	BB	CC	BC	CC	CC	BB	CC	BB	BB	CC
10	BC	BB	BB	CC	BB	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
11	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
12	BC	BB	BC	CC	BB	BB	BB	DD	BC	CC	BB	BB	CC	BB	BB	CC
13	BB	BB	BB	BC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC

14	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
15	CC	BB	BB	CC	BB	BB	BB	EE	BC	CC	CC	BB	CC	BB	BC	CC
16	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
17	CC	BB	BB	CC	BB	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
18	BB	BB	BB	CC	BB	BB	BB	CC	BC	CC	CC	BB	CC	BB	BB	CC
19	CC	BB	BB	CC	BB	BB	BB	CE	BC	CC	BB	BB	CC	BB	BB	CC
20	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BC	BC
21	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	BB	BB	CC	BB	AB	CC
22	CC	BB	BB	BC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
23	CC	BB	BB	CC	BB	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	BC	BB	CC	BB	BB	CC
25	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
26	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
27	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
28	BC	BB	BB	CC	BB	BB	BB	EE	BC	CC	CC	BB	CC	BB	BB	CC
29	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
30	CC	BB	BB	CC	BB	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC
31	CC	BB	BB	BC	BB	BB	BB	DD	BB	CC	BB	BB	CC	BB	BB	CC
32	CC	BB	BB	BC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
33	CC	BB	BB	CC	BB	BB	BB	CD	BC	CC	CC	BB	CC	BB	BB	CC
34	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
35	CC	BB	BB	BC	BB	BB	BB	DD	BC	CC	BC	BB	CC	BB	BB	CC
36	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
37	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	BC	BB	CC	BB	BB	CC
38	BC	BB	BB	CC	BB	BB	BB	CE	BC	CC	CC	BB	CC	BB	BB	BC
39	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC
40G	BC	BB	BB	CC	BB	BB	BB	DD	BB	BC	BC	BB	CC	BB	BB	CC
41G	BC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
42G	CC	BB	BB	BC	BB	BB	BB	CE	CC	CC	BC	BB	CC	BB	BB	CC
43G	BC	BB	BB	BC	BB	BB	BB	DE	BC	CC	BC	BB	CC	BB	BB	CC
44G	BC	BB	BB	BC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
45G	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
NEXT																
MP15	400M KIPAHULU								3	1						
01	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CD	BB	CC	BB	BB	CC
02	BC	BB	BB	CD	BB	BB	BB	DE	BB	CC	CD	BB	CC	BB	BB	CC
03	CC	BB	BB	CD	BB	BB	BB	DD	AB	CC	CC	BB	CC	BB	BB	CC
04	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
05	BC	BB	BB	BD	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
06	BB	BB	CC	CD	BB	BB	BC	DD	BB	CC	CD	BB	CC	BB	BB	CC
07	BC	BB	CC	CD	BB	BB	BC	EE	AB	CC	CD	BB	CC	BB	BB	CC
08	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	BB	BB	BB	DD	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
10	CC	BB	BB	DD	BB	BB	BC	DD	AB	CC	CC	BB	CC	BB	BB	CC
11	BB	AB	CC	CD	BB	BB	BB	DD	AB	CC	BC	BB	CC	BB	BB	CC
12	CC	BB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
13	BC	BB	BB	CC	BB	BB	BB	EE	BB	CD	CC	BB	CC	BB	BB	CC
14	CC	BB	BB	DD	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC
15	BB	BB	BB	CD	BB	BB	BC	DD	BB	CC	CD	BB	CC	BB	BB	CC
16	BB	BB	BB	CD	BB	BB	BC	DD	BB	CC	CD	BB	CC	AB	BB	CC
17	BC	BB	BB	CC	BB	BB	BB	DD	BC	CC	CC	BC	CC	AB	BB	CC
18	BB	BB	BB	DD	BB	BB	BC	EE	BB	CC	CC	BB	CC	BB	BB	CC

19	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
20	BB	BB	BC	DD	BB	BB	BC	DE	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BB	CD	BB	BB	BB	DD	BB	CD	BC	BB	CC	BB	BB	CC
22	BB	BB	BB	CC	BB	BB	BB	DE	AB	CC	CC	BB	CC	BB	BB	CC
23	CC	BB	CC	CD	BB	BB	BB	DD	AB	CC	CC	BB	CC	BB	BB	CC
24	BB	BB	BB	DD	BB	BB	BC	DE	BB	CC	BC	BB	CC	BB	BB	CC
25	BB	BB	BB	CC	BB	BB	BB	DD	BB	CD	BB	BB	CC	BB	BB	CC
26	BC	BB	CC	DD	BB	BB	BB	EE	AB	CC	CC	BB	CC	BB	BB	CC
27	BB	BB	BB	CD	BB	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC
28	BB	BB	BB	CC	BB	BB	BB	DE	BB	CD	CC	BB	CC	BB	BB	CC
29	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BC	CC	BB	BB	CC
30	BB	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC

NEXT

MP16 600M KIPAHULU

3 1

01	BC	BB	BB	CD	BB	BB	BB	EE	BC	CD	CC	BC	CC	BB	BB	CC
02	BB	BB	BB	DD	BB	BB	BB	DE	BC	CD	BC	BB	CC	BB	BB	CC
03	BB	BB	BC	DD	BB	BB	BB	EE	BB	CC	CD	BC	CC	BB	BB	CC
04	BB	BB	BB	DD	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
05	BB	BB	BB	DD	BB	BB	BB	EE	AB	CD	CC	BB	CC	BB	BB	CC
06	BB	BB	BB	CC	BB	BB	BB	EE	AB	CC	CC	BB	CC	BB	BB	CC
07	BB	BB	BB	CC	BB	BB	BB	DE	AB	CC	CC	BB	CC	BB	BB	CC
08	BB	BB	BB	CD	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	BB	BB	BB	CD	BB	BB	BB	EE	AB	CC	CC	BB	CC	AB	BB	CC
10	BB	BB	BC	CC	BB	BB	BB	DE	BC	CC	CC	BC	CC	BB	BB	CC
11	BB	BB	BC	CD	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
12	BB	BB	BB	CD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
13	BC	BB	BB	CD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
14	BB	BB	BB	CD	BB	BB	BB	DD	BC	CD	CC	BC	CC	BB	BB	CC
15	BB	BB	BB	CC	BB	BB	BB	DE	AB	CD	CC	BC	CC	BB	BB	CC
16	BB	BB	BC	CD	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
17	BB	BB	BB	CC	BB	BB	BB	EE	AB	CC	CC	BB	CC	BB	BB	CC
18	BB	BB	BB	DD	BB	BB	BB	DD	BB	CC	CC	BB	CC	AB	BB	CC
19	AA	BB	AA	BD	BB	AB	BB	DD	BB	CC	CC	CC	CC	BB	BB	BB
20	BC	BB	BC	CD	BB	BB	BB	EE	AB	CC	CC	BC	CC	BB	BB	CC
21	BB	BB	BC	CD	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
22	BB	BB	BC	CD	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	BB	BB	DD	BB	BB	BB	DE	BB	CD	AB	BB	CC	AB	BB	CC
24	BB	BB	BB	CC	BB	BB	BB	CD	BB	CD	CC	BC	CC	BB	BB	CC
25	BB	BB	BB	DD	BB	BB	BB	EE	BB	DD	CC	BB	CC	BB	BB	CC
26	BB	BB	BB	DD	BB	BB	BB	DE	BB	CC	CC	BC	CC	BB	BB	CC
27	BB	BB	BB	CD	BB	BB	BB	DD	AB	CC	CC	BC	CC	BB	BB	CC
28	BC	BB	BB	DD	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
29	CC	BB	BB	DD	BB	BB	BB	DE	BB	CD	AB	BB	CC	BB	BB	CC
30	CC	BB	BB	DD	BB	BB	BB	DE	BB	DD	AB	BB	CC	BB	BB	CC

NEXT

MP17 800M KIPAHULU

3 1

01	BB	BB	BB	CD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
02	BB	BB	BC	CD	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
03	BC	BB	BC	CD	BB	BB	BB	EE	BB	CD	CC	BB	CC	BB	BB	CC
04	BB	BB	BB	DD	BB	BB	BB	EE	AB	CC	CC	BB	CC	BB	BB	CC
05	BB	BB	BC	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
06	CC	BB	BB	CC	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC

07	BB	BB	BB	DD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
08P	BB	BB	BC	DD	BB	BB	BB	DD	BB	CD	CC	BB	CC	BB	BB	CC	
09P	BC	BB	BC	DD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
10	BC	BB	BB	DD	BB	BB	BB	EE	BB	CD	CC	BB	CC	BB	BB	CC	
11	BC	AB	BB	DD	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC	
12	CC	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
13	CC	BB	BC	CC	BB	BB	BB	EE	BB	CD	CC	BB	CC	BB	BB	CC	
14	BB	BB	BC	CC	BB	BB	BB	EE	BB	CD	CC	BB	CC	AB	BB	DD	
15	BB	BB	BC	CC	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
16	BC	BB	BB	CC	BB	BB	BB	CD	BB	CD	CC	BB	CC	BB	BB	CC	
17	BC	BB	BB	CC	BB	BB	BB	DD	AB	CD	BC	BB	CC	BB	BB	CC	
18	BB	BB	BC	CC	BB	BB	BB	EE	BB	CD	BC	BB	CC	BB	BB	CC	
19	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC	
20P	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
21P	BC	BB	BB	CD	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC	
22P	CC	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
23	BB	BB	BC	CC	BB	BB	BB	DE	AB	CC	CC	BB	CC	BB	BB	CC	
24	BC	BB	BB	CC	BB	BB	BB	DE	BB	DD	CC	BB	CC	BB	BB	CC	
25	BB	BB	BB	CC	BC	BB	BB	CD	BB	CD	CD	BB	CC	BB	BB	CC	
NEXT																	
MP18	400M EAST MAUI								4	1							
01	BB	BB	BB	AC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC	
02	BC	BB	BB	AC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
03	CC	BB	BD	CC	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC	
04	CC	BB	BB	CC	BB	BB	BB	CD	BB	CE	CC	BB	CC	BB	BB	CC	
05	CC	BB	BD	CC	BB	BB	BB	DE	BB	CE	CC	BB	CC	BB	BB	CC	
06	BB	AB	BD	CC	BB	BB	BC	DF	BB	CC	CC	BB	CC	BB	BB	CC	
07	AB	BB	BD	CC	BB	BB	BB	CC	BB	CC	BC	BB	CC	BB	BB	CC	
08	BB	BB	BB	AA	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC	
09	BB	BB	BC	CC	BB	BB	BB	FF	BB	CC	CC	BB	CC	BB	BB	CC	
10	BC	BB	BB	AC	BB	BB	BB	DD	BB	CE	CC	BB	CC	BB	BB	CC	
11P	BB	AB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC	
12P	BB	BB	BC	CC	AB	BB	BC	DE	BB	CC	CC	BB	CC	BB	BB	CC	
13P	BC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC	
14	BB	BB	BB	AC	BB	BB	BB	CC	BB	CE	BC	BB	CC	BB	BB	CC	
15	BC	BB	BC	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
16	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
17	BB	BB	BB	CC	AB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC	
18	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
19	BC	BB	BB	BC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
20P	BC	BB	BB	CC	BB	BB	BB	CD	BB	CC	BC	BB	CC	BB	BB	CC	
21P	AB	BB	BC	CC	AB	BB	BC	FF	BB	CC	CC	BB	CC	BB	BB	CC	
22	BC	BB	BB	CC	BB	AB	BB	DF	BB	CE	CC	BB	CC	BB	BB	CC	
23	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC	
24	BB	BB	BD	AA	BB	BB	BC	CC	BB	CC	BC	BB	CC	BB	BB	CC	
25	BB	BB	BC	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
26	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC	
27P	BB	BB	BC	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
28P	CC	BB	BB	BC	BB	BB	BB	EE	BB	CC	BC	BB	CC	BB	BB	CC	
29P	BB	BB	BD	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC	
30P	BB	BB	BD	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC	
31	BB	BB	BB	CC	AB	BB	BB	EE	BB	CC	BC	BB	CC	BB	BB	CC	

32	BC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
33	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
34	BB	BB	BB	AC	BB	BB	BB	DD	BB	CC	CC	BC	CC	BB	BB	CC
35	BC	BB	BB	AC	BB	BB	BB	DD	BB	CE	CC	BB	CC	BB	BB	CC
36	BC	BB	BB	AC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
37	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
38	AB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC

NEXT

MP19	600M	EAST	MAUI			4	1									
01	CC	BB	BB	BC	BB	BB	BB	DE	BC	CD	CC	BB	CC	BB	BB	CC
02	CC	BB	BB	CC	BB	BB	BB	DE	BB	CD	CC	BB	CC	BB	BB	CC
03	BB	BB	BB	CC	BC	BB	BB	DE	BB	CD	CC	BB	CC	BB	BB	CC
04	BB	AB	BB	BC	BB	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
05	BB	BB	CC	BC	BB	BB	BB	DF	BB	CC	CC	BB	CC	BB	BB	CC
06	BC	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
07	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
08	BC	BB	BB	CC	BB	BB	BB	DF	BB	CE	CC	BB	CC	BB	BB	CC
09	BB	BB	BB	CC	BB	BB	BB	DF	BB	CD	CC	BB	CC	BB	BC	CC
10	CC	BB	BB	CC	BB	BB	BB	EE	BC	CC	CC	BB	CC	BB	BB	CC
11	BC	BB	BB	BC	BB	BB	BB	CD	BB	BC	CC	BB	CC	BB	BB	CC
12	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
13	CC	BB	BB	CC	BC	BB	AC	EE	BB	CC	CC	BB	CC	BB	BB	CC
14	BB	BB	BB	CC	BB	BB	BC	CD	AB	CE	CC	BB	CC	BB	BB	CC
15	BB	BB	BC	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
16	BB	BB	BB	BB	BC	BB	BB	CD	BB	CD	BC	BB	CC	BB	BB	CC
17	BB	BB	BB	CC	BC	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
18	BB	BB	BB	BC	BB	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
19	CC	BB	BB	CC	BB	BB	BC	DD	BB	CC	CC	BB	CC	BB	BB	CC
20	BC	AB	BC	BC	BB	BB	BB	FF	BB	DE	CC	BB	CC	BB	BB	CC
21	CC	BB	BB	CC	BB	BB	BC	DD	BB	CC	BC	BB	CC	BB	BB	CC
22	BB	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	BB	BC	CC	BC	BB	BB	DF	BB	CE	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	CC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
25	BB	BB	BB	CC	BC	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
26	CC	BB	BB	CC	BB	BB	BB	CC	BB	CC	CC	BB	CC	BB	BB	CC
27	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
28	BC	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
29	BC	AB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
30P	BB	BB	BB	CC	BB	BB	BB	FF	BB	CC	CC	BB	CC	BB	BB	CC
31P	BC	BB	BC	CC	BC	BB	BB	DF	BB	CE	BC	BB	CC	BB	BB	CC
32P	BB	BB	BC	CC	BB	BB	BB	EE	BB	AC	CC	BB	CC	BB	BB	CC
33P	BC	BB	BB	EE	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
34P	BB	BB	BB	CC	BB	BB	BB	CD	AB	CC	CC	BB	CC	BB	BB	CC
35P	BB	BB	BB	CC	BB	BB	BC	DE	BC	CC	CC	BB	CC	BB	BB	BC
36P	BC	BB	BB	CC	BC	BB	BB	EE	BB	CE	CC	BB	CC	BB	BB	CC
37P	BC	AB	BB	BC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
38P	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
39P	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
40P	BC	BB	BB	CC	BB	BB	BB	DE	BB	CD	CC	BB	CC	BB	BB	CC

NEXT

MP20	800M	EAST	MAUI			4	1									
01	BC	BB	BC	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	BC

02	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
03	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CD	BB	BB	CC
04	CC	BB	BC	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
05	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
06	BC	BB	BB	CC	BB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC
07	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
08	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
10	BB	BB	BB	CC	BC	AB	BB	DE	BB	CD	CC	BB	CC	BB	BB	CC
11P	BB	BB	BC	CC	BC	BB	BB	DD	BB	BC	CC	BB	CC	BB	BB	CC
12P	CC	BB	BB	CC	BC	BB	BB	EE	BB	CC	BC	BB	CC	BB	BB	CC
13P	BC	BB	BB	CC	BC	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
14	BB	BB	BB	CC	BC	BB	BB	DD	BB	CD	BC	BB	CC	BB	BB	CC
15	BC	AB	BB	CC	BC	BB	BB	DD	BB	CC	CD	BB	CC	BB	BB	CC
16	BC	BB	BB	CC	BC	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
17P	BC	BB	BB	CC	BB	BB	BB	CE	BB	CC	CC	BB	CC	BB	BB	CC
18	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
19	BB	BB	BC	CC	BC	BB	BB	DD	BB	AC	BB	BB	CC	BB	BB	CC
20	BB	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	BC	BB	BC	CC	BB	BB	BB	DE	BB	CC	BC	BB	CC	BB	BB	CC
22	BC	BC	BB	CC	CC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	AB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
24	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
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32	CC	BB	BB	CC	BC	BB	BB	DF	BB	CC	CC	BB	CC	BB	BB	CC
33P	CC	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
34	BC	BB	BB	BB	BB	BB	BB	DD	BB	AC	CC	BB	CC	BB	BB	CC
35P	BB	BB	BB	CC	BB	AB	BB	EE	BB	BC	CC	BB	CC	BB	BB	CC
36P	BC	BB	BB	CC	CC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
37P	BC	BB	BB	CC	BC	BB	BC	CC	BB	CC	CC	BB	CC	BB	BB	CC
38P	BB	BB	BB	CC	CC	BB	BB	CE	BB	CC	BC	BB	CC	BB	BB	CC
39P	BB	BB	BC	CC	BB	BB	BB	DE	BB	AC	CC	BB	CC	BB	BB	CC
40P	BC	BB	BB	BC	BB	BB	BB	DE	BB	BC	CC	BB	CC	BB	BB	CC
NEXT																
MP21	1000M	EAST	MAUI					4		1						
01	BC	BB	BC	CC	BC	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	CC
02	BB	BB	BB	CC	BB	BB	BB	CD	BB	CC	CC	BC	CC	BB	BB	CC
03	BC	BB	BB	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
04	CC	BB	BC	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
05	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	AC
06	CC	BB	BB	CC	BC	BB	BB	DD	CC	CC	CC	BB	CC	BB	BB	BC
07	CC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
08	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
09	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
10P	BB	BB	BB	CC	BC	BB	BB	DD	BB	BC	CC	BB	CC	BB	BB	CC
11	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC

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16	BC	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
17	BC	BB	BB	CC	BB	BB	BB	EE	BB	CD	CC	BB	CC	BB	BB	CC
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19	BC	BB	BB	CC	BC	AB	BB	DD	BC	BB	CC	BB	CC	BB	BB	CC
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24	BB	AB	BB	CC	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	CC
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34P	BC	BB	BB	CC	BB	AB	BB	DD	BB	CD	CC	BB	CC	BB	BC	CC
35P	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
36P	BB	BB	BB	CC	BC	AB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
37P	CC	BB	BB	CC	BC	BB	BB	CD	BB	CC	CC	BB	CC	BB	BB	CC
38P	BC	BB	BB	CC	BC	BB	BB	CE	BB	CD	CC	BB	CC	BB	BC	CC
39P	BC	BB	BC	CC	BC	BB	BB	DD	BB	BC	CC	BB	CC	BB	BB	BC
40P	BC	BB	BB	CC	CC	BB	BB	CD	BB	DF	CC	BB	CC	BB	BB	CC
NEXT																
MP22	1200M	EAST	MAUI					4		1						
01	CC	BB	BB	DD	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BB
02	BC	BB	BC	DD	BC	BB	BB	DD	BC	CC	CC	BB	CC	BB	AB	BB
03	CC	BB	BB	CC	BC	BB	BB	DD	BB	CC	BC	BB	CC	BB	BB	BC
04	CC	BB	BB	CC	BC	BB	BB	DE	BC	CC	CC	BB	CC	BB	BB	BC
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09	BC	BB	BC	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	BC
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12	CC	BB	BB	CC	BC	BB	BB	DF	BB	CC	CC	BB	CC	BB	CC	CC
13	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	BC	BB	CC	AB	BB	CC
14P	BB	BB	BB	CC	BC	BB	BB	DG	BB	CC	CC	BB	CC	BB	BC	CC
15	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BC	BC	CC
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21	BB	BB	BB	BC	BC	BB	BB	FF	BB	CC	CC	AB	CC	BB	AB	CC

22P	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BC	CC
23	BC	BB	BB	CC	BC	BB	BB	DG	BC	CC	CC	BB	CC	BB	BC	CC
24	BB	BB	BB	BC	BB	BB	BB	DE	BB	CC	CC	AB	CC	BB	BC	CC
25	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BC	CC
26	BC	BB	BB	CC	BC	BB	BB	DD	BC	CC	CC	BB	CC	BB	BB	CC
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29	BC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	CC	CC
30	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
31P	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
32P	CC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	CC	CC
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38	BB	BB	BB	CC	BC	BB	BB	DF	BC	CC	CC	BB	CC	BB	BB	BC
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40	CC	BB	BB	CC	BB	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
NEXT																
MP23	1400M	EAST	MAUI						4		1					
01	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	AA	CC	BB	BB	CC
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16	BB	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
17	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
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20	BC	BB	BB	DD	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
21	CC	BB	BB	AC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
22	BC	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
23	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	AA	CC	BB	BB	CC
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25	CC	BB	BB	CC	BC	BB	BB	AD	BB	CC	CC	BB	CC	BB	BB	CC
26	BB	BB	BB	CC	BB	BB	BB	DE	BB	CC	CC	BB	CC	BB	BB	CC
27	BB	BB	BB	CC	BC	BB	BB	DE	BB	CC	CC	AA	CC	BB	BB	CC
28	BB	BB	BB	DD	BC	BB	BB	DG	BB	CC	CC	AB	CC	BB	BB	AC
29	BC	BB	BB	CC	BC	BB	BB	DD	BB	CC	CC	BB	CC	BB	BB	CC
30	CC	BB	BB	CC	BB	BB	BB	EE	BB	CC	CC	BB	CC	BB	BB	CC
31	BB	BB	BB	DD	CC	BB	BB	AE	BB	CC	CC	AB	CC	BB	BB	CC

```

32  BB BB BB CC BC BB BB DD BB CC CC BB CC BB BB CC
33  BB BB BB CC BC BB BB DG BB CC CC BB CC BB BB CC
34  BC BB BB CC BC BB BB CC BB CC CC BB CC BB BB CC
35  BC BB BB DD BC BB BB CE BB CC CC BB CC BB BB CC
36  BC BB BB CC BB BB BB DD BB CC CC BB CC BB BB CC
37  CC BB BB CC BB BB BB DD BB CC CC BB CC BB BB CC
38  CC BB BB CC BB BB BB DD BC CC CC BB CC BB BB CC
39  CC BB BB CC BB BB BB AA BB CC CC BB CC BB BB CC
40  BC BB BC CC BC BB BB DD BB CC CC BB CC BB BB CC

```

NEXT

END;

STEP VARIAB:

FULLOUT, PCRIT=2;

END;

STEP HDYWBG:

LEVENE, EXACTP;

END;

STEP SIMDIS:

NEI, ROGERS;

END;

STEP COEFOUT:

BELOW=1, ABOVE=2;

BELOW=3, ABOVE=4;

BELOW=7, ABOVE=8;

END;

STEP HIERARCHY:

NLEVEL=1;

LOCALITIES

POPULATIONS

HALEAKALA

0200M

0400M

0600M

0800M

1000M

1200M

1400M

1600M

1800M

2000M

1950GAP

1950EXTRA

CRATER

POP1

POP2

KIPAHULU

400M
600M
800M
EAST MAUI
0400M
0600M
0800M
1000M
1200M
1400M

END;

STEP SIMAVE:
LEVEL=1, COEF=1;
LEVEL=1, COEF=2;
END;

STEP FSTAT:
OUTPUT=3;
END;

STEP WRIGHT78:
NOHRCHY;
END;

STEP HETXSQ:
SUBDIV=0;
END;

STEP CLUSTER:
COEF=1, COPHEN;
COEF=3;
COEF=7;
END;

STEP DISWAG:
COEF=9, ROOT=1, PLASOUT, ADDCRIT=0, MAXTREE=5, NOOPT;
END;

APPENDIX E

GENOTYPE FREQUENCY DATA FOR DIFFERENT POPULATIONS OF METROSIDEROS SPECIES

The raw data includes different populations of *Metrosideros* species studied on the island of Oahu.

(Individual trees are assayed for four enzymes encoded by six loci.

GENETIC STRUCTURE ANALYSIS OF OHAU POPULATIONS OF OHIA
NOTU=14, NLOC=6, NALL=8 CRT;
(6(1X,A5))
PGI-1 PGM-1 PGM-2 PER-1 LAP-1 LAP-2

STEP DATA:

DATYP=2, NCOL=18, ALPHA;
((6X,9(1X,2A1,1X,I2)) / 6X,9(1X,2A1,1X,I2))
OP1 PUPUKEA-1(RUGOS)
PGI-1 AA:01 BC:01 CC:35 CE:03

PGM-1 AA:01 BB:03 BC:03 BE:05 CC:09 CD:03 CE:10 DE:02 EE:04

PGM-2 AA:02 AB:06 AC:01 BB:20 BC:10 CC:01

PER-1 BB:09 BC:18 CC:12 CD:01

LAP-1 CC:08 CD:01 CE:01 DD:11 DE:01 EE:18

LAP-2 BB:07 BC:21 CC:12

OP2 PUPUKEA-2(DYING)
PGI-1 CC:31 BC:01 AA:01 CE:03

PGM-1 CC:13 CD:03 CE:05 DD:01 EE:04 BC:05 BB:02 BD:01 BE:04
AA:01 DE:01

PGM-2 BB:24 AB:03 BC:10 CC:03

PER-1 CC:12 BC:09 BB:19

LAP-1 DD:22 CD:03 CC:10 EE:05

LAP-2 AA:05 AB:02 BB:05 BC:26 CC:02

OP3 PUPUKEA-3
PGI-1 CC:36 CE:02 AC:02

PGM-1 CC:26 CD:04 CE:02 DD:01 EE:01 BC:02 BB:03 AD:01

PGM-2 BB:35 AB:01 BC:04
 PER-1 CC:19 BC:13 BB:08
 LAP-1 DD:27 CD:01 CC:06 EE:06
 LAP-2 BB:18 BC:13 CC:06 AB:02 AA:01
 OP4 WAINAEA-1
 PGI-1 CC:27 CE:20 CF:01 EE:02
 PGM-1 CC:24 CD:03 CE:04 BC:04 BB:05
 PGM-2 BB:30 AB:01 BC:06 AA:01 BD:02
 PER-1 CC:16 BC:03 BB:01 CD:16 BD:01 DD:13
 LAP-1 DD:27 CD:05 CC:07 EE:01
 LAP-2 BB:16 BC:17 CC:07
 OP5 WAINAEA-2
 PGI-1 CC:11 CE:20 EE:06 CF:01
 PGM-1 GG:02 DD:01 CD:04 BC:04 BB:01 AB:04 CE:03 AC:06 BD:01
 GC:03 AA:01 AD:01 CC:07
 PGM-2 BB:22 AB:09 BC:06 EB:01
 PER-1 CC:29 BC:09
 LAP-1 CC:02 CD:08 DD:28
 LAP-2 BB:30 BC:04 CC:04
 OP6 WAINAEA-3
 PGI-1 CC:16 CE:14 EE:04 CF:01
 PGM-1 GG:01 GD:01 AF:01 BE:01 EF:01 EE:01 DF:01 CD:01 BC:09
 BB:03 AB:02 AC:02 GC:03 AA:02 CC:08 HC:02
 PGM-2 BB:20 AB:10 BC:02 CC:05
 PER-1 BB:02 BC:07 CC:28
 LAP-1 CC:05 CD:02 DD:30
 LAP-2 BB:33 BC:04
 OP7 TANTALUS (TRIMUL)
 PGI-1 CC:23 CE:01 CD:09 DD:02
 PGM-1 CC:14 CD:04 CE:04 DD:03 BC:02 BB:01 BD:04 BE:02 DE:01

PGM-2 BB:24 AB:05 BC:05 AC:01
 PER-1 CC:16 BC:13 BB:05
 LAP-1 DD:14 CD:10 CC:04 EE:01 BB:03 BD:03
 LAP-2 BB:33 BC:02
 OP8 TANTALUS (TRIMUL)
 PGI-1 CC:34 CD:06
 PGM-1 CC:36 CD:01 BC:03
 PGM-2 BB:40
 PER-1 CC:27 BC:12 BB:01
 LAP-1 DD:33 CC:04 BB:03
 LAP-2 BB:31 BC:02 CC:04 AA:03
 OP9 TANTALUS (RUGOSA)
 PGI-1 CC:43 CE:01 DD:01
 PGM-1 CC:11 CD:04 CE:10 EE:05 BC:02 BD:03 BE:06 AA:01 DE:02
 DF:01
 PGM-2 BB:18 AB:01 BC:17 CC:06 AA:02 AC:01
 PER-1 CC:27 BC:01 BB:01 AB:01
 LAP-1 DD:05 DE:01 CC:01 EE:04 CE:01
 LAP-2 BB:04 BC:05 CC:03
 OP10 MAUNALANI
 PGI-1 CC:19 CE:12 EE:01 CD:03
 PGM-1 CC:26 CE:02 EE:01 BC:01 BB:03 BE:01 AD:01
 PGM-2 BB:31 AB:04
 PER-1 CC:21 BC:13 BB:05
 LAP-1 DD:19 CD:06 CC:01 BB:02 BD:04 BC:03
 LAP-2 BB:14 BC:19 CC:02
 OP11 AIEA-1
 PGI-1 CC:25 BC:02 CD:01 DD:02
 PGM-1 CC:19 CD:05 BC:02 BB:01 AA:01 AC:02

PGM-2 BB:29 AA:01
 PER-1 CC:17 BC:11 BB:02
 LAP-1 DD:15 CD:08 BB:01 BD:05 BC:01
 LAP-2 BB:29 AA:01
 OP12 AIEA-2
 PGI-1 CC:20 BC:01 CD:06 DD:02 BB:01
 PGM-1 CC:24 CD:05 DD:01
 PGM-2 BB:27 AB:01 AA:02
 PER-1 CC:15 BC:07 BB:07 CD:01
 LAP-1 DD:22 CD:04 BD:04
 LAP-2 BB:26 AA:04
 OP13 AIEA-3
 PGI-1 CC:21 BC:04 CD:05
 PGM-1 CC:10 CD:08 CE:04 DD:01 EE:01 AA:01 DE:03 AC:01 AB:01
 PGM-2 BB:25 BC:05
 PER-1 CC:14 BC:07 BB:07 CD:02
 LAP-1 DD:17 CD:07 CC:06
 LAP-2 BB:13 AB:11 AA:06
 OP14 AIEA-4
 PGI-1 CC:23 CD:03 DD:03 BB:01
 PGM-1 CC:13 CD:08 CE:02 DD:02 EE:02 BB:01 BE:01 AC:01
 PGM-2 BB:24 BC:06
 PER-1 CC:20 BC:07 BB:03
 LAP-1 CC:05 CD:08 DD:17
 LAP-2 AA:03 AB:09 BB:18
 END;
 STEP VARIAB:
 FULLOUT,PCRIT=2;
 END;

STEP HDYWBG:
LEVENE, EXACTP;
END;

STEP SIMDIS:
NEI, ROGERS;
END;

STEP COEFOUT:
BELOW=1, ABOVE=2;
BELOW=3, ABOVE=4;
BELOW=7, ABOVE=8;
END;

STEP FSTAT:
OUTPUT=1;
END;

STEP WRIGHT78:
NOHRCHY;
END;

STEP HETXSQ:
SUBDIV=0, CONTAB;
END;

STEP CLUSTER:
COEF=2, COPHEN;
COEF=4;
END;

STEP DISWAG:
COEF=9, ROOT=1, PLASOUT, ADDCRIT=0, MAXTREE=5, NOOPT;
END;

APPENDIX F

PHOTOGRAPHIC ILLUSTRATIONS OF ENZYME SYSTEMS

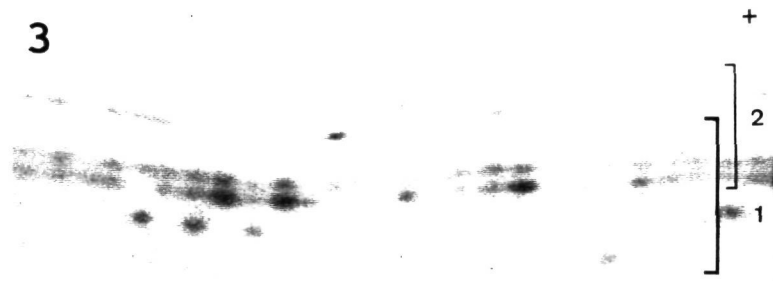
The following are the photographs of gels demonstrating some of the allozymes detected in *Metrosideros* populations:



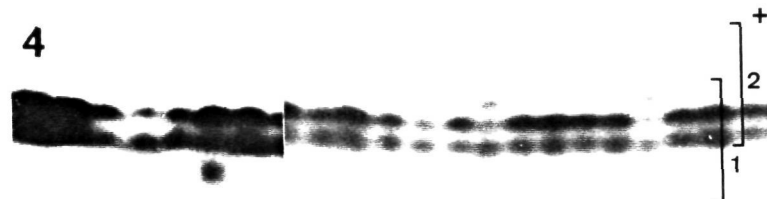
ACO (EC 4.2.1.3) - is a monomer with two loci (overlapping)



ALD (EC 4.1.2.13) - is a tetrameric enzyme with one locus (heterozygotes with 5 bands are crowded and appear smeared)



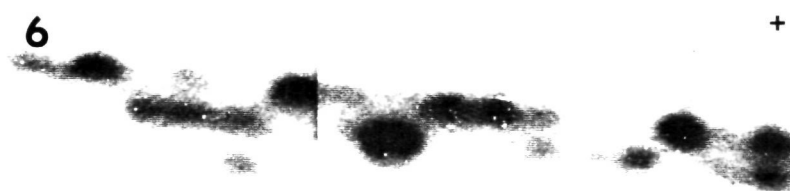
LAP (EC 3.4.11.-) - is a monomer with two loci (sometimes show overlapping)



MDH (EC 1.1.1.37) - is a dimeric enzyme with two loci (overlapping)



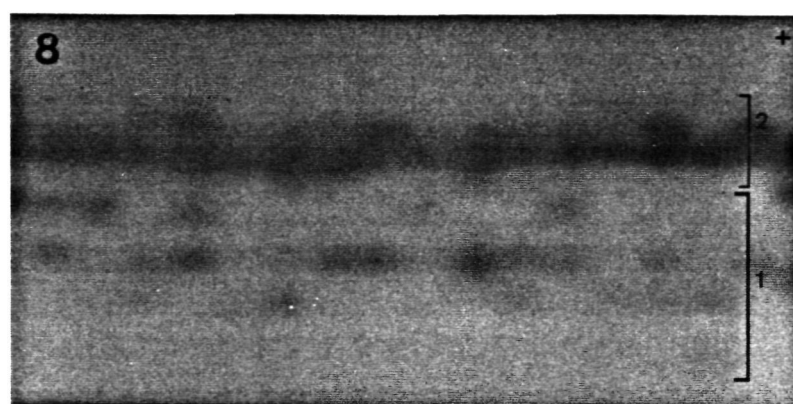
IDH (EC 1.1.1.42) - is a dimer with one locus



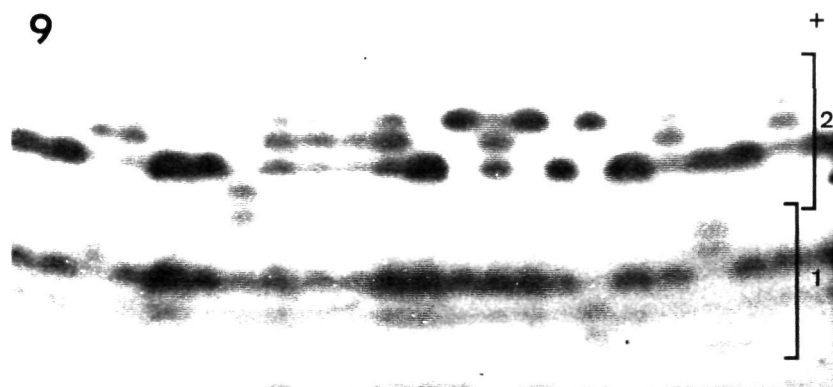
PER (EC 1.11.1.7) - is a monomer with one locus



PGI (EC 5.3.1.9) - is a dimeric enzyme with one locus



PGM (EC 2.7.5.1) - is a monomeric enzyme with two loci (slightly overlapping)



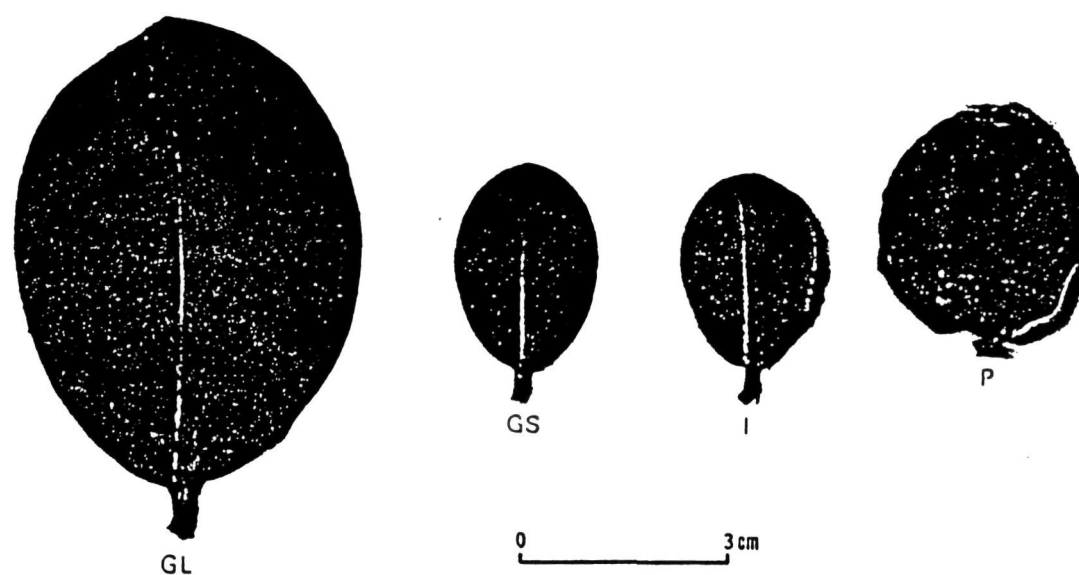
6PGD (EC 1.1.1.44) - is a dimer with two loci (well separated)



SkDH (EC 1.1.1.25) - is a monomer with one locus

APPENDIX G

LEAF VARIATION IN METROSIDEROS POLYMORPHA ALONG THE
ALTITUDINAL GRADIENT ON MT. HALEAKALA



GL: large leaf variant of variety *glaberrima*; GS: small leaf variant of variety *glaberrima*; I: variety *incana*; and P: variety *polymorpha*.

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