MEASURES OF FITNESS AND GENETIC VARIATION IN THE ENDANGERED HAWAIIAN GENUS *HESPEROMANNIA*

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Literature review and thesis proposal

Introduction: Island species at risk

It has been stated that island populations are at greater risk of extinction than those found in continental areas (Frankham 1997, 1998). There are several explanations for this gloomy trend. The most obvious is the greater susceptibility of island endemics to perturbation from alien plants, animals, pathogens, as well as humans. The sensitivity of island taxa to alien species is due to their evolution in isolation from such perturbations as aggressively growing plants, herbivorous animals, etc. (Carlquist 1980). The Hawaiian Islands are home to more endangered species of plants than any other state in the nation. More than one third of the federally listed threatened or endangered plant species in the United States are endemic to the Hawaiian Islands (FWS://ecos.fws.gov/webpage and IUCN www.IUCN.org). Currently there are 282 Hawaiian plant species federally listed as endangered with 10 additional taxa listed as threatened (http://ecos.fws.gov/webpage/).

Other factors that greatly contribute to the risk of extinction for island species are not as readily apparent. In general, endemic island species have fewer populations and the populations are smaller in size than comparable continental species. Small populations are inherent in island endemics due to initial bottleneck events and reduced available habitat upon arrival (Frankham 1998). Rare plants, endemic to islands have an added risk of extinction because of the extremely reduced numbers of individuals and populations. Populations may be severely disintegrated and isolated due to habitat fragmentation and disturbance or decline in the number of individuals. In Hawaii, there

are 32 taxa that are represented by between two and ten wild individuals and 12 taxa whose wild populations consist of a single individual (USFWS 1991, CPC 1992). Specific examples include: *Hibiscadephus woodii* with only 4 individuals left in the wild, and *Cyanea pinnatifida* with a single wild individual remaining (USFWS). There are also several examples of species that are completely extirpated from the wild that survive only in botanical gardens such as *Kokia cookei* that continues to exist only by cuttings grafted on to a related species rootstock (USFWS).

Such small populations put a species at risk for several reasons. First, small populations are more susceptible to demographic and environmental stochastic events (Mills and Smouse 1994; Pimm et al.1988; Lande1999). Environmental occurrences (e.g. periodic drought or hurricane) and demographic stochasitic events (e.g. randomly skewed sex ratios) have a more marked effect when populations are small (Mills and Smouse 1994; Pimm et al.1988). However, there is evidence suggesting that the genetic characteristics of small populations have a greater effect on the survivability of a population than demographic or environmental stochastic events (Newman and Pilson 1997; Frankham 1995,1998). In many cases species fitness can be directly linked to the degree of genetic variation (Charlsworth and Charlsworth 1987; Barrett and Kohn 1991; Ellstrand and Elam 1993). Lammi et al. (1999) found that the smaller the population of a rare orchid, the lower the genetic variability. Small, isolated populations resulting in reduced gene flow among populations are at a greater risk than other endemics for this reason. Low gene flow will cause small populations to be more subject to the effects of inbreeding and genetic drift. Therefore, genetic factors have become the focus of many conservation efforts in theory and in practice in developing management strategies for

various rare species (Barrett and Kohn 1991; Les et al.1991; Ellstrand and Elam 1993; Glover and Abbot 1995; Fischer and Matthies 1997; Newman and Pilson 1997; Matolweni et al.2000; Gemmill et al.1998; Tansley and Brown 2000).

Populations that are small in size are often subject to inbreeding and its associated consequences. Inbreeding is the successive crossing of closely related individuals and resulting in reduced heterozygosity in the progeny. These offspring often experience a reduction in fitness referred to as inbreeding depression. The increased occurrence of homozygosity for rare recessive alleles resulting from inbreeding leads to reduced fitness (and ultimately the loss of adaptability in the face of changing environments) and potential ecological range (Lande 1999). Inbreeding has been proven to decrease fitness both in field and laboratory settings. However, the effects of inbreeding depression are more pronounced in the harsher environmental conditions experienced by wild populations compared to *ex situ* or laboratory specimens (Frankham 1995, 1997).

The decline in fitness associated with inbreeding depression is caused by exposed (i.e., homozygous) deleterious recessive alleles. Although more deleterious alleles such as recessive lethals may be purged via natural selection, the accumulation of mildly deleterious alleles within the population can result in lowered germination and survival rates (Husband and Schemske 1996). The expression of some of these mildly deleterious alleles may not have an effect on fitness until after reproductive maturity causing the perpetuation of harmful genes in succeeding generations (Frankham 1995; Husband and Schemske 1996; Frankham1998). In this light, a species or population might experience a greater extinction threat at the onset of inbreeding because the measure of inbreeding depression decreases over time as the more detrimental alleles are purged through

selection. Husband and Schemske (1996) also found that the degree of inbreeding depression experienced depends on the breeding system. For instance, the magnitude of inbreeding depression in a naturally self-fertilizing species going through prolonged inbreeding is much less than that of a normally outcrossing species that is suddenly faced with inbreeding (Charlsworth and Charlsworth 1987).

Small populations are also often exposed to the effects of genetic drift. Genetic drift is the random fluctuation of allele frequencies that are independent of influences from natural selection (Ellstrand and Elam 1993; Frankham 1998). The role of genetic drift in reducing the genetic variation of a population increases as population size decreases. There will be little or no change in allele frequencies in large populations due to chance events. Genetic bottlenecks are considered severe forms of genetic drift and are may be caused by initial and repeated founder events each time a new island or remote population site is colonized. These random occurrences can cause certain alleles to become fixed or completely removed from a population over successive generations. Over time, the genetic variability of a population would be reduced (Barrett and Kohn 1991). Mildly deleterious alleles have the same chance of being fixed as advantageous alleles in the absence of natural selection. In this circumstance, chance may cause populations to become homozygous for recessive alleles and experience a reduction in fitness (Newman and Pilson 1997). If a substantial portion of these slightly deleterious alleles become fixed, the population could become “genetically inviable” (Lande 1999).

Another consequence of genetic drift is the increase in the genetic differentiation between populations of a species (Ellstrand and Elam 1993; Tremblay and Ackerman 2001). Morden and Loeffler (1999) found that a small and isolated subpopulation of the

endangered Hawaiian mint *Haplostachys haplostachya* was higher in homozygous alleles and was more genetically differentiated than the other larger subpopulations. The isolated population also showed signs of reduced fitness, although the cause is likely a mixture of drift, inbreeding depression, and poor habitat conditions. Reduction of genetic variation and an increase in population differentiation is attributed to genetic drift. A similar situation was found in *Lychnis viscaria* where the smaller, peripheral populations were more genetically differentiated among populations and within population variation was lower than that of larger and more central populations (Lammi et al. 1999). However, there was no detectable decline in fitness for the peripheral populations.

The role of genetics in conservation

We assume most endemic island groups were founded by either a single or very few initial colonizers (Carlquist 1980). Therefore, the effects of genetic drift and inbreeding are compounded when a species or population arises from one or a few founder individuals (Newman and Pilson 1997, Hedrick and Kalinowski 2000). In light of these critical but concealed attributes, the role of genetics in the conservation of rare species has increased and the information gained has become a valuable tool in assessing the management and sustainability of endangered species. Genetic information can be key to understanding a species or population by providing insight into gene flow, population differentiation, hybridization, and also to taxonomic relationships, species distributions and species delineations. The information acquired by genetic studies is consistent with the proposed strategy of most recovery plans to undertake basic biological research (USFWS, CPC 1994). Basic genetic research is lacking for most endangered

Hawaiian plants (CPC 1994). The USFWS (1994) recovery plan for the Wahiawa Plant Cluster states that the knowledge of "genetic variability must be established…to develop effective strategies for enhancing existing populations and establishing new ones."

It is important to collect propagules for germplasm storage and later reintroductions that represent the full range of genetic variation in a particular taxon especially in the case of rare species (Barrett and Kohn 1991, Holsinger and Gottlieb 1991, CPC 1994, Glover and Abbott 1995). Thus, genetic information should be considered in order to collect the samples from appropriate populations. Holsinger and Gottlieb (1991) stated that taxonomic distinctiveness is an important factor for determining conservation priority between rare taxa and hybrids. For instance, Motley (personal communication) found that *Clermontia oblingifolia* ssp. *mauiensis* (Campanulaceae), listed as an endangered in 1992, is actually a hybrid of two sympatric species. If this were the case, it would be acceptable to delist this particular taxa. The level and direction of gene flow and population differentiation within a species could help to determine which populations to collect propagules from. For example, collections of the Hawaiian mint *H. haplostachya* mentioned above (Morden and Loeffler 1999) would be inappropriate from the isolated subpopulation due to the low genetic variation, lower frequency of rare alleles and the reduced fitness of the population compared to the other two populations (Morden and Loeffler 1999). Assessment of the genetic variation in the endangered Mauna Kea silversword, *Argyroxiphium sandwicense* subsp. *sandwicense* (Asteraceae) reveals that there still remains a fair amount of genetic variation among the progeny even though this taxon has undergone a severe bottleneck (Friar et al. 2000). In consequence, reintroduction efforts did benefit from this

knowledge. These studies emphasize the need for genetic sampling before germplasm collections and reintroduction efforts begin.

In addition to collection and propagation, rare taxa also need to be protected in their remaining environments. Genetic information is useful in determining the type of management strategy for such taxa. Tansley and Brown (2000) found that in the endangered South African *Leucadendron elimense* (Proteaceae), small and isolated populations still contained high levels of genetic variability. This genetic information combined with the knowledge of large and long-lived seed banks for this species led Tansley and Brown (2000) to conclude that this species is adapted to survival in small populations. They predicted that *L. elimense* could be managed in a series of small reserves for protection with little cost and management effort as an alternative to large reserve areas. Likewise, Kwon (1999) found that populations of kauila, *Colubrina oppositifolia* and *Alphitonia ponderosa*, contain levels of genetic variation believed to be similar to pre-disturbance levels. These populations are also presumably reduced in size. This type of information can be extremely useful in developing collection protocols.

The genetic consequences associated with small population sizes of rare plants, such as inbreeding depression and genetic drift, can potentially have a large effect on the survivability of a particular population or taxa. Island taxa are particularly at risk due to the nature of insular floras (Carlquist 1980, Frankham 1995). The effects of these genetic conditions are not always obvious and the management of such affected taxa can be difficult to carry out if there are uncertainties about the cause of rarity. For that reason genetic information should play a fundamental part in the conservation and management strategies of rare plant taxa.

Conservation Genetics of the Hawaiian Species of *Hesperomannia*

This study begins with the charge by the US Fish and Wildlife Services (USFWS), Center for Plant Conservation (CPC) and the World Conservation Union (IUCN) to conduct basic biological research into the endangered Hawaiian genus *Hesperomannia,* (Asteraceae: Vernonieae). All three species are rare and locally clustered in their respective locations. Each is listed as endangered species by USFWS and also listed as "Critically Endangered" by the IUCN. The IUCN lists *Hesperomannia arborescens* and *H. arbuscula* as being threatened by continuing decline (i.e. either observed, projected, or inferred) in numbers of mature individuals. The population structures are severely fragmented in that no subpopulation is estimated to contain more than 50 individuals (IUCN website). *Hesperomannia lydgatei* is listed as "Critically Endangered" by the IUCN due to the fact that it is known to exist (significantly) at only a single location and the habitat is in continuing decline (observed, inferred or projected in its area of occupancy; IUCN www.redlist.org). The USFWS (1994, 1995, 1998) lists the current threats to these species as alien plants, feral animals, disease and insect predation, trampling or over collection by humans, and the potential stochastic affects associated with a small number of populations. None of the three species has been successfully propagated by cuttings, and each species is restricted in range although the degree of rarity varies between species.

Taxonomic History

The endemic Hawaiian genus *Hesperomannia* (Asteraceae, Vernonieae) was first described by Asa Gray in 1882. The genus was named after its first collector, Horace

Mann, who discovered a plant on the summit of Lana`i. At the time, there was a genus in the family Simaroubaceae already named *Mannia* in honor of a G. Mann who worked in Africa. As a result, the prefix *hespero,* meaning evening or west, was used to distinguish the honor of this genus in the Western Hemisphere to H. Mann (Gray 1866; Brigham 1868; Wagner et al.1990).

This first species of *Hesperomannia* was named *arborescens* for the arborescent habit of the plant, an uncommon feature for a member of the Asteraceae family. A second species of *Hesperomannia*, collected on West Maui "about 1200' above Lahaina" by E. Bishop was described by Hillebrand (1888) as *H. arbuscula* also in reference to its arboreal habit, but distinguishing it from the former species. Hillebrand (1888) also named a new variety of *arborescens*, var. *oahuensis,* collected in the Wai`anae range on O`ahu. *Hesperomannia lydgatei* was first collected in the Wahiawa/ Kanaele drainage basin on Kaua`i by J. M. Lydgate in 1908 and was named by Forbes after its collector in 1909. The genus *Hesperomannia* was first classified as part of the Asteraceae tribe Mutisieae where it remained even in the most recent systematic treatment of the genus in *The Manual of flowering plants of Hawai`i* (Wagner et al. 1990).

The genus was thought to have originated from one of several South American genera of the same tribe due to similar morphological characters. Kim et al. (1998) found molecular evidence that supports closer affinities to the tribe Vernonieae. More specifically *Hesperomannia* is closely related to a group of African species in the genus *Vernonia* subsection *Strobocalyx*. This makes the origin of the genus unusual among the Hawaiian flora. It is speculated that dispersal likely occurred via southeast Asia and the Pacific island chains west of Hawai`i (Kim et al. 1998).

Degener (1933-1937) revised the genus to include three additional species. *Hesperomannia oahuensis*, the "hairy hesperomannia", was named for the tomentose plants growing in the Wai`anae Range on O`ahu. Degener claimed this species was distinct from Hillebrand's *H. arborescens var. oahuensis* (also in the Waianae Range). He also named two new species, *H. bushiana* (with two varieties, *bushiana* and *fosbergii*) and *H. swezeyi*. The first refers to a small population restricted to Halawa Ridge on O`ahu with slightly smaller flower heads, whereas *H. swezeyi* was a species named for plants growing east of Nu`uanu Pali, in the Ko`olau Mountains of O`ahu.

Carlquist (1957) provided another systematic treatment of the genus based on the anatomy of all species. This treatment divided *H. arbuscula* into two subspecies: subsp. *arbuscula* a sprawling shrub restricted to Maui, with sparse hairs on the undersides of leaves; and subsp. *oahuensis*, the same taxon Hillebrand described as being *H. arborescens* var. *oahuensis* and that Degener referred to as *H. oahuensis*. *Hesperomannia arborescens* was divided into three subspecies: subsp. *arborescens* based only on the specimens collected on Lana`i; subsp. *bushiana* from Halawa ridge, O`ahu; and subsp. *swezeyi* in reference to all other *H. arborescens* on O`ahu.

St. John (1978, 1983) also reviewed the genus. His treatment named a new variety, *H. arbuscula* var. *pearsallii* from O`ahu and a new species, *H. mauiensis* from Iao Valley, Maui. However, the most recent treatment of *Hesperomannia* by Wagner et al. (1990) considered that all varieties and subspecies distinguished previously were artificial divisions and only the original three species distinctions were retained. These are *H. arborescens, H. arbuscula,* and *H. lydgatei.*

Description and distribution of species

Three species of *Hesperomannia* are recognized in the Manual of Flowering Plants of Hawaii (Wagner et al. 1990, 1999); *H. arborescens*, *H. arbuscula*, and *H. lydgatei*. The main characters used to distinguish the three species are presented in Table 1.1.

Hesperomannia arborescens is characterized by having lanceolate to oblanceolate leaves that are 2-4 times as long as wide and being glabrous or very sparsely puberulent along veins and the midrib (Wagner 1990). The leaf margins are often entire, but can also be serrated. The flower heads are erect to ascending flower heads at anthesis and are solitary or in clusters and have four to seven circular series of involucral bracts. This species is found in montane wet forests on sloping, well drained soil around 2000' elevation. It is known from various collections along the Ko`olau mountain range on O`ahu, around the Wailau cliffs on Moloka`i, and was once known from Lana`i where it was first collected by Mann but is now evidently extinct on that island. This is by far the most abundant species of *Hesperomannia*. Populations tend to be locally dense and several populations are regenerating in the wild (personal observation). It is estimated there are ~2000+ mature individuals on O`ahu alone (Joel Lau, Natural Hawaiian Heritage Program, personal communication). However, this species is still considered rare and was first listed as endangered on March 28, 1994 (ecos.fws.gov/webpage/).

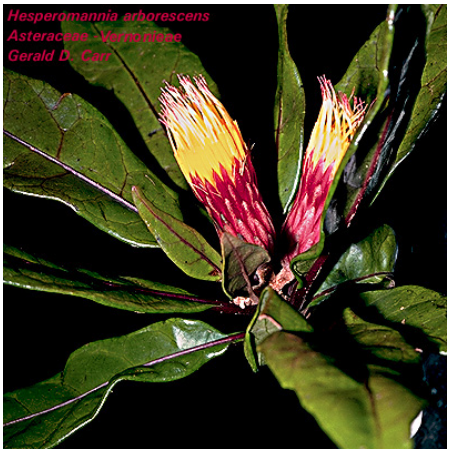

Figure 1.1 *Hesperomannia arborescens*

Table 1.1 Defining characters for the three species of *Hesperomannia*

	H. arborescens	*H. arbuscula*	*H. lydgatei*
Leaves	Lanceolate to oblanceolate (sometimes elliptic); blades 2-4 times long as wide; glabrous or minutely puberulent along midrib	Elliptic to broadly elliptic to elliptic ovate; blades 1-2 times long as wide; adaxial surface glabrous to sparsely pubescent, abaxial surface densely tomentose (sometimes glabrous)	Elliptic ovate to broadly oblanceolate; blades 3-5 times long as wide; glabrous (or nearly so)
Flower Heads	Erect to ascending, 5-7cm high; solitary or in clusters of 2-10	Erect to ascending, 4.5-5.5 cm high; usually in clusters of 4-5	nodding at anthesis; 4-5 cm high; usually in clusters of 4-5
Bracts	In 4-7 series, 2-3.5 cm in length; involucre 2-3.5 cm high	In 4-6 series, 2-2.5 cm in length; involucre 2-2.5 cm high	In 4-5 series, 4-5 cm in length; involucre 4-5 cm high
Pappas	Yellowish brown or tinged purple	Yellowish brown or tinged purple	Yellowish to pale brown

Hesperomannia arbuscula is also characterized by having erect flower heads at anthesis. This species is distinct in having elliptic to elliptic ovate leaves that are1-2 times as long as wide. The adaxial surface is sparsely pubescent and the underside of the leaf is densely tomentose. The margins of the leaves range from entire to serrate to dentate. Flower heads are in clusters of four to five, and individual flower heads have between four and six involucral bract series. According to Wagner et al. (1990), *H. arbuscula* occurs in the Wai`anae Mountain range of O`ahu and in the West Maui mountains in mesic to wet forest above 1000' elevation. This species is the most rare in terms of total numbers of individuals. Previous estimates counted ~80 individuals across the total range of the species. However, this estimate includes individuals found on Maui that are hypothesized to be *H. arborescens* in this study. Excluding individuals found on West Maui, there are fewer than 40 individuals remaining. This species was first listed as endangered on October 29, 1991 (ecos.fws.gov/webpage/).

Figure 1.2 *Hesperomannia arbuscula*.

Hesperomannia lydgatei is the most distinct species in the genus having pendant flower heads at anthesis (Wagner 1990). The leaves are elliptic-obovate to broadly oblanceolate, usually 3-5 times longer than wide, and are glabrous (or nearly so) with

entire margins. Flower heads are in clusters of four to five, and individual heads have four to five involucral bract series. This species is restricted to the island of Kaua`i where it is predominantly found in low wet forest in the Wahiawa/ Kanaele drainage area with a few solitary individuals scattered in other parts of the island. Although very localized, this species has some regeneration in the one large population. This species was listed as listed as endangered on September 20, 1991 (ecos.fws.gov/webpage/).

Figure 1.3 *Hesperomannia lydgatei*.

Purpose of this study

The main purpose of this study is twofold. First, this research will assess the degree of genetic variation within and among representative populations of all three species of *Hesperomannia*. From the degree of population differentiation and the amount of genetic variability within and among populations and species, inferences will be drawn to determine if inbreeding and/or genetic drift are related to the low numbers of individuals and small population sizes. The degree of population genetic differentiation between the three species will also be used to determine species relationships. Second, this basic research should provide enough information to make recommendations for the

conservation and management of *Hesperomannia* species in Hawai`i in regards to their protection, collection, monitoring, and propagation of each species and their respective populations.

Formal Hypotheses

Based on knowledge of the former and current distribution and ranges of each species four formal hypotheses are proposed.

I. Each species of *Hesperomannia* is distinguishable at the genetic level; unique genetic markers distinguish each species.

This hypothesis is supported by two factors: 1) each species is recognized as being a taxonomically distinct entity; 2) each of the three species has a distinct geographic range that does not overlap with the others. Notably, *H. lydgatei* is restricted to the island of Kaua`i where the other two species are not found. Therefore, there are likely to be detectable genetic differences between each of the three species.

II. Each species of *Hesperomannia* possesses low overall genetic diversity.

Each species is rare, consisting of small population sizes with low numbers of individuals. The known species are slow growing trees that are presumably outcrossing. Pollinators have not been observed and may be extinct. Therefore, it is predicted that each population will have low genetic variation, resulting in low overall variation for

each species. Because the populations are small in size and there are few extant populations, and no apparent pollination vector, inbreeding and genetic drift are likely to have occurred. Three secondary hypotheses (one related to each species) are proposed.

a. *Hesperomannia arborescens* populations on different islands are genetically distinct (O`ahu, Moloka`i).

These populations are locally dense, yet low in total numbers of individuals and occur on separate islands. The geographic separation and the apparent lack of a pollinator would suggest that populations on different islands would show some evidence of genetic drift among associated alleles.

b. *Hesperomannia lydgatei* retains considerable genetic variation within the one large remaining population.

Hesperomannia lydgatei is reduced to a single large population in the Wahiawa/ Kanaele Drainage area on Kaua`i. This population consists of ca. 250 individuals (USFWS 1994) with six smaller subpopulations sampled. Each subpopulation may have experienced some effects of genetic drift in allele frequencies and therefore show some degree of differentiation.

c. *Hesperomannia arbuscula* is the species with the least amount of genetic variation.

This species is the most restricted geographically and has the fewest numbers of individuals, ca. <50 individuals. Preliminary observations of pollen fertility revealed very low viability compared to the other two species. This species also has very little to no regeneration/recruitment in the wild whereas populations of the other two species consist of individuals at all stages of growth (personal observation).

III. The West Maui plants referred to as *H. arbuscula* by Wagner et al. (1990, 1999) are genetically distinct from *H. arbuscula* of the Wai`anae Mountains, O`ahu.

The West Maui individuals are currently identified as *H. arbuscula* by Wagner et al. (1991). All other taxonomic treatments had separated Maui individuals from the individuals found in the Waianae Mountains of O`ahu either by species, subspecies, or variety (Degener 1944, Carlquist 1957, St. John 1978). Additionally, individuals collected from Honokohau and Waihe`e populations have nearly glabrous leaves that are narrower compared to the wider, tomentose leaves of *H. arbuscula* in the Wai`anae range. Furthermore, West Maui populations are found in wet forest (as are the *H. arborescens* populations on O`ahu and Moloka`i) in contrast to the drier, mesic habitat of Wai`anae *H. arbuscula* plants. These factors support treatment of the West Maui plants as taxonomically distinct from *H. arbuscula* on O`ahu. I hypothesize that the genetic markers will reinforce the morphological and habitat differences of the West Maui

populations of Honokohau and Waihe`e and support the their treatment as *H. arborescens* rather than *H. arbuscula*.

IV. Pollen viability and seed germination rates are correlated with the amount of genetic variation found in populations.

It is expected that the level of genetic variation will be reflected in the estimated pollen viability and seed germination observations. This assumption is based on the theoretical prediction that lower genetic variation will result in lower fitness (Charlsworth and Charlsworth 1987; Husband and Schemske 1996; Frankham 1995, 1997;Lande 1999). This study will use pollen stainability as an estimation for viability and seed germination observations for measures of fitness.

Materials and Methods

DNA Collections

Leaf material for several populations of the three species of *Hesperomannia* will be collected for DNA extraction. One leaf per accessible individual will be sampled per population. Leaves will be stored and refrigerated until DNA is extracted. Due to the rarity of all three species of *Hesperomannia*, locations will be selected based on the population's accessibility and vulnerability to sampling. Mature individuals will be sampled in each population. When populations are limited in number, juveniles and seedlings will also be sampled. Voucher specimens for populations that are known to

have not been previously collected will be deposited in the Bishop Museum Herbarium (BISH), Honolulu, Hawaii.

DNA Extraction

DNA will be extracted from fresh leaf material using a modified version of the 2% CTAB extraction method (Morden et al. 1996). The extraction buffer consists of: 2% Cety-trimethyl Ammonium Bromide (CTAB), 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20mM EDTA, 2% PVP-40 (polyvinylpyrrolidone 40,000 MW), and 0.2% β-mercaptoethanol (added immediately prior to each extraction). Approximately 1.5-2.0 grams of fresh leaf tissue are added to the extraction buffer and ground in a mortar with pestle (preheated to 60-65^{o}C) and sterile sand. The grindate will be incubated for 15 minutes at 60-65^{o}C with occasional mixing. The DNA will then be extracted with chloroform and centrifuged at 3000 rpm in a Sorvall RT-6000 centrifuge for 10 minutes. The top aqueous phase will be removed to a new tube and gently mixed with an equal volume of 2-propanol (4^{o}C) to precipitate the DNA. The mixture will be incubated at -20^{o}C for at least 30 minutes to further precipitate the DNA. The tubes will be centrifuged (as above) for 5 minutes to pellet the DNA. The pellet will be allowed to dry for approximately 5 minutes and then washed with 5-10 ml wash buffer (76% EtOH and 10 mM NH_4Oac) and centrifuged as above for 5 minutes. The supernatant will be decanted and the pellet air dried for ca. 5 minutes before resuspending in 4.0 ml TE (10 mM Tris-HCl (pH 8.0), 1mM EDTA). The DNA will then be further purified by cesium chloride (CsCl) density gradient centrifugation, where 3.9g CsCl and 50 μl ethidium bromide (EtBr) are added to the tubes (density ca. 1.55 gm/ml) and ultracentrifuged overnight at

55k rpm. DNA bands will be removed with pasture pipettes, EtBr removed with two washes of saturated 2-butanol, and CsCl will be removed by a TE dialysis (Sambrook). All DNA samples will be accessioned into the Hawaiian Plant DNA Library (HPDL), and stored at –20^{o}C (Morden et al. 1996, Randell and Morden 1999).

RAPD Procedure

RAPD reactions will consist of 25 µl reactions containing: ca. 25ng of DNA, 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X *Taq* DNA polymerase buffer (10mM Tris-HCl (pH 9.0 at 25^{o}C), 50 mM KCl and 0.1% Triton X-100; Promega), 1.5mM $MgCl_2$, 0.25 mg BSA, 2 µM random 10-mer oligonucleotide primer (Operon Technologies), and ca. 1 unit of *Taq* DNA Polymerase (Storage Buffer A from Promega). The reactions are then covered with 25 µl of mineral oil. The RAPD-PCR reactions will be performed in a DNA thermocycler. Reaction conditions that have been demonstrated to work previously in similar studies are as follows: an initial denaturation cycle of 94^{o}C for 3 minutes, 35^{o}C for 30 seconds and 72^{o}C for 2 minutes, followed by 43 cycles of 95^{o}C for 45 seconds, 35^{o}C for 30 seconds, and 72^{o}C for 2 minutes. The reaction will end with one final cycle of 95^{o}C for 45 seconds, 35^{o}C for 30 seconds and 72^{o}C for 6 minutes. Operon primers will be screened until there are approximately 20 primers that show clear, reproducible, scorable bands. PCR amplified products will be visualized with EtBr after being electrophoresed on a 1.5% agarose gel. The sizes of the bands will be determined by comparison to a pBS marker (pBS plasmid digested with restriction enzymes *Dra*I, *Pvu*II, and *Kpn*I, to provide a standard range from 0.448 to 2.96 kb.

Because the RAPD technique can be difficult to reproduce (Rieseberg 1996), several precautions must be taken to ensure a dependable product. Each primer for use in the final analyses will be tested for reproducibility with each population. The thermocycler used in the reactions must be reliable and consistent in their performance. The concentration of DNA needs to be standardized and consistent for each reaction to make sure there is no ephemeral amplification. Finally, the reaction components need to be sterile and free of contamination in order to ensure reproducibility.

Pollen Viability Analysis

Pollen will be collected from fresh floral material, when available, for pollen viability analysis. Pollen will be stained with aniline blue in lactophenol to estimate its viability (see Hauser & Morrison 1964). The pollen will be allowed to stain for 24 hrs before viability assessments will be made. Pollen grains stained more deeply (dark blue colored) will be considered viable whereas unstained or lightly stained, or very reduced sized grains will be counted as unviable. Optimally, approximately 300 grains will be counted for each specimen but if fewer are available they will also be counted. The results will be scored as percentages and compared within and among each species. The percent viability of populations and individuals will be used as a measure of fitness to be compared to the results of the genetic variability assessments.

Seed Germination Studies

Immature and mature fruiting heads will be collected from several individuals in each population visited (as available). All seed material will be taken to the Lyon

Arboretum Micropropagation Facility where the immature achenes will be sterilized in a solution of 10% Clorox and Tween 20 for 20 to 30 minutes. Embryos will then be extracted in a sterile hood environment and placed on an agar growth media for germination and growth (Murashige and Skoog 1962). Germination and survivability will be documented. The percent germination per individual and species will be used as an estimate of fitness to be compared to the results of the genetic variability and pollen viability assessments. Any viable seeds or cuttings will be accessioned into the Lyon Arboretum collections.

Chapter II

Measures of Fitness and Genetic Variation in the Endangered Hawaiian Genus *Hesperomannia*

Introduction

Hawaii has been referred to as the endangered species capital of the United States (Royte 1995). Yet, there have only been a handful of studies on the 282 federally listed Hawaiian plant species (USFWS; http://ecos.fws.gov/webpage/). Little is known about population structure or the genetic relationship within and among populations of rare plant species in Hawaii. Many of these species exist only in very reduced or fragmented populations, the explicit consequences of which are largely unknown. However, it is assumed that small populations are at risk of extinction from random stochastic events, either demographic or environmental.

Small populations are also inherently at risk of losing the genetic variation that is essential for a species to adapt to environmental change (Barret and Kohn 1991; Ellstrand and Elam 1993; Frankham 1995; Frankham et al. 2002; Reed and Frankham 2003). Low gene flow among small populations is expected to result in inbreeding and genetic drift. Inbreeding is defined as the successive crossing of closely related individuals resulting in reduced heterozygosity in the progeny. The increased occurrence of homozygosity of recessive alleles in inbred progeny results in a reduction in fitness identified as inbreeding depression (Charlesworth and Charlesworth 1987). Low gene flow among populations is expected to result in genetic isolation that could result in genetic drift. Drift is the random fluctuation in allele frequencies, independent of natural selection (Elstrand and Elam 1993; Frankham 1998), and is more pronounced in smaller

populations. The risk of genetic drift is the eventual loss of genetic variation as alleles become fixed in the population by chance. Any reduction in genetic variation can impair the ability of a species or population to withstand change over time (Lande 1999; Frankham et al. 2002). Thus, genetic information can offer additional insight into the causes of rarity and can enhance plans for rare species recovery. The knowledge of these genetic factors is becoming key in directing conservation efforts of rare species throughout the world (Barrett and Kohn 1991, Les et al. 1991, Ellstrand and Elam 1993, Glover and Abbott 1995, Fischer and Matthies 1997, Newman and Pilson 1997, Gemmill et al. 1998, Tansley and Brown 2000).

This study was intended to provide basic genetic and biological information about the Hawaiian endemic genus *Hesperomannia* A. Gray (Asteraceae, tribe Vernonieae). Basic genetic and biological studies such as this have been suggested by the US Fish and Wildlife Service (USFWS), The Center for Plant Conservation (CPC), and the World Conservation Union (IUCN). There are currently three recognized species in the genus, *H. arborescens* A. Gray, *H. arbuscula* Hillebrand, and *H. lydgatei* C. Forbes (Wagner et al. 1990), all three of which are currently listed as U.S. federally protected endangered species (DOFAW, http://ecos.fws.gov.webpage/) and are considered critically endangered by the IUCN.

Hesperomannia species are small trees or sprawling shrubs. The flowering heads contain 25-40 disk florets and are subtended by 4-7 series of involucral bracts. Current taxonomic treatments report *Hesperomannia arbuscula* occurring in mesic to wet forests from a few, small, scattered populations in the Wai`anae Mountain Range on O`ahu and from a few valleys on West Maui. *Hesperomannia arborescens* occurs in wet forest in

the Ko`olau Mountain Range on O`ahu and a few populations in Northern Moloka`i. *Hesperomannia lydgatei* is found only in the wet forest of Wahiawa/ Kanaele Bog Drainage in South Kaua`i with a few scattered individuals on the north side of the island.

As collections were being made for this study, it was apparent to the author and several field experts (Hank Oppenheimer, Maui Land and Pineapple Co.; Steve Perlman, NTBG; personal communication) that populations of *H. arbuscula* from West Maui did not share similar habit and leaf morphology with *H. arbuscula* populations from the Wai`anae Mountain Range on O`ahu. The population collected from northern Moloka`i shared a comparable morphology and habit with the West Maui plants. Preliminary genetic analyses confirmed that these populations formed a genetically distinct group.

The taxonomic history of *Hesperomannia* in the Maui Nui island group (Kaho`olawe, Lana`i, Maui, and Moloka`i) refers to three different species. The type specimen for the genus was collected on Lana`i Hale by Horace Mann and was described by A. Gray (1866) as *H. arborescens*. A second species was later described by Hillebrand (1888) from West Maui as *H. arbuscula*. Subsequently, Carlquist (1957) monographed the genus and treated *H. arborescens* from Lana`i as *H. arborescens* subsp. *arborescens* and *H. arbuscula* from West Maui as *H. arbuscula* subsp. *arbuscula*. Finally, St. John (1983) named a third species *H. mauiensis* from I`ao Valley, West Maui. Wagner et al. (1990, 1999) identified the specimens from Moloka`i and a small population from West Maui as *H. arborescens*. This research identifies all the specimens of *Hesperomannia* from the Maui Nui island group (extant populations on West Maui and Moloka`i in addition to the type specimen from Lana`i Hale) as a single genetically and morphologically distinct species. Thus, following taxonomic nomenclature priority,

this species will be referred to as *H. arborescens* Gray throughout this paper with the names *H. arbuscula, H. arborescens* subsp. *arborescens*, and *H. arbuscula* subsp. *arbuscula* treated as synonyms of *H. arborescens*.

Designation of the Maui Nui populations as all *H. arborescens sensu strictu* requires reconsideration of names assigned to the tomentose, sprawling shrubs of mesic forests in the Wai`anae Mts., O`ahu (formerly *H. arbuscula*) and the glabrous, small trees of O`ahu (formerly *H. arborescens*). The tomentose plants of the Wai`anae Mts., O`ahu were first described as *H. arborescens* var. *oahuensis* by Hillebrand (1888). These specimens were later distinguished at the species level by Degener (1938) as *H. oahuensis*. Carlquist (1957) renamed these plants as *H. arbuscula* subsp. *oahuensis*. Finally, St. John (1978) named a new variety, *H. arbuscula* var. *pearsallii*. This research treats all tomentose plants of the Wai`anae Mts. of O`ahu as one species. Thus, they will be referred to here as *H. oahuensis* (Hillebr.) Degener.

The glabrous, tree like species of *Hesperomannia* found predominantly in the Ko`olau Mts. of O`ahu was first described by Degener (1933) as *H. swezeyi*. Degener also described a second species with two varieties from the Halawa area in the Ko`olau Mts. as *H. bushiana* var. *bushiana* and *H. bushiana* var. *fosbergii*. Carlquist (1957) designated these two taxa as subspecies of *H. arborescens*, subsp. *swezeyi* and subsp. *bushiana*. Finally, Wagner et al. (1990) lumped all the individuals of *Hesperomannia* in the Ko`olau Mts. into *H. arborescens*. Because preliminary analyses of this study have determined these plants to be distinct from *H. arborescens* on Maui Nui, they will be referred to hereafter as *H. swezeyi* Degener.

The purpose of this study was to address the question of why these species are rare. Specifically, this research focused on determining the degree of genetic variation and differentiation within and among populations of each species and population using random amplified polymorphic DNA (RAPD) markers, pollen viability estimates, and seed germination observations. Pollen viability and seed germination studies are a direct means of assessing fitness. And, in conjunction with genetic variability studies, may contribute insight into the underlying causes of reduced population numbers and reduced fitness. This information may provide evidence of inbreeding and or genetic drift, conditions that may contribute to the rarity of these species. Ultimately, this information will be used to make informed recommendations for the conservation and management of these species in their protection, collection, monitoring, and propagation.

Materials and Methods

Field Sites

Due to the low numbers of individuals, collections made from all the populations were restricted to 1-2 leaves and flowering and fruiting heads when available per accessible individual in order to have minimal impact on wild populations. Voucher specimens were collected only from the more remote populations and only if there were no or few previous collections. Populations were generally visited only once because of their remote locations with the exception of the Makaha and Wai`anae Kai populations of *H. oahuensis* that were more easily accessible.

Leaf tissue and flowering and fruiting heads were collected from three populations of *H. oahuensis* in the Wai`anae Mountains on O`ahu (Table 2.1). All three

populations are in mesic forest ca. 1800-2000 ft. elevation. The population located along the Wai`anae Kai trail on State land, in the middle of the Wai`anae Mountain Range, consisted of 11 individuals at the time of leaf collection. One seedling was observed in the field. However, several mature plants have died since leaf collections were made. Appoximately1 Km to the Northwest of this population, in upper Makaha Valley managed by the Board of Water Supply, was another population consisting of 15 individuals at the time of leaf collection. No regeneration of new individuals was observed from 2000-2002 and several plants have since died. The third population is located in Palawai Gulch within The Nature Conservancy's Honouli`uli preserve in the Wai`anae Mountain Range. This population consisted of nine individuals at the time of DNA collection, two of which were seedlings determined to be vulnerable and hence not sampled.

Hesperomannia arborescens was sampled from four populations, three on West Maui and one on Moloka`i, in montane wet forest at ca. 2000 ft. elevation (Table 2.1). West Maui populations were sampled from Honokohau Valley (21 individuals sampled), Waihe`e Valley (17 individuals sampled) and I`ao Valley (3 individuals sampled). The Moloka`i population is located on the North facing cliffs of Wailau Ridge (27 individuals sampled). All populations are on State land except the Honokohau Valley population managed by Maui Land and Pineapple Company. The three large populations appeared healthy and regeneration was evident with all stages of growth observed (seedlings, juveniles, and adults).

Populations of *Hesperomannia swezeyi* on O`ahu, are generally found along the Ko`olau Mountain Range in wet forest at ca. 2000 ft. elevation. Samples included in this

Table 2.1 Individuals of *Hesperomannia* sampled for RAPD DNA analyses, pollen viability estimates, and germination observations. Population size, N, was estimated wherever possible; populations where estimates were not possible are indicated (?). HPDL numbers are accession numbers in the Hawaiian Plant DNA Library (Morden et al. 1996).

Species, Island, Location	N	# Sampled	Pollen analysis	Germination monitoring	HPDL #
Hesperomannia oahuensis					
O`ahu, Wai`anae Mts.					
• Palawai*	9	7	N	Y	3877-3883
• Wai`anae Kai*	<11	11	Y	Y	1960-1968, 2669, 3914
• Makaha*	<15	15	Y	Y	2752-2764, 2952, 2975
Hesperomannia arborescens					
Maui Nui: West Maui, Moloka`i					
• Waihe`e Valley	18	17	Y	N	3860-3876
• Honokohau Valley	28	21	N	N	2813-2833
• I`ao Valley	3	3	N	N	3911-3912
• Wailau Ridge	30	27	N	N	3884-3910
Hesperomannia swezeyi					
O`ahu, Ko`olau Mts.					
• Kawailoa #1	?	1	Y	Y	2666
• Kawailoa B	?	3	Y	N	2976-2978
• Kawailoa F	?	2	N	N	3915, 3916
• Kawailoa H	?	8	Y	N	2924-2931
• Schofield Barracks East Range (SBE)	100's	23	Y	Y	2901-2923
• Poamoho	?	3	N	N	2665, 3919-3920
• Halawa Ridge	?	1	Y	N	2768
O`ahu, Wai`anae Mts.					
• Lower Ka`ala NAR (LKN)	2	2	Y	Y	2667-2668
Hesperomannia lydgatei					
Kaua`i					
• Wahiawa/ Kanaele Bog	>61	61	Y	Y	3591-3651

*Individuals from these populations have died during the course of this study.

study came from eight locations in the Ko`olau Mountains (Table 2.1). Four populations are managed by the Schofield Army Environmental Unit: (Schofield Barracks East Range (SBE 23 individuals), Kawailoa populations #1 (1 ind.), B (3 ind.), F (2 ind.), and H (8 ind.), Poamoho trail (3 ind.), Halawa (1 ind.) and Lower Ka`ala NAR (LKN) (2 ind.) The northernmost population sampled was Poamoho and the southernmost population was Halawa. The LKN population was recently discovered in the Wai`anae Mountains.

Hesperomannia lydgatei is restricted to a single large population located in the Wahiawa/ Kanaele Bog Drainage Basin in the southern Kaua`i at ca. 1700 ft. elevation. Several small subpopulations are scattered along various stream tributaries within the large population. A total of 61 individuals from six subpopulations were collected, subpopulation "1" being most upstream and subpopulation "6" being the farthest downstream. The Wahiawa drainage basin is owned by the McBryde Sugar Company.

DNA Collection

One leaf per safely accessible and healthy individual was sampled per population. Primarily, mature individuals were sampled in each population. However, juveniles and seedlings that where visually determined to be healthy were sampled in very small populations. Voucher specimens were deposited in the Bishop Museum Herbarium (BISH), Honolulu, Hawaii. The population size, number of plants sampled and Hawaiian Plant DNA Library (HPDL; Morden et al. 1996, Randell and Morden 1999) number are listed in Table 2.1.

DNA Extraction

DNA was extracted from fresh leaf material using a modified version of the CTAB extraction (Morden et al. 1996; Doyle and Doyle 1987). The extraction buffer consisted of 2% Cetyl-trimethyl Ammonium Bromide (CTAB), 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20mM EDTA, 2% PVP-40 (polyvinylpyrrolidone 40,000 MW), and 0.2% β-mercaptoethanol (added immediately prior to each extraction). Approximately 1.5-2.0 grams of fresh leaf tissue was macerated with a mortar and pestle, extraction buffer, and sterile sand. The resulting grindate was incubated for 15-60 min. at 60-65^{o}C with occasional mixing. The DNA was then extracted with chloroform and centrifuged at 3000 rpm in a Sorvall RT-6000 centrifuge for 10 min. The top aqueous phase was removed to a new tube and gently mixed with an equal volume of cold (4^{o}C) 2-propanol to precipitate the DNA. The mixture was incubated at -20^{o}C for at least 30 min. to further precipitate the DNA. The tubes were centrifuged as above for 5 min. to pellet the precipitate. The pellet was allowed to air dry for approximately 5 min. and then washed with 5-10 ml wash buffer (76% EtOH and 10 mM NH_4Oac) and centrifuged as above for 5 min. The supernatant was decanted, the pellet air dried for ca. 5 min., and resuspended in 4.0 ml TE [10 mM Tris-HCl (pH 8.0), 1mM EDTA]. DNA was further purified by cesium chloride (CsCl) density gradient centrifugation, where 3.9g CsCl (final density 1.55-1.58 gm/ml) and 50 μl of 10 mg/ml ethidium bromide (EtBr) were added to the tubes and ultracentrifuged overnight at 55k rpm. DNA bands were removed with pasture pipettes, EtBr removed with two washes of water-saturated isobutanol, and CsCl was removed by dialysis in TE (Sambrook et al. 1989). All DNA samples were accessioned

into the Hawaiian Plant DNA Library (HPDL; Morden et al. 1996, Randell and Morden 1999) and stored at –20°C.

RAPD Procedure

RAPD reactions consisted of 25 μl reactions containing ca. 25ng of DNA, 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X *Taq* DNA polymerase buffer [10mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl and 0.1% Triton X-100, Promega], 1.5mM $MgCl_2$, 0.25 mg BSA, 0.2 μM random 10-mer oligonucleotide primer (Operon Technologies, Alameda, Calif. USA), and ca. 1 unit of *Taq* DNA Polymerase (Storage Buffer A; Promega, Madison, Wisconsin, USA). PCR reactions were performed in a DNA thermocycler using the following reaction conditions: an initial denaturation cycle of 94°C for 3 min., 35°C for 30 seconds and 72°C for 2 min., followed by 43 cycles of 95°C for 45 seconds, 35°C for 30 seconds, and 72°C for 2 min., and one final cycle of 95°C for 45 seconds, 35°C for 30 seconds and 72°C for 6 min. PCR amplified products were electrophoresed on 1.5% agarose gels, stained with EtBr and visualized with a UV light source. Size of amplification products was estimated using either a pBS plasmid (Stratgene, La Jolla, CA, USA) digested with restriction enzymes to produce fragments in a size range from 0.448-2.96 kb or the 100kb ladder (Promega). Gels were digitally photographed using a Kodak DC 290 camera. Digital photos of RAPD gels were analyzed using Kodak ID Image Analysis Software (Eastman Kodak Company 2000, Scientific Imaging Systems, Rochester, NY USA). Negative control reactions were run without DNA for all PCR amplifications to ensure reaction components were uncontaminated.

A total of 31 RAPD primers from Operon Technology kits A-D were screened using a subset of DNA with two individuals from three separate populations. The first 9 primers that showed scorable and consistent amplification for each individual in the primer screening were used in the final analyses with all individuals. Bands from reproducible amplification phenotypes were scored for either presence (1) or absence (0) at each locus. Each distinct RAPD marker represents a single genetic locus of a two allele system (present and absent) and co-migrating markers produced by the same primer were considered homologous (Rieseberg 1996). Absence of a marker within individuals, although present in others, was assumed to indicate that this individual was a null/null homozygote; markers presence indicates the locus was either homozygous or a present/null heterozygote. Absence of a marker from an entire population was assumed to indicate null/null homozygosity rather than the loss of the locus within the population. Other assumptions associated with RAPD marker analysis are described in Lynch and Milligan (1994).

Pollen Viability Analysis

Mature flowering heads were collected (when available) from natural populations (Table 2.1) for pollen viability estimates. When only one or a few flowering heads were present on an individual, one to several florets were removed while the flowering head was allowed to remain on the plant. On average, 1-2 disk florets were dissected and used in pollen assays. Pollen was stained with aniline blue in lactophenol to approximate its viability (see Hauser & Morrison 1964). The pollen was allowed to stain for 24 hrs before viability assessments were made. Pollen grains stained more deeply

(dark blue colored) were considered viable while unstained or lightly stained grains were counted as unviable. Pollen grains that appeared to be reduced size or misshapen were also considered unviable. Optimally, approximately 300 grains were counted for each specimen. The results were scored as percentages and averaged for each species. *Hesperomannia* species generally flower from early spring into late fall. The percent viability of populations and individuals provided a measure of fitness to be compared to the assessments of genetic variability.

Seed Germination Observations

Mature and immature seed heads were collected whenever possible to test frequency of germination from the different species. Visits to the remote populations of *H. lydgatei* and *H. arborescens* were infrequent due to expense and collecting conditions. Although very few collections were made from these populations, better estimates of flowering seasons were made for plants from these locations. Populations of *H. oahuensis* at Wai`anae Kai and Makaha were the most easily accessible and were visited during three successive growing seasons (2000 – 2002).

All seed material was processed at the Lyon Arboretum Micropropagation Facility. The development of efficient seed processing procedures (including sterilization, embryo extractions, and initial micropropagation work) were done in collaboration with Nellie Sugii, Lyon Arboretum Researcher and Director of the Micropropagation Facility with subsequent lab work carried out by Lyon Arboretum staff and volunteers. Immature achenes were sterilized in approximately 50 ml of 10% Clorox with a few drops of Tween-20 for 20-30 min. Embryos were then extracted in a sterile

hood environment and placed on an agar growth media prepared as in Murashige and Skoog (1962). Previous experience indicated that achenes that sink in the sterilization solution contain a much higher frequency of viable embryos (whereas those that float are not viable (Sugii, Lyon Arboretum Micropropagation personal communication). Thus recently, only embryos from achenes that sank were extracted. Achenes that floated were discarded. Germination and survivability was documented for the duration of this study. The percent germination per individual and species was used as an estimate of fitness for comparison to the results of the genetic variability assessments.

Genetic Data Analyses

Percent polymorphic loci (%P) for each species and population were determined using excel. These values give an accurate estimate of the amount of genetic variation contained within species and their populations with and provide a measure of comparison to heterozygosity values. All heterozygosity measurements are estimates due to the dominant nature of RAPD markers. Estimating heterozygosity from RAPD or other data with dominant markers may not accurately represent the allele frequencies from small populations. The assumptions of Hardy-Weinberg equilibrium are violated by the small population size. However, most natural populations are not in H-W equilibrium.

Average expected heterozygosity (H_e) was estimated for each locus in each population (H_S) and species (H_T) according to the equation:

$$H = 1 - (p^2 + q^2),$$

where q is the frequency of the null allele and p is the frequency of the dominant allele. The value obtained ranges from 0 to 1, 0 inferring that all individuals are genetically identical.

The average heterozygosity per subpopulation, $\hat{H}_S$, was determined from the H_e for each locus. The average heterozygosity for all the subpopulations in a species, $\hat{H}_S$, was weighted by the numbers of individuals within each population. A weighted average was used in this case because there are large differences among population sizes. Additionally, the average heterozygosity for all the populations combined, H_T, was calculated for each species and for the genus. These values were then used to calculate Wright's F-statistics (1978), including H_S/H_T and F_{ST} values. H_S/H_T displays the proportion of genetic diversity found within subpopulations. This ratio was calculated for each subpopulation relative to the species and also for each species relative to the entire genus.

F_{ST} is a measure of the amount of genetic differentiation occurring among populations as it compares the smallest subdivision (i.e. the population or subpopulation) to the broadest category (i.e. a metapopulation combining the alleles of all the subpopulations in the species or genus) (Hartl and Clark 1997) and follows the equation:

$$F_{ST} = \frac{(H_T - H_S)}{H_T}$$

The estimated heterozygosity for the metapopulation will always be larger than that of the average of the subpopulations because the larger population combines all the respective subpopulation allele frequencies and assumes random mating (Hartl 2000). F_{ST}

values also range from 0 to 1. A value of 0 infers there is a high degree of gene flow among populations, while 1 indicates populations are genetically isolated.

Genetic similarity indices were estimated using both Gower (1971) and Nei & Li (1979) similarity coefficients for populations and species of *Hesperomannia* sampled using Multivariate Statistical Package (MVSP; Kovach Computing Services 1986-1999). The Gower similarity coefficient is defined as:

$$S = \Sigma S_{ij} / \Sigma W_{ij},$$

where i and j represent the two individuals being compared. S_{ij} is the score for the sample and W_{ij} is defined as the weight of the sample. When both samples lack a marker, (i.e. a null-null comparison), W_{ij} it is given a value of 0, in which case the score is also 0. When both samples possess a character (i.e. a present-present comparison) the score and weight are both 1. When only one sample possesses a character (i.e. a null-present comparison) the score is 0 but the weight is 1.

In contrast, the Nei & Li similarity coefficient is defined as:

$$NLc_{ij} = 2a/[(a + b) + (a + c)]$$

Where NLc is the resulting similarity coefficient for the comparison of individuals i and j, *a* represents a present-present comparison, and *b* and *c* represent a present-absent comparison for a particular marker. The Nei & Li similarity coefficient measures only the compared presence of bands; no score is given for the shared absence of bands between two individuals (Nei and Li 1979, Lamboy 1994). This coefficient measures the shared proportion of markers based on the assumption that the two samples share a common ancestor. This equation also gives more weight to shared presence of bands (i.e. *a*).

For both similarity coefficients, the values range from 0 to 1, where 0 indicates there are no markers in common and 1 indicating complete genetic identity. Relationships within and among populations and species were then projected from both similarity matrixes using Principle Coordinate Analysis and Cluster Analysis with MVSP software (Kovach Computing Services) (Kwon and Morden 2002). The differences in average similarity values between the two coefficients can be accounted for by the fact that the Nei & Li coefficient measures only the shared presence of bands whereas the Gower coefficient also measures the shared absence of bands for two individuals.

An assessment of the correlation of geographic and genetic distances within species was performed using a software addition called GenAlEx (Peakall and Smouse 2001). Pairwise genetic distances were calculated based on pairwise binary, dominant RAPD data using the method described in Huff et al. (1993). Geographic coordinates were roughly estimated from topographic maps. Pairwise linear geographic distances were calculated as the square root of $(X_1-X_2)^2+(Y_1-Y_2)^2$, where X and Y are latitudinal and longitudinal coordinates, respectively. Spatial analyses of geographic and genetic distance measurements (i.e., a mantel test) were then performed, with calculations undergoing 999 permutations.

Results

RAPD Data Analyses: Among species variation

Of the 31 primers tested for amplification, nine produced clear and consistent products that were used on all individuals (Table 2.2). These nine primers yielded 202 scorable RAPD markers with a range of 12-35 loci (average 22) identified for each

Table 2.2 Primer, nucleotide sequence, number of scored bands per primer and range of scored products (kb) used for genetic variability of *Hesperomannia*.

Primer	*Nucleotide Sequence (5'-3')*	*# Scored bands*	*Range of scored bands (kb)*
A-07	GAAACGGGTG	12	420-1500
A-09	GGGTAACGCC	16	400-1960
A-10	GTGATCGCAG	33	400-1200
A-12	TCGGCGATAG	28	600-2500
B-05	TGCGCCCTTC	23	650-2500
B-10	CTGCTGGGAC	24	490-2200
B-11	CTAGACCCGT	15	550-1900
B-18	CCACAGCAGT	35	350-2200
C-02	GTGAGGCGTC	16	420-2200
	Average # bands	**22**	

RAPD primer (Table 2.2.). Ten of the 202 (4.9%) markers were present in all individuals of all species and 192 (95%) markers were present, although polymorphic across the genus. There were 20 markers that were diagnostic of *H. oahuensis* (i.e. present only in this species), the most of all species examined, and an additional six markers that were uniquely absent from this species (i.e. present in at least one population in each of the other three species). Each of the other species had less than half this number of diagnostic markers: 10 were present in *H. swezeyi*, 6 in *H. lydgatei*, and 5 in the *H. arborescens* (Table 2.3). (A data matrix of the presence or absence of each RAPD marker produced by the 9 primers for each species is provided in Appendices A-D).

Polymorphic Loci

RAPD marker statistics, including the percent polymorphic loci within each population and species, are listed in Table 2.3. Populations with only 1 individual were not included in this analysis. The most variable species was *H. oahuensis* with 72.0%

Table 2.3 RAPD marker statistics for each *Hesperomannia* species. Only populations with >1 individual were included in this analysis.

	# Individuals sampled	# Polymorphic Loci	% Polymorphic Loci	# Unique Loci	# Uniquely absent
H. oahuensis, **Wai`anae Mts., O`ahu**					
Makaha	15	98	67.1		
Wai`anae Kai	11	102	69.9		
Palawai	7	56	38.4		
Species Total	**33**	**146**	**72.3**	**20**	**6**
H. arborescens					
Waihe`e, WM	17	65	56.0		
Honokohau, WM	21	52	44.8		
I`ao, WM	3	30	25.9		
Moloka`i	27	64	55.2		
Species Total	**68**	**116**	**57.4**	**5**	**6**
H. swezeyi, **O`ahu**					
SBE	23	73	58.4		
Kawailoa #	3	31	24.8		
Kawailoa F	2	27	21.6		
Kawailoa H	8	57	45.6		
LKN	2	35	28.0		
Poamoho	3	32	25.6		
Species Total	**41**	**125**	**61.9**	**10**	**7**
H. lydgatei, **Kaua`i**					
Subpop. 1	5	38	37.3		
Subpop. 2	10	48	47.1		
Subpop. 3	11	59	57.8		
Subpop. 4	7	51	50.0		
Subpop. 5	7	30	29.4		
Subpop. 6	21	70	68.6		
Species Total	**61**	**102**	**50.5**	**6**	**11**
Genus Total	**203 (4 species)**	**192**	**95.0**		

polymorphic loci compared to the least variable. Polymorphism in the other three species ranged from 50.5% (*H. lydgatei*) to 57.4% (*H. arborescens*) to 61.9% (*H. swezeyi*).

Heterozygosity Estimates

The expected mean heterozygosity (H_e) was calculated for each population and species by averaging the H_e for each RAPD locus. The average estimated heterozygosity per population, $\hat{H}_S$, and the total species heterozygosity, H_T, varied across the four species. The average expected population and species level heterozygosity values for the genus were high, $\hat{H}_S$ 0.117 and H_T 0.170, compared with RAPD data for other Hawaiian species (see Tables 2.4 and 2.13). At the species level, *Hesperomannia oahuensis* had the highest mean and the widest range of heterozygosity among its three study populations, H_S, while the other three species showed similar means and averages over their respective populations (Fig. 2.1.). *Hesperomannia lydgatei* had the lowest average estimated total heterozygosity value of the four species, H_T = 0.092, while the *H. oahuensis* showed the highest estimated total heterozygosity value H_T = 0.163.

The heterozygosity values obtained for each subpopulation were used to compare the genetic diversity within and among each species and also for the genus. Within population variation was estimated from the ratio of $\hat{H}_S / H_T$. This ratio was consistent for all the species/groups in that it shows 78-85% of the genetic diversity is held within populations of each species (Table 2.4.).

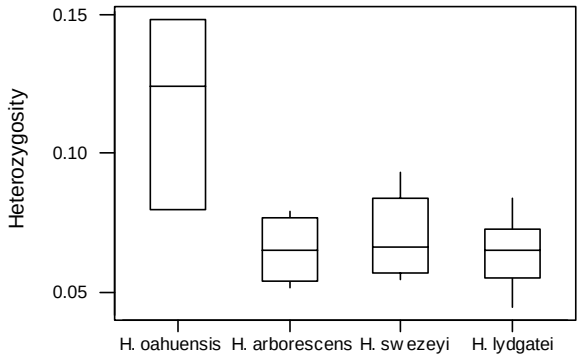

Figure 2.1. The average estimated heterozygosity ($\hat{H}_S$) per population for the four *Hesperomannia* species and the range of heterozygosity among populations within each species.

The G_{ST} values for the four species varied from 0.152 to 0.218. Wright (1978, in Hartl and Clark 1997) determined that values from 0.05-0.15 indicate moderate genetic differentiation among populations and values from 0.15-0.25 indicate great genetic differentiation. When all populations across the four species in the genus are combined, the G_{ST} value is quite high, 0.295, indicating that the four species are highly genetically differentiated.

Table 2.4 Hierarchical structure of genetic diversity in species of the genus *Hesperomannia*. $\hat{H}s$ were determined with a weighted average using the population size.

Species-Population	Island	# Individuals	$\hat{H}_S$	H_T	$\hat{H}_S/H_T$	G_{ST}
H. oahuensis						
Makaha	O`ahu	15	0.124			
Wai`anae Kai	O`ahu	11	0.148			
Palawai	O`ahu	7	0.08			
Species Total		**33**	$\hat{H}_S$ **= 0.123**	**0.163**	**0.843**	**0.157**
H. arborescens						
Waihe`e	West Maui	17	0.079			
Honokohau	West Maui	21	0.06			
I`ao	West Maui	3	0.052			
Moloka`i	Moloka`i	27	0.071			
Species Total		**68**	$\hat{H}_S$ **= 0.07**	**0.113**	**0.782**	**0.218**
H. swezeyi						
SBE A	O`ahu	23	0.081			
Kawailoa #	O`ahu	3	0.058			
Kawailoa F	O`ahu	2	0.055			
Kawailoa H	O`ahu	8	0.093			
LKN	O`ahu	2	0.072			
Poamoho	O`ahu	3	0.061			
Species Total		**41**	$\hat{H}_S$ **= 0.07**	**0.131**	**0.828**	**0.172**
H. lydgatei						
Subpop 1	Kaua`i	5	0.062			
Subpop 2	Kaua`i	10	0.059			
Subpop 3	Kaua`i	11	0.069			
Subpop 4	Kaua`i	7	0.068			
Subpop 5	Kaua`i	7	0.045			
Subpop 6	Kaua`i	21	0.084			
Species Total		**61**	$\hat{H}_S$ **= 0.069**	**0.092**	**0.848**	**0.152**
Genus Total		**203 (4 species)**	$\hat{H}_S$ **= 0.117**	**0.170**	**0.705**	**0.295**

Genetic Similarity Indices

Genetic similarity within and among populations and species was calculated using both Gower and Nei & Li's similarity coefficients. Average genetic similarity among the species differed according to the index used but both coefficients showed similar trends among populations and species. The Gower similarity indices consistently resulted in higher average similarity values than those obtained using Nei & Li's similarity coefficient. Both similarity indices consistently show *H. lydgatei* as having the highest average similarity values (0.79 Nei & Li; 0.89 Gower) and *H. oahuensis* with the lowest average similarity (0.68 Nei & Li; 0.80 Gower). The average similarity for all the individuals sampled in the genus was lower than for any one species (0.63 Nei & Li; 0.79 Gower).

The average similarity values within and among the four species are listed in Table 2.5. Both the Gower and Nei & Li similarity indices identify *H. arborescens* and *H. oahuensis* populations as being the least similar (0.54 Nei & Li; 0.73 Gower), and *H. oahuensis* and *H. swezeyi* as being the two most similar species (0.67 Nei & Li; 0.80 Gower). Populations were compared for similarity within each species (Tables 2.6-2.9). Surprisingly, the two geographically closest populations of *H. oahuensis* are the least similar (0.63 Nei & Li), whereas the two most distant populations are most similar (0.65 Nei & Li). However, these differences in similarity are very small indicating that all populations are closely related. The relationships among populations within the other three species follow a geographical trend with closer populations most often being more similar.

Table 2.5 Within and among species genetic similarity for the four species of Hesperomannia. Gower similarities are above the diagonal and Nei & Li similarities are below (in bold). Average genetic similarity for the genus: 0.629 Nei & Li; 0.794 Gower.

	H. oahuensis	***H. arborescens***	***H. swezeyi***	***H. lydgatei***
H. oahuensis	**0.680**/0.797	0.726	0.797	0.753
H. arborescens	**0.535**	**0.746**/0.863	0.770	0.789
H. swezeyi	**0.668**	**0.591**	**0.740**/0.846	0.787
H. lydgatei	**0.563**	**0.594**	**0.608**	**0.788**/0.894

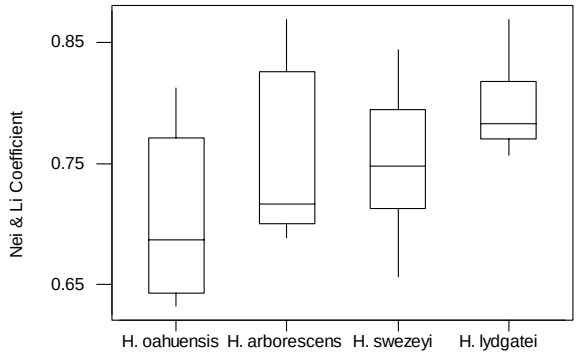

Figure 2.2. The average ranges of genetic similarities for each *Hesperomannia* species based on the Nei & Li similarity coefficient.

Principle coordinate analyses (PCO) and cluster analyses were performed for the entire genus and for all species separately. The PCO plot of the entire genus resulted in three distinct groupings that represent *H. lydgatei*, all O`ahu populations (including both *H. oahuensis* and *H. swezeyi*), and *H. arborescens* populations. The cluster dendogram for the entire genus (not shown) indicated very similar results to the PCO plot. These data indicate that *H. arborescens* is at least as genetically distinct from each of the other three species as the other three species are from each other (Fig. 2.3). The first PCO axis accounts for the distinction of *H. arborescens*, from the two species on O`ahu (*H. oahuensis* and *H. swezeyi*) and *H. lydgatei*. The second axis indicates the distinction between the two O`ahu species and *H. lydgatei*.

Genetic variation among the *H. swezeyi* and *H. oahuensis* populations appear to overlap in Fig. 2.2. Examination of additional axes in the PCO analysis suggested these two species may be distinguished, therefore a separate PCO analysis with only these two species was done (Fig. 2.4.). Individuals of *H. swezeyi* and *H. oahuensis* are split into three distinct groupings where the first axis distinguishes *H. oahuensis* populations from *H. swezeyi* populations and the second axis distinguishes the large SBE population of *H. swezeyi* from the others in this species. Although the populations of *H. oahuensis* form tightly clustered groupings, these groups are continuous and overlap to some degree. However, the relations among these groups are consistent with a North (Makaha) to South (Palawai) distribution of the populations. No such relationship was evident for *H. swezeyi* populations with the exception that the Schofield East Range (SBE) population was distinct from all others (Fig. 2.4).

An additional PCO of all the populations recognized as *H. arbuscula* by Wagner et al. (1990) which include the Maui Nui populations, resulted in 2 distinct groups, one for populations on O`ahu and one representing those on Maui Nui (Fig. 2.5). The first axis represents 24% of the variation and firmly separates Maui Nui from those populations of *H. oahuensis* on Oahu. The second axis represents 10% of the variation and shows the variation within the two species groups.

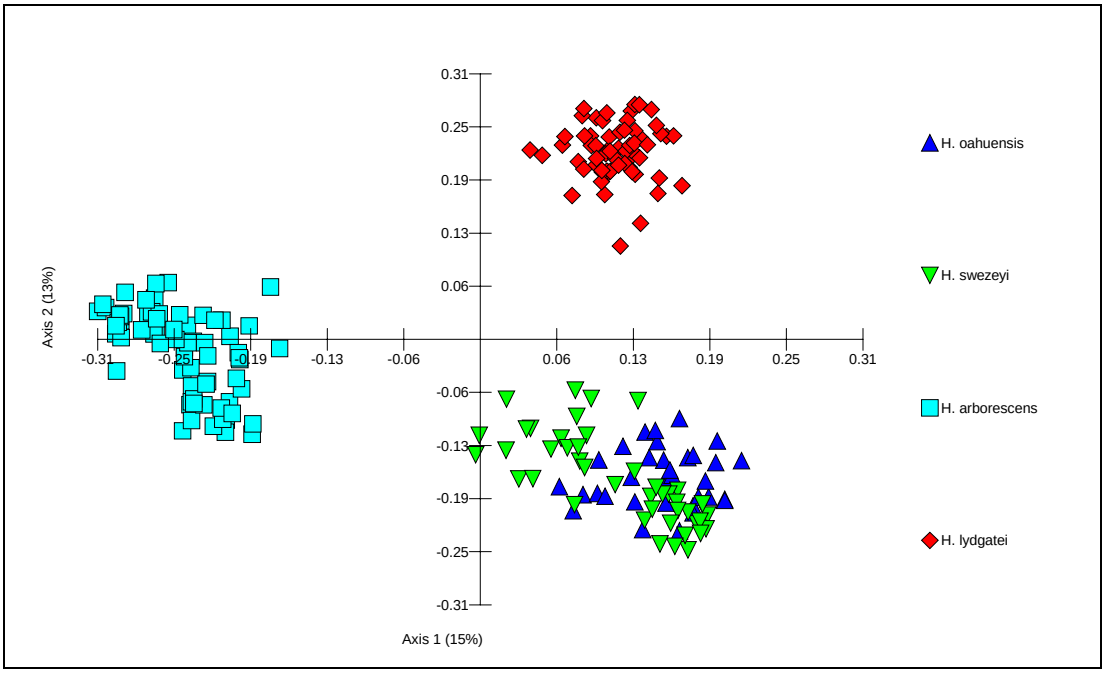

Figure 2.3. PCO scatter plot of each individual sampled in the genus *Hesperomannia*. The first and second axes represent 15% and 13% of the variation respectively.

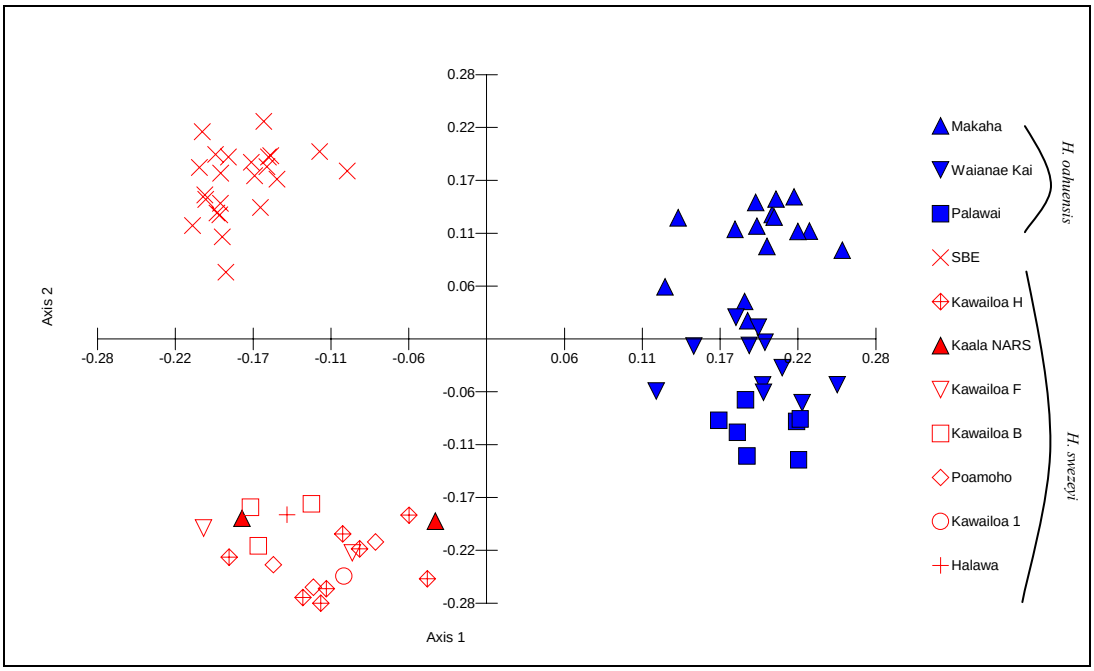

Figure 2.4. PCO scatter plot of *H. oahuensis* and *H. swezeyi*, from the Ko`olau and Wai`anae Mountain Ranges on O`ahu respectively. Solid shapes = Wai`anae Mts., line shapes = Ko`olau Mts. Blue = *H. oahuensis*, Red = *H. swezeyi*.

Mantel tests were performed on the genus as a whole and for *H. oahuensis*, *H. arborescens*, and *H. swezeyi* to determine if there was any relationship between genetic and geographic distance. This test was not performed for *H. lydgatei* due to the lack of distance measurements between the six subpopulations within the one large population sampled. A graph of genetic vs. geographic distance calculated by the mantel test is shown in Fig. 2.6. The slope of the regression line is indicative of the degree of correlation between geographic and genetic distances, while the R^2 value reflects how well the regression line fits the scattered points. For the genus as a whole there is moderate correlation between geographic and genetic distances (m= 0.4561, R^2=0.23), suggesting species that are closer to each other geographically are also more similar genetically.

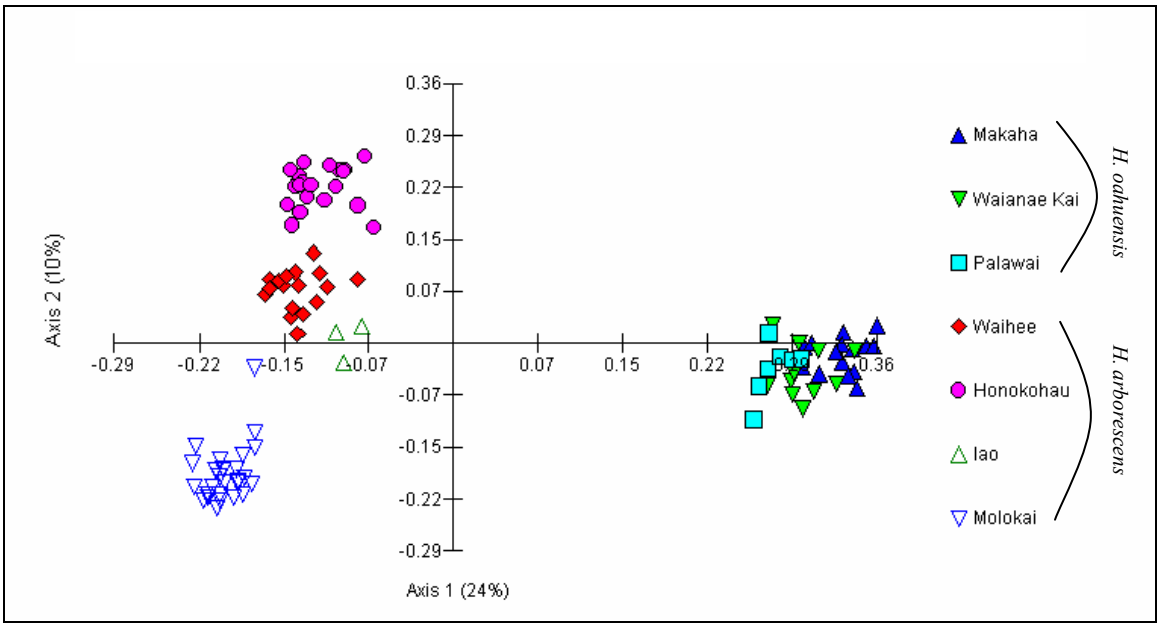

Figure 2.5 PCO analysis of *H. oahuensis* populations on O`ahu and *H. arborescens* populations on Maui Nui, both species were previously recognized by Wagner et al. (1990) as *H. arbuscula*. (Gower General Similarity Coefficient)

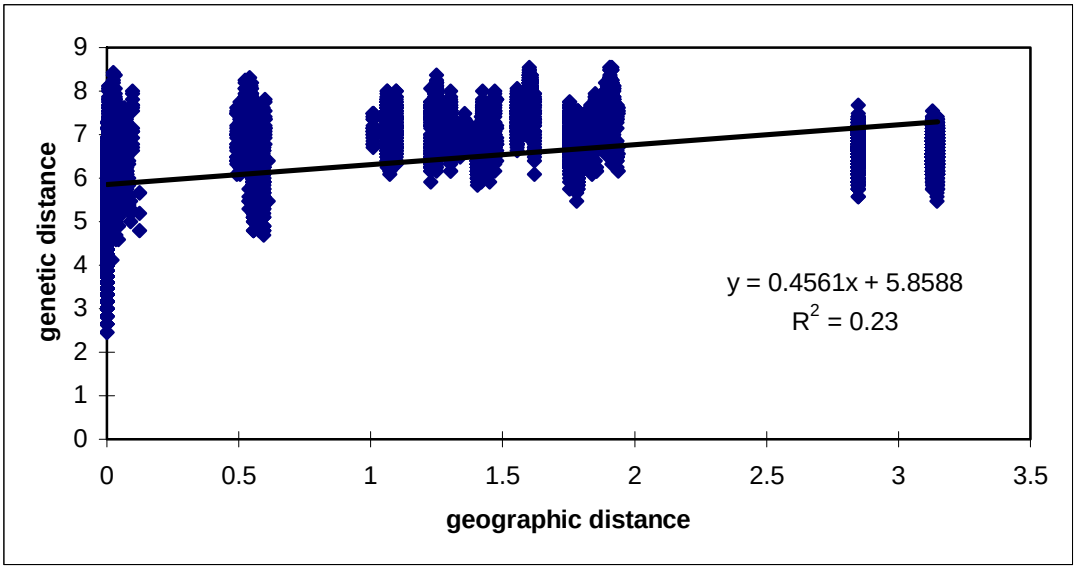

Figure 2.6 Spatial correlation: geographic versus genetic distance for the genus *Hesperomannia*. All individuals sampled are represented. Geographic distances were estimated using a topographic map. P=0.001

Key to the four species of *Hesperomannia* A. Gray

The above results indicate the four species are clearly distinguishable genetically. However, they are also distinguishable morphologically. Therefore, a key to the species is provided based on diagnostic characters that can be used in the field.

1. Heads nodding at anthesis; leaf blades 3-5 times long as wide, glabrous; **K** …….. ……..…….…….……*H. lydgatei*
1. Heads erect to ascending at anthesis; leaf blades 1-4 times long as wide, pubescent or nearly glabrous; **L, WM, Mo, O** (2).

2(1). Plants with lower leaf surfaces, petioles and apices densely tomentose; **O (Wai`anae Mts. only)**…….. *H. oahuensis*

2. Plants glabrous or pubescent only on lower leaf surfaces near veins of younger leaves; **L, WM, Mo, O** (3).

3(2). Leaf blades oval or oblanceolate to obovate, 1-2 times long as wide, puberulent, especially along lower 1/3 to 1/2 portion of midrib on young leaves (and apical buds) sometimes glabrous; **WM, Mo, L** …… *H. arborescens*

3. Leaf blades lanceolate to oblanceolate, or sometimes elliptic, 2-3 (4) times long as wide, glabrous or nearly so; **O (Mostly in Ko`olau Mts.)** ....……. *H. swezeyi*

RAPD Data Analyses: Within Species Variation

***Hesperomannia oahuensis* (Figs. 2.7-2.8)**

The previous taxonomic treatment of *Hesperomannia arbuscula* (Wagner et al. 1990, 1999), included pubescent populations from the Wai`anae Mountains, O`ahu and some slightly pubescent populations from West Maui. In contrast, plants with glabrous or nearly glabrous leaves from Oahu, Moloka`i and Lana`i were classified as *H. arborescens*. As previously demonstrated, all analyses confirm that populations on West Maui are very similar to those on Moloka`i, but distinct from O`ahu populations. The PCO analysis of *H. oahuensis* and *H. arborescens* (Fig. 2.5) provides further support for the formal recognition of *H. oahuensis* as a distinct species.

Hesperomannia oahuensis exhibited a high degree of genetic variation compared to the other species. The average percent polymorphic loci for this species was 72.3%, with 26 unique loci, six of which were unique in being null/null homozygous for markers present in each of the other three species. The average population heterozygosity ($\hat{H}_S$) and the total species heterozygosity (H_T) are the highest in the genus, (0.12 and 0.16 respectively, Table 2.4). The Palawai population had the lowest heterozygosity (0.08), whereas Wai`anae Kai showed a high degree of heterozygosity (0.148). The $\hat{H}_S/H_T$ ratio indicates that 84.3% of the genetic variation is contained within the three populations. Differentiation among populations was moderate with a G_{ST} value of 0.157 (Table 2.4). Average genetic similarity within populations for this species was the lowest in the genus (Table 2.6). Genetic similarity among the three populations ranged from 0.632-0.651 using the Nei & Li coefficient. The PCO analysis for this species shows the range of genetic variation both within and among all the populations (Fig. 2.9). This figure

indicates that the three populations each have distinct genetic markers with the Palawai population having the highest genetic similarity. The Cluster analysis (Fig. 2.10) shows the similarities within and among the three populations.

A mantel test (Fig. 2.11) indicated there is no correlation between genetic and geographic distance for the three populations of *H. oahuensis*. The slope is close to zero (m= 0.06), with little scatter about the regression, the R^2 value suggests that little genetic drift has occurred for these populations (R^2 = 0.0622).

Figure 2.7 *Hesperomannia oahuensis* in Makaha Valley, Wai`anae Mts. O`ahu, Hawai`i.

Figure 2.8. *Hesperomannia oahuensis* flowering head, Wai`anae Kai, O`ahu

Table 2.6. Genetic similarity values within and between populations for *H. oahuensis.* Gower similarities are above the diagonal and Nei & Li are below, in bold. Average genetic similarity for the species: **0.68 Nei & Li**; 0.78 Gower.

H. oahuensis	**Makaha**	**Wai Kai**	**Palawai**	
Makaha	**0.758**/0.85	0.76	0.79	Makaha
Wai Kai	**0.632**	**0.723**/0.81	0.78	Wai Kai
Palawai	**0.651**	**0.646**	**0.812**/0.89	Palawai

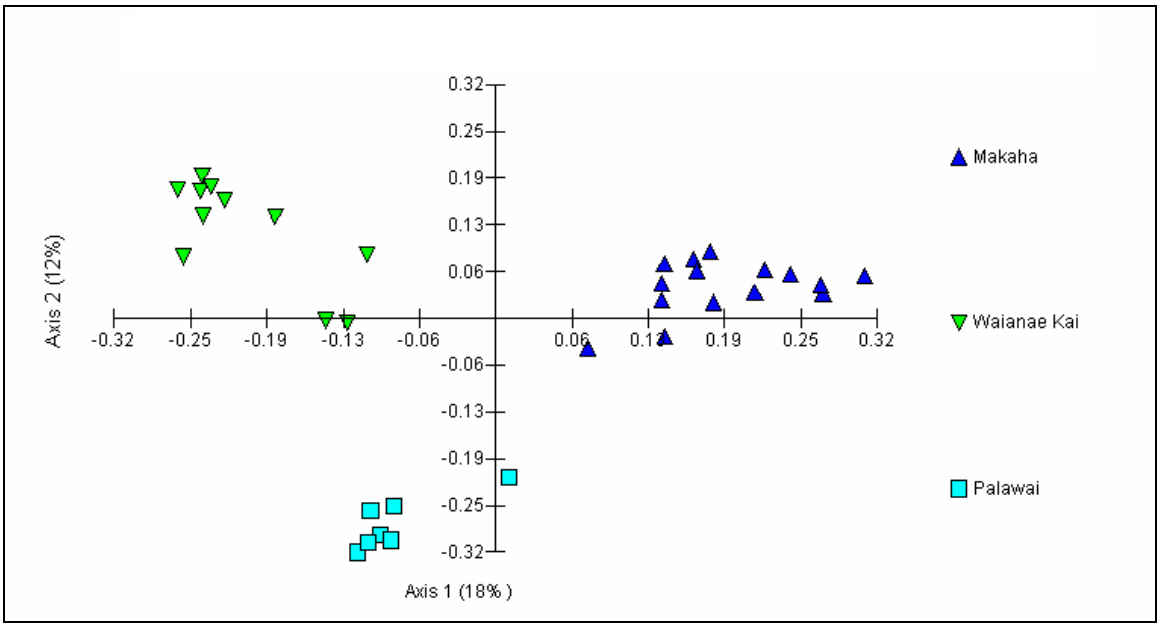

Figure 2.9. PCO analysis of *Hesperomannia oahuensis* individuals based on Gower general similarity coefficient. Axes 1 and 2 represent 18% and 12% of the variation respectively.

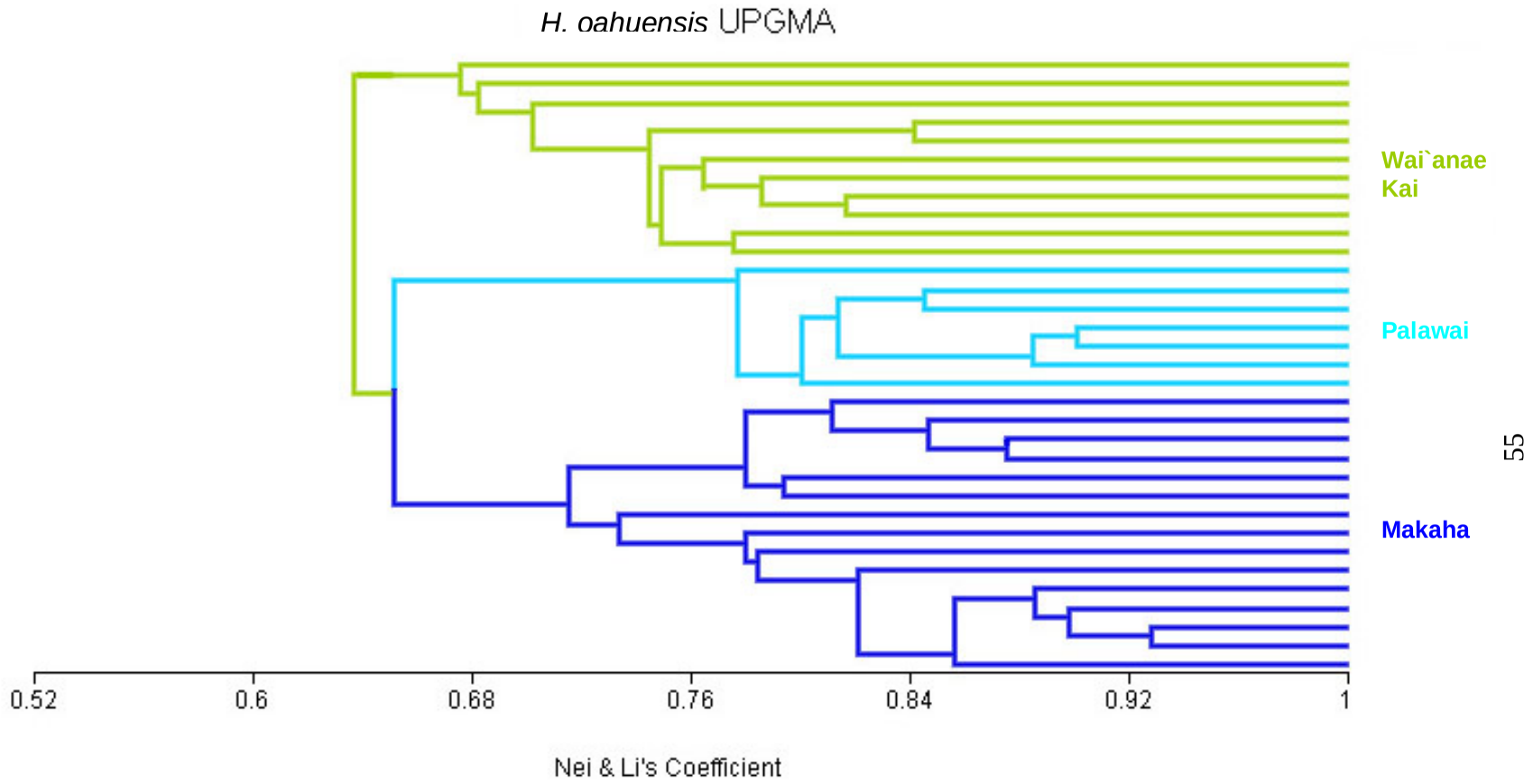

Figure 2.10. Cluster analysis UPGMA dendogram for *Hesperomannia oahuensis* on O`ahu based on the Nei & Li similarity coefficient.

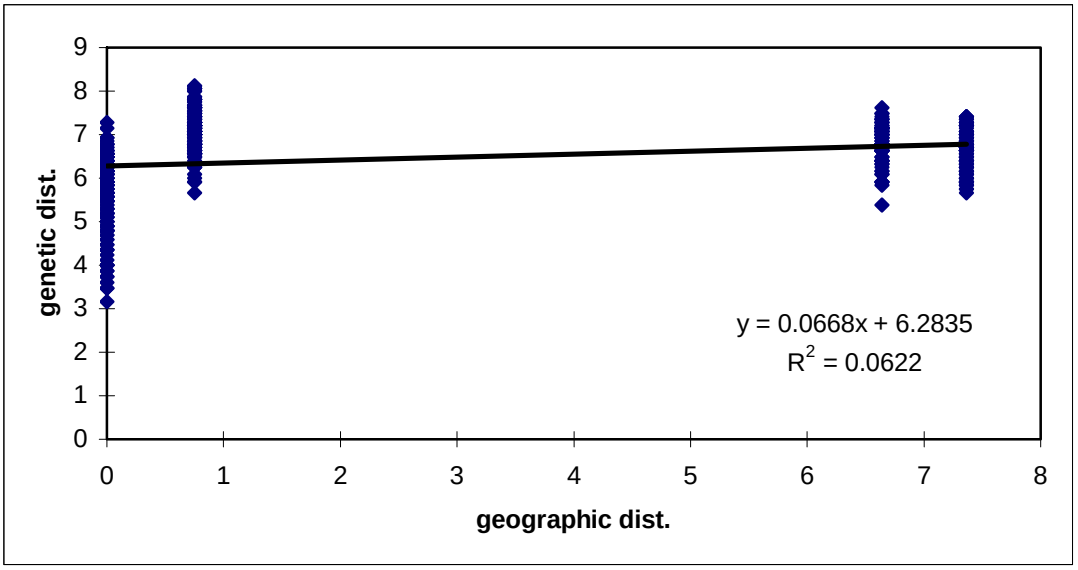

Figure 2.11. Spatial correlation: geographic versus genetic distance for three populations of *H. oahuensis* in the Wai`anae Mts. on O`ahu. P=0.001

Hesperomannia arborescens **(Fig. 2.12)**

Four populations were sampled from this species, three from West Maui and one from Moloka`i. The average percent polymorphic loci per population was 57.4% (Table 2.3). Average heterozygosity within populations, $\hat{H}_S$, was 0.07 (range 0.052 to 0.079, Table 2.4), nearly the same as for those populations within *H. swezeyi* and *H. lydgatei*. Whereas the total heterozygosity for this species, H_T, for this species was lower (H_T = 0.113) compared to the other three species. The $\hat{H}_S/H_T$ values indicate that 78.2% of the genetic diversity is held within populations and that populations are greatly genetically differentiated (G_{ST} = 0.218) (see Wright 1978). Both the PCO and the Cluster analyses for this species reflect the similarity values both within and among the four populations, using the Gower and Nei & Li similarity coefficients respectively (Figs. 2.13 and 2.14). Of the different populations, Waihe`e and I`ao populations were most similar, (0.720 Nei & Li; 0.852 Gower), and Honokohau and I`ao were least similar (0.689 Nei & Li; 0.838 Gower). However, the range of similarities among populations was very small (0.689 to 0.720 Nei & Li; 0.835 to 0.852 Gower; Table 2.7).

Two combinations of the Mantel test were performed on this species (Figs. 2.15 and 2.16). The first test was done with all four populations which includes 3 from West Maui and 1 from Moloka`i. This test shows there is no correlation between geographic and genetic distance. The slope of the regression line was close to zero, (m = 0.02). The test also shows relatively high genetic drift as evidenced by the high R^2 value (R^2 = 0.4072). However, it would not be reasonable to expect that rare plant populations would exchange genetic material across islands. Therefore, a second mantel test was performed

Figure 2.15. *Hesperomannia arborescens* Waihe`e Valley, West Maui, Hawai`i.

using only the three populations on West Maui. The second test shows a high correlation between geographic and genetic distance, though it appears the populations are experiencing a high degree of genetic drift (m = 0.5921, R^2 = 0.6722).

Table 2.7. Similarity values for *H. arborescens* populations. Gower similarities are above the diagonal and Nei & Li are below, in bold. Average genetic similarity for the species: **0.746 Nei & Li**; 0.863 Gower.

	Honokohau	**I`ao**	**Waihe`e**	**Moloka`i**	
Honokohau	**0.869**/0.934	0.834	0.846	0.834	Honokohau
I`ao	**0.689**	**0.814**/0.901	0.852	0.841	I`ao
Waihe`e	**0.701**	**0.720**	**0.819**/0.905	0.835	Waihe`e
Moloka`i	**0.703**	**0.713**	**0.699**	**0.845**/0.911	Moloka`i

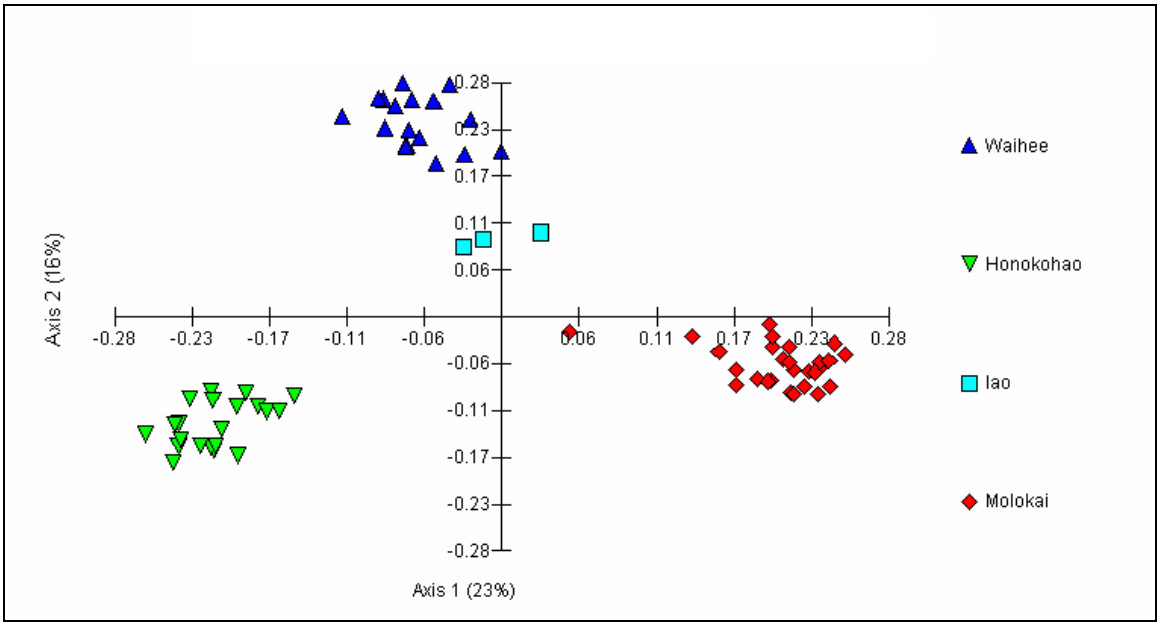

Figure 2.13. PCO analysis of *Hesperomannia arborescens* based on the Gower general similarity coefficient.

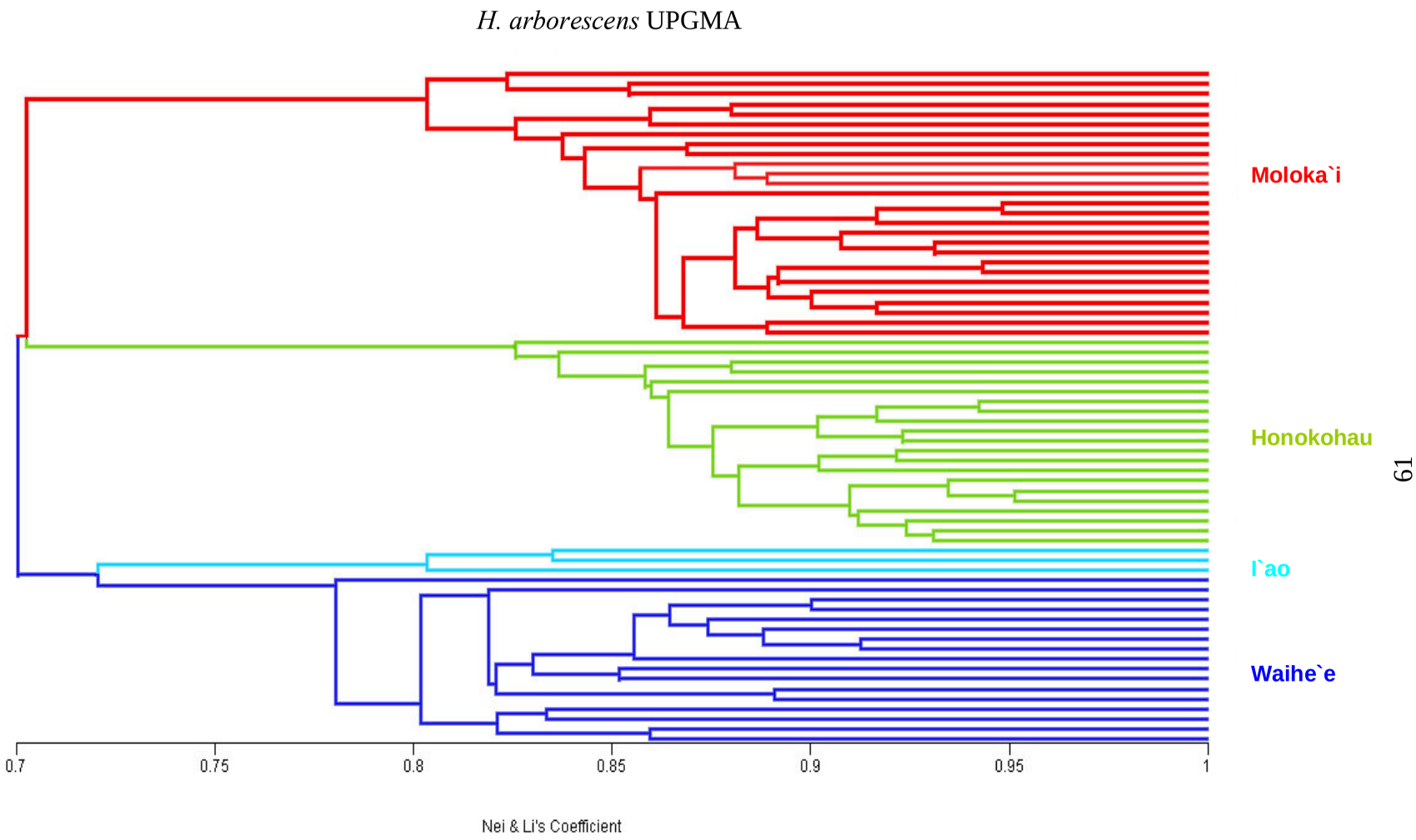

Figure 2.14. Cluster analysis UPGMA dendogram for *Hesperomannia arborescens*, based on the Nei & Li similarity coefficient.

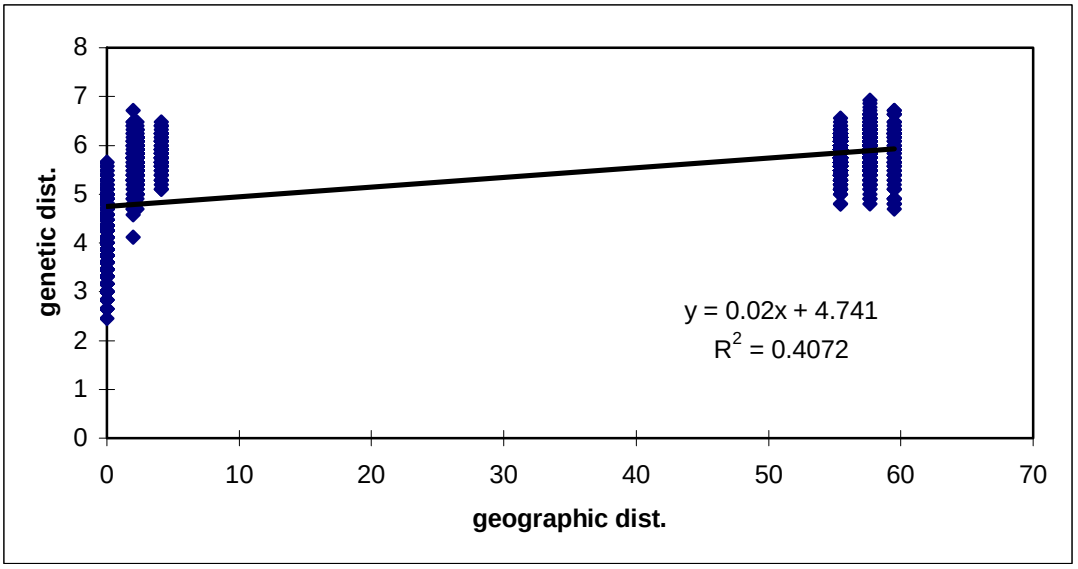

Figure 2.15. Spatial correlation: geographic versus genetic distance for 4 populations of *H. arborescens* on West Maui and Moloka`i. P=0.001

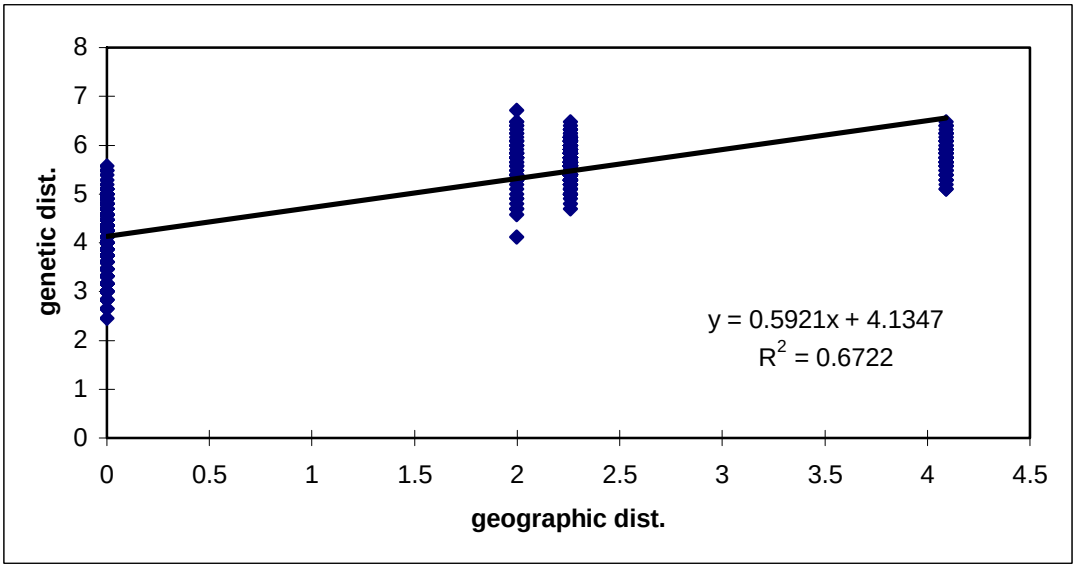

Figure 2.16. Spatial correlation: geographic versus genetic distance for *H. arborescens*, West Maui populations only. P=0.001

Hesperomannia swezeyi

The recent taxonomic treatment of the populations of glabrous plants on O`ahu placed them within *H. arborescens,* the same species as found on Moloka`i and West Maui (Wagner et al. 1990, 1999). As previously demonstrated, those individuals are better characterized as a distinct species (see Fig. 2.3), first described as *H. swezeyi*. Aside from the unique genetic markers, *H. swezeyi* is distinguishable from *H. arborescens* by leaf size, shape, and the amount of pubescence. Therefore, only O`ahu populations were considered in the analysis of *H. swezeyi*.

Hesperomannia swezeyi exhibited a moderate amount of genetic variation compared with the other species. Average heterozygosity value within populations, $\hat{H}_S$ was 0.07 (range 0.055 to 0.093, Table 2.4). Among populations, the total heterozygosity (H_T) was 0.131. The $\hat{H}_S$ / H_T and G_{ST} values indicate that 82.8% of the genetic diversity is held within populations and that genetic differentiation among populations is great (Table 2.4). Both the PCO scatter plot based on the Gower similarity coefficient (Fig. 2.17) and the Cluster analysis based on the Nei & Li similarity coefficient show that the Schofield Barracks East Range (SBE) population is genetically distinct from the other populations. This population has the highest similarity (0.844 Nei & Li; 0.911 Gower, Table 2.8) and the second highest heterozygosity value (0.081) compared to the other populations sampled in this species (Table 2.4).

The Mantel test shows that when all sampled populations are included there is no correlation between genetic and geographic distance and little genetic drift between populations, as evidenced by the low slope of the regression line (m=0.0233, R^2= 0.132); (Fig. 2.19). However, when the two individuals found in the Wai`anae Mountain Range

are removed there is a significant correlation between genetic and geographic distance (Fig. 2.20), where populations closer geographically are also more genetically similar (slope = 17.501). Genetic drift between these populations is also low (R^2=0.1589).

Table 2.8. Similarity values for *H. swezeyi* populations. Gower similarities are above the diagonal and Nei & Li are below, in bold. Average genetic similarity for the species: **0.740 Nei & Li**; 0.846 Gower.

	SBE	Kawailoa H	Ka`ala NARS	Kawailoa F	Kawailoa 1	Poamoho
SBE	**0.844**/0.911	0.804	0.786	0.819	0.832	0.805
Kawailoa H	**0.665**	**0.806**/0.884	0.818	0.853	0.857	0.845
Ka`ala NARS	**0.656**	**0.713**	**0.741**/0.827	0.830	0.854	0.826
Kawailoa F	**0.684**	**0.748**	**0.724**	**0.765**/0.866	0.879	0.879
Kawailoa 1	**0.712**	**0.760**	**0.768**	**0.791**	**0.827**/0.898	0.878
Poamoho	**0.670**	**0.744**	**0.728**	**0.792**	**0.797**	**0.827**/0.894

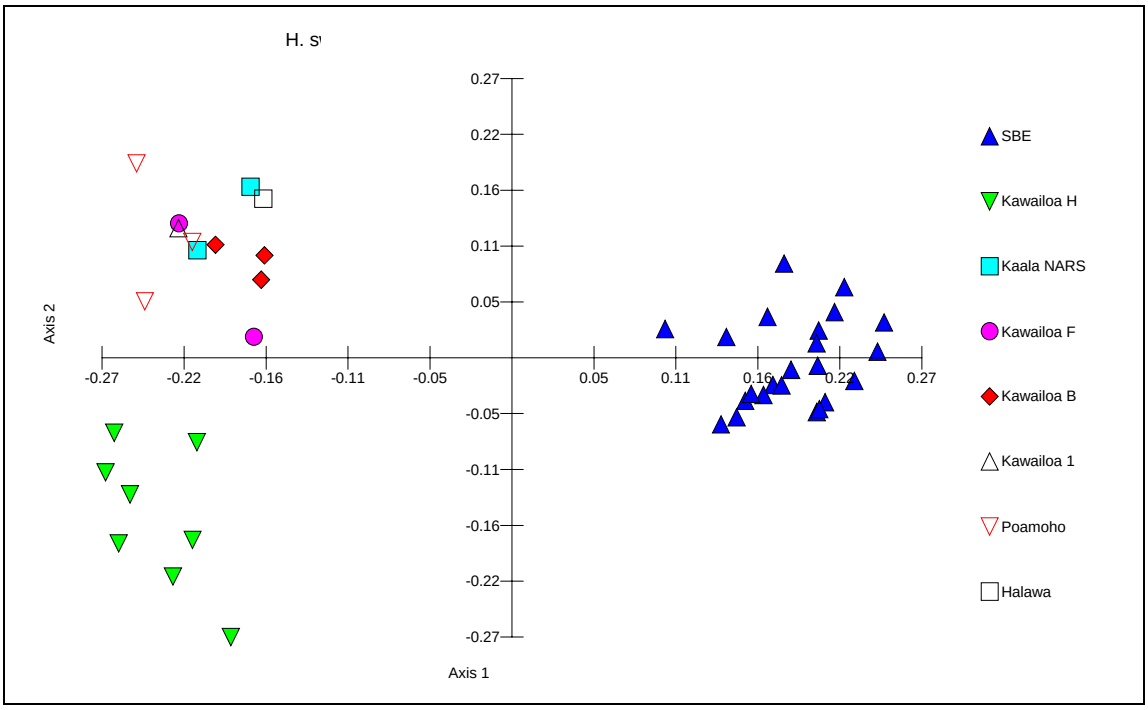

Figure 2.17. Principle coordinate analysis scatter plot of *Hesperomannia swezeyi* based on the Gower general similarity coefficient.

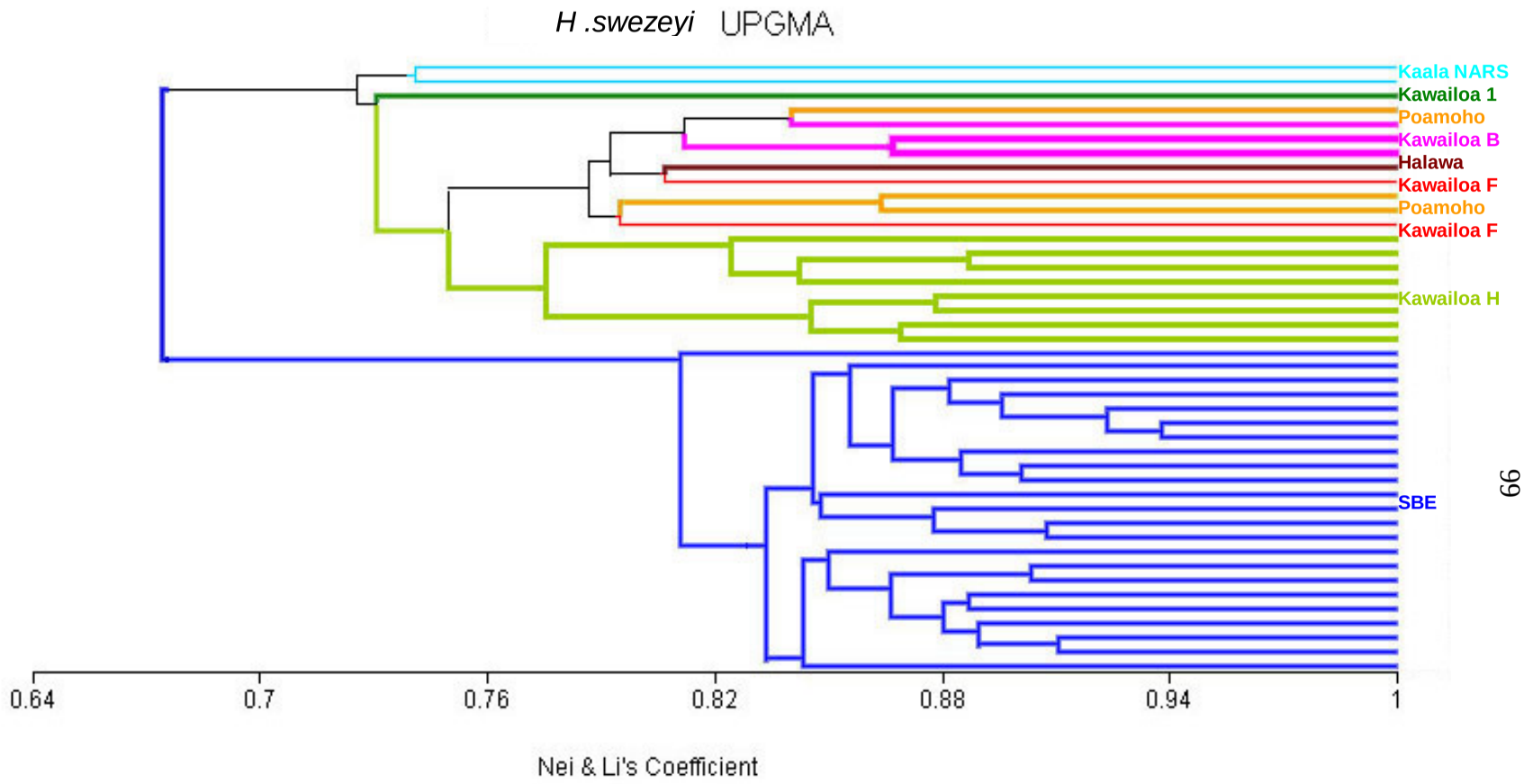

Figure 2.18. Cluster analysis UPGMA dendogram of *Hesperomannia swezeyi* based on the Nei & Li similarity coefficient.

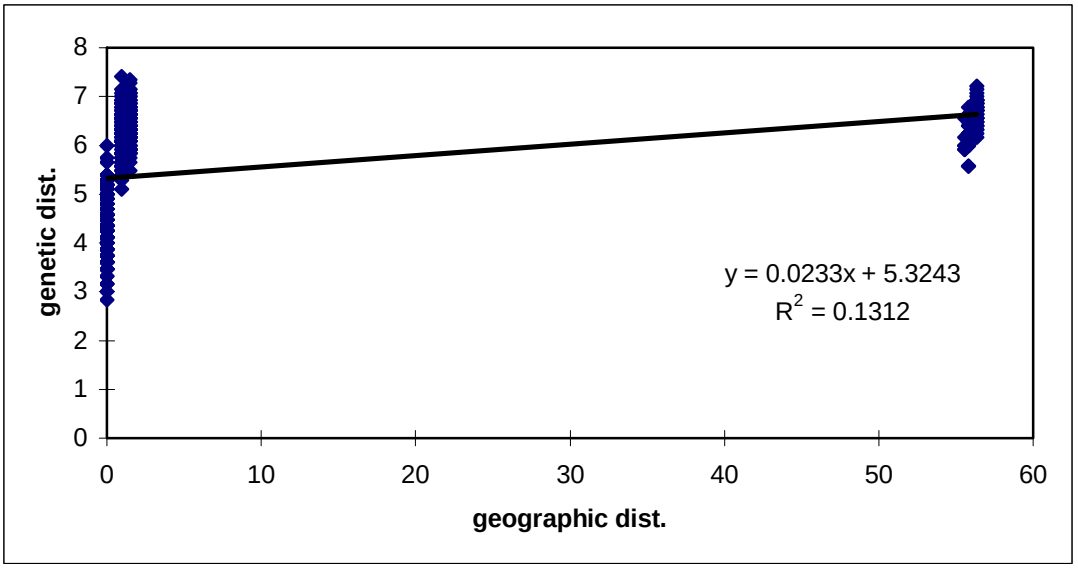

Figure 2.19. Spatial correlation: geographic versus genetic distance for *H. swezeyi* on O`ahu. P=0.001

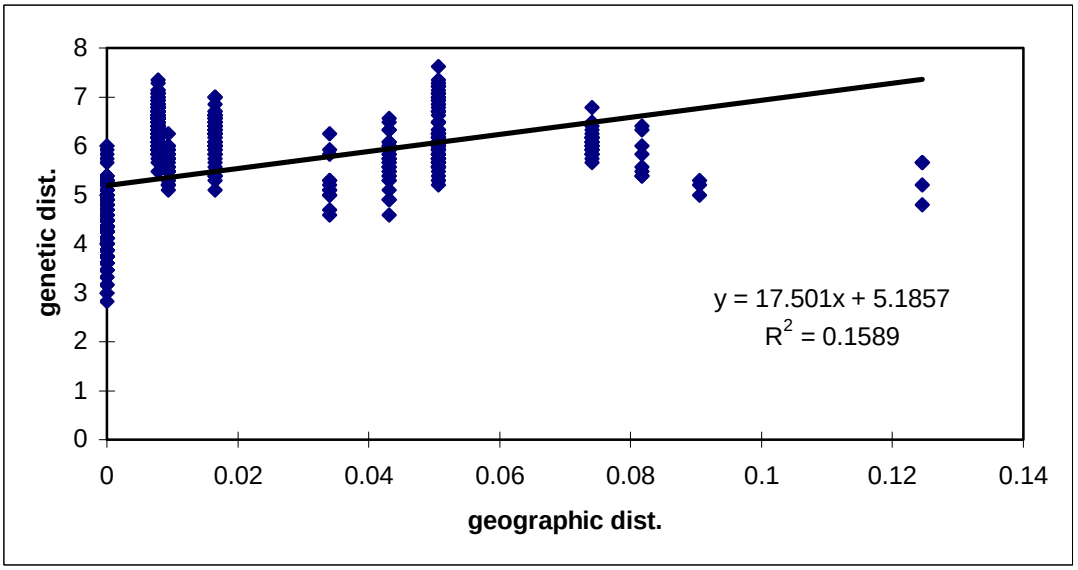

Figure 2.20. Spatial correlation: geographic versus genetic distance for *H. swezeyi*, in the Ko`olau Mts., O`ahu only. P=0.001

Hesperomannia lydgatei **(Fig. 2.21)**

Compared to the other species within the genus, *H. lydgatei* exhibited the lowest amount of genetic variation. This species had the lowest percent polymorphic loci, P = 50.5% (Table 2.3). Average subpopulation level heterozygosity ($\hat{H}_S$ 0.069, Table 2.4) was similar to that found in *H. arborescens* and *H. swezeyi*, (range 0.045 to 0.082). However, the total species heterozygosity for all the subpopulations combined, H_T, was the lowest in the genus at 0.092 (Table 2.5). The $\hat{H}_S$ / H_T and G_{ST} values show that 84.8% of the genetic diversity is held within the subpopulations and that genetic differentiation among the subpopulations was moderate (15.2%). This species also exhibited the highest average genetic similarity among subpopulations, (0.788 Nei & Li; 0.894 Gower, Table 2.9). Populations 4 and 5 were the most similar, (0.829 Nei & Li; 0.915 Gower), while subpopulations 1 and 6 were the least similar (0.757 Nei & Li; 0.875 Gower). The principle coordinate scatter plot shows genetic differentiation among two groups of subpopulations; subpopulations 1, 2 and 3 cluster closely together as do subpopulations 4, 5 and 6 (Fig. 2.22). Despite some overlap of individuals from different subpopulations in the scatter plot, each subpopulation is somewhat distinct genetically. The cluster analysis shows a similar trend of subpopulation distinction but with considerable overlap (Fig. 2.23). No Mantel test was performed on data from this species.

Figure2.21. *Hesperomannia lydgatei*, Wahiawa drainage, Kaua`i, Hawai`i.

Table 2.9. Similarity values for *H. lydgatei* subpopulations, Gower similarities are above the diagonal and Nei & Li are below, in bold. Average genetic similarity for the species: **0.788 Nei & Li**, 0.894 Gower.

	subpop 1	**subpop 2**	**subpop 3**	**subpop 4**	**subpop 5**	**subpop 6**
subpop 1	**0.831**/0.912	0.920	0.901	0.889	0.888	0.886
subpop 2	**0.815**	**0.834**/0.920	0.905	0.884	0.886	0.885
subpop 3	**0.759**	**0.782**	**0.782**/0.905	0.902	0.915	0.898
subpop 4	**0.770**	**0.782**	**0.766**	**0.820**/0.902	0.945	0.901
subpop 5	**0.784**	**0.783**	**0.770**	**0.829**	**0.869**/0.945	0.902
subpop 6	**0.757**	**0.770**	**0.761**	**0.804**	**0.811**	**0.808**/0.902

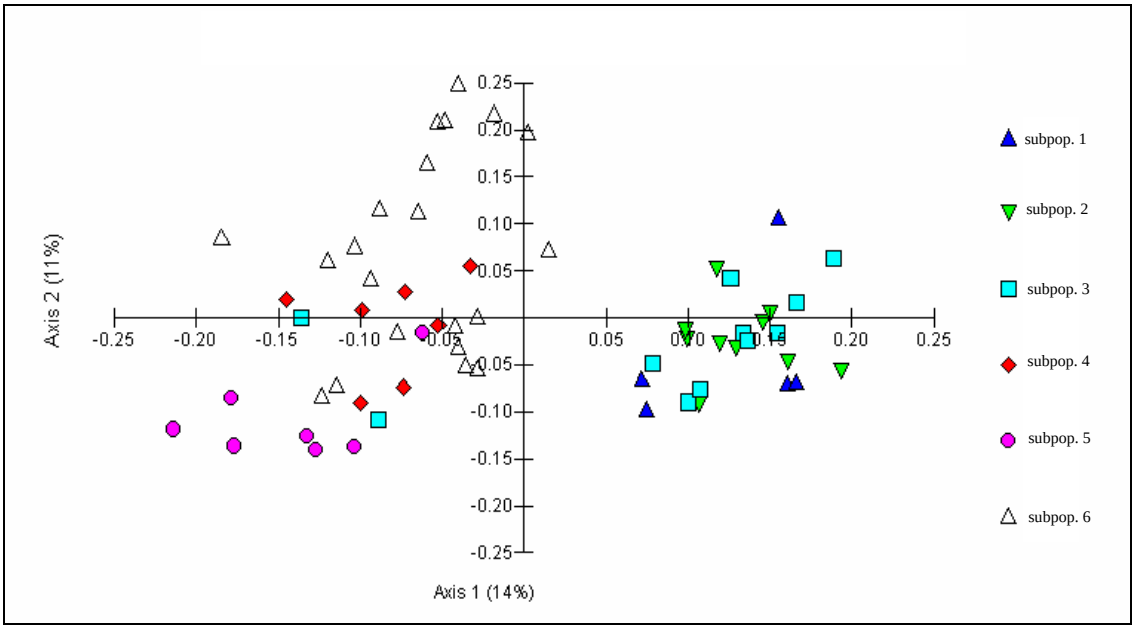

Figure 2.22. Principle coordinate analysis scatter plot of *Hesperomannia lydgatei* based on the Gower general similarity coefficient.

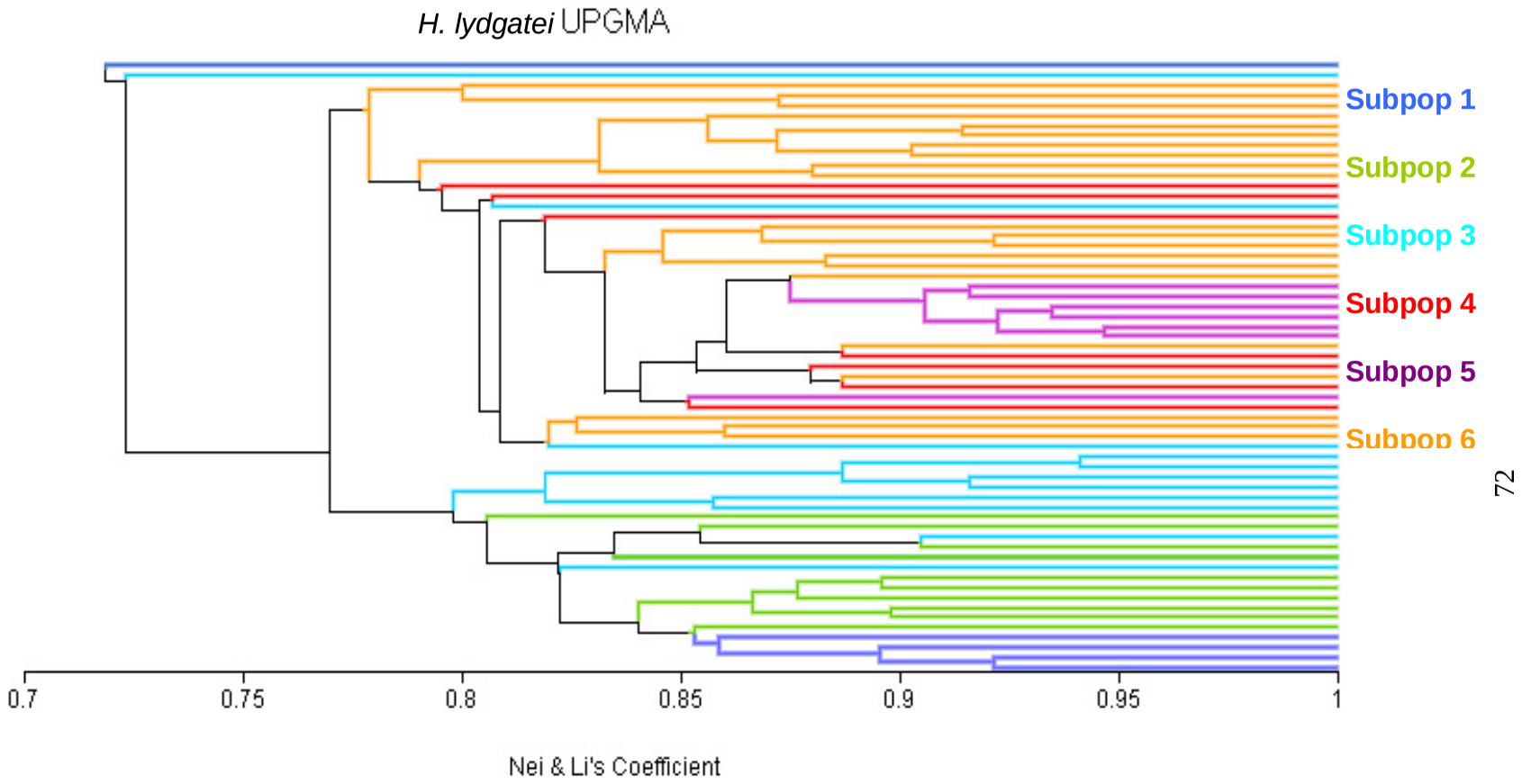

Figure 2.23. Cluster analysis UPGMA dendogram of *H. lydgatei*, based on the Nei & Li similarity coefficient.

Pollen Viability Analysis

Pollen viability estimates based on stainability in analine blue were calculated for each species (Table 2.10). Because of flowering seasonality and limited collection opportunity for *H. arborescens* on West Maui and Moloka`i, only one mature flowering head was collected from each of these populations. The Wai`anae and Makaha populations of *H. oahuensis* were relatively accessible and permitted repeated sampling for three consecutive years (2000-2002). The range of pollen stainability in *H. oahuensis* was the greatest among all species (8.9- 85.7%), and the average (44.6%) was the lowest of any species (Table 2.10). The other species averaged 75.6% (*H. swezeyi*), and 90.6% (*H. lydgatei*). Pollen viability for the one sample of *H. arborescens* was estimated at 98%.

Table 2.10. Estimated pollen viability of *Hesperomannia* species, range of viability within species and number of individuals sampled.

	H. oahuensis	*H. arborescens*	*H. swezeyi*	*H. lydgatei*
% Viable Pollen	44.6% (8.9-85.7%)	98.0%	75.6% (16.3-98.2%)	90.6 % (84.0-99.0%)
# Individuals Sampled	12	1	17	8
# Samples averaged	17	1	17	8

Seed Germination Observations

A total of 2334 achenes were collected from eleven individuals of *H. oahuensis* over the course of this study (2000-2002). Only 61 (2.6%) of the embryos extracted from these achenes germinated. In contrast, both *H. swezeyi* and *H. lydgatei* achenes had higher germination rates. Of the 552 achenes extracted from fruiting heads of 13 *H. swezeyi* individuals, 92 (16.7%) germinated. Similarly, of the 48 achenes extracted from three fruiting heads of *H. lydgatei*, six (12.5%) germinated. No fruiting heads were observed in any of the four *H. arborescens* populations visited in late 2000 and early 2001.

For those achenes that germinated, survivorship was documented throughout the study. Of the 2.6% of embryos of *H. oahuensis* that germinated just 2 seedlings survived for approximately 2 years in micropropagation (Table 2.11). One seedling was planted in the greenhouse, and as of January 2003 was approximately 18 inches tall (located at the Army Environmental greenhouse). In contrast, 30 of the approximately 92 embryos that germinated for *H. swezeyi* survived for 1 year. However, most of those plants died within16 months of germinating. Only one surviving individual remained at the Army Environmental greenhouse as of January, 2003. The six embryos of *H. lydgatei* that germinated died within a few months of germination.

Table 2.11. Average germination (%) for extracted embryos of *H. oahuensis, H. swezeyi* and *H. lydgatei.*.*

	H. oahuensis	*H. swezeyi*	*H. lydgatei*
Average % germination	2.6%	16.7%	12.5%
# individuals sampled	11	13	3
# embryos extracted	2334	552	~48
Survivorship	2 seedlings survived for approx. 2 years (collected 5/17/2001), 1 alive as of 1/03	30 seedlings lived for 1 year, most died at 1yr.4 mos., 1 ind. survived since 11/24/00 as of 1/03	6 germinated & died within a few months

*No Fruiting heads of *H. arborescens* were present when the populations were visited.

Discussion

The Genus *Hesperomannia*

The genus *Hesperomannia* displayed a high degree of genetic polymorphism compared to other Hawaiian plant species for which RAPD studies have been done (95.0 % polymorphic loci for the genus; Table 2.12). Three of the species within the genus also exhibited a higher level of genetic polymorphism compared to other rare Hawaiian species studied (57.4 %-72.3.0 % polymorphic loci). *Hesperomannia lydgatei* was the exception, exhibiting similar, low levels of polymorphism (50.5 %) compared to other rare species such as *Haplostachys haplostachya* (50.8 %) and *Colubrina oppositifolia* (47.0%).

Table 2.12. Percent polymorphic loci among Hawaiian species studied with RAPD markers. Table adapted from Kwon (1999).

Species	% Polymorphic Loci	Reference
Hesperomannia oahuensis	**72.3**	Harbin this chapter
Hesperomannia arborescens	**57.4**	Harbin this chapter
Hesperomannia swezeyi	**61.9**	Harbin this chapter
Hesperomannia lydgatei	**50.5**	Harbin this chapter
Hesperomannia (genus)	**95.0**	Harbin this chapter
Alphitonia ponderosa	41.3	Kwon & Morden 2002
Colubrina oppositifolia	47.0	Kwon & Morden 2002
Haplostachys haplostachya	50.8	Morden & Loeffler 1999
Touchardia latifolia	74.9	Loeffler & Morden 2003
Dubautia scabra	44.0	Caraway 2001
Dubautia ciliolata	55.0	Caraway 2001
Dubautia laxa	60.8	Caraway 2001
Dubautia railliardioides	61.4	Caraway 1997
Labordia sp.	>80.0	Motley 1996
Argyroxiphium sandwicense subsp. *sandwicense*	21.4	Friar & Robichaux 1996

The expected average population level heterozygosity value for the genus was comparatively high ($\hat{H}_S$ =0.117). Average expected level of heterozygosity for the genus combined was also high (H_T =0.170). Within the genus, the average expected population heterozygosity values for *H. arborescens*, *H. swezeyi,* and *H. lydgatei* were lower than the other Hawaiian species studied for which values were available (Table 2.13).

Each of the *Hesperomannia* species has lower similarities than the more common Hawaiian species *Touchardia latifolia*. The hierarchical structure of the genus *Hesperomannia*, based on heterozygosity values, revealed that 70.5% of the genetic variation is contained within the four separate species. The partitioning of genetic diversity for the genus as a whole indicates that the four species are greatly differentiated, G_{ST} = 0.295 (Wright 1978); 29.5% of the genetic variation occurs within the respective species.

Table 2.13. Comparison of expected heterozygosity and Nei & Li's mean genetic identity of Hawaiian species. $\hat{H}_S$ is the average expected heterozygosity at the population level; H_T is the expected heterozygosity at the species level. All values based on RAPD data.

Species	$\hat{H}_S$	H_T	I
Hesperomannia oahuensis	**0.123**	**0.163**	**0.68**
Hesperomannia arborescens	**0.070**	**0.113**	**0.75**
Hesperomannia swezeyi	**0.070**	**0.131**	**0.74**
Hesperomannia lydgatei	**0.069**	**0.092**	**0.79**
Hesperomannia (genus)	**0.117**	**0.170**	**0.63**
*Alphitonia ponderosa**	0.085		
*Colubrina oppositifolia**	0.136		
*Touchardia latifolia***			0.81
Chamaesyce skottsbergii complex**	0.104	0.129	

* Kwon 2002. ** Loeffler and Morden 2003.

This conclusion was supported by the Mantel test. The spatial analysis performed indicates that the four species are in a moderate equilibrium that somewhat follows the stepping stone model of isolation by distance (Kimura 1953, Kimura and Weiss 1964). Thus, more closely spaced populations also exhibit less genetic differentiation than more widely spaced populations. After a certain threshold, there is no correlation between genetic and geographic distance. It would not be expected for the four species to have a continuous linear relationship between genetic and geographic distance because presumably there would be little or no exchange of genetic material across populations on separate islands.

Hesperomannia oahuensis

Based on the genetic and morphological differences of the Wai`anae Kai, Makaha and Palawai populations, it can be concluded that *H. oahuensis* is restricted to the Wai`anae Mountain Range on O`ahu. There is no genetic basis for including the populations from West Maui in this species, and there are also morphological distinctions such as those used in the key that was presented in the results. Thus, it is reasonable to treat these two taxa as separate and distinct species. This delineation restricts *H. oahuensis* to perhaps only five populations in the Wai`anae Range. This study included the largest known populations, Makaha (15 individuals) and Wai`anae Kai (11 individuals), and it is estimated that sampled individuals comprise approximately 80% of all known individuals (33 of ca. 42 individuals). The 33 individuals sampled represent 3 populations. The other two known populations (Pahole and Palikea) contained a total of 9 individuals combined at the start of this study and now collectively contain 2 individuals.

None of the plants were healthy enough during this study to take leaf samples for DNA analyses because leaves were lacking or taking a leaf would have been detrimental to the survivability of the plant (Dan Sailer, TNC; Talbert Takahama, DLNR DOFAW pers. comm.). Since the beginning of this study, several individuals in the Wai`anae Kai and Makaha populations have died, presumably due to severe drought conditions and possibly human disturbance. As of June 2003, the populations were reduced to seven and eight individuals, respectively, and total numbers for the species are approximately 19 individuals. This species is by far the most rare and biologically least vigorous. The populations lack any substantial recruitment; only three juveniles (less than 25 cm in height) were observed from the three populations during a four year period including this study (1999-2002). Estimated pollen viability is low, averaging 44.6% across all individuals with one individual as low as 14%. Germination rates were similarly low for embryos extracted from achenes and survivorship of seedlings was poor. Nearly all seedlings died within the first few months and few survived to 1 year. There is only one juvenile, grown from an extracted embryo, that has survived *ex situ* since 2001. Therefore, the potential for recruitment in the wild is extremely poor and propagation *ex situ* at this time has been unsuccessful.

Despite apparent low fitness, this species showed the highest genetic variability within the genus. Overall, *H. oahuensis* had 72.3% polymorphic markers compared to 50.5%-61.9% in the other species. Average heterozygosity values of *H. oahuensis* were comparatively high within the genus, $\hat{H}_S$ of 0.123 and H_T of 0.163 compared with much lower values in the other three species ($\hat{H}_S$ ranges of 0.069-0.07 and H_T ranges of 0.092-0.131). This species also showed the lowest genetic similarity, 0.68 Nei & Li, among

populations. The G_{ST} value confirms that much of the genetic diversity for this species lies within the different populations. PCO analysis further revealed that the three populations are quite differentiated.

The high genetic differentiation of the populations and low within species similarity values suggest that these populations are experiencing genetic drift. This could be due to initial founder events of the different populations or through reproduction under prolonged genetic isolation. The species has the highest estimated total heterozygosity level in the genus and all three populations have relatively high estimated population heterozygosity. The two largest populations also have higher percent polymorphic loci (67.1% and 69.9%), inferring that genetic drift is occurring without extensive inbreeding taking place. However, the effects of both genetic drift and inbreeding are likely combined *in situ* (Newman and Pilson 1997).

These findings appear to be in contrast with the results of the Mantel test, which indicates that there is no correlation between geographic and genetic distances and relatively low measurements of genetic drift are apparent (m= 0.0668, R^2= 0.0622). There are several possible explanations for this apparent discrepancy. Hutchinson and Templeton (1999) suggest first that these results could arise from new invasions forming several populations where material came from the same source population. Second, they suggest that these populations have undergone relatively recent population isolation i.e., a recent reduction in gene flow perhaps due to a sudden loss of pollinator or vegetative changes causing reduced gene flow. A second possible explanation is that geographic distance plays no role in genetic distance due to populations arising from a single fertile

individual randomly disbursed where separate populations were founded by single individuals. In such cases, the results show that genetic drift has been significant.

Despite having the highest levels of genetic diversity within the genus, these elevated levels of variation (compared to the other species) are clearly not related to higher fitness levels. The comparative attenuation in fitness of this species may still be due to the loss of essential genetic variability. Populations of *H. oahuensis* in the Wai`anae Mountain Range on O`ahu occur in mesic forests that in recent years, and presumably periodically, have experienced severe drought. The higher genetic variation detected in these populations is likely an adaptation to the harsher environmental conditions experienced by this species (Frankham 1995, 1997). Therefore, variability in this species may be severely reduced compared to historic levels.

Hesperomannia arborescens

As mentioned, preliminary analyses of the data revealed populations of *H. arbuscula* from West Maui and the population of *H. arborescens* from Moloka`i are very similar to each other and also genetically distinct from the O`ahu populations of each species. All data indicate that any taxonomic ties to the two species found on O`ahu are tenuous. The group as a whole is as dissimilar from each of the other three species as those species are from each other. This species includes populations of the formerly recognized *H. arbuscula* on West Maui and populations of *H. arborescens* on Moloka`i.

Hesperomannia arborescens exhibited a moderate degree of genetic variation among the populations compared to the other three species. The percent polymorphic loci for this species was moderate compared to the other species as were estimated population

and species level heterozygosity values. Accordingly the G_{ST} value indicated most of the genetic diversity is contained among populations with PCO analysis showing each population being genetically distinct. The average genetic similarity for this species was moderate, 0.746 Nei & Li. The relationships among the populations are reflected in the cluster analysis, based on the Nei & Li similarity coefficient. All evidence indicates that the Waihe`e and I`ao populations are the most closely related. The Honokohau and I`ao populations are the least similar; both are more closely related to the Moloka`i population than to each other. However, all the similarity values comparing populations are high. Therefore, although the Moloka`i population is separated by a large geographical distance, the high similarities of all the populations reinforce the taxonomic delineation of this species from those on the older islands.

The first Mantel test concluded that there is no correlation between geographic and genetic distance, and that genetic drift is high ($m = 0.02$, $R^2 = 0.4072$) when all four populations are considered. This is to be expected since it is unlikely that inter-island gene flow among these rare plant populations is occurring. The high degree of drift is a factor of the isolation of the four populations, as this is the only species with a distribution on more than one island. A second Mantel test examined only those populations on West Maui, a group more likely to have had recent gene flow. This test showed a strong correlation between geographic and genetic distance and fits the classic example of the isolation by distance model described by Kimura (1953) and Kimura and Weiss (1964) where populations that are closer in geographic proximity also have higher genetic similarity. This correlation is evidenced by the high slope of the regression line in Fig. 2.11 ($m= 0.5921$). Genetic drift was determined to be high between the Maui

populations ($R^2 = 0.6722$) suggesting that these populations likely arose from a similar genetic source or had historical gene flow, but are not experiencing gene flow currently.

These results indicate that there is likely both inbreeding and genetic drift occurring in *H. arborescens*. However, the populations still contain a good deal of genetic variation, genetic drift is has likely had a greater effect than inbreeding. These populations, except Ι`ao, appear to be stable *in situ*. All stages of growth were observed in the field, including seedlings. Thus, despite the evident genetic drift the larger populations appear to be doing well. However, because only one flowering head was collected for pollen analysis and no fruit was collected for germination observations, it is difficult to compare the fitness of this species to the others in the genus.

Hesperomannia swezeyi

Hesperomannia swezeyi is found primarily in the Ko`olau Mts., O`ahu, with two individuals recently found in the Wai`anae Mountain Range. From the PCO analysis for all the species of *Hesperomannia* (Fig. 2.3) it appears that *H. swezeyi* and *H. oahuensis* should be treated as a single entity. However, PCO analysis of just those populations on O`ahu reveals their relationship as two closely related, but distinct, species. In addition to the genetic distinction, these species are also distinct morphologically, as mentioned in the key, and occupy ecologically distinct habitats: *H. swezeyi* occurring primarily in wet forest and *H. oahuensis* occurring in mesic forests.

Hesperomannia swezeyi demonstrated a wide range of genetic variation as indicated in genetic similarity values and visualized in the PCO and cluster analyses. This species is by far the most abundant in the genus. Current estimations of the total number

of individuals on O`ahu are over 2000 (Joel Lau, Hawaiian Natural Heritage Program, personal communication 2001). This species exhibited a moderate degree of genetic variation relative to the other species with 61.9% polymorphic loci and species level heterozygosity levels approximately the same as the *H. arborescens* and *H. lydgatei*. The G_{ST} value indicates that 82.8% of the genetic diversity is held within populations and that the populations are greatly differentiated, $G_{ST} = 0.172$.

The mantel test for populations of *H. swezeyi* from the Ko`olau Mts. shows an extremely high correlation between geographic and genetic distances. Even though it is obvious from the data that geographically close populations are most similar, the consistency of moderate scatter around the regression line indicates that gene flow, even among close populations, is restricted. Therefore there is persistent genetic drift even though limited gene flow is occurring.

The populations from Schofield Barracks East Range and Kawailoa “H” are highly differentiated from the other individuals sampled. These two populations and populations Kawailoa #, Kawailoa F, and Poamoho are all found in the Ko`olau Mountains. If these two populations were experiencing inbreeding to account for their separation from the other populations we would expect to see a relative deficiency in both heterozygosity and percent polymorphic loci. However, these parameters were within the range found in other populations in the Ko`olau Mts. There was also no visible evidence of inbreeding depression in these populations as plants appeared vigorous and there were numerous seedlings and juveniles present. Estimated pollen viability was high, 75.6% and embryo germination rates were also high compared to the other species studied.

Differentiation among the populations of *H. swezeyi* is likely a result of genetic drift. *Hesperomannia swezeyi* populations in the Ko`olau mountain range are often separated by drainages and or ridges. Genetic drift could occur following a population bottleneck after initial founder events or it could occur over successive generations due to isolation, and would intensify in smaller populations (Ellstrand and Elam 1993, Frankham 1998).

The relative abundance of this species on O`ahu represents an additional explanation for the apparent population differentiation observed. It is possible this study lacked sufficient sample size to account for the full range of genetic variability represented in the wild. Populations of this species are not fully isolated from each other as there are several populations in close proximity. This apparent genetic distinction may be a factor of sampling heavily in one area and not enough in another. It is possible that if more populations were sampled, the gaps in genetic differentiation among populations would be filled.

However, the most likely explanation for the differentiation among populations is genetic drift. Several populations in the Ko`olau range and one in the Wai`anae range were small, (two and three individuals), leading one to believe that the populations have not recently had a continuous geographically based genetic pattern. The larger populations hold a greater amount of genetic variation, yet this variation does not include the several markers found in the smaller populations. Although this species is relatively abundant, it is possible that prolonged isolation of populations likely resulted in an eventual reduction in genetic variability due to both genetic drift and inbreeding.

Hesperomannia lydgatei

Hesperomannia lydgatei exhibited the lowest amount of genetic variation compared with the other species based on the percent polymorphic loci and the estimated species level heterozygosity. This might have been predicted based on previous knowledge of the species. *Hesperomannia lydgatei* is essentially known from a single large population in the Wahiawa Drainage area of SE Kaua`i (USFWS 1994). There are reports of one or a few scattered individuals on the Northern side of the island in Limahuli Valley and near Kokee (Steve Perlman NTBG & Kerri Fay, personal communication), but these were not available for analysis in this study.

As expected, similarity values show that subpopulations 1 and 6 are the least similar being located at the extremes of the entire population's geographical range. There was further limited genetic differentiation of subpopulations based on geography of the subpopulations, with subpopulations 1, 2 and 3 being genetically distinct from subpopulations 4, 5 and 6.

Low genetic variation and high similarities throughout the large population indicates that the sampled individuals are the result of some degree of inbreeding. This population was severely reduced by hurricane Iniki in 1992. Before the hurricane the population was estimated to contain approximately 280 individuals. However, there were less than 100 individuals when field collections were made for this study (September 2000). Many of the trees sampled for this study may have germinated following the hurricane. In which case it could be possible that the high degree of similarity and low

degree of genetic variation could be attributed to regeneration from a few closely related individuals.

Hesperomannia lydgatei is highly restricted in range and numbers of individuals, and this study suggests there is some evidence of inbreeding and drift taking place based on the genetic similarity trend over geographical distances and by the lower overall genetic diversity at the subpopulation and species level. However, due to the current restricted distribution of this species, there is some evidence of a reduction in fitness compared to the other species in the genus. Although, all stages of growth were observed in the field and estimated pollen viability was among the highest of those measured.

Conclusions: recommendations for conservation

Small population size is one of the major concerns in the conservation of all the species in this genus. Overall, there is an apparent positive correlation between population size and genetic variability as evidenced by the lower frequency of percent polymorphic loci per population as the size of the population decreases. The estimated population heterozygosity, $\hat{H}_S$, for all species also seems to follow this trend. However, as revealed in PCO analyses some of the smaller populations may contain unique genetic markers not found in the larger populations.

Because each of the species in the genus is rare, the reduction in population size and the subsequent loss of genetic variability due to genetic drift and inbreeding is only likely to intensify over time (Barrett and Kohn 1991, Newman and Pilson 1997). Several of the populations included in this study contain just two or three individuals. These small populations occur even in what is considered to be the most abundant species, *H.*

swezeyi. With the eventual reduction in genetic variation over time, the populations will be prone to corresponding reductions in fitness and the ability to adapt to new and or changing environments (Elstrand and Elam 1993, Husband and Schemske 1996, Frankham 1995, 1997, Lande 1999).

With such small and restricted populations, it should be realized that not all of the individuals contribute to regeneration, especially considering there are no documented pollinators for *Hesperomannia*. Thus, the effective population size is often much smaller than the total number of individuals. As an example, there are obvious differences in fitness among individuals within populations of *H. oahuensis*. Some individuals consistently had low or high pollen viability and most of the embryos that germinated were collected from the same individuals that provided progeny the previous year. Thus, conservation efforts for the genus *Hesperomannia* in Hawai`i should focus on preventing or stopping further reductions in population sizes.

Based on this research, the current Federal status of Endangered is appropriate for all species in this genus. However, conservation efforts should have a different focus for each species. Tomentose plants of the Wai`anae mountain range on O`ahu should be considered *Hesperomannia oahuensis* and should remain listed as endangered in order to provide federal protection. Recognizing that this species has a very small range with very few individuals will emphasize the grave situation of these populations. This species is the most critical in terms of needing urgent conservation efforts. Over the course of this study, the numbers of individuals has drastically declined due to a number of factors including low recruitment, competition with invasive species, human and animal disturbance, and persistent drought. The top priority for this species should be fencing or

otherwise protecting the remaining individuals from animals. The number of individuals should also be increased through propagation of seed to foster genetic variability and provide material for asexual propagation. Successful asexual propagation would serve as *ex situ* germplasm storage and a protection against complete loss of the species. Because germination is so low for this species and because there is little to no recruitment in the field, the probability of successful propagation would be enhanced if all seed were collected from flowering individuals whenever possible.

The first priority for the Maui Nui populations of *Hesperomannia* is to recognize them as *H. arborescens.* With the taxonomy clearly defined, conservation efforts can be directed to the most critical populations, such as the I`ao population on West Maui. There is a need to continue to search for additional populations in the West Maui mountains and also in the wet forests of the North coastal cliffs of Moloka`i. This would provide a better estimate of the species range and numbers of individuals. Even though the populations appear to be stable, the high degree of genetic drift and the great degree of genetic diversity held among populations of *H. arborescens* emphasize the need to preserve each population, i.e., each population may contain unique loci needed for the conservation of this species. Therefore, each population should be monitored for any drastic reductions in numbers. Finally, efforts to propagate this species ex situ should be made. To date, there has never been an effort to do this. Fruiting heads, both immature and mature, should be collected for propagation and micropropagation. If successful, the *ex situ* individuals would provide a genetic safety net against complete loss and may also someday be used for out-planting in restoration sites.

Hesperomannia swezeyi appears to be the most abundant and vigorous species in terms of numbers of populations and fitness, as estimates of numbers of individuals are comparatively high and numerous seedlings and juveniles have been seen in the wild. However, the range of this species may currently be smaller than historical collections indicate. The results of this study indicate that the relative abundance and fitness of this species does not warrant a federal delisting of this species, especially when the degree of population differentiation and genetic variability are considered. Conservation efforts for this species should go towards habitat protection and monitoring of existing large populations for any drastic reduction in numbers. The larger populations should be protected, but it would also be beneficial to protect or collect propagules from any new individuals in smaller populations if they are found to hold genotypes not present in the larger populations. *Hesperomannia swezeyi* could also help in the recovery of the other species because of its relative abundance and fitness, by providing a good resource for developing more successful propagation methods for cuttings and seed.

Finally, *Hesperomannia lydgatei* should have a high priority for conservation efforts. The fact that this species occurs predominantly in one large population is cause for concern. Any number of stochastic events such as disease, hurricane or drought could lead to the complete loss of this species. Conservation efforts should focus on monitoring the current population for drastic changes in numbers and the propagation of this species with intentions to restore populations to the historical range and provide a germplasm storage base. This study shows that even though there is essentially one population left, there is still a fair amount of genetic variation within each subpopulation. Increasing the

number of individuals *in situ* and *ex situ* will aid in maintaining and potentially increasing genetic diversity in the future.

Chapter 3

Hypotheses Revisited

The following formal hypotheses were proposed at the beginning of this research based on the former and current distribution and ranges of each species and the estimated numbers of individuals in each of the respective populations. Conclusions regarding acceptance or rejection of hypotheses of this study follow.

I. Each species of *Hesperomannia* is distinguishable at the genetic level; unique genetic markers distinguish each species.

This hypothesis was supported. Additionally, a fourth species, *Hesperomannia arborescens*, occurring on West Maui and Moloka`i, was distinguished based on the degree of genetic differentiation from the other species and the high genetic similarities among those populations. Each species, including *H. arborescens*, contained unique loci not found in the other species.

II. Each species of *Hesperomannia* possesses low overall genetic variation.

When compared to other molecular studies of Hawaiian plant taxa, this hypothesis was not necessarily upheld for the genus *Hesperomannia*. The frequency of polymorphic loci for the genus based on RAPD data was high (95%) compared to other taxa (21.4->80.0 see Table 2.12.). Certain species, however, did exhibit lower genetic variation than others. Overall, the four taxa studied here showed higher frequencies of polymorphic loci (range 50.5-72.3) than other rare Hawaiian taxa such as *Argyroxiphium sandwicense* spp.

sandwicense (21.4 %) (Friar and Robichaux 1996), *Alphitonia ponderosa* (41.3 %), and *Colubrina oppositifolia* (47.0 %) (Kwon 1999).

a. *Hesperomannia arborescens* populations on different islands are genetically distinct (O`ahu, Moloka`i).

This hypothesis was supported, though not on the basis of the explanation provided in the proposal (Chapter 1). The O`ahu plants were shown in this study to have a RAPD fingerprint so distinct from the Moloka`i plants that they both warrant species level recognition (*H. swezeyi* and *H. arborescens*, respectively). Analysis of RAPD data also revealed genetic variation that distinguished the two larger populations and some of the smaller populations of *H. swezeyi* sampled on O`ahu (see Figure 2.10.).

b. *Hesperomannia lydgatei* retains considerable genetic variation within the one large remaining population.

This hypothesis was supported to a limited degree. The six smaller subpopulations sampled in the Kanaele Bog Drainage area did show some subdivision based on subpopulation and geographic associations. There is a division between subpopulations 1-3 and 4-6 that is visible in the PCO and cluster analyses, as based on similarity indices (Figures 2.12-13.). However, this species showed the highest degree of similarity among individuals (0.788 Nei & Li). Therefore, population subdivisions are not large when compared to other species.

c. *Hesperomannia arbuscula* is the species with the least amount of genetic variation.

The data gathered in this study do not support this hypothesis. In fact, *Hesperomannia oahuensis* (*H. arbuscula sensu* Wagner et al. 1990) exhibited the highest genetic variation in the genus according to RAPD data. This species exhibited the highest frequency of polymorphic loci and estimated heterozygosity at the population and species level and had the highest number of unique loci. Conversely, this species exhibited the lowest degree of fitness as measured by estimated pollen viability and seed germination. This species is also the most critical in terms of low numbers of individuals and populations. The comparatively high degree of genetic variation in this species is possibly due to evolution in a harsher environment compared to the other species. Thus, the genetic variation found in this species today may be residual from a time when this species was more abundant.

III. The West Maui plants referred to as *H. arbuscula* by Wagner et al. (1990, 1999) are genetically distinct from *H. arbuscula* of the Wai`anae Mountains of O`ahu.

This hypothesis was based on the previous classification of these individuals as a distinct species, subspecies or variety by Carlquist (1957) and St. John (1978). These plants are also more similar in habit and habitat to *H. swezeyi* than to *H. oahuensis*. The plants from West Maui have a tree-like habit and are found in wet forest rather than a sprawling habit in a more mesic environment like that of *H. oahuensis*. The hypothesis was found to be somewhat incorrect, for the West Maui and Moloka`i plants were a distinct taxonomic

entity based on several morphological distinctions (see Key to 4 species in Chapter two) and their high similarity among the populations studied. Nomenclatural priority requires this species to be referred to as *H. arborescens* rather than *H. arbuscula*.

IV. Pollen viability and seed germination rates are correlated with the amount of genetic variation found in populations.

This hypothesis was not supported. These results showed that *H. oahuensis* had the lowest pollen viability and seed germination rates yet had the most genetic variability compared to the other species. When considered with the indication that this species has undergone genetic drift, the relatively low fitness levels observed suggest that although *H. oahuensis* is more genetically variable, current levels of genetic variability are possibly much lower than historical levels. It is also possible that drift has resulted in the loss of critical alleles from each population. Such loss could be especially detrimental if these populations are self-incompatible. However, this possibility is yet to be explored. The higher fitness levels observed in *H. swezeyi* and *H. lydgatei* contrasted with their moderate degree of genetic variability. These two species also exhibited regeneration in the field. Thus, *H. swezeyi* and *H. lydgatei* may have a gene pool that is less eroded and less affected by genetic drift than that of *H. oahuensis*.

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