

BIOSYSTEMATICS OF THE ENDEMIC HAWAIIAN SPECIES OF LYSIMACHIA
(PRIMULACEAE)

by

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Abstract

The endemic Hawaiian species of Lysimachia were examined using a combination of analyses of metric and non-metric morphological characters, allozyme variation and crossing studies. The purpose was to produce a taxonomic revision and to investigate the degree of genetic divergence and type of reproductive isolation that has accompanied speciation. Principal components analysis of metric characters produced an ordination of OTU's, that was used as a first step toward understanding the range of variation. However, greater reliance was placed on non-metric characters for the taxonomic revision. Sixteen species and four subspecies, including three previously undescribed species are now recognized.

Allozyme variation was analyzed in 15 taxa from 48 populations. Genetic identities ranged from 0.71-1.00, indicating high genetic similarity and supporting the hypothesis of a monophyletic origin. These values are intermediate to those of other insular genera. There is a low correlation between morphological variation and allozyme variation. Total genetic diversity, H_t , within species, ranges from 0.02-0.22. Genetic diversity *within* populations does not decrease in an entirely linear manner from the oldest to the youngest island. However, there is a step-wise decrease in genetic diversity *among* taxa within an island from the oldest through to the youngest island.

All interspecific crosses that were attempted produced fertile seeds. Pollen stainability was quite variable in all species and in the F_1 hybrids of most species pairs. The reduced stainability of the parents involved in the crosses makes it difficult to interpret the significance of reduced stainability of some hybrids. Approximately 80% of self-pollinations within flowers failed. This appears to be due to protogyny, not to self-incompatibility.

Adaptive radiation in Lysimachia has resulted in species that have diverged in corolla pigmentation, leaf size and shape, and ecological preferences. Speciation has occurred without the types of divergence between species that often accompanies speciation on continents, i.e., internal, post-zygotic barriers to reproduction and low genetic identities. This pattern of morphological and genetic variation is similar to that seen in other insular groups.

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Dedication

This dissertation is dedicated to the memory my father, Dr. John W. Marr (1914-1989), a kind and gentle man, and plant ecologist, who treated all people with dignity.

You taught me how to love,
What it took, what it took,
You never said too much,
But still you showed the way,
And I knew, from watching you.

(From the song, "Everthing I Own", written by David Gates for his father.)

And to my mother, Ruby and sister Linda, for all of their love and encouragement.

Chapter 1

Introduction

1.1 Overview of insular evolution

Oceanic islands are volcanic in origin and have never been connected to continents; their significance is seen in observations of the biota of the Galapagos Islands, which were important in helping Charles Darwin to formulate his ideas concerning both the fact and the mechanisms of evolution. It is this isolation from source areas of propagule dispersal that is perhaps the most important difference between evolutionary processes on continents and those on islands (Hubbell, 1968). Darwin, however, apparently did not realize that the separation of two populations that had once belonged to the same gene pool could result in the formation of new species, preferring instead to invoke natural selection as the primary force of evolution (Carson, 1987), although Darwin's reliance on geographic variation does rely on a tacit assumption of isolation.

Two additional features of oceanic islands that influence evolutionary processes are their relatively recent origin and the close proximity of a diversity of habitats in a small area (Crawford et al., 1987b). Because habitats of an individual island are generally much younger than continental ones, the events that lead to speciation are usually more recent and it is easier to discern the processes responsible for divergence among species (Carson, 1987). Internal

reproductive barriers among island plants in general appear to be lacking and it is usually easy to produce artificial F_1 and later generation hybrids as well as backcrosses. This makes it possible to examine the nature of reproductive isolation between species. Furthermore from the frequency of various traits among the progeny of such crosses, it may be possible to determine the genetic basis of morphological differences.

After the passage of time, the descendants of founding populations may diverge into several species. This process is frequently referred to as "adaptive radiation," defined by Hubbell (1968) as "the separation of the descendants of one ancestral stock into numerous species, adapted to live in a variety of new situations by changes in form, function, and ecological tolerances and requirements." Carlquist (1974) has presented some of the most important trends in insular evolution in his 24 "Principles of dispersal and evolution." Those that are most relevant to the present study are summarized as follows: (1) Because of the rarity of dispersal events, most taxa, especially those that grow away from coastal areas, are probably established at a single time and there is no further genetic input from the ancestral species. (2) Founding populations must overcome the restriction of genetic material that is a product of the small size of the initial population if effects such as inbreeding are to be countered. This often results in selection for outcrossing mechanisms. (3) Adaptive radiation is inevitable where a small number of founding taxa encounter a broad range of

ecosystems. (4) New growth forms evolve among plants on oceanic islands, especially a tendency toward increased stature. (5) Pollination relationships correspond to, and change with respect to, availability of insects and other pollination agents on islands. (6) Some mutations that would be lethal or disadvantageous in continental environments have a more neutral value in the less competitive environment of an oceanic island.

1.2 Affinities of the Hawaiian flora

The Hawaiian Islands are perhaps the most isolated oceanic islands in the world. They lie about 2,000 miles from California, 3,400 miles from Japan, 450 miles to the nearest small island and 850 miles south to the next island chain. Prior to the arrival of people, propagules that gave rise to the native flora arrived by floating in sea currents, on natural rafts, attached to the feathers or in the gut of birds, or were carried by the wind (Carlquist, 1974). The approximately 270 native Hawaiian angiosperm genera have affinities with all parts of the Pacific basin: 40.1% are Indo-Pacific, 18.3% are American, 16.5% Austral, 2.6% Boreal, 12.5% pantropic and cosmopolitan and 10.3% are obscure in origin Fosberg (1983).

1.3 Dissertation objectives

The endemic species of Lysimachia were chosen for this study of insular evolution with two objectives in mind: first

to produce a taxonomic revision; second, to attempt to understand the evolutionary processes that resulted in the divergence among species. The need for a taxonomic revision was evident from the discrepancy in the number of species recognized by two recent treatments. St. John (1987) published diagnoses of 44 new endemic species and four new combinations, to add to the 10 previously described. Wagner, et al. (1990) treated the genus as consisting of 10 endemic species, but noted that a thorough biosystematic study was needed. The second objective had two parts: one, to arrive at an estimate of the level of genetic divergence that has accompanied adaptive radiation in Lysimachia as measured by electrophoretically detectable allozyme variation; and two, to evaluate the nature of reproductive isolating mechanisms among species.

The results of this study are presented in three chapters. In Chapter Two, several areas relating to the classification of the group are discussed including taxonomic history, a multivariate analysis of morphological characters, which provides insight into patterns of variation, and a taxonomic revision at the end of the chapter based primarily upon qualitative non-metric characters. The non-metric characters proved to be more useful in grouping populations together based on the possession of shared combinations of characters. In Chapter Three the results of allozyme analysis are presented. These results were used to estimate the level of genetic variation within, and divergence among populations

of the same species as well as between species. The nature of reproductive isolation among species was evaluated by making artificial crosses in the greenhouse and recording the pollen stainability (as an estimate of fertility) of F_1 hybrids. These results are presented in Chapter Four.

1.4 Previous studies of Hawaiian Lysimachia

Prior to the present research, there had been no investigation of any aspect of the biology of Lysimachia apart from their alpha taxonomy. Even this information was presented in a somewhat disorganized manner. Gray (1862) published the first description of the Hawaiian species. Hillebrand (1888), Heller (1897) and Knuth (1905) provided keys and descriptions as additional species were discovered. After 1905, several more species were described but there were no further comprehensive treatments of the Hawaiian species until Wagner et al. (1990) produced a revised and expanded treatment in the Manual of the Flowering Plants of Hawai'i. At the time of his death in 1991, long-time student of the Hawaiian flora, Dr. Harold St. John had a manuscript in preparation that included a key and lengthy description of 72 species. This manuscript was never published; however, a copy was loaned to me by the Bishop Museum in Honolulu. Many of the specimens that St. John named in this paper were collected as many as 60-95 years preceeding his manuscript. In some cases, St. John applied different names to specimens that bore the same collection number. In other cases, specimens that

were collected several years apart, but from the identical location, were given different specific epithets. Despite these serious deficiencies, I found his treatment of the taxonomic history to be especially useful. The page proofs of a second, not professionally published manuscript written in 1983 by Otto and Isa Degener, was also made available to me: "Plants of Hawaii National Parks Illustrative of Plants and Customs of the South Seas". This, the third edition, was never formally published, but was effectively published because copies were sent to many botanical institutions worldwide. The only other analysis of Hawaiian Lysimachia are chromosome counts of one species by Skottsberg (1953) and of two species by Carr (1978) and pollen analyses by Selling (1947), Huynh (1970) and Bennell and Hu (1983).

Nothing was known about the reproductive compatibility system of Hawaiian Lysimachia, or of the existence of mechanisms that might promote outcrossing such as dichogamy or heterostyly. I have not found any information regarding pollination of Lysimachia, apart from a note on a herbarium label that the flowers were more fragrant at night (this agrees with my observations of plants grown in the greenhouse). Zimmerman (1978) lists three insect species whose host plants are different species of Lyismachia, but no pollinators are mentioned.

As far as I have been able to determine, there have been two previous attempts to grow Hawaiian Lysimachia under artificial conditions. This is documented in an exchange of

letters written between 1938-1940, that are stapled to an herbarium specimen (*Degener 17,672* at NY) collected from the Waianae Mtns. One set of correspondence is between Hawaiian plant collector Otto Degener and English plant anatomist J.H. Priestly, and concerns an attempt to cultivate this species in a glasshouse from cuttings sent by Degener. Dr. Priestly was evidently interested in this species because it reportedly lacked secondary phloem and he wanted to investigate its potential suitability for physiological studies on translocation of plant foods. Cuttings were also sent to Leonard Croizat at Harvard. There is no further record of the success or failure of these attempts at cultivation.

1.5 Geographical affinities of endemic Hawaiian Lysimachia

The combination of characters found in the endemic Hawaiian Lysimachia is not found in any of the other approximately 180 species distributed worldwide. This makes it difficult to speculate on their geographical origin or sister group. Hawaiian Lysimachia are characterized by their shrubby habit, having alternate leaves, regularly dehiscent capsules, axillary flowers, connate filaments adnate to the base of the corolla, tetraporate pollen and basifixed anthers. The most common corolla color is red, that of one species is green and another is white. Chromosome numbers of $2n=72$ (Carr, 1978) are known for two of the endemic species. Lysimachia glutinosa Rock (mistakenly identified as L. kalalauensis Skottsb.) and L. hillebrandii J. D. Hook. ex A.

Gray. There are no counts for any other species. The indigenous L. mauritiana Lam. has $2n=20$ (Carr, 1978).

A monophyletic origin for the endemic Hawaiian species (excluding the widespread L. mauritiana) is supported by two lines of evidence. First, the pollen of the seven endemic Hawaiian species that have been observed is tetracolporate, whereas that of non-Hawaiian species is tricolporate (Huynh, 1970, 1971; Bennel and Hu, 1983). The most parsimonious explanation for this is that it was a character that evolved once, presumably from an ancestor with tricolporate pollen. Second, despite the remarkable range of morphological variation among the endemic species, they nevertheless have more in common with each other than they do with any non-Hawaiian species because all are woody, have a variable (5-10) number of floral parts, (non-Hawaiian species are five (rarely six)-merous) and most Hawaiian Lysimachia have a reddish corolla, while extra-Hawaiian species generally have a yellowish or white corolla.

Handel-Mazetti (1928) considered that L. alpestris Champ. (a south China species) "is nearly allied to the shrubby red-flowered (i.e. endemic Hawaiian) species." However, pollen studies of Huynh (1970, 1971) did not support this nor could Chen and Hu (1979) find any characters to support such a relationship. Based on some pollen similarities, Bennell and Hu (1983) very tentatively speculated that the Hawaiian species were derived from section Alternifoliae (Subgen. Lysimachia) through L. mauritiana.

Lysimachia mauritiana however is distinct from the endemic species in being a somewhat fleshy perennial herb, having $2n=20$, distinct filaments that are versatile and an irregularly dehiscent capsule. This species has always been placed in a different subgenus from the endemic species.

The single North American west coast species of Lysimachia, L. thyrsiflora L. ($2n=40$, 54) is an herbaceous species with opposite or whorled leaves and yellow flowers that occur in short, dense, pedunculate racemes in the leaf axils (Hickman, 1993). Such a plant is unlikely to be similar to the ancestor of the Hawaiian species.

Wagner et al. (1990) and St. John (unpublished manuscript) suggested that Malesia (the Malay Peninsula, and all islands north of Australia and eastward to the easternmost Solomon Islands (Carlquist, 1974)) is a likely source region for the Hawaiian Lysimachia. Eight species, L. capillipes Hemsl., L. decurrens Forst. f., L. japonica Thunb., L. laxa Baudo, L. mauritiana Lam., L. montana (Reinw.) Bakh, L. peduncularis Wall ex. Kurz and L. sikokiana Miq., are found in the Malesian area (Van Steenis, 1972). All are herbaceous with axillary inflorescences and alternate leaves. They have yellow corollas, except for L. decurrens Forst. f. which is either white or red and L. mauritiana which is white or pink. St. John (unpublished manuscript) and Hu Chi Ming (personal comm.) suggest that L. laxa may be the species most closely related to the Hawaiian species. Of the species listed above, L. laxa and possibly L. capillipes, both in subgenus

Idiophyton, are the only ones that have in common with the Hawaiian species basifixed anthers with lateral dehiscence, and filaments connate and adnate to the corolla. Marginal leaf veins, which are present in all Hawaiian species, are lacking in L. capillipes. Ligneous stems are found in some species of subgenus Idiophyton (Chen and Hu, 1979), further support for a connection between a species similar to L. laxa and the Hawaiian species.

Based on corolla pigmentation alone, L. mauritiana and L. decurrens have more in common with the endemic Hawaiian species. However, these two species differ in having versatile anthers and the filaments are not connate at the base.

Chromosome numbers are available for approximately 35 non-Hawaiian species, but are not especially useful in elucidating the extra-Hawaiian ancestor. Unfortunately counts are not available for L. laxa or L. capillipes. At least 19 different sporophytic numbers have been reported: $2n=16, 18, 20, 24, 28, 30, 32, 34, 36, 40, 42, 60, 84, 92, 98, 100, 102, 108, 112$ (Ornduff, 1967, 1968; Federov, 1974; Moore, 1974, 1977; Goldblatt, 1981, 1984, 1985, 1988; Goldblatt and Johnson, 1990). The count of $2n=36$ is for L. nummularia, a yellow-flowered species with opposite leaves native to Europe. Its geographical distribution and morphological characters make it highly unlikely that this species is closely related to the Hawaiian ones, despite the fact that a single polyploid event would yield the same chromosome number as the Hawaiian

species. Base numbers of $x=5,6,7$ have been suggested by Ko et al. (1986) and Tanaka and Hizume (1980).

To date, one can only speculate on the extra-Hawaiian ancestor of the Hawaiian Lysimachia. A more accurate identification of the ancestor of the Hawaiian species could provide information useful for classification, as well as providing insights into other processes involved in speciation and evolution, such as changes in breeding system, pollinator syndrome and ecological adaptation.

Chapter 2

Analysis of morphological variation and taxonomic revision of the endemic Hawaiian Lysimachia

2.1 Introduction

2.1.1. Taxonomic position of endemic Hawaiian Lysimachia

Lysimachia is one of the largest genera of the Primulaceae, consisting of approximately 180 species of upright or sprawling perennial or annual herbs, shrubs or subshrubs. The center of diversity is in southwest China, where there are 122 (110 endemic) species (Chen and Hu, 1979). The remaining species grow in temperate areas of the northern hemisphere, the Southeast Asian tropics, South America, Africa and Australia (Bennell and Hu, 1983). Lysimachia and Anagallis are considered by Hutchinson (1969) to be the most primitive members of the family because of the contorted corolla lobes and the presence in some species of staminodes that alternate with the stamens. The presence of secretory cells in Lysimachia is an otherwise uncommon feature in the Primulaceae. This may be an indication that this genus is most closely related to another Primulalean family, the Myrsinaceae (Cronquist, 1981), thus also placing Lysimachia at or near the phylogenetic base of the family.

Lysimachia has been divided into six subgenera and 18 sections, based primarily on the work of Handel-Mazzetti (1928). In a taxonomic revision of the Chinese species, this

work included a subgeneric classification of the entire genus and emphasized floral structure, particularly the androecium. The subgeneric classification was modified somewhat by Chen and Hu (1979).

Two subgenera occur in the Hawaiian islands. Subgenus Palladia (Moench) Hand.-Mazz. is represented by a single indigenous coastal species, Lysimachia mauritiana. Subgenus Lysimachiopsis (Heller) Hand.-Mazz., consists exclusively of species endemic to the Hawaiian islands. Only the endemic species were examined in the present study. These have always been considered to belong to a single subgroup within the genus (Handel-Mazzetti, 1928), but there has been some disagreement regarding their affinities and subgeneric position. Heller (1897) concluded that the shrubby habit and urceolate, reddish corollas of the endemic Hawaiian species (characters otherwise not found in the genus, at least among the species known at that time) were sufficiently distinctive to create a separate genus, Lysimachiopsis Heller. Knuth (1905) did not regard the shrubby habit as a sufficient basis for the creation of a separate genus and returned the Hawaiian species to Lysimachia. Knuth (1905) did, however, create section Fruticosae Knuth solely for the endemic Hawaiian species. Handel-Mazzetti (1928), combined section Cilicina Klatt (a prior name for Fruticosae Knuth, published in 1866, which Handel-Mazzetti cited as a synonym) with section Rosulatae Champ. to form subgenus Lysimachiopsis (Heller) Handel-Mazzetti. Rosulatae contains one Asian species, L.

alpestris, a yellow-flowered prostrate herb. Synonymy of Cilicina with Fruticosae was unwarranted because Fruticosae was typified to consist only of the Hawaiian species. Whereas Cilicina Klatt appears to have been a heterogeneous group as Ray (1956) included only part of Cilicina Klatt within subgenus Lysimachia.

Studies of pollen morphology from representatives of all sections shed further light on the distinctness of the endemic Hawaiian species. Seven endemic Hawaiian species were examined and all had tetracolporate pollen, whereas non-Hawaiian species possessed tricolporate grains, although some species may occasionally form tetracolporate grains (Huynh, 1970,1971; Bennell and Hu, 1983). Pollen grains of Hawaiian species were the largest in the genus, especially those of L. forbesii Rock (Huynh, 1970,1971). Huynh (1970) observed that the pollen type of L. alpestris differed from the endemic Hawaiian species. Based on this observation Huynh (1970) split subgenus Lysimachiopsis along sectional lines and elevated the sections to subgeneric status: subgen. Sandwicensia Huynh for section Fruticosae and subgen. Nullicaulis Huynh for section Rosulate. Sandwicensia was an unnecessary name because Lysimachiopsis was already validly published at the subgeneric level to include only the Hawaiian species. Although subgenus Sandwicensia was mistakenly retained by Bennel and Hu (1983), the endemic Hawaiian Lysimachia are properly classified in subgenus Lysimachiopsis (Heller) Handel-Mazzetti.

Wagner et al. (1990), recognized nine extant and one extinct species (L. forbesii Rock) of Lysimachia endemic to the Hawaiian Islands. The extant species are L. daphnoides (A.Gray) Hillebr., L. filifolia C.N. Forbes & Lydgate, L. glutinosa Rock, L. hillebrandii Hook. f. ex A. Gray, L. kalalauensis Skottsb, L. lydgatei Hillebr., L. maxima (R. Knuth) St. John, L. remyi Hillebr., and L. venosa (Wawra) St. John.

According to the taxonomic treatment of Wagner et al. (1990), L. hillebrandii is distributed on Kauai, Oahu, Molokai and Maui, and L. remyi on Molokai and Maui alone. However, using a combination of foliar and corolla characters, plants from Molokai fit the description for L. hillebrandii more closely than they do that of L. remyi, and plants from Maui fit the description of L. remyi not L. hillebrandii. As circumscribed by Wagner et al. (1990), L. hillebrandii and L. remyi as well as L. filifolia encompass a broad range of variation. These names are retained in the revised taxonomic treatment presented here, however not in the same sense as in Wagner et al. (1990). In other words, several additional taxa are recognized either at the specific or the subspecific rank within the concept of these species as proposed by Wagner et al. (1990). In order to avoid confusion, whenever these three species are discussed in the broadest sense, they are referred to as L. hillebrandii s.l., L. remyi s.l. and L. filifolia s.l.

Species are found on all of the major islands except Kahoolawe, Niihau and Hawaii. Species of Lysimachia occur in a wide variety of these vegetation types including montane bogs, waterfall spray zones, subalpine mesic shrublands, montane dry and wet forests and lowland mesic shrublands, ranging in elevation from 250 m to 2300 m as classified by Gagne and Cuddihy (1990).

Hawaiian Lysimachia differ from each other in leaf and calyx lobe size and shape, phyllotaxy, corolla size, shape and pigmentation, and the extent of pubescence. Most species have a reddish-purple corolla, but that of L. glutinosa is white to cream and L. kalalauensis is green with a reddish base and veins (as was L. forbesii). All species are perennial woody shrubs. Most are scandent or even upright, with the exception of L. filifolia s.l., which is pendulous. The hermaphrodite flowers are sweetly fragrant, especially in the evening, and are presumably insect pollinated.

2.1.2. Objectives

In the tentative taxonomic treatment of the most recent flora of the Hawaiian islands, Wagner et al. (1990) observe that Lysimachia is "greatly in need of careful monographic work, especially the Lysimachia hillebrandii-L. remyi complex on the younger islands" (i.e. Maui and Molokai) which "virtually form a broad continuum of variation". Contributing to the nomenclatural uncertainty and taxonomic confusion is the fact that St. John (1987) described 44 new endemic

species, bringing to 54 the total number of names published. Many of these are referable to either L. hillebrandii s.l. or L. remyi s.l. according to the classification of Wagner et al. (1990).

The purpose of the present analysis of morphological variation was to evaluate the tentative taxonomic treatment of Wagner et al., (1990) and to determine whether or not there are breaks in the continuous range of variation that had been described, especially in the L. hillebrandii s.l.-L. remyi s.l. complex. A multivariate tool, principal components analysis (PCA), was used as the first step in a quantitative description of the variation in order to provide a framework for organizing taxonomic decisions. However, ultimately, the distribution of variation in non-metric, qualitative characters made the greatest contribution to the revised classification.

2.2 Methods and Materials

2.2.1. Source of specimens for measurements

Field collections were made in the summers of 1990, 1991 and 1992. Priority was placed on seeking populations that included the full range of variation, as understood from examination of herbarium specimens, without necessarily following the system of classification of Wagner et al. (1990). The single known population of L. venosa is almost inaccessible and was not visited. Unsuccessful searches were made of the type localities of L. forbesii (last collected in

1934) as well as several other taxa (sensu St. John, 1987) that have not been collected for 50-80 years.

The Operational Taxonomic Units (OTU's) in this study were individual plants. Collection locations and population codes are presented in Table 2.1. Because the islands of Maui and Oahu both resulted from two geographically and chronologically separate volcanic events, collection designations are subdivided into the Waianae Mtns. and Koolau Mtns. for Oahu, and East Maui and West Maui for Maui. Sample size varied, but an attempt was made to collect at least 10 flowering specimens from each population. In some populations, no plants were flowering, still others consisted of fewer than ten individuals. A number of populations that were represented only by herbarium specimens had a sample size of fewer than ten, sometimes one.

2.2.2. Metric measurements

Measurements were made on plants grown in the greenhouse from seed or cuttings, pressed specimens from collections done for this study, and specimens borrowed from the following herbaria: BISH, F, GH, MASS, MO, NY, PTBG, RSA, US, and W (abbreviations from Holmgren et al., 1990).

The choice of characters measured reflects to some extent the observation of Wagner et al. (1990) that leaf spacing, leaf shape, calyx and corolla size and shape and pedicel length are the characters that show the greatest variation in

Table 2.1 Collection locations of populations of endemic Hawaiian *Lysimachia* sampled for morphometric analysis. Populations marked with an asterisk are represented by herbaria specimens only. Specific epithets and species codes (in parenthesis) are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. glutinosa</u>	GKALP	Kauai	1300	Kokee; Kalua-Puhi trail.
<u>L. glutinosa</u>	GKALR	Kauai	1260	Kokee; Road between Kalalau and Puu O Kila Lookouts.
<u>L. glutinosa</u>	GKALL	Kauai	1230	Kokee; Below Kalalau Lookout.
<u>L. glutinosa</u>	GHONO	Kauai	1230	Kokee; Honopu Trail.
<u>L. kalalauensis</u>	KHONO	Kauai	1110	Kokee; Edge of Honopu Valley.
<u>L. kalalauensis</u>	KAAPU	Kauai	1140	Kokee; Awaawapuhi Trail.
<u>L. kalalauensis</u>	KKALL	Kauai	1230	Kokee; Rim of Kalalau Valley.
<u>L. kalalauensis</u>	KMAKA	Kauai	1015	Kokee; North of Makaha Valley Road.
<u>L. kalalauensis</u>	KNUAP	Kauai	1080	Kokee; Upper Nualolo Valley, SE Branch.
<u>L. kalalauensis</u>	KKOHU	Kauai	955	Kokee; Maile Flats Trail.
<u>L. kalalauensis</u>	KWAIA	Kauai	1110	Kokee; Waialae Ridge.
<u>L. hillebrandii</u>	OLIMA	Kauai	650	On ridgetop SW of Limahuli Falls.
<u>L. hillebrandii</u>	OWAIN	Kauai	615	Wainiha Pali; SW of Kulana'ililia.

Table 2.1 continued on next page.

Table 2.1 continued. Collection locations of populations of endemic Hawaiian Lysimachia sampled for morphometric analysis. Specific epithets are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. pendens</u>	NSPTR	Kauai	800	Kokee; Below and E of Kalalau Lookout, North-South ridge.
<u>L. daphnoides</u>	DSECO	Kauai	1230	Kokee; Alakai Swamp Trail.
<u>L. daphnoides</u>	DBIGB	Kauai	1230	Kokee; Alakai Swamp.
<u>L. filifolia</u>	FKONE	Kauai	615	Head of N fork of Wailua River.
<u>L. filifolia</u>	FKTWO	Kauai	615	Head of N fork of Wailua River.
<u>L. filifolia</u>	FKTHR	Kauai	615	Head of N fork of Wailua River.
<u>L. filifolia</u>	FKFOU	Kauai	615	Head of N fork of Wailua River.
<u>L. filifolia</u>	FKFIV	Kauai	615	Head of N fork of Wailua River.
<u>L. filifolia</u>	FOONE	Oahu	250	Koolau Mtns.; Waiahole Ditch Trail.
<u>L. filifolia</u>	FOTWO	Oahu	250	Koolau Mtns.; Waiahole Ditch Trail.
<u>L. filifolia</u>	FOTHR	Oahu	250	Koolau Mtns.; Waiahole Ditch Trail.
<u>L. hillebrandii</u>	HPUKS	Oahu	710	Waianae Mtns.; Ridge SE of Puu Kaa.
<u>L. hillebrandii</u>	HPUKE	Oahu	800	Waianae Mtns.; Ridge E of Puu Kaa.
<u>L. hillebrandii</u>	HKAPU	Oahu	540	Waianae Mtns.; Kapuna Gulch.

Table 2.1 continued on next page.

Table 2.1 continued. Collection locations of populations of endemic Hawaiian Lysimachia sampled for morphometric analysis. Specific epithets are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. hillebrandii</u>	HPAHO	Oahu	540	Waianae Mtns.; Pahole Gulch.
<u>L. hillebrandii</u>	HWAIK	Oahu	800	Waianae Mtns.; Kamaileunu Ridge in Makaha Valley; No of small peak N of Puu Kawiwi.
<u>L. hillebrandii</u>	HPALI	Oahu	740	Waianae Mtns.; N fork of Palwai Gulch.
<u>L. hillebrandii</u>	HLJAL	Oahu	830	Waianae Mtns.; Ridge between Lualualei and Nanakuli Valleys.
<u>L. remyi</u>	WMBLG	W. Maui	340	Iao Valley; Black Gorge.
<u>L. remyi</u>	WMNAK	W. Maui	500	Iao Valley; Nakalaloa Stream.
<u>L. remyi</u>	WMMN2	W. Maui	1050	Hanaula.
<u>L. remyi</u>	WMMN3	W. Maui	1075	Hanaula.
<u>L. remyi</u>	WMUKE	W. Maui	1170	Hanaula; rim of Ukemehame canyon.
<u>L. remyi</u>	WMMPP	W. Maui	860	Manawainui Plant Preserve.
<u>L. remyi</u>	WMLIL	W. Maui	1130	Lihau, ridge west of summit.
<u>L. remyi</u>	WMLIS	W. Maui	1260	Lihau summit.
<u>L. remyi</u>	WMHPL	W. Maui	985	Halepohaku, ridge SW of summit.

Table 2.1 continued on next page.

Table 2.1 continued. Collection locations of populations of endemic Hawaiian Lysimachia sampled for morphometric analysis. Specific epithets are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. remyi</u>	WMHPS	W. Maui	1050	Halepohaku summit.
<u>L. lydgatei</u>	WMHPY	W. Maui	1000	Halepohaku, ridge SW of summit.
<u>L. remyi</u>	WMHEL	W. Maui	1230	Helu, E of summit.
<u>L. remyi</u>	EMPAL	E. Maui	2000	Ridge SE of Paliku.
<u>L. remyi</u>	EMHPA	E. Maui	2090	Ridge SE of Paliku.
<u>L. remyi</u>	EMKUI	E. Maui	2215	Ridge N of Kuiki.
<u>L. remyi</u>	EMKUK	E. Maui	2280	Kuiki, rim of Kipahulu Valley.
<u>L. remyi</u>	EMLWA	E. Maui	2155	Above Lake Waianapanapa.
<u>L. remyi</u>	EMKAW	E. Maui	1785	W Kaupo Gap, below spring.
<u>L. remyi</u>	EMKPR	E. Maui	2300	Kalapawili Ridge; rim of Kipahulu.
<u>L. remyi</u>	EMKAE	E. Maui	1845	Kaupu Gap below Paliku.
<u>L. remyi</u>	EMWAI	E. Maui	2030	Koolau Gap, Waikau Cabin site.
<u>L. remyi</u>	EMKOT	E. Maui	1970	Koolau Gap, treeline.
<u>L. remyi</u>	EMKOB	E. Maui	2920	Koolau Gap, below treeline.

Table 2.1 continued on next page.

Table 2.1 continued. Collection locations of populations of endemic Hawaiian Lysimachia sampled for morphometric analysis. Specific epithets are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. remyi</u>	EMKIP	E. Maui	1000	Kipahulu valley.
<u>L. hillebrandii</u>	MKAWE	Molokai	920	West fork Kawela Gulch.
<u>L. hillebrandii</u>	MKOLE	Molokai	1040	W of Puu Kolekole, S of road.
<u>L. hillebrandii</u>	MMAKA	Molokai	970	Makakupaia; ridge S of Onini gulch.
<u>L. hillebrandii</u>	MONIN	Molokai	850	Makakupaia; bottom of Onini Gulch.
<u>L. hillebrandii</u>	MWAIK	Molokai	1050	Waikolu; SE of Puu Kaeo.
<u>L. hillebrandii</u>	MKAUN	Molokai	920	Small ridge between Kauanakakai and Kupaia Gulches.
<u>L. maxima</u>	MMAXI	Molokai	950	Pelekunu; N of Ohialele.
<u>L. hillebrandii</u>	EMKAL	E. Maui	1510	Haleakala; lower Kaupo Gap.
<u>L. forbesii</u>	FORBE*	Oahu	600-710	Koolau Mtns.; Punaluu Valley/Castle Trail.
<u>L. hillebrandii</u>	PUNAL*	Oahu	unknown	Koolau Mtns.; Castle Trail.
<u>L. hillebrandii</u>	HALAW*	Oahu	unknown	Koolau Mtns.; Middle Halawa Ridge.
<u>L. hillebrandii</u>	KALIB*	Oahu	400-500	Koolau Mtns.; Kalihi Pali.
<u>L. hillebrandii</u>	MOANA*	Oahu	unknown	Koolau Mtns.; Moana Valley Pali.

Table 2.1 continued on next page.

Table 2.1 continued. Collection locations of populations of endemic Hawaiian Lysimachia sampled for morphometric analysis. Specific epithets are sensu Wagner et al. (1990).

Species	Pop. Code	Island	Altitude (meters)	Location
<u>L. hillebrandii</u>	WAIHO*	Oahu	unknown	Koolau Mtns.; Waihole Gulch.
<u>L. hillebrandii</u>	KOOLY*	Oahu	710	Koolau Mtns.; Olympus-Waimanalo Pali.
<u>L. hillebrandii</u>	NUUAN*	Oahu	unknown	Koolau Mtns.; Lateral ridge in Nuuanu Valley.
<u>L. hillebrandii</u>	KULIO*	Oahu	unknown	Koolau Mtns.; Puu-O-Kona (Crest of Kuliouou Valley).
<u>L. hillebrandii</u>	LANAI*	Lanai	unknown	Mtns. at east end.
<u>L. filifolia</u>	OLOKE*	Kauai	unknown	Olokele Valley.
<u>L. kalalauensis</u>	HANAP*	Kauai	unknown	Hanapepe Valley.
<u>L. glutinosa</u>	KAHIL*	Kauai	unknown	Kahili Ridge.
<u>L. hillebrandii</u>	PALAW*	Oahu	740	Waianae Mtns.; S. Palawi Gulch.
<u>L. hillebrandii</u>	HAPAP*	Oahu	800	Waianae Mtns.; Puu Hapapa.
<u>L. hillebrandii</u>	KALEN*	Oahu	760-900	Waianae Mtns.; Puu Kalena.
<u>L. hillebrandii</u>	KAALA*	Oahu	770-1140	Waianae Mtns.; Mt. Kaala, N slope.
<u>L. hillebrandii</u>	KANEH*	Oahu	830	Waianae Mtns.; Puu Kanehoa, near summit.
<u>L. hillebrandii</u>	WATAW*	Oahu	800	Koolau Mtns.; Waiawa.

L. hillebrandii s.l. From each OTU, measurements were taken on three leaves, (representative of the most frequent form) and whenever possible, one to four flowers. Vegetative measurements included leaf length (LEAFLM), leaf width at the widest point (LEAFWM), petiole length (PETLM), upper leaf surface pubescence (ULPUBE), lower leaf surface pubescence (LLPUBE), stem pubescence (STEMPUBE), minimum internode length (MINNTRND), maximum internode length (MAXNTRND), and the standard deviation of the internode lengths (STDNTRND). This last measurement assesses the evenness of distribution of leaves along a stem and is based on six adjacent internodes measured from the mid-portion of mature stems. Floral measurements included calyx length (CALLENM), calyx width (CALWIDM), calyx pubescence (CALPUBE), corolla length (CORLENM), corolla width (CORWIDM), filament length (FILLENM), style length (STYLENM), pedicel length (PEDLENM), and pedicel pubescence (PEDPUBE). The high density of hairs of the most pubescent plants precluded an actual count. A value of "one" was assigned to glabrous surfaces and "five" to the most pubescent. For each OTU an average of the above characters was used in all further analyses.

2.2.2.1. Analysis of metric data

The inconsistent occurrence of flowers and fruits indicated that more than one PCA would be needed to describe the data adequately. Thus, three data sets were analyzed by PCA. The first data set (1036 OTU's) included vegetative

characters of all specimens. The specimens in the remaining data sets are subsets of the specimens in the first. The second data set (405 OTU's) comprised specimens that bore flowers. PCA of this data set used two different combinations of characters: 1) floral alone; 2) vegetative and floral. The third data set (636 OTU's) comprised OTU's of taxa that were not distinct from each other based on the ordination of OTU's in data sets one and two. By eliminating distinct taxa, subtle relationships among the remaining taxa may be revealed. These belong to the L. hillebrandii s.l.-L. remyi s.l. complex, L. filifolia s.l. and L. lydgatei. PCA of this data set used all vegetative and calyx characters.

The statistical package SYSTAT (Wilkinson, 1990) was used for the principal components analyses (PCA). Ordination of the scores from the first two PCA axes was visualized using SYGRAPH (Wilkinson, 1990).

2.2.3 Non-metric observations

A number of qualitative, non-metric characters differed among populations of the L. hillebrandii s.l.-L. remyi s.l. complex. Observations were made of the following characters: shape of leaf blade, veins pellucid or not, prominence of areoles, leaf color, calyx lobe size, color and shape, pedicel color and position and presence of wax on leaves and stems. The angle of divergence between the primary vein and the secondary vein was measured on plants from 10-15 leaves of each group. Most of these characters are non-continuous and

to include them in the PCA through arbitrarily assigned values would mean violating assumptions of the method. This does not detract from their importance, however. In many cases, characters such as color and shape of organs contribute more significantly to our perceptions of differences among species than do quantitative differences.

2.3 Results

2.3.1. Principal Components Analysis

Using only vegetative characters, some groups are evident in Figure 2.1. Lysimachia forbesii, L. glutinosa, and to a lesser degree, L. filifolia s.l., L. kalalauensis, and L. maxima form somewhat distinct clusters. OTU's of L. hillebrandii s.l. and L. remyi s.l. are distributed throughout the plot. The first component had high loadings for LEAFLM, LEAFWM and PETLM, the second component had high loadings for ULPUBE, LLPUBE and STDNTRND (Table 2.2). The first two PCA axes represented 66% of the variation.

Non-flowering specimens were removed, leaving data set two, and the PCA was repeated. The ordination of OTU's using floral characters alone (Figure 2.2) differs from that using vegetative characters alone (Figure 2.1) in that OTU's of the same species form more distinct clusters in the former than in the latter. Lysimachia daphnoides, L. glutinosa, L. forbesii, and to a lesser extent L. maxima and some OTU's of L. filifolia, are distinct. Again, there is considerable overlap in the ordination of OTU's of L. remyi s.l. and L. hillebrandii s.l. All characters except CALPUBE and PEDPUBE had high loadings on the first PCA axis, CALPUBE and PEDPUBE had high loadings on the second PCA axis (Table 2.3). The first two PCA axes represented 75.1% of the variation.

The ordination of OTU's based on PCA of both vegetative and floral characters of data set two (Figure 2.3), was

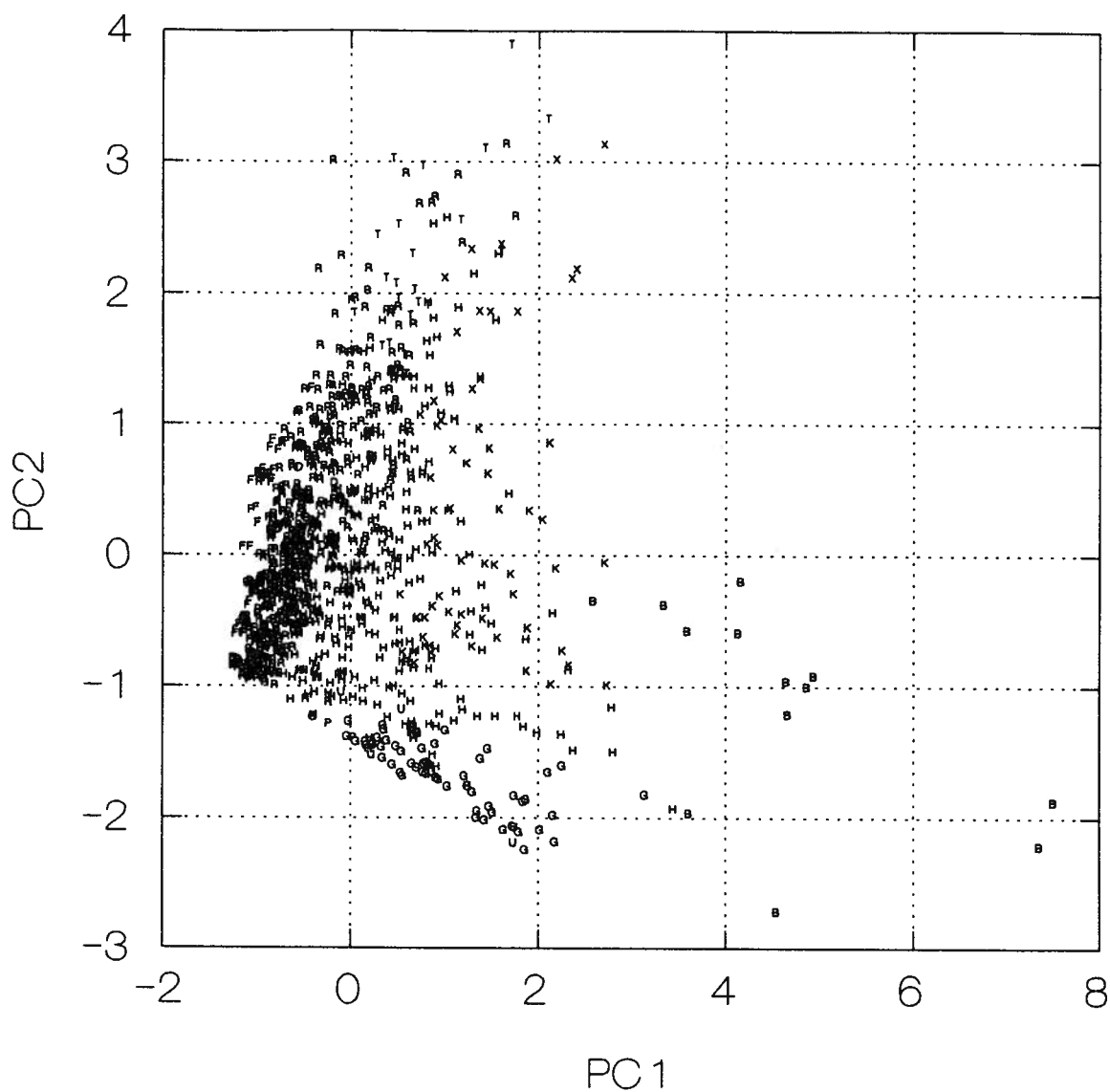


Figure 2.1. Plot of principal components 1 and 2 using vegetative measurements. Each symbol represents a single OTU. Abbreviations: B=L. forbesii, D=L. daphnoides, F=L. filifolia s.l., G=L. glutinosa, H=L. hillebrandii s.l., K=L. kalalauensis, R=L. remyi s.l., T=L. lydgatei, X=L. maxima.

Table 2.2. Principal components analysis of Hawaiian Lysimachia using only vegetative characters. Measurements taken from 1036 OTU's. A plot of OTU's in relation to the first two principal components is shown in Figure 2.1.

Variable	Component Loadings		
	1	2	3
STEMPUBE	0.206	0.815	0.286
ULPUBE	0.274	0.712	0.345
LLPUBE	0.222	0.841	0.292
LEAFLM	0.853	-0.335	0.150
LEAFWM	0.882	-0.275	0.122
PETLM	0.806	-0.249	0.320
MINNTRND	0.672	-0.303	0.112
MAXNTRND	0.668	0.284	-0.670
STDNTRND	0.442	0.438	-0.767
Eigenvalues	3.415	2.494	1.476
% Variance	37.9	27.7	16.4
% Cum. Var.	37.9	65.6	82.0

Table 2.3. Principal components analysis of Hawaiian Lysimachia using floral characters. Measurements taken from 405 OTU's. A plot of OTU's in relation to the first two principal components is shown in Figure 2.2.

Variable	Component Loadings	
	1	2
CALPUBE	0.109	0.862
PEDPUBE	-0.039	0.893
PEDLENM	0.696	-0.126
CORLENM	0.965	-0.038
CORWIDM	0.866	-0.077
CALLENM	0.854	0.116
CALWIDM	0.838	-0.258
STYLENM	0.851	0.221
FILLENM	0.868	0.070
Eigenvalues	5.087	1.697
% Variance	56.2	18.9
% Cum. Var.	56.2	75.1

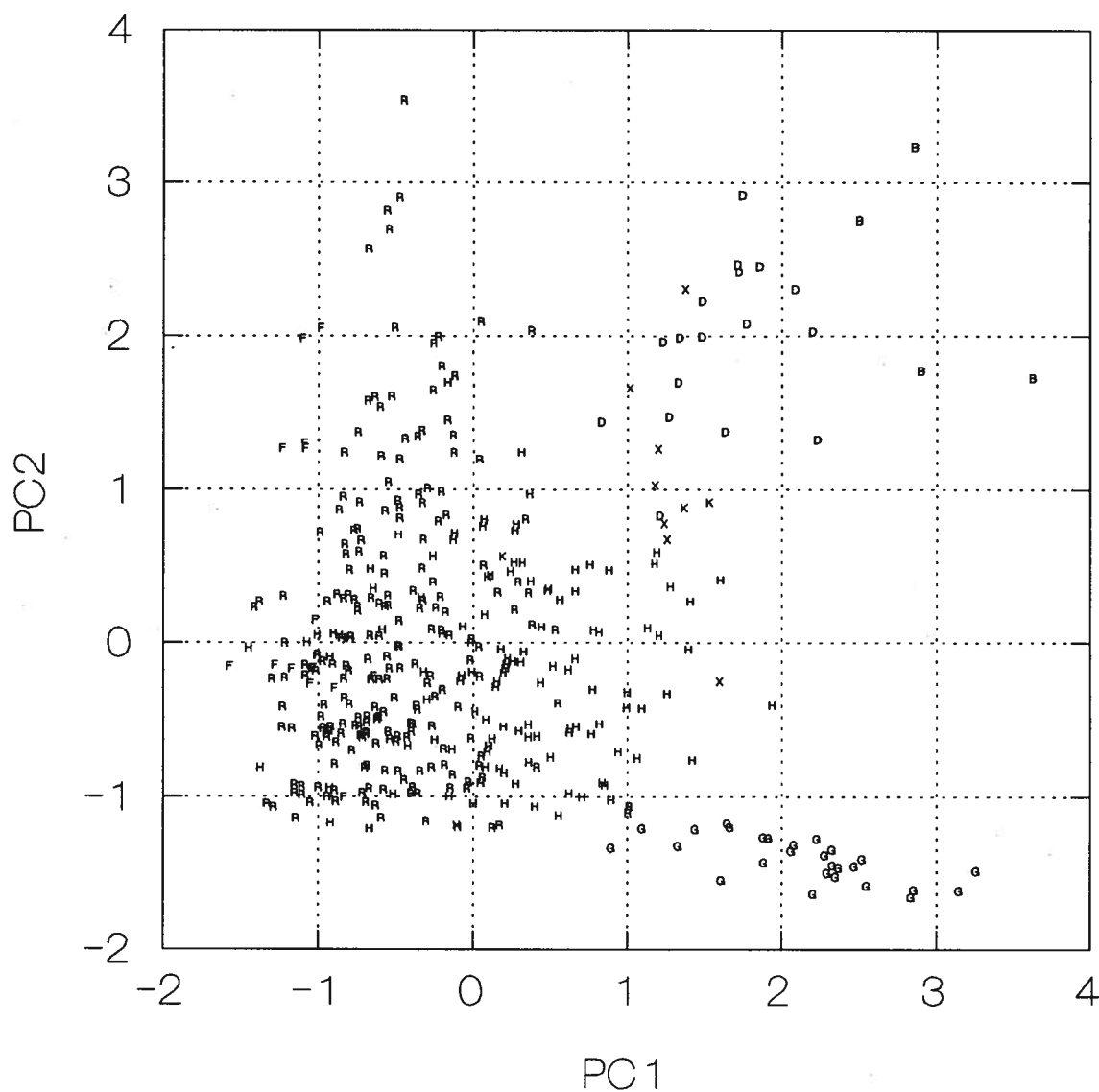


Figure 2.2. Plot of principal components 1 and 2 using floral measurements. Each symbol represents a single OTU. Abbreviations: B=L. forbesii, D=L. daphnoides, F=L. filifolia s.l., G=L. glutinosa, H=L. hillebrandii s.l., K=L. kalalauensis, R=L. remyi s.l., T=L. lydgatei, X=L. maxima.

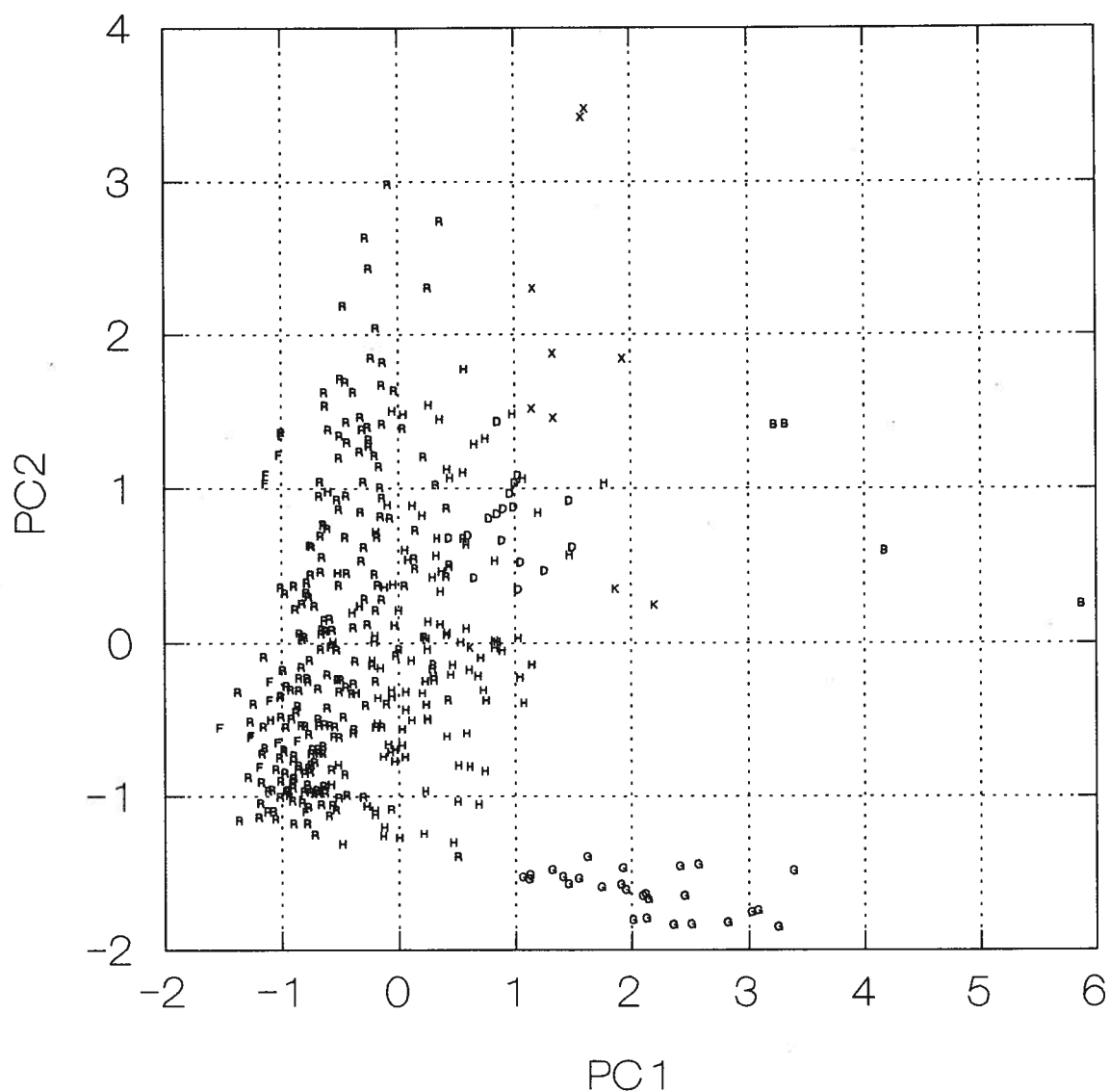


Figure 2.3. Plot of principal components 1 and 2 using floral and vegetative measurements. Each symbol represents a single OTU. Abbreviations: B=L. forbesii, D=L. daphnoides, F=L. filifolia s.l., G=L. glutinosa, H=L. hillebrandii s.l., K=L. kalalauensis, R=L. remyi s.l., T=L. lydgatei, X=L. maxima.

Table 2.4. Principal components analysis of Hawaiian Lysimachia using vegetative and floral characters. Measurements taken from 405 OTU's. A plot of OTU's in relation to the first two principal components is shown in Figure 2.3.

Variable	Component Loadings			
	1	2	3	4
STEMPUBE	0.094	0.832	0.051	0.064
ULPUBE	0.209	0.681	0.119	0.069
LLPUBE	0.096	0.849	0.058	0.023
CALPUBE	0.132	0.683	0.205	0.184
PEDPUBE	-0.054	0.783	0.341	0.113
PEDLENM	0.704	-0.070	-0.119	-0.194
CORLENM	0.921	-0.093	0.202	-0.181
CORWIDM	0.761	-0.092	0.297	-0.396
CALLENM	0.875	0.040	0.138	0.077
CALWIDM	0.786	-0.215	0.133	-0.306
STYLENM	0.819	0.110	0.236	-0.407
FILLENM	0.783	-0.013	0.332	-0.231
LEAFLM	0.880	-0.183	-0.172	0.227
LEAFWM	0.864	-0.046	-0.203	0.269
PETLM	0.661	-0.085	-0.169	0.614
MINNTRND	0.595	-0.162	-0.216	0.505
MAXNTRND	0.415	0.387	-0.770	-0.214
STDNTRND	0.221	0.467	-0.714	-0.420
Eigenvalues	7.207	3.474	1.765	1.418
% Variance	40.0	19.3	9.8	7.9
% Cum. Var.	40.0	59.3	69.1	80.0

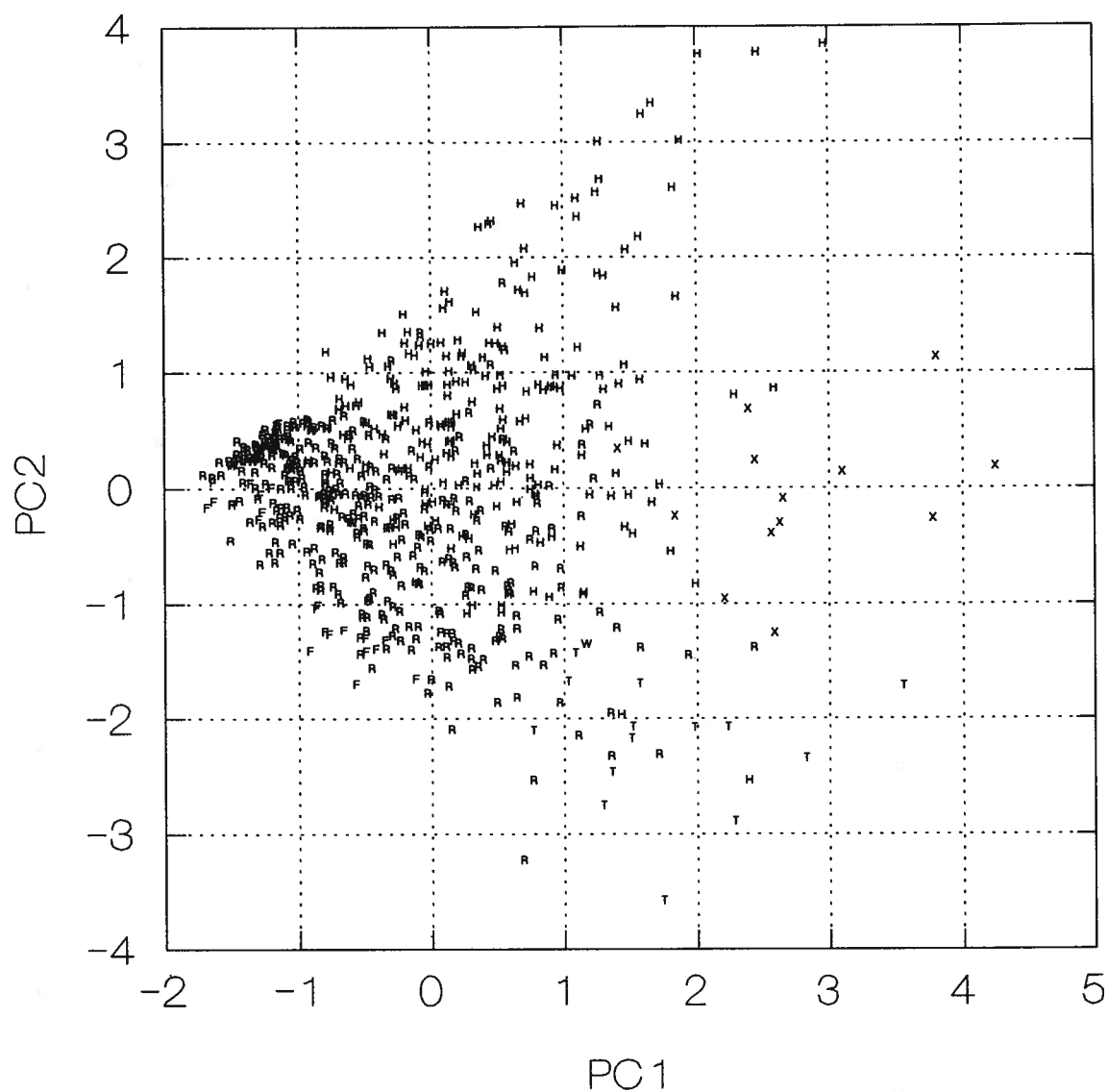


Figure 2.4. Plot of principal components 1 and 2 using vegetative and calyx measurements. Each symbol represents a single OTU. Abbreviations: F=L. filifolia s.l., H=L. hillebrandii s.l., R=L. remyi s.l., X=L. maxima, T=L. lydgatei.

Table 2.5. Principal components analysis of Hawaiian Lysimachia using vegetative and calyx characters. Measurements taken from 636 OTU's. A plot of OTU's in relation to the first two principal components is shown in Figure 2.4.

Variable	Component Loadings			
	1	2	3	4
STEMPUBE	0.548	-0.649	0.038	0.095
ULPUBE	0.511	-0.602	0.159	0.071
LLPUBE	0.567	-0.661	0.089	0.048
CALPUBE	0.416	-0.620	0.322	-0.162
CALLENM	0.586	-0.103	-0.540	0.287
CALWIDM	0.433	0.164	-0.496	0.580
LEAFLM	0.733	0.431	0.088	0.238
LEAFWM	0.739	0.418	0.251	0.050
PETLM	0.529	0.301	0.650	-0.018
MINNTRND	0.338	0.472	0.470	0.181
MAXNTRND	0.753	0.299	-0.272	-0.479
STDNTRND	0.686	0.168	-0.443	-0.540
Eigenvalues	4.101	2.435	1.661	1.076
% Variance	34.2	20.3	13.8	9.0
% Cum. Var.	34.2	54.5	68.3	77.3

similar to that using floral characters alone (compare Figures 2.2 and 2.3), however, L. daphnoides was not as distinct. On the first PCA axis there was less overlap between L. hillebrandii s.l. and L. remyi s.l., than there was using vegetative or floral characters alone. There were two groups of OTU's of L. filifolia s.l., evident in Figure 2.3 that did not form as close a cluster as in Figure 2.2. One group is distinct from all other OTU's, the second overlaps with L. remyi s.l. On the first PCA axis PEDLENM, CORLENM, CORWIDM, CALLENM, CALWIDM, STYLENM, FILLENM, LEAF LM, LEAFWM, PETLM, and MINNTRND had high loadings; ULPUBE, LLPUBE, CALPUBE, and PEDPUBE had high loadings on the second PCA axis (Table 2.4). The first two PCA axes represented 59% of the variation.

For data set three, OTU's of L. glutinosa, L. kalalauensis, L. daphnoides and L. forbesii were removed from data set one because they were distinct from the other taxa in either quantitative vegetative or floral characters, as well as possessing unique suites of non-metric characters. The PCA was repeated and the resulting scores plotted in Figure 2.4. Many OTU's of L. lydgatei and L. maxima were distinct from the other species. Although there was substantial overlap between OTU's of L. remyi s.l. and L. hillebrandii s.l., they occupy different areas of the scatterplot. On the first PCA axis LEAF LM, LEAFWM, PETLM, STDNTRND and MAXNTRND had high loadings; ULPUBE, LLPUBE, CALPUBE and STEMPUBE had high loadings on the second PCA axis (Table 2.5). The variation represented by the first two PCA axes is 54%.

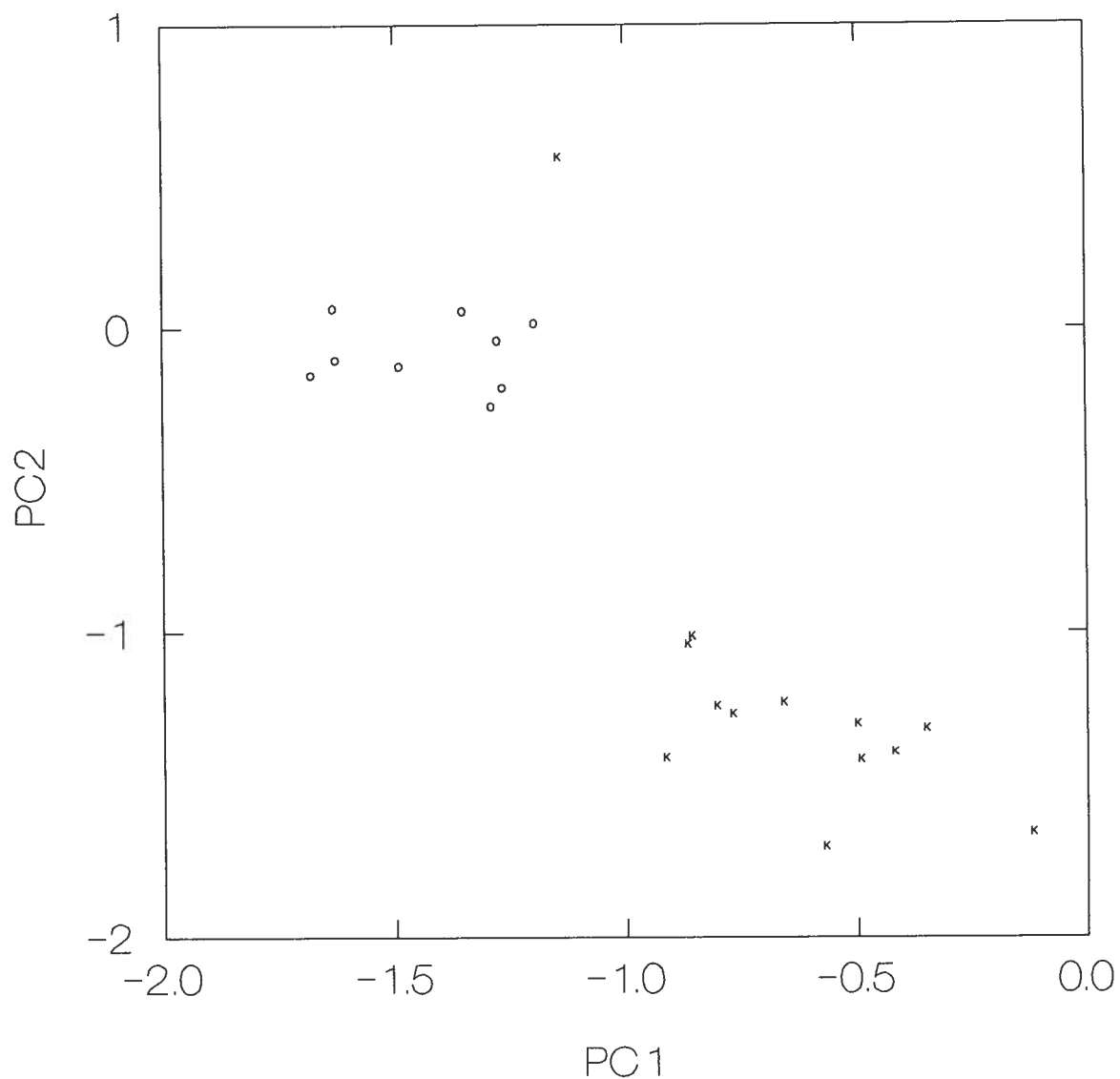


Figure 2.5. Plot of principal components 1 and 2 using vegetative and calyx measurements of *L. filifolia* s.l. Each symbol represents a single OTU. Abbreviations: K=OTU's from Kauai, O=OTU's from Oahu. OTU labelled "K" closest to those labelled "O", came from Olokele Valley, the remaining "K" OTU's came from the headwaters of the Wailua River.

The high density of OTU's whose ordination is similar to that of L. filifolia s.l. obscures their position in Figure 2.4. When plotted alone however, two groups are clearly evident (Figure 2.5).

2.3.2 Distribution of non-metric characters

Qualitative morphological characters and selected quantitative characters defined five groups of populations in L. hillebrandii s.l. (Table 2.6), and three groups in L. remyi s.l. (Table 2.7). In Figure 2.6, OTU's of L. hillebrandii s.l. and L. remyi s.l. are plotted according to the group designations from Tables 2.6 and 2.7. Lysimachia lydgatei is included with Group G of L. remyi s.l. because the geographical distribution and morphology of this species are very similar to those of OTU's of L. remyi s.l.

Table 2.6. Distribution of selected metric and non-metric characters among groups of populations previously classified as L. hillebrandii s.l.

Character	Group A*	Group B	Group C	Group D	Group E
Leaf length (mm)	55-65	45-60	60-80	50-65	45-55
Leaf width (mm)	8-11	8-11	25-36	16-24	12-24
Upper leaf color	dark green oblance.	light green narrowly elliptic-oblance.	light green elliptic	light green elliptic	light green elliptic
Leaf shape					
Leaves pubescent	no	yes/no	no	no	yes
Tertiary veins pellucid	no	no	no	yes	no
Leaf areoles prominent	no	no	no	yes	no
Leaf apex recurved	yes	no	no	no	no
Angle between primary and secondary vein	20-35°	15-35°	35-40°	35-45°	35-55°
Calyx lobe length(mm)	4-5	5-8	4-5.5	4-5	5-8
Calyx lobe color	green	green	dark red	green	green
Calyx lobe shape	ovate	lanceolate	lanceolate	ovate	lanceolate
Corolla lobe length(mm)	10-11	11.5-14	6-8.5	11-13	12-16
Pedicel color	green	green	dark red	green	green
Pedicel position	erect	erect	pendulous	erect	erect
Stems pulverulent	yes	no	no	no	no
Habitat	lowland mesic Kauai	lowland mesic Molokai	lowland wet Kauai	lowland mesic Oahu	montane wet Oahu
Island	Kauai	Molokai	Kauai	Oahu	Oahu

* Population Group: A= NSPTR; B= MKawe, MKole, MmaKa, Monin, Mwaik, Mkaun; C= OLIMA, OWAIN; D= HPUKS, HPUKE, HKAPU, HPAHO, HWAik, HPALI, HLUAL; E= MOANA, WAIHO, KOOLY, NUUAN, KULIA, PALAW, WAHIA, WAIW, PUNAL, HALAW, KALIB. See Table 2.1 for population locations.

Table 2.7. Distribution of selected metric and non-metric characters among groups of populations previously classified as *L. remyi* s.l.

Character	Group F*	Group G	Group H
Leaf length (mm)	20-55	30-45	20-30
Leaf width (mm)	2-17	8-14	4-6
Upper leaf color	dark green	dark green	light green
Leaf shape	linear-elliptic	ovate	linear-oblong.
Tertiary veins pellucid	no	yes	no
Leaf margins revolute	no	yes	no
Leaf areoles prominent	no	no	no
Leaf apex recurved	no	no	no
Angle between upper secondary veins and primary vein	45-60°	25-30°	20-30°
Calyx lobe length (mm)	4-7	5.5-7	5-6
Calyx lobe color	green	green	green
Calyx lobe shape	lanceolate	lanceolate	lanceolate-
Phyllotaxy	alternate	alternate/whorled	alternate
Corolla lobe length (mm)	8-13	10-11	8-10
Habitat	montane mesic	montane wet	montane mesic
Island	Maui	Maui	Maui

* Population Group: F=WMMHPL, WMMN2, WMMN3, WMUKE, WMMPP, WMLIL, WMLIS, WMHPS, WMHPY, WMHEL; G=EMKOT, EMKOB, EMKAL; H=WMMNAK, WMBLG, EMHPA, EMKAW, EMLWA, EMKPR, EMKUI, EMPAL. See Table 2.1 for population locations.

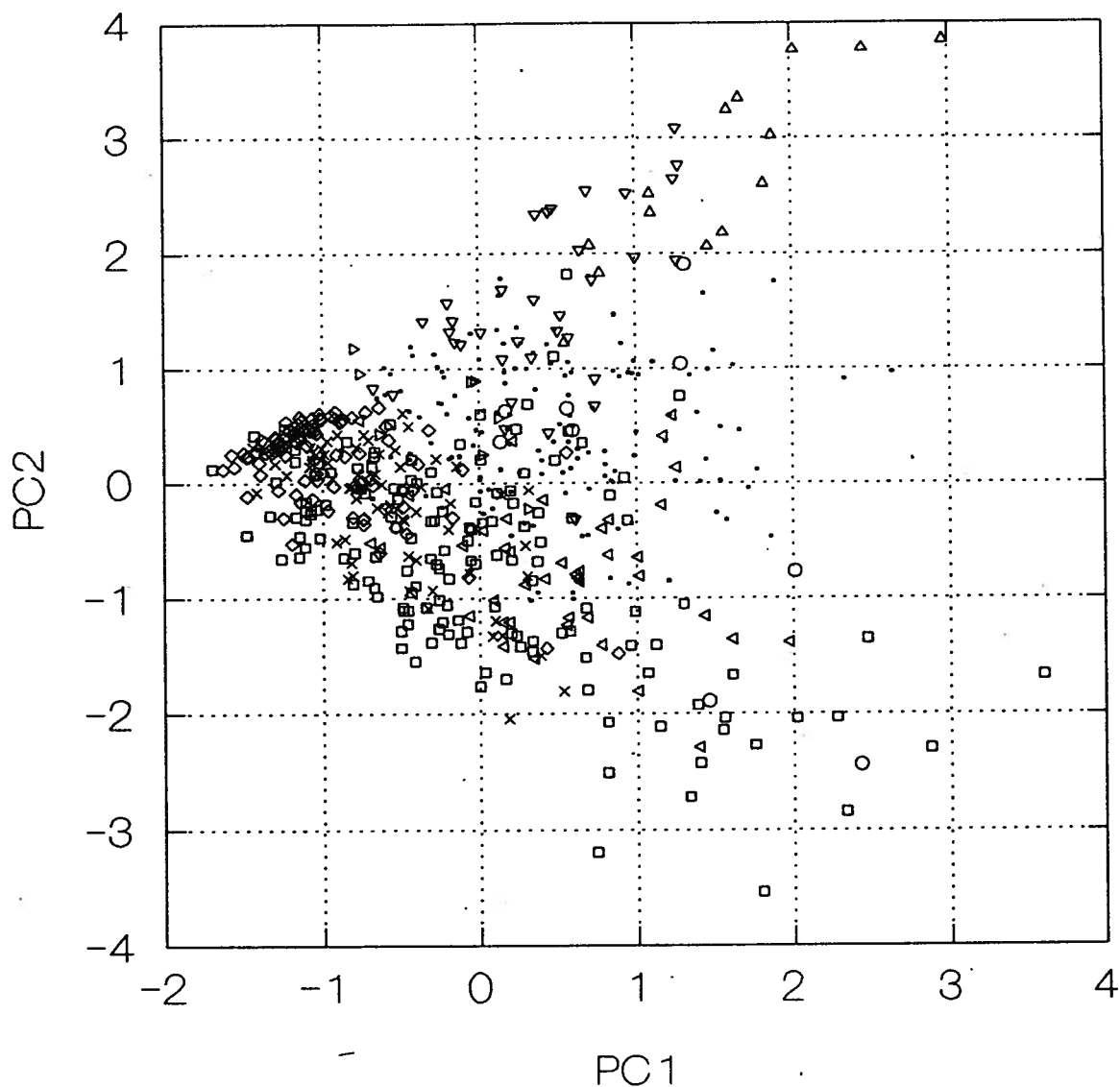


Figure 2.6. Plot of principal components 1 and 2 using vegetative and calyx measurements of *L. hillebrandii* s.l. and *L. remyi* s.l. Each symbol represents a single OTU belonging to group designations for *L. hillebrandii* s.l. ($\blacktriangle$ = Group A; $\cdot$ = Group B; $\triangle$ = Group C; ∇ = Group D; $\circ$ = Group E) or *L. remyi* s.l. ($\blacktriangle$ = Group F; $\square$ = Group G; $\diamond$ = Group H; $\times$ = Group G X Group F).

2.4. Discussion

2.4.1 Taxonomic concepts

Categories recognized here as species are based entirely upon morphological characters. Interspecific F_1 hybrids are highly fertile (Chapter 4), and crossability cannot be used as criteria to delineate species. Species are defined here as interbreeding groups of populations that share the same unique suite of morphological characters. These combinations of characters are not found in any other species and are taken as evidence that each species is monophyletic and reproductively isolated from every other species. Subspecies are considered to consist of geographically discrete populations within the overall distribution of the species; they differ in vegetative, but not floral characters.

2.4.2 Taxonomic Treatment

The results from the multivariate analysis provided the starting point for the taxonomic revision. However, relying on PCA alone to make taxonomic decisions can be misleading because qualitative characters may distinguish taxa that are not differentiated in an ordination. Although PCA was used as the first step, non-metric, morphological characters ultimately contributed more significantly to the taxonomic revision than did the results of PCA.

Based on the PCA, the species as classified by Wagner et al. (1990) fall into three categories. The first category includes those that are clearly distinct: *L. glutinosa*, *L.*

kalalauensis, L. daphnoides, and L. forbesii. Differences in floral morphology (Figure 2.2) and vegetative morphology (Figure 2.1) define these species relatively well. In the second category are L. maxima and to a lesser degree L. lydgatei, whose ordinations also form clusters that set them apart from other groups of OTU's (Figure 2.4). In a third category are L. remyi s.l., L. hillebrandii s.l. and L. filifolia s.l.. In spite of occupying common areas in an ordination, L. remyi s.l. and L. hillebrandii s.l. have qualitative features that separate these taxa into groups of populations, each of which differs in a number of characters from all other groups (Tables 2.6 and 2.7); these groups require taxonomic clarification. In the case of L. filifolia s.l., a taxonomic revision is suggested because PCA separated this taxon into two clusters of OTU's (Figure 2.5).

2.4.2.1. Taxonomic revision of L. filifolia s.l.

Wagner et al. (1990) acknowledged that the classification as one species, of narrow leaved plants from the headwaters of the Wailua River, and upper Olokele Valley (the type location) on Kauai, and from Waiahole Gulch on Oahu as L. filifolia s.l., may have been a somewhat artificial grouping. The single OTU from Olokele clusters more closely with those from Waiahole (Figure 2.5) than it does with those from the Wailua River. Leaves of OTU's from the Wailua River are 2-4 mm wide and pubescent, whereas leaves of plants from Waiahole Gulch and Olokele are 0.8-1.2 mm wide and glabrous. St. John

(unpublished manuscript) noted that the type specimen had viscid leaves. This character would distinguish it from plants at the other two locations of L. filifolia s.l., however, viscid leaves are not mentioned in the type description nor have I been able to detect glands on my examination of the type specimen. Thus a revised classification of L. filifolia (sensu Wagner et al., 1990) would include L. filifolia C.N. Forbes & Lydgate, from Olokele Valley and Waiahole Gulch, and the new species L. pendens Marr from the Wailua River.

2.4.2.2. Taxonomic revision of the L. hillebrandii s.l.-L. remyi s.l. complex

From Tables 2.6 and 2.7 and Figure 2.6, it is apparent that there are suites of characters that separate populations of the L. hillebrandii s.l.-L. remyi s.l. complex into eight groups. The differences among these groups are regarded here to be sufficient for the recognition of six species and three subspecies within the L. hillebrandii s.l.-L. remyi s.l. complex. In the following discussion, the characters used to distinguish among these taxa are highlighted.

Three groups of populations are distinguishable within L. remyi s.l. The dimensions and shape of the calyx lobes, corolla lobes, and pedicels are variable within these groups and some combinations of these characters are unique to one group. However, observations of plants in the greenhouse indicate that based on these floral characters alone it is

often impossible to determine to which group a particular plant belongs. There are however, vegetative characters that are specific to each group, and based on these, a plant from any population on Maui can be assigned correctly to the appropriate group. Because these groups differ consistently in vegetative characters, but not floral characters, they are treated here as three subspecies of L. remyi.

Populations that belong to Group F are found only on West Maui. Perhaps the most useful character that distinguishes Group F from Groups G and H is the angle of divergence of the secondary veins. In Group F and in L. lydgatei, this angle varies from 20-30° for the lower veins, but increases to 40-60° for the upper veins, whereas the angle of divergence for leaves of Group G and H is the same for all veins and varies from 15-40°. Leaf size, shape, pubescence and color are also useful in many cases. In populations of Groups G and H these characters are much less variable. Two patterns of variation are seen in populations of Group F. Plants from some populations differ markedly from each other in having leaves that range from linear and glabrous to elliptic and densely tomentose, approaching what could be classified as L. lydgatei, while in other populations these characters are more uniform. In some populations most plants fit the description of L. lydgatei, but also include plants that are nearly glabrous and with narrower leaves that, therefore, would be classified as L. remyi s.l. One interpretation of the pattern of variation is that morphologically diverse populations have

resulted from hybridization between L. lydgatei and a taxon that is nearly glabrous, with narrow leaves and a small calyx. Supporting this interpretation is the fact that on leeward Maui summits narrow-leaved plants grow on the more windswept, sparsely vegetated aspects, while L. lydgatei is found on the adjacent, protected slopes under a low canopy forest. This suggests that ecological factors may contribute at least to a difference in phenotypic expression. It is unclear however, whether or not this is controlled by specific genes. In the "common garden" of the greenhouse, with an admittedly small sample size, progeny that were grown from the seed of plants with narrow leaves also have narrow leaves. However, progeny of L. lydgatei were morphologically variable and include plants with nearly glabrous, narrow leaves, as well as plants with broader tomentose leaves. This observation, and the fact that most populations from West Maui are morphologically diverse, argues in favor of treating these populations as belonging to a single phenotypically diverse taxon, that includes OTU's previously classified as L. lydgatei. Populations of Group F and L. lydgatei are here reclassified as L. remyi subsp. remyi.

The remaining populations of L. remyi s.l. belong to Groups G and H. A number of vegetative characters separate these. OTU's of Group G have ovate leaves that are dark green, with pellucid veins and revolute margins. Leaves of Group H are linear to oblanceolate, are light green and not only are the veins not pellucid, they are often nearly

obscured by a thick cuticle. Plants of populations that belong to Group G fit the description of L. caliginis St. John, and are here reclassified as L. remyi subsp. caliginis (St. John) Marr comb. nov. Plants of Group H fit the description of L. kipahuluensis St. John and are here reclassified as L. remyi subsp. kipahuluensis (St. John) Marr comb. nov.

Populations of L. hillebrandii s.l. are found on Kauai, Oahu, and Molokai. The five groups of populations in Table 2.6 can be broadly subdivided further into populations of OTU's with narrowly elliptic to oblanceolate leaves 8-11 mm wide (Groups A and B) and those with elliptic leaves 12-36 mm wide (Groups C, D and E). In addition, the angle of divergence between the primary and secondary veins in the leaves is 15-35° in Groups A and B, and 35-55° in Groups C, D, and E. Although Groups A and B share similar leaf shapes and dimensions, they differ in a number of features (Table 2.6). Plants of Group A are glabrous, have dark green leaves, often with the apex recurved, waxy deposits on the young leaves and shoots, a thick cuticle that nearly obscures the veins and ovate calyx lobes. Plants of Group B are pubescent or glabrate, have light green leaves, lack waxy deposits, have lanceolate calyx lobes, and visible veins. Group A was unknown prior to this study and is here designated as L. scopulensis Marr. Populations of Group B are segregated as L. remyi subsp. subherbacea (St. John) Marr.

Although there is some overlap in the ordination of OTU's of Group C, D, and E (Figure 2.6), there are combinations of characters that are unique to each (Table 2.6). Leaves of Group D are distinct from all other groups because the areoles are well defined based on prominent tertiary and quaternary veins. In other taxa, the quaternary, and often the tertiary, veins are obscured. Calyx lobes of Group D are broadly ovate, whereas those of Groups C and E are lanceolate. Corolla lobes of Group D are longer than those of Group C, and often are shorter than those of Group E. Characters that distinguish Group C are linear calyx lobes, dark red pigmentation for the entire length of the calyx and pedicel, and the pendulous pedicel. The calyx and pedicel of some OTU's of several other taxa are occasionally pigmented as well, but never for the full length. OTU's of Group D have an ovate, green calyx and the pedicel is green and upright. Leaves, stems and calyx lobes of OTU's from Group E are moderately to densely pubescent and the calyx lobes are lanceolate to narrowly ovate. By comparison, leaves and stems of Groups C and D are glabrous. The location and description of OTU's of Group C fit that of the type specimen of L. ovoidea St. John. Likewise, OTU's of Group D match the description and location of the type specimen of L. waianaeensis St. John. OTU's of Group E fit the type description of L. hillebrandii Hook. f. ex A. Gray, a name which is retained only for plants from the Koolau and southern Waianae Mtns, and one location on Molokai.

2.4.3. Summary of Taxonomic Revision

A total of 16 species, one with four subspecies are recognized in the following key and species descriptions. The distribution of species by island is as follows: Kauai, 12 species (Figure 2.7); Oahu, five species (Figure 2.8); Molokai three species (Figure 2.9); Maui one species and three subspecies (Figure 2.10). All species on Kauai are endemic to that island except for L. filifolia and L. hillebrandii. Three of the five species on Oahu are endemic to Oahu, with one occurring also on Molokai and the second with the same subspecies of L. remyi as occurs on Molokai. One of the three species on Molokai is restricted to that island. One subspecies of L. remyi is restricted to West Maui, the other two subspecies that are found on Maui occur on both East and West Maui.

The taxonomic conclusions for most taxa are based on the results presented in this chapter with the following exceptions. Lysimachia iniki Marr sp. nov. was not included because material of this new taxon became available as the study was ending. Lysimachia venosa was not included based on the lack of specimens. Lysimachia haupuensis St. John and L. kahiliensis St. John, are treated here as distinct species but would have been classified as L. hillebrandii s.l. in the key of Wagner et al. (1990). These species are represented only by their type specimens.

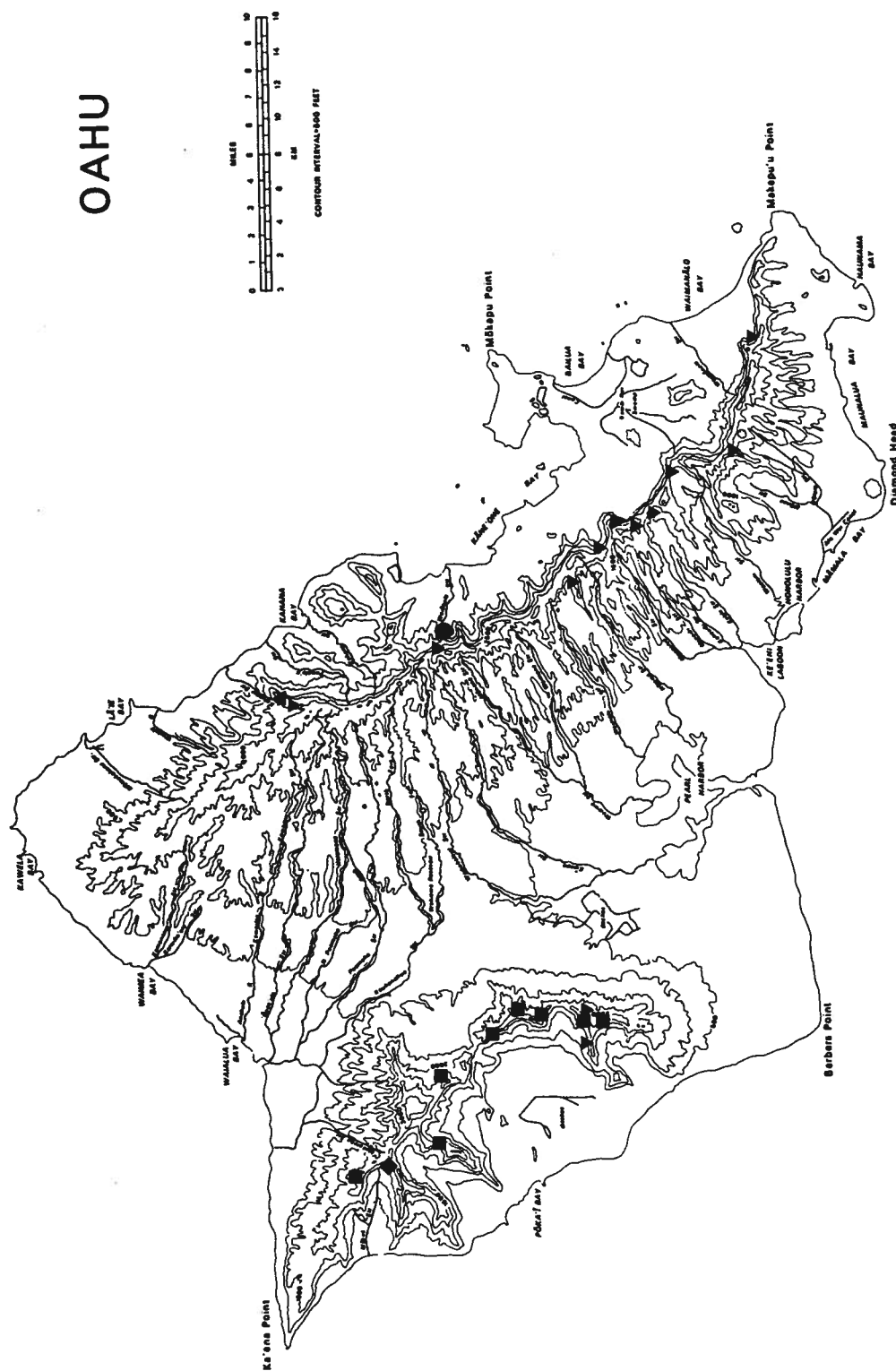


Figure 2.8. Distribution of endemic Hawaiian species of Lysimachia on the island of Oahu: Δ = L. forbesii; $\blacklozenge$ = L. remyi subsp. subherbacea; $\blacksquare$ = L. waianaeensis; $\bullet$ = L. hillebrandii; $\circ$ = L. filifolia.

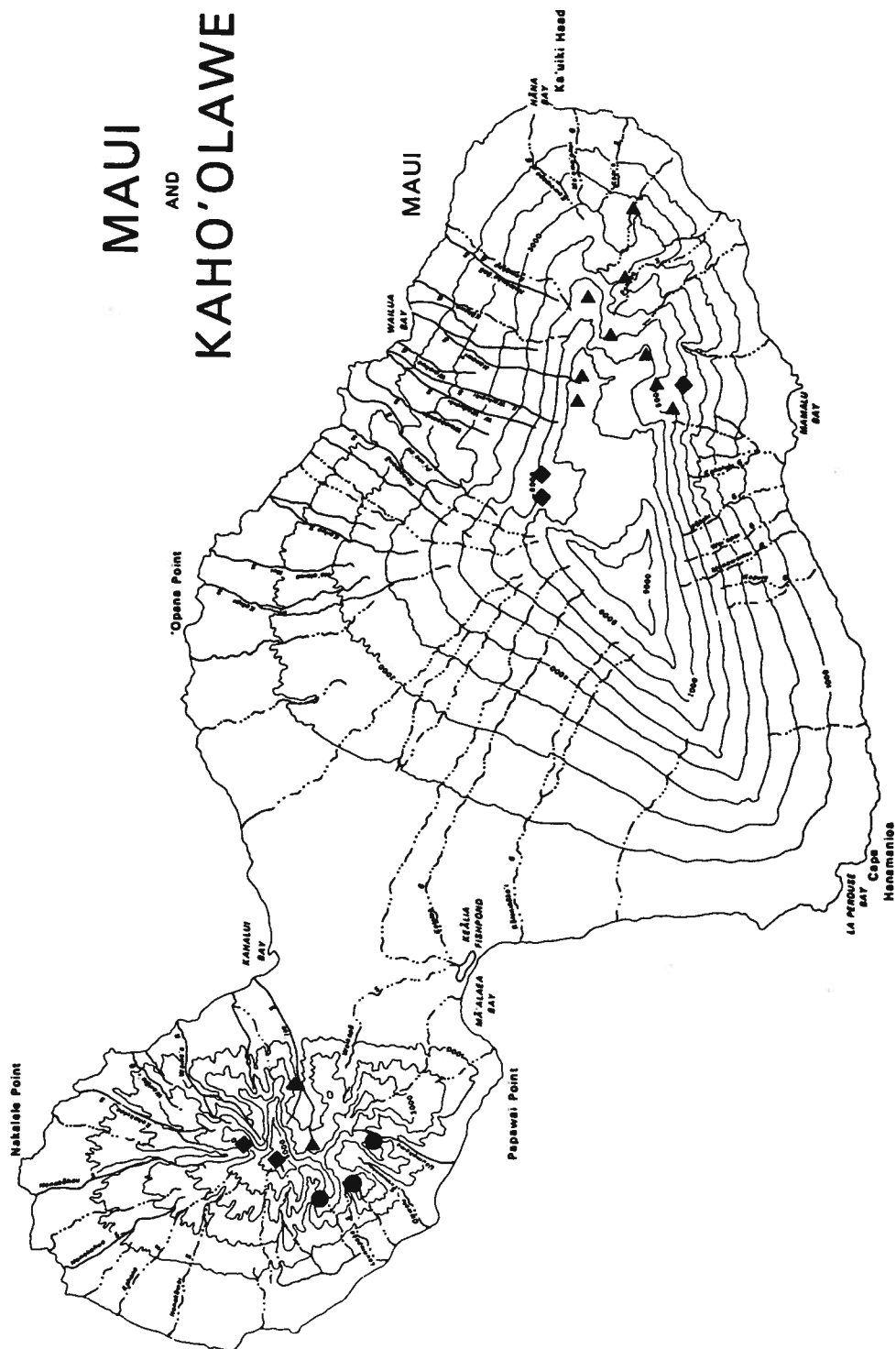


Figure 2.10. Distribution of endemic Hawaiian species of *Lysimachia* on the island of Maui; ● = *L. remyi* subsp. *remyi*; ▲ = *L. remyi* subsp. *kipahuluensis*; ◆ = *L. remyi* subsp. *caliginis*.

2.5. Key and descriptions of endemic Hawaiian Lysimachia

Lysimachia subgen. Lysimachiopsis (Heller) Handel-Mazzetti, Notes, Royal Botanic Garden, Edinburgh 16:121-122, 1928.

Lysimachiopsis Heller, Minn. Bot. Studies 1:875, 1897;

Lysimachia section Fruticosae Knuth, Das Pflanzenreich. IV.

237 (Heft 22): 309-312, 1905; Lysimachia subgen. Sandwicensia Huynh, Candollea 25:288-289, 1970, nom. illeg.-TYPE (lectotype here chosen): Lysimachia hillebrandii var. hillebrandii (as var. ♂) Hook. f. ex A. Gray.

Erect, ascending, or prostrate perennial herbs, sometimes subshrubs or shrubs. Leaves entire, opposite, whorled, or alternate, usually glandular-dotted. Flowers hypogynous, actinomorphic, 5(6-10)-merous, in terminal racemes, corymbs, panicles, or solitary in the leaf axils; calyx imbricate or valvate, persistent, deeply parted; corolla rotate to urceolate, deeply parted, tube very short, yellow, white, purple, reddish-purple, or green, the lobes contorted in bud; staminal filaments slightly to nearly completely adnate to corolla, often more or less basally connate; anthers basifixed or versatile, opening by apical pores or longitudinal slits; ovary superior, placentation free-central; capsule 5-10-valved or irregularly dehiscent, ovoid to globose; seeds numerous, oblong, orbiculate or angular, testa crustaceous.

Key to endemic Hawaiian species.

- 1a. Corolla green on upper portion, dark red at the base, petal margins erose; calyx lobes narrowly lanceolate, usually more than 12 mm long.(2)
- 1b. Corolla creamy white or reddish-purple, petal margins entire; calyx lobes ovate to linear, usually less than 12 mm long, or if longer than 13 mm, then usually at least 5 mm wide and ovate.(3)
- 2a. Leaves (80-)160-200(-260) mm long, (20-)55-65(-95) mm wide; upper and lower surface densely tomentose, the hairs multicellular; calyx and corolla lobes more than 20 mm long. Oahu.3. L. forbesii
- 2b. Leaves (50-)60-80(-100) mm long, (15-)25-32(-45) mm wide; upper and lower surface glabrous or glabrate, the hairs unicellular; calyx and corolla lobes less than 20 mm long. Kauai.9. L. kalalauensis
- 3a. Corolla white, (15-)19-24(-30) mm long; leaves shiny, light green above, slightly lighter below, glabrous, (50-)80-120(-160) mm long; entire plant usually viscid, especially young shoots. Kauai4. L. glutinosa
- 3b. Corolla red, or base red and upper 5-7 mm white, (5-)6-18(-20) mm long; leaves dull, light or dark green, glabrous or pubescent, (15-)20-80(-100) mm long; plants not viscid or if viscid, then viscid-hirsute to viscid-hirtellous.(4)

- 4a. Leaves less than 2 mm wide, or if 2-4 mm wide, then the leaves narrowly lanceolate and the stems pendulous, hanging from cliffs.(5)
- 4b. Leaves more than 2 mm wide; stems upright, not hanging from cliffs.(7)
- 5a. Leaves 2-4 mm wide, narrowly lanceolate. Kauai.
.....12. L. pendens
- 5b. Leaves less than 2 mm wide, usually 1 mm wide, filiform.(6)
- 6a. Plants pendulous, hanging from wet cliffs. Kauai, Oahu.
.....16. L. filifolia
6. Plants upright, streamsides and bogs. East Maui.
.....13b. L. remyi subsp. kipahuluensis
- 7a. Leaves, stems and pedicels densely viscid-hirsute to viscid-hirtellous; leaves sometimes glabrate but the margins remaining viscid-hirsute.(8)
- 7b. Leaves stems and pedicels glabrous or pubescent but not viscid.(9)
- 8a. Leaves (25-)35-38(-42) mm wide, broadly ovate to orbicular, cupped upwards; secondary veins prominent; upper portion of corolla cream to white. Kauai.
.....7. L. iniki
- 8b. Leaves (7-)10-13(-19) mm wide, oblanceolate to oblong;

- secondary veins obscure; upper portion of corolla salmon pink to dark red. Kauai.1. L. daphnoides
- 9a. Calyx lobes lanceolate, 13-16 mm long, 4-6 mm wide; leaves obovate (50-)75-80(-100) mm long; plants glabrous. Kauai.15. L. venosa
- 9b. Calyx lobes ovate to lanceolate, 3-11 mm long, 1.5-4 mm wide, or if greater than 4 mm wide, then ovate; leaves various (15-)20-60(-95) mm long; plants glabrous or pubescent.(10)
- 10a. Leaves whorled, 3-4 per node, more than 20 mm wide obovate or elliptic; stems rusty tomentose, glabrate with age. Molokai.10. L. maxima
- 10b. Leaves alternate, or if whorled, then less than 20 mm wide, shape various; stems glabrous or pubescent. ..(11)
- 11a. Leaves elliptic, ovate or obovate and usually more than 16 mm wide, apex usually rounded, often abruptly acuminate.(12)
- 11b. Leaves linear, lanceolate or oblanceolate, and mostly less than 16 mm wide, if elliptic, then less than 16 mm wide or densely rusty tomentose, apex attenuate, acuminate or sometimes acute.(14)
- 12a. Pedicel and calyx lobes dark red for entire length; pedicel pendulous; calyx lobes linear, 1.5-2 mm wide;

- petioles (6-)7.5-12(-15) mm long. Kauai.
11. L. ovoidea
- 12b. Pedicel and calyx lobes entirely or mostly green;
 pedicel erect; calyx lobes lanceolate to ovate, 2-5 mm
 wide; petioles (2-)3-8(-15) mm long.(13)
- 13a. Leaves with poorly defined areoles (tertiary veins
 obscure); calyx lobes lanceolate, (4-)5-8(-9) mm long;
 pedicel, calyx lobes, stem and lower leaf surface
 densely dark brown pilose to tomentose. Kauai, Oahu,
 Molokai.6. L. hillebrandii
- 13b. Leaves with well defined areoles, (tertiary and higher
 order veins prominent); calyx lobes usually broadly
 ovate, (3-)4-5(-8) mm long; plants nearly glabrous.
 Oahu.16. L. waianaeensis
- 14a. Leaves with prominent glands when dried, elliptic. Kauai.
5. L. haupuensis
- 14b. Leaves without internal glands that are prominent when
 dried, shape various.(15)
- 15a. Young leaves and stems pulverulent (powdery appearance
 due to fine wax crystals), otherwise glabrous; leaves
 dark green, the tips recurved; calyx lobes glabrous,
 broadly ovate. Kauai.14. L. scopulensis
- 15b. Leaves and stems not pulverulent, slightly to densely
 pubescent; leaves light or dark green, the tips flat;

calyx lobes glabrous or pubescent, lanceolate,
occasionally ovate.(16)

- 16a. Angle of divergence of secondary leaf veins acute,
nearly parallel to primary vein; calyx lobes 9-11 mm
long; leaves narrowly obovate, 35-50 mm long, 9-12(-15)
mm wide. Kauai.8. L. kahiliensis
- 16b. Angle of divergence of secondary leaf veins more obtuse;
calyx lobes usually less than 10 mm long, if longer than
10 mm, then the leaves more than 50 mm long, leaves
various. Oahu, Molokai, Maui.13. L. remyi

1. *Lysimachia daphnoides* (A. Gray) Hillebr., Fl. Hawaiian Isl. 285, 1888. *Lysimachia hillebrandii* Hook.f. var. β *daphnoides* A. Gray, Proc. Amer. Acad. Arts and Sci. 5:329, 1862. *Lysimachiopsis daphnoides* (A. Gray) Heller, Minn. Bot. Studies 1:875, 1897.-TYPE: Sandwich (Hawaiian) Islands, Kauai, Mts., U.S. Exploring Expedition under Capt. Wilkes 1838-1842, (holotype: US!).

Lysimachia longa St. John, Phytologia 64:46, 1987.-TYPE: Kauai, bog at head of Wahiawa stream, 19 Oct., 1895, Heller 2736 (holotype: BISH!; isotypes: BISH!, F!, GH!, MASS!, MO!, NY!, P[3]!, US!).

Erect, upright shrubs usually less than 1 m tall, branching largely from the base, rarely above; stems green when young becoming dark reddish-brown, densely viscid-hirtellous when young becoming densely reddish-brown tomentose, occasionally glabrous. Leaves alternate, (0.5-)3-7(-11) mm apart, sessile or petioles less than 1 mm long; blades oblanceolate to oblong, thickly coriaceous, (20-)30-35(-52) mm long, (6-)10-13(-21) mm wide, base obtuse, apex acute, margins slightly revolute; upper surface light green, usually viscid-hirtellous, but sometimes glabrous, the hairs white in younger leaves becoming brown, lower surface lighter green than upper, densely viscid-hirtellous; base of lamina, primary vein and sometimes secondary veins red; secondary veins often obscure. Flowers solitary in leaf axils, 6-8-merous, campanulate;

pedicels (20-)25-32(-110) mm long, erect, densely reddish-brown viscid-hirtellous; calyx lobes green, sometimes red at the base, sparsely viscid-hirtellous, lanceolate to narrowly ovate (6-)7.5-11 mm long, (2-)3-4(-4.5) mm wide; corolla lobes dark maroon to salmon pink obovate, (13-)15-18(-21) mm long, (7-)11-13(-16) mm wide; filaments (7-)8.5-11(-14) mm long, anthers 2-2.5 mm long; style (8-)10.5-13(-14) mm long. Capsules 7-10 mm long. Seeds dark brown, irregularly shaped, 1-1.4 mm long.

Phenology. Flowering Jan.-Nov.

Distribution and Habitat. Kauai. Montane wet sedgelands of Alakai Swamp usually restricted to low hummocks that rise slightly higher than the surrounding bog vegetation, 1230-1400m. Growing with Oreobolus, Rhynchospora, Carex, Panicum, Metrosideros, Cheirodendron, Melicope, Dubautia, Cibotium, Dicranopteris, Adenophorus, Vaccinium, Styphelia, Viola, Lycopodium and Coprosma.

Comments. The diagnostic characters of this species include the densely viscid-hirtellous leaves, stems and pedicels. These characters are also found in L. iniki. The differences between these two species are discussed under L. iniki.

There is some confusion regarding the origin of the collection of this species from Wahiawa Bog. Specimens labelled *Heller 2736* were collected on different dates and from different locations. According to their labels, specimens at P[3], BISH, F and US were collected on 19 Oct.

1895 and specimens at MASS and F were collected on 12 Aug., 1895 "in and near a bog at the head of the Wahiawa". A specimen at G was collected on 14 Aug. 1895, "along the Hanapepe River, near the falls". A specimen at MO was collected on 24 Aug. 1895 "between Hanapepe and Wahiawa Rivers". Heller (1897), notes that these collections came from 800 m, which is higher than the 480 m elevation of Wahiawa Bog, but somewhat lower than the Alakai Swamp. A recent inventory of Wahiawa Bog failed to find this species (Tim Flynn, personal comm.). Given these considerations, it seems most likely that *Heller 2736* did not come from Wahiawa Bog and instead came from the eastern part of the Alakai Swamp, or no longer occurs in Wahiawa Bog due to habitat alterations.

In the Hawaiian language *L. daphnoides* is identified as "lehua makanoe" or "kolokolo kuahiwi" (Hillebrand, 1888). *Representative Specimens examined.* KAUAI: eastern Alakai Swamp ("Sincocks Bog"), *Perlman 10631* (MO,PTBG), *Davis 133* (BISH), *Warshauer 3354* (BISH); western Alakai Swamp, *Forbes 888.K* (BISH,US,W), *Herbst 2175* (F,GH,MO,NY,PTBG,W), *Takeuchi 92a* (GH), *Fay 322* (BISH,F,NY), *Fay and Bulmer 332* (PTBG), *Hillebrand s.n.* (GH), *Takeuchi 92b* (BISH), *Selling 2905* (BISH), *Hobdy 153* (BISH), *Van Royen 11701* (BISH), *Marr 250,251,252*, *Marr 504-Marr 522* (UBC), *Lorence 5700* (MO,PTBG), *Lorence 6355* (PTBG), *Wawra 2122* (W), *Wawra s.n.* (W), *Sinclair s.n.* (K).

2. *Lysimachia filifolia* C.N. Forbes & Lydgate, Occasional Papers of Bernice P. Bishop Museum, 6(3):74-75, 1916.

Lysimachiopsis filifolia (C.N. Forbes & Lydgate) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-

TYPE: Kauai, upper Olokele Valley, Jan. 1912, *Lydgate 2* (holotype: BISH!).

Lysimachia waiaholeensis St. John, Phytologia 64(1):50, 1987.-

TYPE: Oahu, Waiahole gulch, 250 m, 26 July 1926, *Degener 17666* (holotype: NY!).

Lysimachia funkiae St. John, Phytologia 64(1):44, 1987.-TYPE: Oahu, Waiahole gulch, 250 m, 24 Jan. 1984, *Funk 211* (holotype: BISH!).

Decumbent, delicate shrubs, branching profusely up to 60 cm long, reddish-brown to green, pilose, eventually glabrate; stems red, nearly glabrous. Leaves alternate, (1-)3-5(-9) mm apart, petioles 0.1 mm long; blades filiform, coriaceous, (12-)25-40(-50) mm long, 0.5-1.2 mm wide, base attenuate, apex attenuate, upper surface glabrous, dark green, lower surface pilose when young, glabrate, dark green; secondary veins obscure. Flowers solitary in leaf axils, 5-7-merous; pedicels 17-27(-32) mm long, pendulous, glabrous or minutely pilose, occasionally red toward calyx; calyx lobes green, linear, to lanceolate, occasionally red toward base

(4-)5(-6) mm long, (1-)1.5-2(2.5) mm wide; corolla lobes dark red, lighter at tips, widely obovate, (5.5-)6-8(-10) mm long, 4.5-6 mm wide; filaments (2.5-)4-5 mm long, anthers 1 mm long; style 3.5-4.5(-5) mm long. Capsules ovoid, 3.5-5 mm long. Seeds dark brown, irregularly shaped, 1-1.5 mm long.

Phenology. Flowering in Jan.-July.

Distribution and habitat. *Kauai.* Known from a single collection made in 1912 from the upper part of Olokele Valley. *Oahu.* Growing in waterfall spray zones, hanging from wet cliffs with Isachne, Eragrostis, Machaerina, Bidens, and Selaginella. Known from only three small sub-gulches of Waiahole Gulch, 250 m.

Comments. As the name implies, a distinguishing character of this species is the extremely filiform leaves. Further collections from the type location would be most useful to verify that plants from Oahu are the same in all regards as the type specimen. Unfortunately no collections from the type location have been made since the initial collection. The land is privately owned and access is not available. The habitat is not stated either on the label on *Lydgate 2* or in the type description. From the description on the specimen label, "far mauka, Olokele Valley" (mauka is the Hawaiian word for "toward the mountain") it is likely that the collection came from the upper part of Olokele Valley, quite possibly in a waterfall habitat similar to the Oahu population. The original description states that the plant is a shrub though

the habit is not specified. Plants from Oahu are finely branching and pendulous. The stem of the type specimen is 0.4 cm wide, thicker than that of the Oahu plants, which suggests that it may have been more upright. St. John (unpublished manuscript) states that L. filifolia has viscid leaves. Wagner et al. (1990, p. 1080) state that the Oahu and Wailua river (L. pendens) plants are sparsely puberulent, while the type is viscid. I have been unable to detect viscid leaves in any specimens of L. filifolia or on those of L. pendens, which was included in L. filifolia sensu Wagner et al. Viscid leaves are not mentioned in the type description. St. John's comment is in reference to the type specimen from Olokele only. If plants from Olokele were viscid and upright, these characters would distinguish them from the Oahu plants.

Representative Specimens examined. KAUAI. Known only from the type specimen. OAHU: Waiahole gulch Obata 90-689, 990-703, 90-705 (BISH), Perlman 11149 (PTBG), Marr 246, 247, 248, Marr 791-Marr 799 (UBC).

3. *Lysimachia forbesii* Rock, Fedde Repert. 12:361, 1914.

Lysimachia longisepala C.N. Forbes, Occasional Papers, Bernice P. Bishop Museum., 4(3):222, 1909, non Forrest (1908).

Lysimachia koolauensis C.N. Forbes, Occasional Papers, Bernice P. Bishop Museum., 6(1):39, 1914. *Lysimachiopsis forbesii* (Rock) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-TYPE: Oahu, Koolau Range, Punaluu Mtns., wet forest, 2300 ft., Sept., 1908, C.N. Forbes & Rock s.n. (lectotype here designated: BISH 576726!; isoelectotype: MO 786053!).

Nomenclatural note. *Lysimachia koolauensis* C.N. Forbes, though published in the same year as *L. forbesii*, was published later than *L. forbesii* Rock (St. John, 1933).

In the original description, flowering specimens collected in Sept. 1908 and fruiting specimens collected eight months later are cited. The specimen chosen here as the lectotype, bears an immature flower and is labelled as a type but bears no date. This specimen matches the original description in having three flowers in the leaf axils, and the calyx lobes exceed the corolla lobes (in older flowers the corolla lobes exceed the calyx), thus it may be the specimen upon which the type description was based. The isoelectotype is dated Sept. 1908, elev. 2300 ft., as stated in the original description, but does not show floral features as well as the one designated as the lectotype. The lectotype bears a note "Sheet no. 3". I have not seen sheets no. 1 or 2.

Sprawling woody shrubs, with stems up to 1.5 m long, usually unbranched; stems dark red, pilose with red hairs, becoming glabrous when older. Leaves alternate, (3-)5-23(-40) mm apart, petioles (23-)31-45(-62) mm long; blades narrowly to broadly elliptic, chartaceous, (80-)160-200(-260) mm long, (20-)55-65(-95) mm wide, base attenuate, apex acuminate, upper surface dark green, glandular punctate when young, becoming glabrous, lower surface much lighter, pilose, the hairs red, multicellular, glandular punctate throughout; primary, secondary, and tertiary veins prominent, especially on lower surface. Flowers 1-5 in leaf axils, (6-)7-8(-9)-merous; pedicels 16-25(-32) mm long, densely tomentose, pendulous; calyx lobes green with red veins, pilose, lanceolate (18-)20-22(-27) mm long, (3-)4-5(-7) mm wide; corolla lobes green with red veins, narrowly elliptic, the margins erose 20-24(-28) mm long, (6.5-)7-10 mm wide; filaments (9-)9.5-13.5(-16.5) mm long, anthers 3 mm long; style (16.5-)17-19(-24) mm long, persistent in fruit. Capsules 10.5-12(-15) mm long. Seeds dark brown, irregularly shaped 1.5-2.5 mm long.

Phenology. Flowering in September.

Distribution and habitat. Oahu. Known only from wet forest along the Castle ("Pig-God") Trail, Punaluu Mtns. in the Koolau Range, 600-710 m. From the collection labels it is unclear if all collections came from a single population. Some specimens were collected "near the top of the trail overlooking the Valley" (presumably Punaluu). Another

collection comes from a "small gulch at the head of Kaluanui". The latter is the valley immediately west of Punaluu; the trail connects the two.

Comment. This species cannot be confused with any other Hawaiian Lysimachia. In all characters, this was the largest species of Lysimachia in the world. Among Hawaiian species it was unique in having multicellular hairs, punctate glands on the leaves, and often more than one flower per axil. Of the extant species, L. kalalauensis is almost certainly the closest relative, because these are the only species that have a green corolla with erose margins.

Lysimachia forbesii was last collected in 1934 and is presumably extinct. During the present study unsuccessful searches were made in the area of the type location, the only place this species was ever found. The ecology of the area has been significantly altered by the invasion of introduced species, yet many native species do persist.

Representative Specimens examined. OAHU: Koolau Mtns. Castle Trail, Rock 815 (P,BISH), Degener 17688 (GH,MO,NY), Rock 8839 (GH,US), Rock s.n. (GH), Rock 12502 (BISH), Degener 17689 (MASS,MO,NY,US), Forbes s.n. (F), Degener 17690 (NY), Forbes and Cooke s.n. (NY), Rock 376 (BISH), Swezey s.n. (BISH), Hosaka 38 (BISH), Rock 1031 (BISH), Forbes s.n (BISH).

4. *Lysimachia glutinosa* Rock, Bull. Torrey Bot. Club 37:297-304, 1910. *Lysimachiopsis glutinosa* (Rock) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-TYPE: Kauai, ridge west of Halemanu, 14-26 Feb. 1909, Rock 1770 (holotype: BISH, photo at NY!;isotypes: BISH!,NY!,P!,US!,W!)

Lysimachia fayi St. John, Phytologia 64(1):44, 1987.-TYPE: Kauai, Mt. Kahili, 550 m, 3 Dec. 1975, Fay 502 (holotype: BISH!).

Lysimachia olokeleensis St. John, Phytologia 64(1):47-48, 1987.-TYPE: Kauai, Olokele Valley, Jan. 1912, Lydgate 9 (holotype: BISH!).

Woody shrubs up to 2.5 m tall, branching primarily from the base, the entire plant viscid, often pulverulent; stems green to reddish-brown. Leaves alternate, (1-)3-13(-35) mm apart, petioles (1-)4.5-10.5(-12) long; blades oblanceolate to broadly obovate or elliptic, coriaceous, (50-)80-120(-160) mm long, (16-)25-35(-49) mm wide, base attenuate, apex acute to acuminate, sometimes abruptly acuminate, upper surface glabrous, light green, shiny, lower surface slightly lighter than above. Flowers solitary in leaf axils, (5-)6-7(-8)-merous, campanulate; pedicels (23-)33-47(-51) mm long, glabrous, erect; calyx lobes green, the margins hyaline, glabrous, lanceolate to ovate, often with prominent branching nerves, (8-)10-12(-16) mm long, (4.5-)5.5-7(-8) mm wide;

corolla lobes white or cream, sometimes reddish-purple at base, obovate, (15-)19-24(-30) mm long, (9-)11-14(-17) mm wide; filaments (6-)8-11(-12) mm long, white, anthers (2-)2.5-4(-5.5) mm long; style (6-)8-9(-10.5) mm long, white, green or red. Capsules (9.5-)10-11(-12) mm long. Seeds dark brown, irregularly shaped, 1.2-1.7 mm long.

Phenology. Flowering January-July.

Distribution and Habitat. Kauai. Mostly restricted to the Kokee area, 1090-1290 m. A single collection from Kahili Ridge, SE Kauai (Fay 502), 550 m is an unusually low elevation for the species. Exact location of Olokele collection is unknown, but is also outside the known principal range of the species. Growing in Lowland Wet Forest dominated by Metrosideros, Dianella, Dicranopteris, Scaevola, Coprosma, Perrottetia, Psychotria, Myrsine, Styphelia, Nestegis, Myrsine, Ilex and Cheirodendron.

Comments. This is the only white flowered species of Hawaiian Lysimachia. It is also distinct in having sessile glands that are responsible for producing a viscid surface. "Viscidness" may be a recessive trait as hybrids between this species and non-viscid species, are not viscid.

A single putative hybrid (Marr 615) collected below Kalalau Lookout, is morphologically intermediate between this species and L. kalalauensis. This individual closely resembles artificial F₁ hybrids between these two species. Plants that appear to be hybrids between this species and L.

scopulensis have also been collected below Kalalau lookout (Wood 1712, 1421) and near Puu Kii (Wood 2396, 2397).

Representative Specimens examined. KAUAI: Kalalau Lookout, Degener 30208, 33496 (BISH), Carlson 3713 (F), Degener 22334, 21561 (BISH, MASS, PTBG), Herbst 1001 (BISH, US), Fosberg 41467 (BISH), Takeuchi 2473 (BISH), Wagner 5005 (BISH, G, RSA), Takeuchi Alakai 91a, 91b (BISH), Carlquist 1986 (RSA), Henrickson 4036 (BISH, RSA, US), Lyon 5019L (BISH), Stone 1616, 3765 (BISH), Plews 129 (PTBG), Lorence 6319 (MO, PTBG), Lorence 6297 (PTBG), Lorence 5126 (BISH, MO, PTBG), Marr 614, Marr 616-Marr 619, Flynn 2769 (PTBG), Stern 2998 (BISH); Kalahu Degener 21465 (BISH, F, NY); Kaluapuhi Tr. Spence 174 (BISH, US), Flynn 160 (PTBG), Marr 254 (UBC); Honopu Trail, Marr 260, Marr 559-Marr 576 (UBC), Flynn 55 (PTBG), Gagne 546 (BISH), Herbst 2154 (F, MO, NY, PTBG), Darwin 1126 (GH, K, PTBG, US), Hobdy 69 (BISH), Herbst 2374 (BISH), Sohmer 6532 (NY); Kilohana, Yuncker 3494 (F, NY), Degener 22334 (BISH, MASS, NY), St. John 19985 (BISH); along road between Kilohana and Puu O Kila, Marr 255, Marr 590-Marr 609 (UBC); Ridge between Puu O Kila and Pihea, Lamoureux 2846 (BISH, F), Carlquist 1317 (RSA, US), Carlquist 1793 (RSA); Kahuamaa Flat Herbst 2055 (F); Road opposite Awaawapuhi trailhead, Gustafson 1014, 1715 (RSA), Lorence 5798 (PTBG); Lehuamakanoi Trail, near road Degener 23949 (W); Kokee area, not specific, Forbes 786.K (NY, P, US, W), MacDaniels 810 (BISH), Stern 2998 (BISH, RSA), Wilder 446 (BISH), Shear s.n. (US), Degener 17667 (K, MO), Neill s.n. (MO), Rock s.n. (K).

5. Lysimachia haupuensis St. John, Phytologia 64(1):45, 1987.-TYPE: Kauai, Haupu Range, along base of cliff, 400 m, 26 Feb. 1927, MacDaniels 883 (holotype: BISH!).

Low branching shrub at least 50 cm tall; stems dark brown, pilose when young. Leaves alternate, 1-10 mm apart, petioles 2 mm long; blades elliptic, coriaceous, glandular, (25-)36-45(-50) mm long, (7-)9-13(-14) mm wide, base acute, apex acute, upper surface dark green, shiny, glabrous, lower surface lighter than above, brown pilose becoming glabrous; secondary veins prominent, tertiary veins obscure. Flowers solitary in leaf axils, 6-merous; pedicels 25 mm long, densely pilose, erect; calyx lobes lanceolate, pilose, 6 mm long, 2 mm wide; corolla lobes red, obovate, 10 mm long, 5 mm wide; filaments 4 mm long, anthers 2 mm long; style 7 mm long. Capsules not seen.

Distribution. Kauai. Possibly extinct. Known from only the single sheet of the type collection made in 1927, Haupu Range.

Comments. This species is difficult to classify due to the limited collections. A character that it shares with only L. scopulensis, also from Kauai, are the prominent (upon drying) internal foliar glands. The leaves of L. scopulensis are much lighter green and are linear, or narrowly lanceolate to obovate. Lysimachia haupuensis also differs from L. scopulensis in having longer, lanceolate shaped calyx lobes and lacking pulverulent leaves and stems. Although L.

haupuensis was collected probably within 1-2 km of L. kahiliensis, another species for which a single collection was made, it differs from the latter in a number of regards including differences in leaf shape, leaf venation and calyx size, thus it would be inappropriate to combine these two taxa, although they do have similar leaf sizes. The leaf shape of L. haupuensis most closely resembles that of collections of L. hillebrandii from the Koolau Mtns on Oahu, yet internal glands are not prominent in dried specimens of L. hillebrandii and leaves of this species have a thicker texture and are much lighter green than those of L. haupuensis.

6. *Lysimachia hillebrandii* Hook. f. ex A. Gray, Proc. Am. Acad. Arts and Sci. 5:329, 1862; *Lysimachia hillebrandii* var. *hillebrandii* (as var. α) Hook. f. ex A. Gray, Proc. Am. Acad. Arts and Sci. 5:329, 1862; *Lysimachiopsis hillebrandii* (Hook. f. ex A. Gray) Heller.; *Lysimachiopsis grayi* O. Deg. & I. Deg., Plants Hawaii Nat. Parks P. 391, 1983.-TYPE: Oahu, Kalihi, *Hillebrand* 183 (lectotype here designated: K!; isolectotype: F!).

Lysimachia macdanielsii St. John, Phytologia 64(1):46, 1987.-TYPE: Oahu, Konahuanui-Olympus Trail, edge of Pali, 710 m, 1 Nov. 1926, *MacDaniels* 89 (holotype: BISH; isotype: BISH!).

Lysimachia mannii St. John, Phytologia 64(1):46, 1987.-TYPE: Oahu, Waiahole, 6 Feb. 1912, *Forbes* 1747.0 (holotype: BISH!).

Lysimachia rotundifolia Hillebr., Fl. Hawaiian Isl. 284, 1888, non Schmidt, 1793, nom. illegit. *Lysimachiopsis ovata* Heller, Minn. Bot Stud. 1:876, 1897. *Lysimachia ovata* (Heller) St. John, Pacific Trop. Bot. Gard., Mem. 1:270, 1973.-TYPE: Oahu, Nuuanu, *Hillebrand* s.n. (holotype: fragment of B at BISH!).

Lysimachia rubrimaculata St. John, Phytologia 64(1):48, 1987.-TYPE: Oahu, Koolau Mtns., Moanalua Valley Pali, *Forbes* s.n., 6 April 1909, (holotype: BISH!).

Lysimachia russii St. John, Phytologia 64(1):49, 1987.-TYPE: Oahu, Waiawa (top of Koolau), Feb. 1930, Russ s.n. (holotype: BISH; isotype: BISH!).

Lysimachia stenophylla St. John, Phytologia 64(1):49, 1987.-TYPE: Oahu, Koolau Mtns., Kalihi Valley, Forbes 1255.O, 2 April 1909, (holotype: BISH!;isotype: MO!).

Lysimachia waiehuensis St. John, Phytologia 64(1):50, 1987.-TYPE: Molokai, Waiehu, Wailau Valley, Sept. 1912, Forbes 559.Mo (holotype: BISH!;isotypes: MO!,P!,W!).

Lysimachia websteri St. John, Phytologia 64(1):50, 1987.-TYPE: Oahu, S. Palawai gulch, 28 Mar. 1948, Webster 1458 (holotype: BISH!).

Nomenclatural Note. This species was originally published in Gray (1862) as one of three varieties of Lysimachia hillebrandii Hook. f. ex A. Gray. This publication provided descriptions of specimens collected during the U.S. South Pacific Exploring Expedition of 1840. The descriptions for this variety of L. hillebrandii were provided by J.D. Hooker "L. hillebrandii Hook. f. in litt.-Oahu and Maui". The specific epithet suggests that a collection made by Hillebrand was the basis for the description of Hooker. Hillebrand, however, did not arrive in Hawaii until 1851. It is unclear whether or not Hooker based his description on specimens (US

2983211, NY s.n.) from the 1840 collection, or upon a specimen sent to him later by Hillebrand. In Hillebrand (1888), specimens cited for var. are restricted to the Koolau Mtns. of Oahu, "bare mountain ridges of Kalihi and Manoa." Knuth (1905) cited *Hillebrand s.n.* and *Wawra 2211* and *2380*, and also restricted var. *typica* R. Knuth, to Oahu. However, *Wawra 2211* is from the Waianae Mtns and is *L. waianaeensis* St. John. The specimen chosen here, *Hillebrand 183*, as the lectotype was collected from Kalihi and sent to Kew in 1865 (according to St. John, unpublished manuscript) and was identified as *L. hillebrandii*. This sheet bears four flowering branches and fits the description of *L. hillebrandii* var. α of Gray (1862). It is here designated as the lectotype of *L. hillebrandii*.

Woody shrubs up to 2 m tall; stems dark brown, densely reddish-brown tomentose at the tip, glabrate. Leaves alternate, (0.5-)3-24(-36) apart, petioles (2-)3-7(-13) mm long; blades narrowly to broadly elliptic or obovate, coriaceous, (24-)36-55(-60) mm long, (6-)12-24(-40) mm wide, base attenuate, apex acute to rounded, often abruptly acuminate, upper surface usually light green, pilose, red at base, lower surface usually slightly lighter than above, pilose to rusty tomentose, especially along the primary vein and petiole. Flowers solitary in leaf axils, (6-)7-8(-9)-merous; pedicels (10-)11-24(-30) mm long, densely rusty tomentose, erect; calyx lobes green, often red toward base, lanceolate to narrowly ovate, (4-)5-8(-9) mm long, (1.5-)2-

3.5(-5) mm wide; corolla lobes red, obovate (9-)12-16 mm long, (5-)6-8(-9) mm wide; filaments (4-)5-7.5(-8.5) mm long; style 6-8(-12) mm long. Capsules (6-)8-10 mm long. Seeds dark brown, irregular, 1.5-2.5 mm long.

Phenology. Flowering Sept.-Feb.

Distribution. Oahu. Koolau Mtns, wet forest at 400-710 m. Southern Waianae Mtns. in lowland mesic forest and cliffs 720-820 m, growing with Dubautia, Bidens, Hedyotis, Eragrostis, Elaphoglossum, Peperomia, Carex, Styphelia, Plantago, Hibiscus, Lysimachia waianaeensis, and Silene. Molokai. Known from a single 1912 collection from Wailau, exact location not given. Kauai. Single collection, with the year and location not given.

Comments. Lysimachia hillebrandii resembles L. waianaeensis in having obovate, ovate or elliptic leaves, however the areoles are not prominent. It is sometimes difficult to observe this feature from herbarium specimens. Useful here is a comment on the label of a specimen (Degener 4138) collected April, 1932 from near Mt. Kaala in the Waianae Mtns.: the leaves are "barely coriaceous, thinner with showier veins than the Punaluu plants". Degener had collected specimens from Punaluu (Koolau Mtns., Oahu) earlier that year. Lysimachia waianaeensis has stems, pedicels, calyx lobes and leaves that are glabrous, whereas those of L. hillebrandii are pubescent; calyx lobes of L. waianaeensis are narrowly ovate, 4-5 mm

long, while those of L. hillebrandii are lanceolate to broadly ovate, 5-8 mm long.

Leaf width is extremely variable in this species. While leaves of most specimens are 12-40 mm wide, a few scattered collections (*Forbes 1255.O, Forbes s.n., Degener 17669, St. John 13004, Faurie 707*) from the Koolau Mtns. have narrower leaves, 6-14 mm wide. The distribution of these specimens overlaps somewhat with that of the broader-leaved specimens and in fact, some sheets (*Mann and Brigham 229, BISH, F*) bear narrow leafed branches as well as broader leafed ones. Specimens from Oahu have leaves less than 32 mm wide, however, those from Molokai are up to 40 mm wide.

The most recent collection of this species from the Koolau Mtns. was a 1980 collection (*Obata 434*), from a population evidently of a single individual. Prior to 1980, L. hillebrandii had not been collected from the Koolau Mtns. since 1937. More recently collections have been made in the Waianae Mtns. but flowering specimens have never been collected from there.

Lysimachia hillebrandii is identified as "puahekili" in the Hawaiian language (Hillebrand, 1888).

Representative Specimens examined. OAHU: Olympus-Waimanalo Pali, *Rock s.n. (BISH), Garber 249 (BISH)*; Punaluu (Pig God Trail), *Degener 17681 (GH,NY,US), Degener 17685 (GH,MO,NY), Degener 17683, 33503 (NY); Suehiro s.n. (BISH)*; Nuuanu, opposite King's Falls, *Mann and Brigham 229 (BISH[2],F[2],GH,*

MASS,MO,NY[2]); Puu-O-Kona (Crest of Kuliouou) Obata 434 (BISH); Kalihi Valley, top of ridge, Swezey s.n. (GH), Christopherson 1270 (BISH), Forbes 229.O (BISH), Forbes 2291.O (K), Hillebrand s.n. (BISH), Faurie 707 (P); NE of Palikea Wood 1812 (PTBG), Perlman 5404 (PTBG); Palawai Gulch Wilbur 622 (BISH); ridge between Nanakuli and Lualualei Marr 1292, 1293 (UBC), Wood 1976, 2917 (PTBG), Wood 1977, 1982 (PTBG,UBC); Middle Halawa Ridge, Degener 17669 (GH,NY); Kalauao-Waimalu Ridge, St. John 13004 (BISH); drainage NE of Palikea, Wood 1817 (PTBG); location not stated (probably Koolau Mtns.), Wawra 2380 (W), U.S. South Pacific Exploring Expedition, 1838-1842, s.n. (NY, US). MOLOKAI: Waiehu, Wailau Valley, Forbes 559.Mo (BISH,MO,P,W). KAUAI: Lydgate s.n. (BISH 586775).

7. Lysimachia iniki Marr sp. nov., TYPE: KAUAI: Headwaters of N. fork of Wailua River, 720 m, 30 Oct. 1992, Lorence 7270 (holotype: PTBG!; isotype: BISH!). Figure 2.11.

Lysimachiae daphnoidi affinis a qua praecipue differt apice albo corollae minoris; foliis latioribus, plus orbiculatis, sursum versis, viridibus, versum basem, non rubris.

Small woody shrubs with pendulous branches 30-150 cm long; stems green, densely hirsute. Leaves alternate, 2-5(-10) mm apart, sessile; blades obovate to orbicular, cupped upward, coriaceous, chartaceous when dry, (35-)37-45(-54) mm long, (25-)35-38(-42) mm wide, base cordate, apex acute to rounded, abruptly acuminate, upper and lower surfaces light green, translucent viscid-hirtellous throughout, becoming glabrate, scattered patches of red pigment on lower surface; veins pellucid. Flowers solitary in leaf axils, 6-7-merous, funneliform; pedicels 15-25 mm long, densely viscid-hirtellous, erect; calyx lobes green, the margins hyaline, densely translucent viscid-hirtellous, lanceolate, 8-10 mm long, 2-3 mm wide; corolla lobes with the upper 5-7 mm white, the lower portion dark red, the inner surface densely glandular-punctate, oblanceolate, 15-16 mm long, 5 mm wide; filaments 10 mm long, dark red, anthers 2.5 mm long; style 8-9 mm long. Capsules 6-7 mm long. Seeds dark brown, irregularly shaped, 1 mm long.

Phenology. Flowering time unknown, fruiting in Oct.

Distribution and habitat. Known only from the wet cliffs of the headwaters of the North fork of the Wailua River, 720 m and above. At least two populations of more than 25 individuals are known. Growing on vertical wet, mossy or rocky cliffs with Machaerina, Isachne, Bidens, Plantago, Hedyotis, Pipturus, Cyrtandra, Dubautia, Athyrium, and Metrosideros.

Comments. The viscid-hirtellous leaves, stems, pedicels and calyx lobes of L. iniki resemble L. daphnoides, from which it differs in having broader, chartaceous (when dry) leaves, pellucid veins, smaller fruit, a shorter calyx, a shorter pedicel, and narrower corolla lobes that are distally white.

Named after Hurricane Iniki, which struck Kauai on Sept. 11, 1992. The force of the hurricane broke off several branches from the cliffs above the headwaters of the Wailua River in the same area where L. pendens grows. These were discovered by Lorence et al. and seeds (from Lorence 7270) sent to UBC where the plant was successfully grown and first seen in flower. In the Hawaiian language "iniki" means "sharp or piercing, as wind or pangs of love" (Pukui and Elbert, 1992).

Representative Specimens examined. KAUAI: headwaters of N fork of Wailua River, Flynn 5276 (PTBG), Perlman 13079 (PTBG).

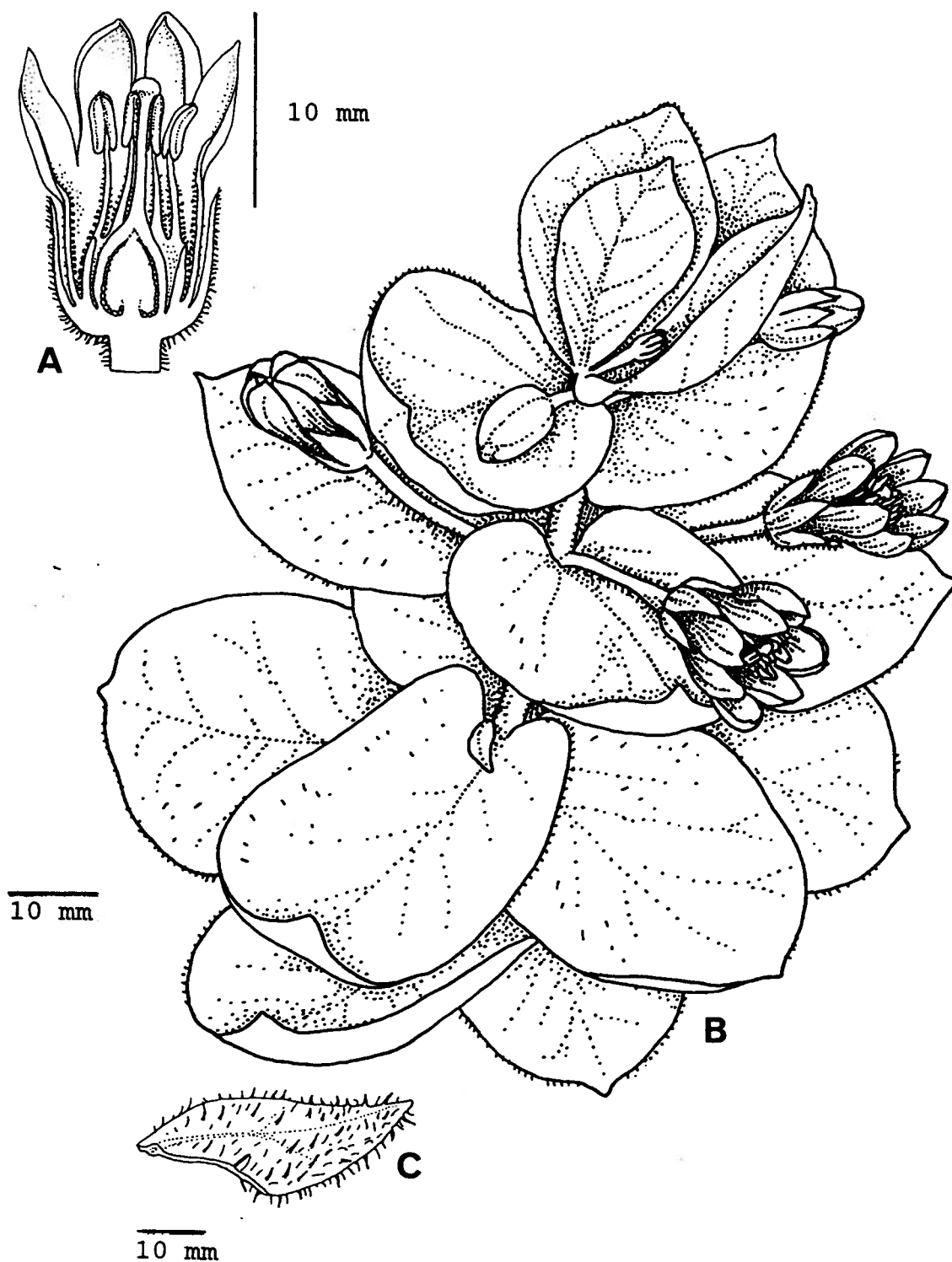


Figure 2.11. *Lysimachia iniki*. A. flower; B. flowering branch; C. leaf.

8. Lysimachia kahiliensis St. John, Phytologia 64(1):44, 1987.-TYPE: Kauai, Kahili Ridge, Aug. 1909, Forbes 271.K (holotype: BISH!;isotype P!,US!)

Upright shrub, at least 40 cm tall; stems reddish-brown, glabrous. Leaves alternate, 1-10 mm apart, petioles 1-2 mm long; blades narrowly obovate, coriaceous, 35-50 mm long, 9-12(-15) mm wide, base attenuate, apex attenuate, upper surface glabrous, lower surface glabrous; angle of divergence of the prominent secondary veins narrowly acute from the primary vein, tertiary veins obscure. Flowers solitary in leaf axils, 6-merous; pedicels 6-17 mm long; calyx lobes green, lanceolate, 9-11 mm long, 3 mm wide; corolla unknown. Capsules 6 mm long. Seeds, dark brown, irregularly shaped 1.5-3 mm long.

Phenology. Unknown.

Distribution. Kauai. Possibly extinct. Collected only once from Kahili Ridge, exact location unknown.

Comment. This is a problematic species because it is known from a single collection. The leaf, with a nearly sessile base, resembles L. daphnoides. Lysimachia kahiliensis differs from the latter in that the secondary veins depart from the primary vein in a more acute angle, the leaves are much less coriaceous, the apex is more acuminate, the base tapers more abruptly, and the entire plant is nearly glabrous.

9. *Lysimachia kalalauensis* Skottsbl., Goteborgs Bot. Tradg., Meddel. 15:429-430, 1944. *Lysimachiopsis hillebrandii* sensu Heller, Minn. Bot. Stud. Bull. 9, May 1897, Plate LVIII, non *Lysimachiopsis hillebrandii* (Hook. f. ex. A.Gray) Heller. *Lysimachiopsis helleri* (Knuth) O. Deg. and I. Deg., Plants Hawaii Nat. Parks, third edition, p. 392, 1983; *Lysimachia hillebrandii* var. *helleri* R. Knuth, Pflanzenr. IV. 237(Heft 22):310, 1905. *Lysimachiopsis kalalauensis* (Skottsbl.) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-TYPE: Kauai, Kilohana Lookout above Kalalau Valley, 20 Aug. 1938, Cranwell, Selling & Skottsberg 3034 (BISH). (Note: The description of the type location is in error. Kilohana Lookout is above Hanalei valley, Kalalau Lookout is above Kalalau Valley. The habitat at Kalalau Lookout is more typical for the species, and there is an extant population there, therefore it is much more likely that the type collection was from this location rather than from Kilohana Lookout).

Lysimachia hanapepeensis St. John, Phytologia 64(1):44, 1987.-TYPE: Kauai, ridge west of Hanapepe, 23 July 1895, Heller 2614A (holotype: K;isotypes: A!,F!,GH!,MO!,NY!,P!,UC!,US).

Lysimachia lamiatilis St. John, Phytologia 64(1):46, 1987. TYPE: Kauai, Wahiawa Marsh, Lydgate s.n. (holotype: BISH!).

Shrub with stems up to 4 m long, branching mostly from the base, with short lateral shoots; stems brown to dark red, densely rusty tomentose when younger, becoming glabrous. Leaves alternate, (1-)3-23(-40) mm apart, petioles (5-)7.5-8.5(-10.5) mm long; blades elliptic, coriaceous, (50-)60-80 (-100) mm long, (15-)25-32(-45) mm wide, base attenuate to rounded, apex acute to abruptly acuminate, upper surface dark green, glabrous, lower surface much lighter, lightly pilose, with scattered reddish-purple streaks; primary vein and secondary veins red, secondary veins prominent, higher order veins often obscured by thick cuticle. Flowers solitary in leaf axils, (5-)6-7(-8)-merous, urceolate, the petals often tightly closed around the exerted style, even until corolla abscission; pedicels (12-)16-33 mm long, lightly pubescent, often pendulous, rarely erect; calyx lobes, green, spotted with streaks of red, the margins slightly hyaline, nerves sometimes visible, linear (7-)13-16(-20) mm long, (2-)3-3.5 (-5) mm wide; corolla lobes obovate, the margins erose, red at base becoming green in upper half, but the veins red and inner surface of corolla remaining red further distally than on the outer surface (13-)15-17 mm long, (6.5-)7-9(-9.5) mm wide; filaments 10-12 mm long, red, anthers (2.5-)3-5 mm long, sometimes elongating beyond the corolla lobes and clasping the style; style 10-12 mm long, red at base, persistent in fruit. Capsules broadly ovoid to subglobose (7.5-)8-10(-11) mm long. Seeds dark brown, irregularly shaped, 2-3.1 mm long.

Phenology. Flowering Feb.- Nov.

Distribution. Kauai. Scattered populations in Lowland Diverse Mesic Forest of western and central Kauai, 970-1260 m. Growing with Metrosideros, Acacia, Zanthoxylum, Melicope, Tetraplasandra, Alyxia, Psychotria, Pouteria, Wikstroemia, Styphelia, Vaccinium, Antidesma, Wilkesia, Remya, Scaevola, Alphitonia, Dianella, Pleomele, Hedyotis, Dodonaea, Dubautia and Nestigis.

Comments. A number of characters distinguish this species from all others. This is the only extant species in which the corolla lobes are distally green with red veins and erose margins. The corolla lobes frequently remain tightly closed around the exerted style; in all other species, with the possible exception of L. ovoidea, the corolla lobes are always reflexed. Leaves of seedlings and sometimes young leaves of older plants often have a silver color. The short lateral shoots is a feature also common in L. waianaeensis.

Representative Specimens examined. KAUAI: Kalalau Lookout Stone 3766, (K,BISH); Makaha Valley road, Flynn 1157, (BISH,PTBG) Flynn 3282, (PTBG), Wagner 6067 (BISH), Lorence 6308 (MO,PTBG), Marr 1235,1236,1237 (UBC); Kalalau Pali, Forbes 1041.K (BISH,P), Flynn 3892,1870 (PTBG), Marr 249 (UBC); Honopu trail, Hobdy 119 (BISH), Wood 1188 (PTBG), Marr 256-257, Marr 540-Marr 558 (UBC); Kaunuohua Ridge, Flynn 3275 (BISH), Stern & Carlquist 1348 (RSA,US), Marr 271 (UBC); Awaawapuhi Trail, Flynn 1459 (BISH,PTBG), Wood 1803 (PTBG), Marr 273,275, Marr 577-Marr 589 (UBC); Puu Kii-dividing ridge

between Kalalau and Pohakuao, *Wood 2395* (PTBG,UBC), *Wood 2390*,
2394 (PTBG); Ridge west of Hanapepe River *Heller 2614*
(F,MASS,NY,P,US); Waialae Ridge, *Marr 620-Marr 635* (UBC);
Kohua Ridge *Marr 266,267,270* (UBC); Kalalau, below Puuokila
Wood 1104 (PTBG).

10. Lysimachia maxima (R. Knuth) St. John, Phytologia 64(1):47, 1987. *Lysimachia hillebrandii* Hook. f. ex A. Gray var. *maxima* R. Knuth in F. Pax and R. Knuth, Pflanzenr. IV. 237 (Heft 22):310, 1905. *Lysimachiopsis maxima* (Knuth) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-TYPE: Molokai, south rim of Pelekunu valley, just north of Ohialele, 950 m, *Hillebrand s.n* (lectotype designated by St. John (1987): fragment of B at BISH!).

Lysimachia ternifolia St. John, Phytologia 64(1):49, 1987.-TYPE: Molokai, Pelekunu Tr., *Forbes 242.Mo* (holotype: BISH; isotype: NY!).

Nomenclatural note. Hillebrand (1888) first described plants from "Molokai! Pali of Pelekunu, and smaller forms, quite glabrate, with sessile leaves from Maunahui; E. Maui! Haleakala; at heights of 3000 to 4000 ft." as *L. hillebrandii* var. ♂, which was not validly published. Knuth (1905) named it var. ♂ *maxima* and cited the Pelekunu and Haleakala collections. I have not seen any specimens from Maui labelled *L. maxima*, however it is possible that the plant cited was *L. remyi* subsp. *caliginis*, which also has densely tomentose stems and whorled leaves.

Sturdy upright shrubs 1-2 m tall; stems light brown or green, densely brown tomentose at tip, retaining pubescence on the older stem. Leaves 3(-4) per node, sometimes alternate,

(0.1-)0.5-43(-70) mm apart, petioles (1-)2-3(-5) mm long, red at base; blades ovate, obovate or elliptic, coriaceous, slightly rugose, margins revolute, (38-)55-60(-95) mm long, (18-)23-30(-50) mm wide, base attenuate, apex acute to abruptly acuminate, upper surface dark green, sparsely pilose, lower surface lighter green, moderately pilose with red streaks; secondary and tertiary veins prominent, pellucid. Flowers solitary in leaf axils, (5-)6(-7)-merous, campanulate; pedicels (16-)25-30 mm long, densely tomentose, erect; calyx lobes green, lanceolate, 9-10.5(-11) mm long, (3-)3.5-4(-4.5) mm wide; corolla lobes obovate, red, lighter at tips (15-)15.5-16(-17.5) mm long, 9-10 mm wide; filaments (6.5-)7.5-8.5(-10) mm long, anthers 1.5-2(-2.5) mm long; style 9-11 mm long. Capsules 8-9 mm long. Seeds dark brown, irregularly shaped, 1-2 mm long.

Phenology. Flowering June-July.

Distribution and Habitat. Molokai. Known from only the leeward side of the island on the western rim of Pelekunu valley in Lowland Wet Forest from a single population of 45-50 individuals, 950 m. Growing with Metrosideros, Dicranopteris, Vaccinium, Psychotria, Lycopodium, Machaerina, Hedyotis, Labordia, Cheirodendron, Dubautia, Sadleria, Elaphoglossum, Broussaisia, Styphelia, Scaevola and Cyrtandra.

Comments. This species has been distinguished by having leaves that are ternately arranged (Wagner et al., 1990). While this is often true, leaves are sometimes alternately

arranged and occasionally there are up to four leaves per node. L. maxima appears to be most closely related to L. remyi in which the leaves are also occasionally ternately arranged. It differs from the latter in having much broader leaves, thicker secondary veins, longer calyx and corolla lobes, secondary and tertiary veins that are more pellucid and in having the upper surface of the leaf darker and the lower surface lighter. The angle of divergence of the secondary veins is also more obtuse in L. maxima than it is in L. remyi. *Representative Specimens examined.* MOLOKAI: western rim of Pelekunu valley, just north of Ohialele, 950 m, Marr 1123-Marr 1135, Marr 1137.

11. Lysimachia ovoidea St. John, Phytologia 64(1):48, 1987.-
 TYPE: Kauai, west side of Wainiha Valley along narrow ridgetop
 separating Wainiha and Manoa drainages, southwest from
 Kalanaililia, 600-700 m, 20 May 1976, Fay 581 (holotype:
 BISH!; isotype BISH!,PTBG!)

Sprawling shrub mostly branching from the base; stems up to 3
 m long, reddish-purple to green, pilose at the tip, becoming
 glabrous. Leaves alternate, (1-)5-36(-48) mm apart, petioles
 (6-)7.5-12(-15) mm long, dark red; blades elliptic,
 coriaceous, waxy, (45-)60-80(-100) mm long, (18-)25-36(-41) mm
 wide, base rounded, apex acute to cuspidate, upper surface
 glabrous, light green, lower surface glabrous, slightly
 lighter; primary vein dark red, secondary veins prominent,
 tertiary veins obscure. Flowers solitary in leaf axils, 5-6-
 merous, barely open, almost urceolate; pedicels (20-)24-30
 (-32) mm long, pendulous, thin at base, broadening toward
 calyx, dark red for full length; calyx lobes dark red,
 narrowly lanceolate, 4-5.5 mm long, 1.5-2 mm wide; corolla
 lobes obovate, deep maroon, the margins lighter, 6-8.5 mm
 long, 4-5.5 mm wide; filaments (2-)3-4 mm long, anthers 1-1.5
 mm long; style (3-)4-5(-5.5) mm long. Capsules 5-6 mm long.
 Seeds dark brown, irregularly shaped, 1-2 mm long.

Phenology. Flowering April-Aug.

Distribution and Habitat. Kauai. Known from only two
 populations, the type location and the ridge between Limahuli

and Hanakapiai Valleys, above Limahuli waterfall, 615-680 m. Growing in Lowland Wet Forest with Metrosideros, Psychotria, Eugenia, Cyanea, Pittosporum, Dicranopteris, Diplopterygium, Ilex, Alyxia, Broussaesia, Xylosma, Scaevola, Dubautia, Freycinetia, Bidens, Vaccinium, Melicope, Cibotium.

Comments. This is the only species in which all individuals of a population have dark red pigment for the full length of the calyx and pedicel. This species and L. kalalauensis are the only species that have pendant pedicels. Leaf shape of L. ovoidea is similar to that of L. waianaeensis and L. hillebrandii, but the areoles are obscure (vs. prominent areoles of L. waianaeensis). Lysimachia ovoidea also has smaller calyx lobes than L. hillebrandii. The growing tips of most Hawaiian Lysimachia present a gradual increase in leaf size from those that are just being initiated to those that are mature. In L. ovoidea, the second youngest leaf is nearly full-sized and significantly larger than the youngest leaf. This is seen also in L. waianaeensis and L. kalalauensis, but the transition is not nearly as abrupt. In L. ovoidea the angle between the petiole and the stem is acute, nearly parallel to the stem; in other species this angle is more obtuse, often nearly 90° to the stem.

Representative Specimens examined. KAUAI: Wainiha-Manoa Ridge, Christensen 283,316 (BISH), Wichman 253 (PTBG,UBC) Marr 1238-Marr 1254 (UBC); Limahuli-Hanakapiai Ridge, Perlman 18 (BISH), Flynn 2164 (MO,PTBG,RSA), Marr 525-Marr 531, Marr 533,534,535 (UBC).

12. Lysimachia pendens Marr, sp. nov.-TYPE: Kauai, headwaters of the north fork of the Wailua river, 720 m, 23 July 1987, Lorence 5349, (holotype: PTBG!). Figure 2.12. (NOTE: This is not intended to effect the valid publication of a nomenclatural entity.)

Lysimachiae waiaholeensi affinis a qua praecipue differt foliis latioribus, lanceolatis, non linearibus; pagina inferiore foliorum brunnea manente et puberula ubi matura; pedicello longiore.

Small, many branched, delicately pendulous or prostrate shrubs; stems 20-60 mm long, densely tan tomentose when young, eventually glabrate. Leaves alternate, (0.1-).5-3.5(-5) mm apart, petioles 1(-2) mm long; blades narrowly lanceolate, soft coriaceous, (20-)25-30(-45) mm long, 2-4 mm wide, base attenuate, apex attenuate, upper surface green glabrous, lower surface green, brown pilose; secondary and higher order veins obscure. Flowers solitary in leaf axils, 5-7-merous; pedicels (6-)9-12(-14) mm long, green, occasionally red below calyx, densely tomentose, erect; calyx lobes narrowly ovate, 4-6 mm long, 2-2.5(-4) mm wide; corolla lobes red to the tips, obovate, 7.5-8.5 mm long, 5-6 mm wide; filaments 3.5-4 mm long, red, anthers 1 mm long; style 3.5-4.5 mm long. Capsules 5-6.5(-7) mm long. Seeds dark brown, irregularly shaped, 1.2-1.8 mm long.

Phenology. Flowering June-July.

Distribution and habitat. Kauai. Growing on vertical wet, mossy or rocky cliffs with Machaerina, Isachne, Bidens, Selaginella, Plantago, Hedyotis, Pipturus, Cyrtandra, Dubautia, Athyrium, and Metrosideros. Known from only several small populations at the headwaters of the north fork of the Wailua River, 720 m.

Comments. This species is a low, almost mat-forming shrub with pendant stems. The characters that distinguish it from L. filifolia are its broader leaves and tomentose leaves, stems and pedicels.

Representative Specimens examined. KAUAI: Headwaters of the north fork of the Wailua river, Wood 95,344 (PTBG), Lorence 7253 (BISH), Perlman 13078 (BISH), Marr 261,262, Marr 470-Marr 503 (UBC).

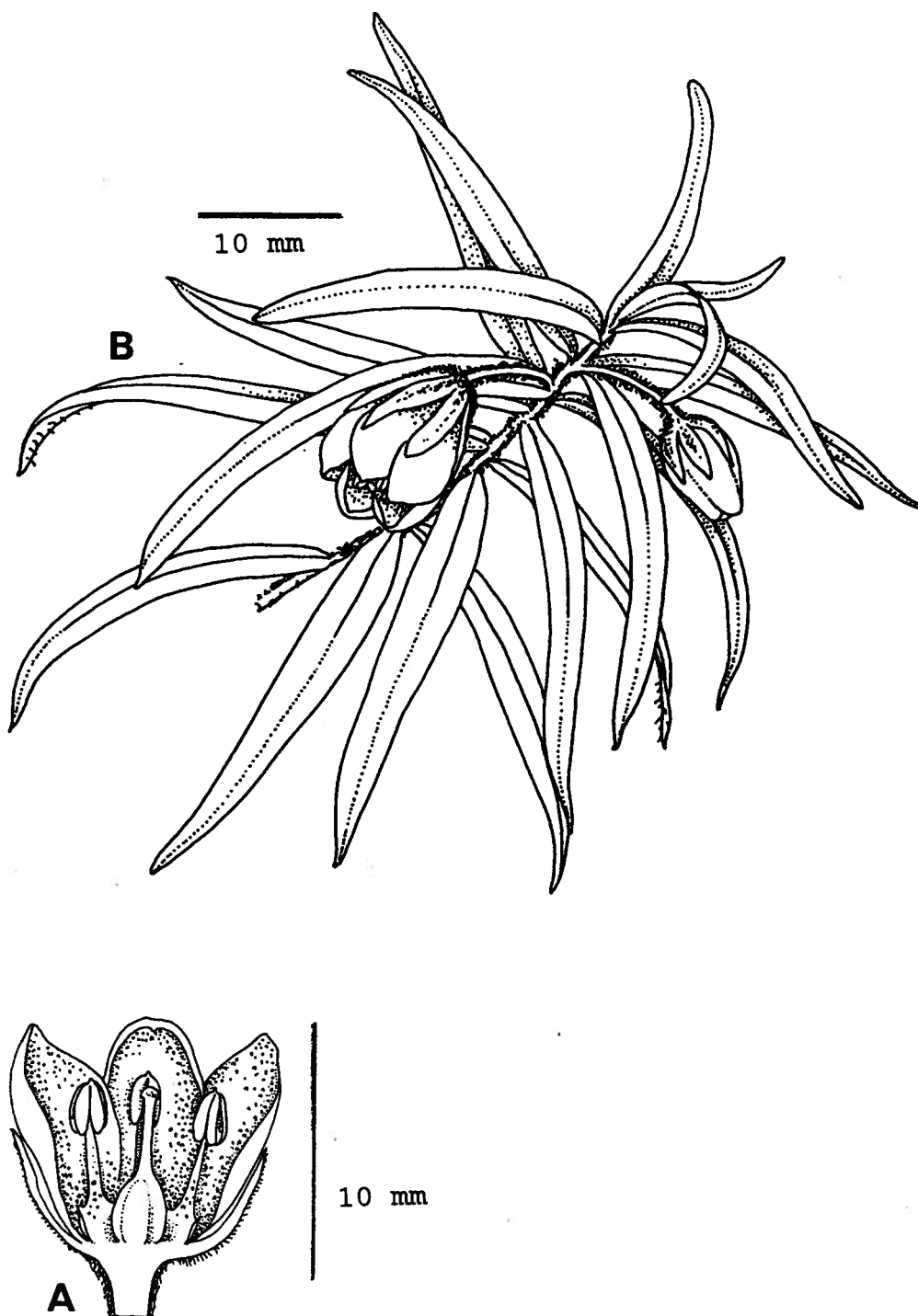


Figure 2.12. *Lysimachia pendens*. A. flower; B. flowering branch.

13. Lysimachia remyi Hillebr., Fl. Hawaiian Isl. 284, 1888.
Lysimachiopsis remyi Heller, Minn. Bot. Stud. 1:876, 1897.
Lysimachiopsis remyi (Hillebr.) Heller, Plants Hawaii Nat.
 Parks, third edition, p. 392, 1983.-TYPE: Maui, Voyage de M.J.
 Remy, 1851-1855, no. 458 (lectotype here designated:
 P!;isolectotype: K!).

Nomenclatural note. Hillebrand (1888) cited 6 collections in his description of Lysimachia remyi: "Maoui! Haleakala, Waihee, Waiehu; Molokai! Halawa, Waikolu, collected also by Remy." Lysimachia hillebrandii var. ♂ angustifolia (Gray, 1862) collected by Remy on Maui is cited in Hillebrand (1888) as a synonym. A sheet from GH appears to be one of the collections cited by Hillebrand. It bears two collections, one labelled "Lysimachia hillebrandii var. linearis H. f., W. Maui: Gulch of Waihee" and the other "Lysimachia hillebrandii Hook. f. var. angustifolia, Molokai: Gulch of Halawa". On this sheet the collection from Waihee lacks fruit and flowers and is otherwise a poor specimen; the collection from Halawa bears fruit and a single flower but has unusually narrow leaves for plants from Molokai, nevertheless the angle of divergence of the secondary veins in the leaves is typical of that of L. remyi subsp. subherbacea. The specimen cited as "Haleakala" by Hillebrand may have been a specimen from the Wilkes 1838-1842 Expedition, (US 76574), labelled "Lysimachia hillebrandii Hook. f. verging to var. angustifolia", however, no collection location is given. This specimen belongs to L.

remyi subsp. caliginis, distributed on both West and East Maui (Haleakala).

I have designated *Remy* 458 as the lectotype because it is a good flowering specimen and would appear to be among the material cited by Hillebrand. The collection location is not stated on the specimen chosen as the lectotype, however, St. John (unpublished manuscript) viewed a duplicate from GH and lists the collection location as Waiehu in his description of L. stene St. John, which is based upon the same collection.

Sprawling to erect shrubs with stems up to 5 m long; stems red or green, glabrous, glabrate or densely reddish-brown tomentose. Leaves alternate or whorled, (0.1-)0.5-30(55) mm apart, petioles (1-)2-10(-12) mm long; blades linear, oblanceolate, ovate, elliptic, rarely orbicular, coriaceous, sometimes undulate and rugose, margins sometimes revolute, (15-)20-60(-95) mm long, (1-)4-17(-33) mm wide, base and apex acute to attenuate or rounded, upper surface light green to dark green, glabrous, glabrate to tomentose, lower surface slightly lighter, glabrous, pilose, or densely light brown tomentose sometimes with scattered red streaks, veins sometimes pellucid. Flowers solitary in leaf axils, 5-8-merous, campanulate, rarely urceolate; pedicels (3-)10-30(-70) mm long, glabrous to pilose, sometimes tomentose, erect; calyx lobes entirely green, or red at the base, linear, lanceolate or ovate, glabrous to densely tomentose (2.5-)4-8(-10) mm long, (1-)1.5-3(-4.5) mm wide; corolla lobes obovate, red

(5-)8-14(-17) mm long, (5-)6-9(-15) mm wide; filaments (3-)4.5-7.5(-11) mm long; style 3-6.5(-7) mm long. Capsules (4-)4.5-8(-10) mm long.

Comments. This is an extremely variable species that encompasses all plants from Oahu, Molokai and Maui that are upright shrubs, have leaves 4-20 mm wide, red corolla lobes less than 15 mm long and calyx lobes less than 10 mm long. Leaf morphology and spacing is often extremely variable among as well as within populations. There are however, groups of populations that share vegetative characters that distinguish them from other groups. Floral characters also differ within populations, but the range of variation overlaps among the different groups of populations. While it is possible to determine which group of populations a specimen belongs to based on leaf morphology, this is not always possible if using floral characters alone. Four subspecies are recognized, based primarily upon vegetative differences.

Key to subspecies

- 1a. Angle of divergence of upper secondary veins in the leaves, more obtuse than lower; leaves dark green, alternate. West Maui.13c. L. remyi subsp. remyi.
- 1b. Angle of divergence of secondary veins in the leaves nearly uniform; leaves dark or light green, alternate or whorled.(2)
- 2a. Leaf veins pellucid; leaves often whorled; stems

usually densely tomentose. East and West Maui.

.....13a. L. remyi subsp. caliginis.

2b. Leaf veins not pellucid; leaves alternate, rarely whorled; stems nearly glabrous or tomentose.(3)

3a. Leaves distantly spaced, (.1-)1-22(-55) mm apart, if closely spaced then nearly ternate, otherwise alternate; leaves (20-)45-60(-95) mm long; corolla lobes (6-)11.5-14 (-17) mm long. Molokai.

.....13d. L. remyi subsp. subherbacea.

3b. Leaves closely spaced, (.5-)1-6(-20) mm apart, alternate; leaves (15-)20-30(-60) mm long; corolla lobes (6-)8-10(-13) mm long. East and West Maui.

.....13b. L. remyi subsp. kipahuluensis.

13a. *Lysimachia remyi* subsp. *caliginis* (St. John) Marr comb. nov.; *Lysimachia caliginis* St. John, Phytologia 64(1):43, 1987.-TYPE: E. Maui, E. of Ukulele, along edge of stream, July 1919, *Forbes 864.M* (holotype: BISH!;isotype: NY[2]!). (NOTE: This is not intended to effect the valid publication of a nomenclatural entity.)

Lysimachia pentophylla St. John, Phytologia 64(1):48, 1987.-TYPE: East Maui, Koolau Gap at treeline, 2 Sept. 1945, *St. John & Mitchell 21266* (holotype: BISH;isotype: BISH!).

Lysimachia kukuiensis St. John, Phytologia 64:45-46, 1987.-TYPE: W. Maui, Puu Kukui, open bog, 1540 m, 18 Dec. 1928, *Ewart 140* (holotype: BISH!).

Upright shrubs, sometimes sprawling, with stems up to 2.5 m long, often growing in dense clumps; stems light brown, often densely reddish-brown tomentose, even when older. Leaves sometimes alternate, but often whorled, with up to 5 leaves per whorl, 0.5-19(-35) mm apart, petioles (1-)2-3(-5) mm long, often dark red; blades lanceolate to ovate, coriaceous, undulate, rugose, the margins revolute, (20-)30-45(-55) mm long, (4-)8-14(-20) mm wide, base acute to attenuate, apex acute to attenuate, upper surface dark green, glabrous, lower surface lighter, slightly pilose, scattered red streaks; veins pellucid, secondary and tertiary veins prominent. Flowers solitary in leaf axils, (5-)6-7-merous, campanulate; pedicels

(8-)13-20(-40) mm long, glabrous to pilose, sometimes tomentose, often red toward calyx, erect; calyx lobes often dark red toward base, otherwise green with prominent reddish-purple veins, lanceolate to ovate, (4-)5.5-7(-8) mm long, 2-3(-4.5) mm wide; corolla lobes dark red, obovate (7-)10-11(-14) mm long, (5-)7-8.5(-12.5) mm wide; filaments red, (3-)5-6(-7) mm long, anthers 1.5-2 mm long; style red, (4-)5-6(-7) mm long. Capsules 5-6.5(-7) mm long, widely ovate. Seeds dark brown, irregularly shaped, 1.3-2.4 mm long.

Phenology. Flowering May-December.

Distribution. East Maui. Montane Wet Forests, growing at or below treeline in Koolau Gap and Kaupo Gap, with Metrosideros, Blechnum, Vaccinium, Styphelia, Dubautia, Coprosma, Stenogyne, Cheirodendron, Machaerina, Rubus, 1660-1970 m. West Maui. Montane wet shrublands and wet forest on summits, growing with Metrosideros, Dicranopteris, Dodonaea, Styphelia, Coprosma, Broussaisia, Sadleria, Lycopodium, Scaevola, Vaccinium, Dianella and Sphenomeris, 1200-1760 m.

Comments. Lysimachia remyi subsp. kipahuluensis, L. r. subsp. caliginis and L. r. subsp. subherbacea differ from L. r. subsp. remyi in having the angle of divergence of the secondary leaf veins nearly equal for all secondary veins, whereas those of L. r. subsp. remyi become more obtuse toward the tip of the leaf. Leaves of L. r. subsp. caliginis and subsp. kipahuluensis are generally less than 45 mm long, whereas those of L. r. subsp. subherbacea are generally

greater than 45 mm long. Lysimachia remyi subsp. caliginis differs from L. r. subsp. kipahuluensis in having leaves that are often whorled, larger, more ovate, darker, rugose, undulate, and with revolute margins. Higher order veins are prominent and pellucid, and the stems are often densely tomentose. Lysimachia remyi subsp. kipahuluensis and L. r. subsp. caliginis form hybrid swarms on East Maui in Kaupo Gap and near the old Waikau Cabin site in Koolau Gap. In these areas the range of leaf shape and size is continuous between the ovate leaves of subsp. caliginis and the linear leaves of subsp. kipahuluensis although these two species are not sympatric now, they may well have been in the past. Decades of destruction of the native vegetation in Haleakala has undoubtedly affected species distributions. Hybridization between L. r. subsp. caliginis and subsp. remyi could also account for some of the variation in leaf shape and spacing in some specimens from West Maui.

Representative Specimens examined. EAST MAUI: Haleakala, Koolau Gap, Marr 327-Marr 344, Marr 1019-Marr 1044 (UBC), Degener 2551 (BISH,GH,MO,NY,P), Degener 2553 (BISH,GH,MO,NY,US), Degener 2552 (GH,NY), Degener 17668 (BISH,GH,MASS,MO,NY), Rock 8632 (GH,BISH), Herbst 1620 (BISH), Carlquist 1933 (RSA), Forbes 1014.M (P), Hobdy 745 (BISH), Warshauer 2803 (BISH), St. John and Mitchell 21266 (BISH), Perlman 10762,10767 (PTBG), Perlman 10769 (MO,PTBG); Location unclear but in Haleakala Crater, Degener 2554 (BISH,GH,K,MASS,

MO,NY,US), Degener 23296 (NY), Hitchcock 14960 (US), Woolford
s.n. (BISH,GH); Pipeline trail, Olinda, Degener 17686
(BISH,GH,MASS,MO,NY,W); East of Ukulele, along edge of
stream, Forbes 864.M (BISH); Without locality: U.S. Exploring
Expedition, under Captain Wilkes (US), as *L. hillebrandii*
Hook.f., verging to var. *angustifolia*. WEST MAUI: Puu Kukui,
Hitchcock 14820 (US), Degener 25074 (NY), Rock 8140 (GH,NY),
Munro s.n. (BISH), Neal s.n. (BISH); Mt. Eke, Degener 2550
(GH,NY,US), Forbes 389.M (BISH); Hanakao, Forbes 62.M (BISH).

13b. Lysimachia remyi subsp. kipahuluensis (St. John) Marr comb. nov. *Lysimachia kipahuluensis* St. John, Pacific Science 25:50, 1971. *Lysimachiopsis kipahuluensis* (St. John) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 391, 1983.-TYPE: E. Maui, Haleakala, Lake Waianapanapa, Kipahulu-Kuhiwa divide, in low thicket, crest of divide, 1720 m, 13 Aug. 1945, H. St. John and A.L. Mitchell 20,980, (holotype: BISH!; isotype: US!). (NOTE: This is not intended to effect the valid publication of a nomenclatural entity.)

Lysimachia angusta St. John, Phytologia 64(1):43, 1987.-TYPE: East Maui, Hana Forest Reserve near N. rim of Kipahulu Valley on steep inner slope of old cinder cone, 1730m, 16 Nov. 1973, Harrison 539 (holotype: BISH; isotype: BISH!).

Lysimachia arta St. John, Phytologia 64(1):43, 1987.-TYPE: E. Maui, Hana Forest Reserve, near Waieleele, NE of Lake Waianapanapa, near rim of Kipahulu valley, 2090 m, 29 June 1973, Harrison 272 (holotype: BISH!).

Lysimachia furcata St. John, Phytologia 64(1):44, 1987.-TYPE: East Maui, NE of Lake Waianapanapa, 2120 m, 23 Nov. 1973, Harrison 488 (holotype: BISH!).

Upright shrubs usually less than 1 m tall, but up to 2 m tall, mostly branching from the base, often growing in dense clumps

several meters square; stems light brown to red, glabrous to minutely pilose. Leaves alternate, 0.5-6(-20) mm apart, petioles (1-)2-3(-4) mm long; blades linear to oblanceolate or narrowly ovate, coriaceous (15-)20-45(-60) mm long, (1-)2-6 (-14) mm wide, base attenuate and apex acute to acuminate, upper surface light green, glabrous, lower surface paler, slightly pilose, secondary veins pellucid, tertiary veins usually obscured by thick cuticle. Flowers solitary in leaf axils (5-)6-7(-8)-merous, sometimes urceolate, but usually campanulate; pedicels (5-)11-17(-31) mm long, often maroon toward calyx, lightly pilose, erect; calyx lobes green, glabrous or pilose, linear to narrowly ovate, occasionally red at base, (3-)5-6(-9.5) mm long, (1-)2-2.5(-4.5) mm wide; corolla lobes red, much lighter toward margins, obovate, (6-)8-10(-13) mm long, (5-)6-7(-9.5) mm wide; filaments (3-)4.5-5.5(-8) mm long, red, anthers (1-)1.5(-2) mm long; style (4-)5-6.5(-7) mm long, red. Capsules widely ovate, 5-6(-7) mm long. Seeds dark brown, irregularly shaped, 0.9-2.1 mm long.

Phenology. Flowering March through November.

Distribution and habitat. East Maui. Montane Wet Forest in Haleakala Crater above Paliku Cabin growing with Metrosideros, Cheirodendron, Coprosma, Melicope, Myrsine, Blechnum, Elaphoglossum, Peperomia, Astelia, Rubus, Dubautia, Vaccinium. Montane Mesic Forest of the east and west sides of Kaupo Gap. Subalpine Mesic Shrublands above treeline and into the forest

around the rim of Kipahulu Valley growing with Metrosideros, Styphelia, Coprosma, Blechnum, Vaccinium, Dubautia, Deschampsia, Lycopodium, Nephrolepis, from 1510-2330 m. Growing with Metrosideros, Broussaisia, Cheirodendron, Vaccinium, Selaginella, Machaerina, Dicranopteris, Acacia, Psychotria, Clermontia and Carex along streamsides or similar high moisture environments in Kipahulu Valley, Waihoi Valley and Hana Forest Reserve from 690-1750 m. West Maui. Along wet cliffs of streamsides growing with Pipturus, Bidens, Artemisia, Selaginella, Hedyotis, Coprosma and Metrosideros 370-900 m.

Comments. Lysimachia remyi subsp. kipahuluensis is distinct in having light green, closely spaced, linear to narrowly ovate leaves, generally less than 6 mm wide, but up to 14 mm wide. Plants with very narrow leaves are scattered along streamsides and in bogs in Kipahulu Valley, Waihoi Valley and the Hana Forest Reserve. Broader leafed plants, more typical of the subspecies also occur in these areas.

Plants from West Maui have somewhat longer corollas (6-9 mm) than do those from East Maui (6-7 mm).

Representative Specimens examined. EAST MAUI: Gulch SE of Paliku cabin, Marr 281, Marr 678-Marr 705 (UBC), Degener 17670, (GH,MASS,MO,NY), Degener 17753 (GH,MO,NY,P), Degener 21400 (BISH,NY), Degener 17754 (NY), Higashino 849 (BISH,US), Henrickson 3509 (BISH,RSA,US), St. John 21117 (GH,RSA,US); South of Kuiki, east side of Kaupo Gap, Degener 17671 (GH,MO,NY,US), Rock 8605 (BISH,GH,NY,P,PTBG,US), Olson 6

(BISH,US); Small gulch between Healani Gulch and Kaupo Gap, Gagne and Montgomery 605 (BISH), Hobdy 500 (BISH); Cliffs SE of Haleakala Mtn., Degener 17755 (BISH,GH,MASS,MO,NY,US); Kalapawili Ridge E of Pohaku Palaha to Lake Waianapanapa, Marr 282-Marr 291, Marr 636-Marr 666 (UBC), Harrison 531, 272 (BISH), Forbes 1195.M (K,US), Henrickson 3915 (BISH,US), Henrickson 3913 (BISH), Perlman 10517 (PTBG); Upper Kipahulu Valley, Higashino 10190 (BISH); S of Paliku cabin, Kaupo Gap, Henrickson 3884 (BISH,RSA,US); Waihoi Valley, Harrison 26 (BISH); Waihonu Stream, Waihoi Valley, Nagata 1049 (BISH); Upper Hana Forest Reserve "mid-camp", stream bank, Harrison 567 (BISH), inner slopes of old cinder cone Harrison 409 (BISH); Kipahulu Valley, Palikea Stream, Wood 3201, 3202, 3212, 3217 (PTBG), Marr 1279-Marr 1284, Marr 1290,1291 (UBC), below upper valley plateau, Marr 1287,1288,1289 (UBC), Kokowai Stream, Anderson s.n. (UBC); Upper Kipahulu valley, Marr 430 (UBC). WEST MAUI: Nakalaloa stream, Marr 432, Marr 434-Marr 442 (UBC); Black Gorge, Marr 1258-Marr 1263 (UBC), Wood 0333 (PTBG).

13c. *Lysimachia remyi* subsp. *remyi*. *Lysimachia stene* St. John, Phytologia 64(1):49, 1987. *Lysimachia hillebrandii* Hook. f. var. *angustifolia* Gray, Proc. Am. Acad. Arts Sci. 5:329, 1862. *Lysimachiopsis angustifolia* (Gray) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 390, 1983.

Lysimachia ciliolata St. John. Phytologia 64:43, 1987.-TYPE: W. Maui, secondary ridge at right hand head of Olowalu, 14 May 1920, *Forbes* 2369.M (holotype: BISH!; isotype: W!).

Lysimachia elliptica St. John, Phytologia 64(1):44, 1987.-TYPE: Maui, ridge left of Lahainaluna Valley, Feb. 1913, *Forbes* 325A.M. (holotype: BISH!).

Lysimachia lata St. John, Phytologia 64(1):46, 1987.-TYPE: Maui, ridge left of Lahainaluna Valley, Feb. 1913, *Forbes* 325B.M. (holotype: BISH!; isotype: BISH!).

Lysimachia lydgatei Hillebr. Fl. Hawaiian Isl. p. 284, 1888. *Lysimachiopsis lydgatei* (Hillebr.) Heller, in Minn. Bot. Stud. 1:876, 1897.-TYPE: W. Maui, on slopes and in gulches back of Lahaina, *Hillebrand* s.n. (holotype: fragment of B at BISH!; isotype: GH,K).

Lysimachia occidentalis St. John, Phytologia 64(1):47, 1987.-TYPE: Maui, Hanaulaiki, 1000 m, 14 March 1972, *Hobdy* s.n. (holotype: BISH!).

Lysimachia pedicellata St. John. Phytologia 64:48, 1987.-

TYPE: Lanai, ridge at head of Maunalei, 14 Oct. 1916, Munro 431 (holotype: BISH!).

Lysimachia pilosula St. John, Phytologia 64(1):48, 1987.-TYPE:

Maui, Hanaula, June 1910, Forbes 114.M. (holotype: BISH!).

Lysimachia scansoria St. John. Phytologia 64:49, 1987.-TYPE:

Lanai, Munro s.n. (holotype: BISH!).

Spreading shrub up to 1.5 m tall, branching mostly from the base, often with very short axillary shoots; stems green, light brown or red, glabrate to densely rusty tomentose. Leaves alternate, (0.1-)0.5-30(-50) mm apart, petioles (1-)1.5-10(-12) mm long; blades linear, lanceolate, oblanceolate, ovate or elliptic, rarely orbicular, coriaceous, (15-)20-55(-90) mm long, (1-)2-17(-33) mm wide, base acute, attenuate or rounded, apex acuminate, acute, or attenuate, upper surface dark green, glabrous to densely brown tomentose, lower surface much lighter, glabrate to densely brown tomentose, especially along the primary vein; secondary veins prominent or obscure, tertiary veins usually obscure. Flowers solitary in leaf axils, (5-)6-8-merous, campanulate; pedicels (3-)10-25(-65) mm long, densely tomentose, usually erect, sometimes pendulous; calyx lobes lanceolate, sometimes dark

red at base, glabrous to densely tomentose (2.5-)4-7(-9) mm long, (1-)1.5-3(-3.5) mm wide; corolla lobes obovate, red, (5-)8-10(-13) mm long, (4-)5-7(-9) mm wide; filaments red (2.5-)3-5(-7) mm long; style red (3-)4-6(-6.5) mm long. Capsules (5-)6-8(-10) mm long. Seeds dark brown, irregularly shaped, 1.3-2.9 mm long.

Phenology. Flowering Feb.- Oct.

Distribution and habitat. West Maui. Montane Mesic Forest on the leeward side of the island. Growing with Metrosideros, Dodonaea, Styphelia, Sadleria, Vaccinium, Coprosma, Cheirodendron, Dubautia, Hedyotis, Myrsine, Pipturus, Alyxia, Astelia, Wikstroemia, Selaginella, Scaevola and Lobelia at 670-1110 m. Lanai. Mountains, 650 m.

Comment. This is easily the most variable taxon of Hawaiian Lysimachia. Leaf length, width and shape vary from linear and glabrous to broadly elliptic or even orbicular and often densely tomentose. At one extreme are plants with nearly glabrous leaves, 15 mm long and 2 mm wide, these would have been classified as L. remyi in the classification of Wagner et al. (1990). At the other extreme are plants such as a specimen from Lihau, *Hobdy 519* (BISH) which has elliptic, densely tomentose leaves, 90 mm long and 30 mm wide. The length, width, and degree of pubescence of the calyx lobes is also quite variable in many populations. Plants with elliptic leaves and tomentose stems, leaves, and calyx lobes were previously classified as L. lydgatei. This species is not

recognized here because of the continuum in form between it and nearly glabrous plants with linear leaves.

Representative Specimens examined. WEST MAUI: Kaulaula Canyon, Hobdy 1212 (BISH); Lihau Hobdy 519 (BISH), Marr 408-Marr 430, Marr 801-Marr 870 (UBC), Hobdy 515,516,823 (BISH), Perlman 8425 (MO,PTBG,UBC); Hanaula, Manawainui Gulch, Degener 17687 (GH,NY,US,BISH), Gustafson 2078,2069,2781 (RSA), Forbes 114.M (BISH,P,US), Nagata 961 (BISH,US), Nagata 1923 (BISH), Hobdy 248 (BISH) Degener 22063 (NY,MASS) Marr 350-Marr 356, Marr 871-Marr 927 (UBC); ridge north of Pohakea Gulch, Degener 2556 (US); Pohakea Gulch, Gustafson 2075 (RSA), Hobdy 2671,2690 (BISH), Nagata 1918 (BISH), Wood 1170 (PTBG); south of Hanaula, Wood 1176,1179 (PTBG); left-hand side of Olowalu, Forbes 2300.M (BISH,W); Olowalu, right hand side of valley, Forbes 2368.M (NY,P), Forbes 2247.M (BISH,K), FORBES 2248.M (BISH); Kahoolewa ridge, Perlman 10581 (PTBG); Waihee Valley, Hillebrand s.n. (GH); Helu summit, Marr 1264-Marr 1268 (UBC), Perlman 10729,10728 (PTBG); Hale Pohaku, Marr 933-Marr 1018 (UBC); Puu Lio, Degener 12909 (GH,NY[2],US); Black Gorge, Degener 23735 (GH,NY); between Kinihapai and Ae streams, Gustafson 3300 (RSA); upper Ukumehame Canyon, Hobdy 1259 (BISH); Wailuku Pali, Forbes 2440.M (BISH); lowlands back of Kaanapali, Rock 8164 (BISH); location uncertain, Forbes s.n. (BISH). LANAI, Mtns. east end, Forbes 221.L (BISH,MO,NY,P,US); Kaiholena, Forbes 387.L (BISH); Manaha, Rock 8096 (BISH,GH,K,NY); ridge above Maunalei, Munro 627 (BISH,W).

13d. *Lysimachia remyi* subsp. *subherbacea* (St. John) Marr comb. nov. *Lysimachia subherbacea* (Hillebr.) St. John, *Phytologia* 64(1):49, 1987. *L. hillebrandii* Hook. f. var. *subherbacea* Hillebr., *Fl. Hawaiian Is.* 283, 1888. *Lysimachiopsis subherbacea* (Hillebr.) O. Deg. and I. Deg. *Plants Hawaii Nat. Parks*, third edition, p. 393, 1983. -TYPE: Molokai, gulch of Halawa, *Hillebrand s.n.*, (lectotype here designated: fragment of B at BISH!). (NOTE: This is not intended to effect the valid publication of a nomenclatural entity.)

Lysimachia attenuata St. John, *Phytologia* 64(1):43, 1987.-TYPE: Molokai, Kahaunui Gulch, 12 May 1928, *Degener 17676* (holotype: GH; isotype: MO!).

Lysimachia fauriei St. John, *Phytologia* 64(1):44, 1987.-TYPE: Molokai, Kamolo, 1,000 m, June 1910, *Faurie 705* (holotype: GH!; isotype: BISH!,P!). (St. John changed the collection number on the P specimen to 705A).

Lysimachia kalaupapaensis St. John, *Phytologia* 64(1):45, 1987.-TYPE: Molokai, Kalaupapa Pali, 520 m, 23 May 1918, *Rock 14030* (holotype: BISH!).

Lysimachia mucronata St. John, *Phytologia* 64(1):47, 1987.-TYPE: Molokai, Wailau Valley-Waiehu, June 1910, *Forbes 528.Mo* (holotype: BISH!; isotype: GH!,K!,P!,W!).

Lysimachia munroi St. John, Phytologia 64(1):47, 1987.-TYPE: Molokai, edge of Waihanau Valley, 770 m, 23 Jun 1927, Munro 127 (holotype: BISH!).

Lysimachia rockii St. John, Phytologia 64(1):48, 1987.-TYPE: Molokai, Mapulehu, ridge to Kamakou 770 m, 22 March 1910, Rock 6146 (holotype: BISH!; isotype: BISH!, P!, US!, W!).

Lysimachia rufa St. John, Phytologia 64(1):49, 1987.-TYPE: Molokai, Puu Kaeo, Waikolu, 28 April 1928, Degener 17679 (holotype: A!; isotype: BISH!, NY!).

Lysimachia waikoluensis St. John, Phytologia 64(1):50, 1987.-TYPE: Molokai, Waikolu, Hanailoilo, 1110 m, 23 Dec 1932, St. John 12348 (holotype: BISH; isotype: BISH!).

Nomenclatural note. Four specimens, two at K and two at P are labelled "Moai; voyage de M.J. Remy, 1851-1855 no 458". This is the same collection number as the type specimen of *L. remyi* from Maui. One specimen from each of these herbaria clearly belong to *L. r.* subsp. *subherbacea* and the letter "b" has been added after 458, perhaps by St. John, who annotated the specimen in 1976 as *L. occidentalis* St. John. The other specimen from K is labelled 458a and the second one from P remains 458. Both of these clearly came from West Maui.

Branching shrubs, acquiring an almost vine like habit; stems brown to red, up to 5 m long, young stems rusty tomentose, older glabrate. Leaves alternate, sometimes almost ternate, (0.1-)1-22(-55) mm apart, petioles (1-)2-3(-7) mm long; blades linear, lanceolate, oblanceolate, or obovate, coriaceous, sometimes slightly undulate, (20-)45-60(-95) mm long, (3-)8-11(-20) mm wide, base acute to attenuate, apex acute to attenuate, upper surface olive green, glabrous above, the veins slightly rugose, lower surface lighter, initially pilose, becoming glabrous, with scattered streaks of red; secondary veins prominent, tertiary veins obscure. Flowers solitary in leaf axils, (4-)5-7-merous, campanulate; pedicels (11-)20-30(-70) mm long, glabrous to densely tomentose, often red toward calyx, pendulous; calyx lobes green, often red at base, lanceolate to narrowly ovate, (3-)5-8(-10) mm long, (2-)3-4 mm wide; corolla lobes obovate, rose pink to dark red at base, lighter toward the tips (6-)11.5-14(-17) mm long, (5-)7.5-9.5(-15) mm wide; filaments red, (4.5-)6-7.5(-11) mm long, anthers 2-3.5 mm long; style red, (5-)6.5-8(-11) mm long. Capsules (5-)6-8(-9) mm long. Seeds dark brown, irregularly shaped, 1-2.1 mm long.

Phenology. flowering March-Nov.

Distribution and Habitat. Molokai. Eastern side of the island at higher elevations, on both leeward and windward sides, 490-1130 m. Found in lowland mesic shrublands and Lowland Mesic Forest dominated by Metrosideros, Styphelia,

Dodonaea, Vaccinium, Wikstroemia, Alyxia, Elaphoglossum,
Dianella, Psychotria, Selaginella, Peperomia, Nestigis,
Chamaesyce, Pipturus, Dubautia, Diospyros, Viola and Coprosma.
 Oahu. Collected in 1993 for the first time from the Waianae
 Mtns., 785-835 m. Growing on cliffs with Dubautia, Bidens,
Lipochaeta, Tetramolopium, Schiedea, Carex, Viola, Panicum,
Hedyotis, Lepidium, Lobelia and Pleomele.

Comments. Leaf and calyx size and shape are somewhat variable
 both within as well as among populations of this species.
 Plants in gulches generally have broader leaves and longer
 stems than those on drier hillsides. A few collections from
 the ridges and gulches of the south side of Molokai resemble
L. maxima in having nearly ternate leaves, but differ in that
 the leaves are not as broad, nor are the calyx and corolla as
 long as that of L. maxima.

Representative Specimens examined. MOLOKAI: Puu Kolekole,
 Gustafson 3002 (RSA), Forbes 166.Mo (BISH), Davis 772, 806
 (BISH), Anderson 514 (BISH), Marr 371-Marr 377, Marr 1138-Marr
 1169; Kamalo, Rock 7024 (BISH); Makakupua, Degener 33508,
 Marr 378-Marr 382, Marr 1194-Marr 1207 (UBC); Wailau Valley
 Faurie 706 (GH,P); Kaulahuki, Evans s.n. (BISH), Degener 17677
 (GH), Degener 33497 (NY); edge of Waihanau Valley, Degener
 33500 (NY), Munro s.n. (BISH); Waikolu, Marr 391-Marr 404,
 Marr 1103-Marr 1122 (UBC), Carlquist 2216 (BISH,RSA), St. John
 23437 (BISH), Degener 23733 (NY), Degener 23734 (K), Gillett
 1948 (US), St. John 12348 (NY); near Laianui, Degener 17678
 (BISH,GH,NY); Hanalilolilo, Lorence 6324 (PTBG); Onini gulch,

Mill 502 (BISH), Davis 846 (BISH), Marr 1208-Marr 1229 (UBC);
 Kalaupapa Rim, Harrison s.n. (BISH), Southworth s.n. (BISH),
 Forbes 24.Mo, (BISH,MO,US), Rock 14030, Krajina 620612006
 (UBC); Kawela, Perlman 6602 (PTBG,UBC), Lorence 5630
 (BISH,MO,PTBG), St. John 19880 (PTBG), Warshauer 2317 (BISH),
 Lorence 5615 (PTBG), Marr 367-Marr 370, Marr 1171-Marr 1193
 (UBC); Ravine N of Puu Makaliilii, Degener 34499 (BISH,NY),
 Degener 17674 (GH,NY); ridge between Kaunakakai and Kupaia
 gulches, Davis 880 (BISH), Cuddihy 1244 (BISH), Marr 1066-Marr
 1102 (UBC); Kahuaawi gulch, Degener 33498 (GH,NY), Degener
 17676 (NY); Halawa Gulch, Hillebrand s.n. (BISH), Rock 6146
 (PTBG); Wailau Valley, ridge to Olokui along seacliff, Wood
 1258 (MO,PTBG); no location given, Remy 457 (P), Remy 458b,
 (K,P), Hillebrand s.n. (US 809323). OAHU: Waianae Mtns.,
 Makua Keaau Forest Reserve, Ohikilolo area, Wood
 2496,2490,2633,2634 (PTBG), Wood 2633,2490 (UBC,PTBG).

14. Lysimachia scopulensis Marr sp. nov. -TYPE: Steep cliffs of Kalalau Valley rim, north of Kahuamaa Flat, 3 Mar. 1991, Wood 634 (PTBG!). Figure 2.13. (NOTE: This is not intended to effect the valid publication of a nomenclatural entity.)

Lysimachiae waianaeensi similis magnitudine et forma calycis, sed foliis paene sessilibus, areolis et venis secundariis obscuris. Folia atro-viridia et saepe pulverulenta, ad apicem deorsum curvata, caulibus semper pulverulentis.

Branching shrubs up to 75 cm tall; stems red or green, pulverulent when young. Leaves alternate, coriaceous, almost succulent, glandular, (0.5-)2-7(-8) mm apart, petioles (2-)3-4.5(-5) mm long; blades linear to narrowly oblanceolate, sometimes narrowly obovate, tips slightly to extremely recurved, (33-)55-65(-86) mm long, (5-)8-11(-23) mm wide base acute, apex acute, upper surface glabrous, dark green, drying olive green, pulverulent when young, lower surface glabrous, dark green, drying lighter than above; primary vein and petiole sometimes dark red above and below, secondary veins prominent, tertiary veins obscure. Flowers solitary in leaf axils, (5-)6(-7)-merous, campanulate; pedicels 20-45 mm long, green, erect; calyx lobes green or red at the base, the tip acute, margins scarious, widely ovate, pulverulent (2.5-)4-5 mm long, (2-)3-4(-4.5) mm wide; corolla lobes obovate, burgundy, 10-11 mm long, 6-7 mm wide; filaments 5 mm long,

anthers 2 mm long; style 5 mm long. Capsules 6.5-7.5(-9) mm long. Seeds dark brown, irregularly shaped, 1.5-2.5 mm long.

Phenology. Flowering March.

Distribution. Kauai. Growing on steep cliffs of Diverse Lowland Mesic Forest in the upper part of Kalalau Valley, 780-880 m. Associated with Hedyotis, Chamaesyce, Hibiscadelphus, Nototrichium, Stenogyne, Poa, Melicope, Lobelia, Myrsine, Remya, Wilkesia, Dubautia, Lepidium, Lipochaeta, Metrosideros, Coprosma, Vaccinium, Rumex, and Exocarpus.

Comments. The most distinguishing characteristics of this species are the recurved leaf tips, the dark green and shiny upper leaf surfaces, and the pulverulent young leaves and stems.

Representative Specimens examined. KAUAI: Kalalau rim, below Puuokila, Wood 1008 (UBC,PTBG), Wood 798, 1233 (PTBG), Wood 2036 (MO,PTBG).

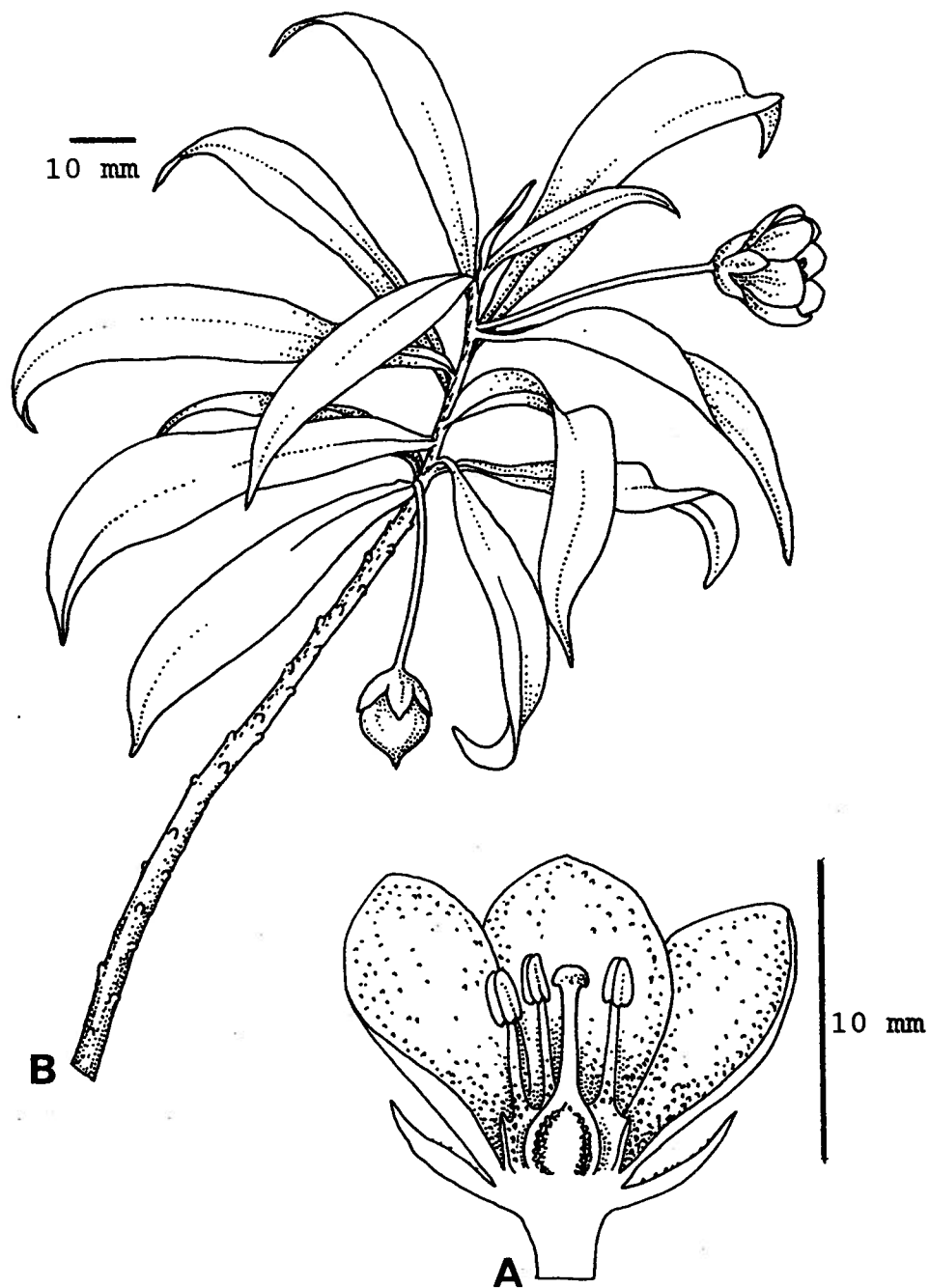


Figure 2.13. Lysimachia scopulensis. A. flower; B. flowering branch.

15. Lysimachia venosa (Wawra) St. John, Phytologia 64:50, 1987. *Lysimachia hillebrandii* Hook. f. ex A. Gray var. *venosa* Wawra, Flora 5:523, 1874. *Lysimachiopsis venosa* (Wawra) O. Deg. & I. Deg., Plants Hawaii Nat. Parks, third edition, p. 393, 1983.-TYPE: KAUAI, summit of Mt. Waialeale, 1600 m, Wawra 2165 (holotype: W!; isotype: W!, fragment at BISH!).

Shrubs 0.5-1 m tall; stems brown, pilose when young, becoming glabrous. Leaves alternate, (3-)10-15(-25) mm apart, petioles 1-6 mm long; blades obovate, coriaceous (50-)75-80(-100) mm long, (12-)20-35(-48) mm wide, base attenuate, apex acuminate, upper surface dark green, glabrous, lower surface lighter than above, glabrous; secondary veins prominent, tertiary veins obscure. Flowers solitary in leaf axils, 6-7-merous; pedicels 15-27 mm long, green, erect; calyx lobes green with prominent dark veins, narrowly lanceolate, 13-16 mm long, 4-6 mm wide; corolla lobes obovate, dark red, 15-19 mm long, 10-11 mm wide; filaments 7 mm long, anthers 3 mm long; style 9-10 mm long. Capsules not seen.

Phenology. Unknown.

Distribution. Kauai. Known from two collections from the type location, where it was last collected in 1911. No recent collections from there despite several visits by botanists in recent years. A 1991 collection (Wood 784) from the headwaters of the North fork of the Wailua River (NE corner of amphitheater) was of a broken branch that had been dislodged

from the cliffs above, possibly from the summit area of Mt. Waialeale.

Comments. The size and shape of the leaves of this species closely resemble L. glutinosa. L. venosa differs in having a dark red corolla, is not viscid, and has longer calyx lobes versus those of L. glutinosa which are ovate.

Representative Specimens examined. KAUAI: Summit of Mt. Waialeale, Rock 8881 (BISH,GH); Wood 784 (PTBG).

16. Lysimachia waianaeensis St. John. Phytologia 64(1):50, 1987.-TYPE: Oahu, Puu Kanehoa, Waianae Mtns. 830 m, 7 Jan. 1934, St. John 14012 (holotype: BISH; isotype: BISH!).

Sprawling shrubs with leader shoots up to 4 m long and much shorter secondary shoots; stems green to light brown, with distinct linear lenticels, usually pilose at tips, glabrate. Leaves alternate, (.5-)2-18(-26) mm apart, petioles (2-)5-8 (-15) mm long, often red; blades elliptic to slightly ovate or obovate, coriaceous, (30-)50-65(-100) mm long, (8-)16-24(-39) mm wide, base rounded, apex abruptly acuminate; areoles prominent, i.e. secondary, and all higher order veins distinct. Flowers solitary in leaf axils, 5-6(-7)-merous, campanulate; pedicels (10-)15-25(-46) mm long, green, glabrous, erect; calyx lobes (3-)4-5(-8) mm long, (1-)2.5-3.5(-5) mm wide, green, usually widely ovate, rarely lanceolate; corolla lobes red, obovate, (9-)11-13(-14) mm long, (6-)7-9(-10) mm wide; filaments (5-)6-7.5(-8) mm long, anthers 1.5-2 mm; style (5-)6-7(-8) mm long. Capsules subglobose, (5.5-)7-9(-10) mm long. Seeds dark brown, irregularly shaped, 1.6-2.3 mm long.

Phenology. Flowering Nov.-May.

Distribution and habitat. Oahu. Lowland mesic forest only in the Waianae Mtns, 540-1140 m, growing with Metrosideros, Acacia, Charpentiera, Cyanea, Selaginella, Bidens, Hedyotis, Alyxia, Pleomele, Wikstroemia, Viola, Sida, Claoxylon,

Chamaesyce, Diospyros, Psychotria, Blechnum, Canthium,
Eragrostis, Hibiscus, Elaphoglossum, Melicope, Dodonaea,
Antidesma, Santalum, Schiedea and Dubautia.

Comment. The most distinct characteristic of this species is the prominent areoles in the leaves. In the southern Waianae Mtns. this species grows sympatrically with L. hillebrandii in gulches NE of Palikea (800-870 m) and the ridge between Nanakuli and Lualualei (720-820 m). The evident lack of hybrids between L. hillebrandii and L. waianaeensis in this area suggests that the two may be reproductively isolated from each other. This species typically lacks pigment on the calyx and pedicel and the calyx is ovate. However, the calyx of an exceptional plant grown in the greenhouse from seed collected in Makaha Valley, on Kamaileunu Ridge (Waianae Kai), was entirely red and lanceolate.

Representative Specimens examined. OAHU: Waianae Mtns., Puu Hapapa, *Krajina 620401115* (BISH), *Degener 17672* (BISH,GH,MO,NY,US), *Degener 12368* (NY), *Degener 21004* (BISH); Makaha Valley, Kamaileunu Ridge, N. of Puu Kawiwi, *Perlman 5053* (PTBG,BISH), *Perlman 6818* (PTBG), *Forbes s.n.* (K,MO,US), *Degener 4138* (BISH,GH,MO,NY,US), *Nagata 1132* (BISH), *Degener 33505* (P); Makaleha Valley, central ridge *Degener 21278* (GH,MO,NY,US), *Obata 279* (BISH); Puu Kaua, *Marr 231* (PTBG), *Marr 233,234*, *Marr 239-Marr 245*, *Marr 778-Marr 788* (UBC), *Perlman 5103* (MO,PTBG), *Perlman 5110* (BISH), *Degener 33507* (NY,W), *Obata s.n.* (BISH), *Takeuchi 2059,3719* (BISH), *Nagata 1155* (BISH), *Montgomery s.n.* (BISH); Makua Valley, *Degener*

17684 (GH,NY,US), *Degener* 17682 (NY); Puu Kanehoa, *Degener*
 17680 (GH); N slope Mt. Kaala, *Degener* 19439 (GH,NY),
Carlquist 2354 (RSA); E ridge Puu Kalena *Hartung* s.n. (BISH),
Fosberg 13011 (BISH,GH), *Kerr* s.n. (BISH); Makua Forest
 Reserve, Ohikilolo, *Wood* 2635 (PTBG), Koiahi, *Wood* 2494
 (PTBG); Pahole NAR, Kukuiula Gulch, *Fosberg* 13072 (BISH),
 Kapuna Gulch, *Marr* 448,449,450 (UBC), Pahole gulch, *Marr* 769-
Marr 777 (UBC); Mokuleia, *Forbes* 1775.0 (BISH); Napepeiau
 Olelo Gulch, *Marr* 443-Marr 447 (UBC); ridge between Nanakuli
 and Lualualei, *Marr* 1295,1296 (UBC); Location unclear: Mtns.,
 Oahu, U.S. Exploring Expedition 1838-1842 (US); Kaala Wawra
 2211 (W).

Chapter 3

Allozyme diversity in endemic Hawaiian *Lysimachia*

3.1 Introduction

The extreme geographical isolation of the Hawaiian Islands makes dispersal of propagules to the islands a rare event. Many plants have evolved in isolation from their closest relatives, resulting in a flora that is 89% endemic at the species level (Wagner et al., 1990). Although the Hawaiian flora and fauna are frequently cited as dramatic examples of adaptive radiation, it is often overlooked that 68% of the 216 native (32 endemic) plant genera have not undergone adaptive radiation or are represented by only two species (Lammers, 1990). Nevertheless, many genera are quite species-rich. For example, the 52 species of the endemic genus *Cyanea* have presumably evolved from a single ancestor (Wagner et al., 1990). For genera in which a monophyletic origin can be demonstrated, the Hawaiian Islands are an ideal location to investigate the initial stages of speciation. In contrast to studies of continental taxa, however, few biosystematic studies of island genera have utilized allozyme or DNA analysis of species to elucidate phylogenetic relationships and genetic divergence associated with primary speciation following long-distance dispersal (Crawford et al., 1987b). Only five genera of Hawaiian angiosperms have been analyzed using allozyme variation to estimate the degree of genetic divergence that is associated with adaptive radiation:

Tetramolopium (Lowrey and Crawford, 1985), Bidens (Helenurm and Ganders, 1985), Dubautia (both n=13 taxa and n=14 taxa) and Wilkesia (Witter and Carr, 1988), and Metrosideros (Aradhya et al., 1991). Species in each of these genera are morphologically and ecologically diverse, yet with the exception of the n=14 species of Dubautia, genetic identities are high. This contrasts with continental taxa where species pairs that have high genetic identities are usually very similar morphologically (Crawford et al., 1987b).

Outside of Hawaii, allozyme variation within and among populations of insular taxa has been studied in genera from the Juan Fernandez Islands (Crawford et al., 1987a, 1990a, 1992, 1993), the Galapagos Islands (Wendel and Percy, 1990; Wendel and Percival, 1990) and the Bonin Islands (Ito and Ono, 1990). Most of these studies addressed questions regarding (continental) ancestor-(insular) derivative relationships of genera possessing a single insular species. While these have been useful in estimating genetic variation within populations, except for Ito and Ono (1990) and Crawford et al. (1992, 1990a), few studies have estimated the level of genetic divergence among insular species of larger (i.e. more speciose) monophyletic lineages.

The endemic Hawaiian species of Lysimachia were chosen for evaluation of genetic variation within and among insular congeneric species for two reasons. First, despite gross morphological differences among the species, several morphological characters suggest that the group is

monophyletic. Secondly, the availability of morphometric data, used to produce a taxonomic revision of the Hawaiian species, permits a comparison of morphological divergence with genetic divergence, as estimated from allozyme variation. Few studies have made such a direct comparison (Hamrick, 1989).

Extensive ecological and morphological divergence have accompanied adaptive radiation of Lysimachia in the Hawaiian Islands. Species grow in a diversity of habitats including montane bogs, waterfall spray zones, subalpine mesic shrublands, montane dry and wet forests and lowland mesic shrublands from 250-2300 m elevation. All are woody shrubs, some with stems up to two cm in diameter. This contrasts with their herbaceous continental relatives, a change of habit that is common in insular evolution (Carlquist, 1974). Growth habit varies from upright to scandent, or sometimes vinelike, with stems up to 5 m long, supported by the surrounding vegetation. The fruit is a dehiscent capsule, thus seeds are dispersed locally by gravity. Founding distant new populations is more problematical. A reasonable vector of dispersal is the strong winds that accompany hurricanes. Seeds stuck in the mud on the feet of birds is also a possible mode of dispersal.

Of the 16 species and 4 subspecies, 3 (L. forbesii Rock, L. haupuensis St. John, and L. kahiliensis St. John) have not been collected for 60 years or more and are probably extinct. The characters that are most useful to distinguish among species include leaf size and shape (Figure 3.1), as well as

calyx and corolla size, shape and pigmentation. Most species have a reddish corolla, however that of L. glutinosa Rock is white and L. kalalauensis Skottsb. is green. Observations of plants in the greenhouse indicate that the species are protogynous. Self-pollinations within flowers fail to set fruit, however plants are self-compatible and geitonogamous pollination is possible.

The extant high islands are the youngest of a 6,000 mile long chain of islands, the Emperor and Hawaiian Chain. These began forming over a hot spot in the Pacific plate at least 70 million years ago (Walker, 1990). The older islands, now converted mostly to coral atolls, were once high islands, undoubtedly with much greater habitat diversity than they presently contain, and could have been the source of propagules for dispersal to the extant high islands. For this reason, it cannot be assumed that Kauai was the site of the original founding event or that species in any extant lineage have been evolving for less than 5.7 million years. In another monophyletic group, the drosophiloid lineage of the Hawaiian Drosophila, it appears that the ancestor of the Hawaiian species arrived in Hawaii 10 million years ago, using sequence data from the alcohol dehydrogenase locus (Thomas and Hunt, 1991).

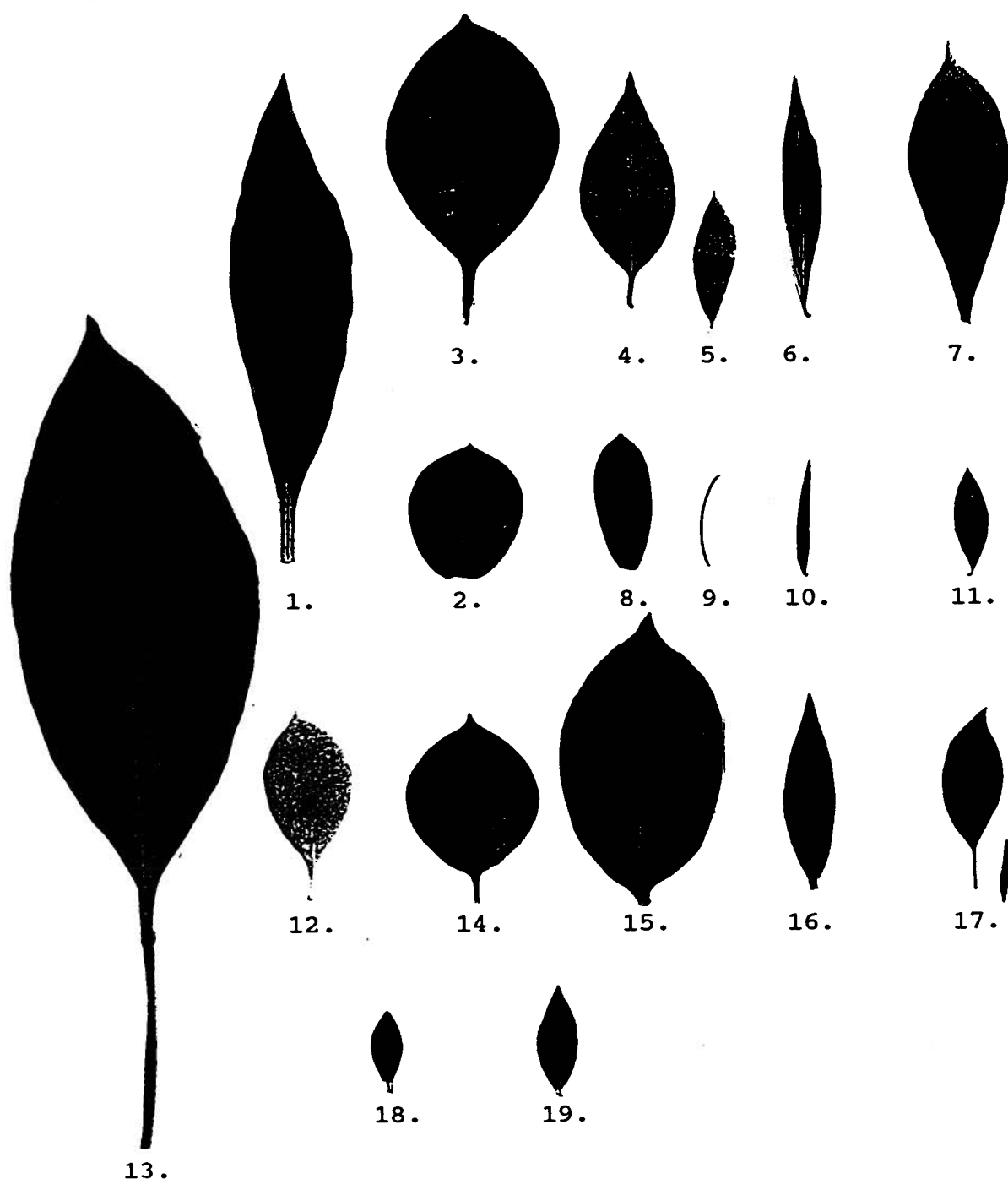


Figure 3.1. Representative leaves of endemic Hawaiian *Lysimachia* by island: Kauai (1-11), Oahu (12-14), Molokai (15-16), Maui (17-19). 1. *L. glutinosa*, 2. *L. iniki*, 3. *L. kalalauensis*, 4. *L. ovoidea*, 5. *L. kahiliensis*, 6. *L. scopulensis*, 7. *L. venosa*, 8. *L. daphnoides*, 9. *L. filifolia*, 10. *L. pendens*, 11. *L. haupuensis*, 12. *L. hillebrandii*, 13. *L. forbesii*, 14. *L. waianaeensis*, 15. *L. maxima*, 16. *L. remyi* subsp. *subherbacea*, 17. *L. remyi* subsp. *remyi*, 18. *L. remyi* subsp. *kipahuluensis*, 19. *L. remyi* subsp. *caliginis*.

Species of Lysimachia are distributed on all of the major islands with the exception of Hawaii, Kahoolawe and Niihau. The number of species per island correlates roughly with the age of the island (dates from MacDonald et al., 1990). Twelve species grow on Kauai, the oldest extant high island (4.5-5.7 million years), four species and one subspecies grow on Oahu (2.1-3.6 million years), one species and one subspecies on Molokai (1.5-1.8 million years), and three subspecies on West Maui (1.3 million years), and one species and two subspecies on East Maui (0.7-0.8 million years).

Fifteen taxa, 11 species and 4 subspecies, were included in the present study, these are: L. daphnoides (A. Gray) Hillebr., L. filifolia C.N. Forbes and Lydgate, L. glutinosa Rock, L. hillebrandii Hook F. ex A. Gray, L. kalalauensis Skottsb., L. maxima (R. Knuth) St. John, L. ovoidea St. John, L. pendens Marr, L. remyi subsp. caliginis (St. John) Marr, L. remyi subsp. kipahuluensis (St. John) Marr, L. remyi subsp. remyi, L. remyi subsp. subherbacea (Hillebr.) Marr, L. scopulensis Marr, and L. waianaeensis St. John. Lysimachia venosa (Wawra) St. John and L. iniki Marr, could not be included because their populations are too inaccessible. The taxa that were sampled represent most of the morphological variation of the genus with the exception of collections from Oahu. Regrettably, two species from the Koolau Mtns., Oahu, may be extinct: L. forbesii (last collected in 1934), and L. hillebrandii (last collected from the Koolau Mtns. in 1980). One population of L. hillebrandii was sampled, but this came

from the Waianae Mtns. The dimensions of the leaves, calyx, corolla, and pollen of L. forbesii were the largest of any species of Lysimachia in the world.

Most taxa are endemic to a single island and are often restricted to a single ecological zone. Several are known from a single location. Populations are small and scattered, typically consisting of from 10-100 individuals. Distributions are typically allopatric, with rare exceptions. One location of sympatry is on the steep slopes and cliffs of upper Kalalau Valley on Kauai. Here the ranges of distribution of L. glutinosa, L. kalalauensis and L. scopulensis overlap somewhat. Hybrids between L. glutinosa and L. kalalauensis, and between L. glutinosa and L. scopulensis have been found in this area. Elsewhere populations of L. glutinosa and L. kalalauensis are quite isolated from each other. In the southern Waianae Mtns. on Oahu, plants of L. hillebrandii grow sympatrically with L. waianaeensis in some populations. Further field observations are needed to determine whether these two species hybridize. In Haleakala crater on East Maui, individuals in two populations display the full range of morphological intermediacy between L. remyi subsp. caliginis and L. remyi subsp. kipahuluensis, and are interpreted as being hybrid swarms. Although the ranges of these two subspecies apparently do not overlap at the present time, they may well have in the past. The structure of the vegetation in this

area was severely damaged by decades of damage from feral goats and pigs.

An important question concerning studies of differences among species in general and insular adaptive radiation in particular is whether or not gross morphological differences are the result of changes at fewer loci than would be expected given the degree of morphological divergence evident (Helenurm and Ganders, 1985; Crawford et al., 1987b). Gottlieb (1984) summarized literature on the genetic basis of morphological differences among species, and found that characters of structure, shape, orientation and presence versus absence were typically controlled by one or two genes. Characters of dimension, weight and number, were usually controlled by numerous genes. Many of the characters that are often used to distinguish one species from another fall within the first category. The most direct means of assessing genetic control of differences among species is, of course, progeny analysis of F_1 and F_2 hybrids as well as backcrosses. Island taxa are ideal for these types of studies because internal reproductive barriers are often lacking. An alternative approach is to use allozyme analysis as an indirect means of measuring genetic variation within and among populations or species by comparing the number and frequency of alleles that code for several different proteins.

3.1.1 Objectives of allozyme analysis

The objectives of this study were to: 1) estimate the degree of genetic variation within and among species using allozymes; 2) to compare these results with published results from other insular taxa, as well as with continental taxa; 3) to compare morphological divergence to allozyme divergence among a group of congeneric species that have undergone adaptive radiation; and 4) to evaluate the taxonomic revision proposed in Chapter 1.

3.2 Materials and Methods

3.2.1 Sample collection.

Young leaves were collected in the field from 1028 individuals in 48 populations (Table 3.1). Plants were sampled randomly and whenever possible at least 30 plants per population were sampled. Vouchers of populations collected have been deposited at UBC. Leaves were refrigerated until they could be sent via express mail to UBC and stored at -80°C until immediately prior to enzyme extraction. Even after one year, enzyme activity of samples stored in this manner was comparable to that of fresh leaves.

Table 3.1 Collection localities for populations of endemic Hawaiian Lysimachia sampled for allozyme analysis.

Species	Pop. Code	Island	Altitude (meters)	Locality
<u>L. glutinosa</u>	GKALR	Kauai	1260	Kokee; Road between Kalalau and Puu O Kila Lookouts.
<u>L. glutinosa</u>	GKALL	Kauai	1230	Kokee; Below Kalalau Lookout.
<u>L. glutinosa</u>	GHONO	Kauai	1230	Kokee; Honopu trail.
<u>L. kalalauensis</u>	KHONO	Kauai	1110	Kokee; Edge of Honopu Valley.
<u>L. kalalauensis</u>	KAAPU	Kauai	1140	Kokee; Awaawapuhi trail.
<u>L. kalalauensis</u>	KMAKA	Kauai	1015	Kokee; North of Makaha Ridge road.
<u>L. kalalauensis</u>	KWALA	Kauai	1110	Kokee; Waialae Ridge.
<u>L. ovoidea</u>	OLIMA	Kauai	650	On ridge SW of Limahuli Falls.
<u>L. ovoidea</u>	OWAIN	Kauai	615	Wainiha Pali; SW of Kulana'ililia.
<u>L. scopulensis</u>	NSPTR	Kauai	800	Kokee; north-south ridge below and E of Kalalau Lookout.
<u>L. daphnoides</u>	DSECO	Kauai	1230	Kokee; Alakai Swamp trail.
<u>L. daphnoides</u>	DBIGB	Kauai	1230	Kokee; Alakai Swamp, SE of trail.
<u>L. pendens</u>	FKONE	Kauai	615	Head of N fork of Wailua River, south wall.
<u>L. pendens</u>	FKTHR	Kauai	615	Head of N fork of Wailua River, west wall.

Table 3.1 continued on next page.

Table 3.1 continued. Collection localities for populations of endemic Hawaiian Lysimachia sampled for allozyme analysis.

Species	Pop. Code	Island	Altitude (meters)	Locality
<u>L. pendens</u>	FKFIV	Kauai	615	Head of N fork of Wailua River, east wall.
<u>L. filifolia</u>	FOTWO	Oahu	250	Koolau Mtns.; Waiahole Ditch Trail.
<u>L. filifolia</u>	FOTHR	Oahu	250	Koolau Mtns.; Waiahole Ditch Trail.
<u>L. waianaeensis</u>	HPUKS	Oahu	710	Waianae Mtns.; ridge SE of Puu Kaua.
<u>L. waianaeensis</u>	HPUKE	Oahu	800	Waianae Mtns.; ridge E of Puu Kaua.
<u>L. waianaeensis</u>	HWAIK	Oahu	800	Waianae Mtns.; Kamaileunu Ridge in Makaha Valley; N of small peak N of Puu Kawiwi.
<u>L. hillebrandii</u>	HLUAF	Oahu	830	Waianae Mtns.; ridge between Lualualei and Nanakuli Valleys.
<u>L. waianeensis</u>	HLUAS	Oahu	830	Waianae Mtns.; ridge between Lualualei and Nanakuli Valleys.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	WMBLG	W. Maui	340	Iao Valley; Black Gorge.
<u>L. remyi</u> subsp. <u>remyi</u>	WMHEL	W. Maui	1230	Helu, E of summit.
<u>L. remyi</u> subsp. <u>remyi</u>	WMMN2	W. Maui	1050	Hanaula.
<u>L. remyi</u> subsp. <u>remyi</u>	WMMN3	W. Maui	1075	Hanaula.
<u>L. remyi</u> subsp. <u>remyi</u>	WMMPP	W. Maui	860	Manawainui Plant Preserve.

Table 3.1 continued on next page.

Table 3.1 continued. Collection localities for populations of endemic Hawaiian Lysimachia sampled for allozyme analysis.

Species	Pop. Code	Island	Altitude (meters)	Locality
<u>L. remyi</u> subsp. <u>remyi</u>	WMLIL	W. Maui	1130	Lihau, ridge west of summit.
<u>L. remyi</u> subsp. <u>remyi</u>	WMLIS	W. Maui	1260	Lihau summit.
<u>L. remyi</u> subsp. <u>remyi</u>	WMHPL	W. Maui	985	Halepohaku, ridge SW of summit.
<u>L. remyi</u> subsp. <u>remyi</u>	WMHPS	W. Maui	1050	Halepohaku summit.
<u>L. remyi</u> subsp. <u>remyi</u>	WMHPY	W. Maui	1000	Halepohaku, ridge SW of summit.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMPAL	E. Maui	2000	Ridge SE of Paliku Cabin.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMHPA	E. Maui	2090	Ridge SE of Paliku Cabin.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMKUI	E. Maui	2215	Ridge N of Kuiki.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMKUK	E. Maui	2280	Kuiki, rim of Kipahulu Valley.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMLWA	E. Maui	2155	Above Lake Waianapanapa.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMKAW	E. Maui	1785	West Kaupo Gap, below spring.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMKPR	E. Maui	2300	Kalapawili Ridge; rim of Kipahulu Valley.

Table 3.1 continued on next page.

Table 3.1 continued. Collection localities for populations of endemic Hawaiian Lysimachia sampled for allozyme analysis.

Species	Pop. Code	Island	Altitude (meters)	Locality
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X	EMKAE	E. Maui	1845	Kaupo Gap, below Paliku Cabin.
<u>L. remyi</u> subsp. <u>caliginis</u>	EMWAI	E. Maui	2030	Koolau Gap, Waikau Cabin site.
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X				
<u>L. remyi</u> subsp. <u>caliginis</u>	EMKOT	E. Maui	1970	Koolau Gap, treeline.
<u>L. remyi</u> subsp. <u>caliginis</u>	EMKOB	E. Maui	2920	Koolau Gap, below treeline.
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	EMKIP	E. Maui	1000	Kipahulu Valley, Palikea stream.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MKAWE	Molokai	920	West fork Kawela Gulch.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MKOLE	Molokai	1040	W of Puu Kolekole, S of road.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MMAKA	Molokai	970	Makakupaia; ridge S of Onini gulch.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MONIN	Molokai	850	Makakupaia; bottom of Onini Gulch.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MWAIK	Molokai	1050	Waikolu; SE of Puu Kaeo.
<u>L. remyi</u> subsp. <u>subherbacea</u>	MKAUN	Molokai	920	Small ridge between Kauanakakai and Kupaia Gulches.
<u>L. maxima</u>	MMAXI	Molokai	950	Pelekunu; N of Ohialele.

Wherever possible, at least two populations of each taxon were sampled. In some cases the limited number of populations sampled is a reflection of their limited distribution.

Lysimachia maxima is known from a single population and L. ovoidea is known from only two populations. Lysimachia filifolia is known from one locality on Oahu and one on Kauai. The two populations from Oahu that were sampled are in adjacent small gulches separated by 30-40 meters. Likewise, L. pendens is known only from a large amphitheater approximately 200 meters across. Populations were sampled from different walls of this amphitheater. Lysimachia remyi subsp. kipahuluensis was collected from both East Maui and West Maui. Collections from East Maui are referred to as L. remyi subsp. kipahuluensis (EM) and collections from West Maui as L. remyi subsp. kipahuluensis (WM). Populations of this subspecies from East Maui were analyzed separately from those from West Maui. Two populations, EMKAE and EMWAI, hybrid swarms between L. remyi subsp. caliginis and L. remyi subsp. kipahuluensis, were excluded from calculations of genetic variation at the island level, however they were included in all other analyses.

3.2.2 Electrophoretic procedures

Leaf tissue was ground in the 0.1 M Tris-HCl, pH 7.5 extraction buffer of Soltis et al. (1983). Extracts were absorbed onto Whatman 3MM chromatography paper wicks. Samples were electrophoresed on 12.5% starch gels using three buffer

systems from Soltis et al. (1983). System 2 was used to resolve isocitrate dehydrogenase (IDH); system 8 to resolve phosphoglucoisomerase (PGI), triosephosphate isomerase (TPI), malic enzyme (ME), leucine amino peptidase (LAP); and system 9 to resolve alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), shikimate dehydrogenase (SKD), menadione reductase (MNR), 6-phosphogluconic dehydrogenase (6-PGD), phosphoglucomutase (PGM) and diaphorase (DIA). The pH of each gel buffer was changed from those of Soltis et al. (1983) as follows: system 2, pH=8.6; system 8, pH=8.1; system 9, pH=6.7.

Gels were electrophoresced in a refrigerated cabinet. System 2 was run for 4 hr at a constant voltage of 150V, System 8 was run for 10 hr at 110V, System 9 was run for 6 hr at 190V. Following electrophoresis, enzymes were visualized according to the protocols of Soltis et al. (1983), with the exception of DIA and MNR which were visualized using protocols of Wendel and Weeden (1989). TPI and DIA were stained using agarose overlays. The most anodal isozyme was designated as "1", likewise, the most anodal allozyme was designated "a".

3.2.3 Data Analysis

Results were analyzed in such a way as to compare the distribution of allozyme variation at several levels: 1) within populations; 2) among populations of the same species; 3) among species; 4) within islands; 5) among islands.

3.2.3.1. Analysis of allozyme diversity

Four measures of genetic variation within populations were calculated: the number of alleles per locus (A), the percent of loci polymorphic (P) (a locus was regarded as polymorphic if there were two or more alleles, each with a frequency of at least 0.01), the mean number of alleles per polymorphic locus (A_p), and the expected heterozygosity (H) (according to Nei, 1987, P. 177, equation 8.1). Measurements of H are calculated from:

$$H = 1 - \sum_{i=1}^m x_i^2$$

where m is the number of alleles, and x_i is the population frequency of the i th allele. Very rare alleles have little effect on the value of H. Maximum diversity occurs when all alleles have equal frequencies. Some hypothetical examples illustrate the effect of the number and frequency of alleles on the value of H for the simplest case where only one locus is analyzed. If only two alleles, "a" and "b", are present at equal frequencies of $a=0.5$ and $b=0.5$, then $a^2 + b^2 = 0.25 + 0.25 = 0.5$, and $H = 1 - 0.5 = 0.5$. However, if $a=0.1$ and $b=0.9$, then $a^2 + b^2 = 0.01 + 0.81 = 0.82$, and $H = 1 - 0.82 = 0.18$. Now consider the effect on H as the number of alleles increases. If $a=b=c=0.333$, then $a^2 + b^2 + c^2 = 0.111 + 0.111 + 0.111 = 0.333$, and $H = 1 - 0.333 = 0.667$. If $a=b=c=d=e=0.2$, then $a^2 + b^2 + c^2 + d^2 + e^2 = 0.04 + 0.04 + 0.04 + 0.04 + 0.04 = 0.2$ and $H = 1 - 0.2 = 0.8$.

Gene diversity statistics (unbiased for sample size), H_s , H_t , D_{st} , and G_{st} were calculated to compare the distribution

of variation within and among populations of the same species. The measure of genetic diversity is H_s , (calculated the same as H above). Diversity among populations is D_{st} , and H_t is the total genetic diversity, therefore $H_t = H_s + D_{st}$. Finally, G_{st} , the proportion of the total genetic diversity that is distributed among populations of a species, is calculated as $G_{st} = D_{st} / H_t$. This latter measure is also known as the coefficient of gene differentiation.

Nei's genetic identities (I) (Nei, 1972), were calculated for each pair of populations. This statistic compares the degree to which two populations share the same alleles at the same frequency. When $I=1$, the two populations share all alleles at identical frequencies. When $I=0$, they have no alleles in common.

Gene diversity statistics (Nei and Chesser, 1983) and Nei's genetic identities (I) (Nei, 1972) were calculated using GENESTAT-PC 3.3 (Lewis, 1993). A phenogram based on UPGMA (unweighted pair-group method with arithmetic mean) clustering of Nei's genetic identities was produced using BIOSYS-1 (release 1.7) (Swofford and Selander, 1989).

3.2.3.2. Principal Components Analysis

Principal components analysis (PCA) was used to characterize and ordinate morphological variation and allozyme variation among populations. The measurements used for PCA of allozyme variation were the allele frequencies at each

allozyme locus from each population. For ADH and DIA, only two alleles were detected. To avoid redundancy, the frequencies of the alternative alleles, *Adh-1a* and *Dia-1b* were not included. The eight vegetative and eight reproductive characters that were used in Chapter Two (page 25), were used in the PCA of morphological characters. Population means of the factor scores were used in all subsequent analyses. Pearson product-moment correlation coefficients were calculated to compare the ordination of populations based on allozyme analysis to that based on morphological characters. SYSTAT (Wilkinson, 1990) was used to compute the PCA's and the Pearson correlation coefficients.

3.3. Results

3.3.1. Electrophoretic Patterns

Eleven isozyme loci, coding for the following eight enzymes could be scored in all populations: three for TPI, two for MDH, and one each for SKD, DIA, PGM, ADH, IDH, and PGI. Inconsistent staining or poor resolution of *Mnr-1*, *Pgm-2*, *Pgi-2*, *Mdh-1*, *Mdh-2*, *Mdh-5*, *Lap-1*, *Me*, *6-Pgd-1*, and *6-Pgd-2* precluded their inclusion in the analysis. Interpretation of the genetic basis of the banding patterns was based on the number of isozymes reported for diploid plants (Weeden and Wendel, 1989) and analysis of F_1 hybrids. Nearly all individuals displayed three, five or seven bands at the two most anodal isozymes of MDH, and the cathodal isozyme of TPI. Because these are dimeric enzymes, the phenotype of diploid homozygous plants should be one band; three bands would be expected for a heterozygous plant. A phenotype of five and seven bands requires the presence of three and four alleles respectively, indicating that the gene for that locus is duplicated. Plants were scored in accordance with this explanation.

Allele frequencies for each population are reported in Appendix 1, Table A3.1. All of the 11 loci that could be scored were polymorphic, as were the 10 that could not be consistently scored. All species shared the same highest frequency allele at four loci, *Adh-1*, *Mdh-4*, *Skd-1*, *Dia-1*. Most species shared the same highest frequency allele at the remaining loci as well. However, at a few loci of some

species, especially those on Kauai, there was a different highest frequency allele. The geographical and taxonomic distribution of loci whose highest frequency allele differed from the most common allele (compared to all other species) is presented in Table 3.2.

The following "private alleles", those that were detected in a single species, were not necessarily diagnostic of the species they occur in because they had very low frequencies: *Pgm-1a*, *Pgm-1b* and *Pgi-1d* in *L. daphnoides*, *Idh-1e* and in *L. kalalauensis*, *Mdh-4c* and *Pgm-1f* in *L. ovoidea*, and *Adh-1b* in *L. waianaeensis*. None were present in all individuals of a population or even in all populations of a species.

Some plants, especially *L. glutinosa*, had an additional zone of stain for MDH, *Mdh-5*. Evidently, at this locus there is a null allele in some species because no stain was detected in many plants. In the progeny of some hybrid crosses involving *L. glutinosa* two bands were detected at *Mdh-5*. These were interpreted as the homodimer of the protein contributed by *L. glutinosa* and the heterodimer between the protein of the allele from *L. glutinosa* and that of the null allele contributed by the other species. This locus could not be scored because of inconsistent staining as well as the possibility that more than one null allele exists.

Table 3.2. Geographic and taxonomic distribution of the highest frequency alleles for loci at which the allele with the highest frequency is different from the most common allele (based on comparison to all other species), for that locus, in taxa of Hawaiian Lysimachia.

Allele	Taxon	Island
Pgi-1a	<u>L. glutinosa</u>	Kauai
Idh-1a	<u>L. glutinosa</u>	Kauai
Idh-1d	<u>L. glutinosa</u>	Kauai
Idh-1b	<u>L. ovoidea</u>	Kauai
Pgm-2e	<u>L. ovoidea</u>	Kauai
Tpi-1a	<u>L. scopulensis</u>	Kauai
Idh-1b	<u>L. pendens</u>	Kauai
Pgi-1a	<u>L. daphnoides</u>	Kauai
Tpi-2a	<u>L. waianaeensis</u>	Oahu
Idh-1b	<u>L. waianaeensis</u>	Oahu
Pgm-2e	<u>L. waianaeensis</u>	Oahu
Tpi-3a	<u>L. remyi</u> subsp. <u>subherbacea</u>	Molokai
Idh-1a	<u>L. remyi</u> subsp. <u>subherbacea</u>	Molokai
Idh-1b	<u>L. remyi</u> subsp. <u>subherbacea</u>	Molokai
Mdh-3b	<u>L. remyi</u> subsp. <u>kipahuluensis</u>	Mauui
Mdh-3b	<u>L. remyi</u> subsp. <u>caliginis</u>	Mauui

Malic enzyme displayed two closely spaced zones of stain. This was difficult to interpret because ME is a tetrameric enzyme with one isozyme present in diploid plants (Weeden and Wendel, 1989). The phenotype of homozygous plants should be a single band. Heterozygous individuals should display five bands, the two homotetramers and three heterotetramers. Duplication of the locus for ME should result in a complicated pattern of at least three interlocus heterotetramers in addition to the two homotetramers in individuals that are homozygous for different alleles at the different loci. The phenotype of individuals heterozygous at one or both of the duplicated loci would be even more complicated. The lack of a suitable explanation for the banding pattern, prevented the inclusion of this enzyme in the analysis, however at least two alleles were detected, one apparently unique to L. glutinosa.

In some cases the banding pattern of *Dia-1* on gels of system 8 differed from that seen using system 9 gels, but was the same as that seen for MNR (when stained on system 9). For example, some plants that appeared heterozygous for DIA using system 9, appeared homozygous when stained using system 8. A similar observation has been made in Daucus carota (Westphal and Wricke, 1991). Wendel and Weeden (1989) report that the stains for DIA and MNR are not necessarily specific for one enzyme; i.e. in some species the same enzyme is visualized using the different stains, while in others, the enzyme that is visualized by the stain for DIA differs from the enzyme

visualized by MNR. In order to be consistent, *Dia-1* was always scored using gels from buffer system 9.

3.3.1.1. Allozyme variation within and among populations of species

Estimates of genetic variation within populations are presented in Table 3.3. The mean number of alleles per locus (A), ranged from 1.1 for a population of *L. remyi* subsp. *kipahuluensis* to 2.1 for a population of *L. remyi* subsp. *remyi*. (The low value of 1.0 from a population of five plants of *L. pendens* undoubtedly reflects the small sample size.) The mean number of alleles per polymorphic locus (A_p), ranged from 2.0 for populations of many species, to 3.0 for a population of *L. glutinosa*. The percent of polymorphic loci (P), ranged from 9% for one population each of *L. glutinosa*, *L. remyi* subsp. *kipahuluensis*, and *L. remyi* subsp. *caliginis*, to 72% for one population of *L. waianaensis*. The expected heterozygosity (H), ranged from 0.01 for populations of *L. remyi* subsp. *caliginis* and *L. remyi* subsp. *kipahuluensis*, to 0.24 for one population each of *L. remyi* subsp. *subherbacea* and *L. waianaensis*.

Table 3.3. Genetic variability in 48 populations of endemic Hawaiian Lysimachia. Mean number of individuals scored for each enzyme (N); mean and standard error for number of alleles per locus (A); mean number of alleles per polymorphic locus (Ap); percentage of polymorphic loci (P); mean and standard error for expected heterozygosity (H); number of unique alleles (U).

ISLAND						
Species						
Pop.	N	A±s.d.	Ap	P(%)	H±s.d.	U
KAUAI						
<u>L. daphnoides</u>						
DBIGB	24.6	1.9±.3	2.7	54	0.135±.052	2
DSECO	8.0	1.5±.2	2.3	36	0.116±.062	0
mean	16.3	1.6	2.5	45	0.126	
<u>L. glutinosa</u>						
GKALR	18.8	1.2±.2	3.0	9	0.062±.062	0
GKALL	17.5	1.7±.3	2.7	36	0.118±.059	0
GHONO	28.4	1.6±.3	2.7	36	0.108±.053	0
mean	21.57	1.5	2.8	27	0.096	
<u>L. kalalauensis</u>						
KHONO	25.1	1.6±.2	2.4	45	0.122±.054	0
KAAPU	25.0	1.6±.2	2.2	54	0.140±.059	0
KMAKA	9.3	1.2±.1	2.0	18	0.075±.052	0
KWAIA	22.5	1.5±.2	2.0	54	0.130±.051	2
mean	20.5	1.5	2.1	43	0.117	
<u>L. ovoidea</u>						
OLIMA	8.4	1.5±.2	2.0	45	0.169±.062	0
OWAIN	37.6	1.5±.2	2.0	54	0.149±.058	2
mean	23.0	1.5	2.0	50	0.159	
<u>L. scopulensis</u>						
NSPTR	7.2	1.4±.2	2.0	36	0.135±.058	0
<u>L. pendens</u>						
FKONE	12.2	1.4±.2	2.0	36	0.077±.035	0
FKTHR	6.5	1.2±.1	2.0	18	0.061±.042	0
FKFIV	5.1	1.0±.0	-	0	0.000±.000	0
mean	7.9	1.2	1.3	27	0.046	

Table 3.3. continued on next page.

Table 3.3. continued. Genetic variability in 48 populations of endemic Hawaiian Lysimachia.

ISLAND						
Species						
Pop.	N	A±s.d.	Ap	P(%)	H±s.d.	U

OAHU-WAIAANAE						
<u>L. waianaeensis</u>						
HPUKE	28.1	1.9±.3	2.3	72	0.239±.065	1
HPUKS	8.5	1.5±.2	2.0	45	0.191±.072	0
HWAIK	23.2	1.5±.2	2.0	54	0.173±.062	0
HLUAS	12.5	1.5±.2	2.7	27	0.171±.089	0
mean	18.1	1.6	2.2	50	0.194	
 <u>L. hillebrandii</u>						
HLUAF	21.5	1.5±.2	2.0	54	0.132±.056	0
 OAHU-KOOLAU						
<u>L. filifolia</u>						
FOTWO	21.8	1.3±.1	2.0	27	0.051±.032	0
FOTHR	21.4	1.3±.2	2.5	18	0.088±.060	0
mean	21.6	1.3	2.3	22	0.070	
 MOLOKAI						
<u>L. maxima</u>						
MMAXI	27.8	1.5±.2	2.2	45	0.148±.065	0
 <u>L. remyi</u> subsp. <u>subherbacea</u>						
MWAIK	26.6	1.7±.2	2.3	54	0.223±.068	0
MKOLE	22.2	1.5±.2	2.2	45	0.167±.073	0
MONIN	22.9	1.5±.2	2.2	45	0.213±.075	0
MMAKA	26.5	1.7±.3	2.6	45	0.219±.079	0
MKAWE	19.9	1.6±.2	2.2	54	0.238±.077	0
mean	23.6	1.6	2.3	49	0.212	
 WEST MAUI						
<u>L. remyi</u> subsp. <u>kipahuluensis</u>						
WMBLG	11.1	1.4±.2	2.0	36	0.087±.043	0

Table 3.3. continued on next page.

Table 3.3. continued. Genetic variability in 48 populations of endemic Hawaiian Lysimachia.

ISLAND						
Species						
Pop.	N	A±s.d.	Ap	P(%)	H±s.d.	U
<u>L. remyi</u> subsp. <u>remyi</u>						
WMHEL	41.4	1.8±.2	2.3	63	0.130±.056	0
WMHPL	29.1	1.8±.3	2.3	63	0.115±.045	0
WMHPY	9.9	1.5±.2	2.0	45	0.106±.052	0
WMMN2	28.1	2.0±.3	2.7	63	0.162±.050	0
WMMPP	27.2	1.6±.2	2.2	54	0.139±.050	0
WMHPS	24.6	1.5±.2	2.2	45	0.089±.037	0
WMLIS	54.5	2.1±.3	2.5	72	0.144±.042	0
WMLIL	16.1	1.7±.3	2.3	54	0.142±.055	0
WMLIM	11.2	1.3±.1	2.0	27	0.055±.033	0
mean	26.9	1.7	2.3	54	0.120	
EAST MAUI						
<u>L. remyi</u> subsp. <u>kipahuluensis</u>						
EMPAL	26.3	1.4±.2	2.0	36	0.075±.042	0
EMHPA	23.0	1.1±.1	2.0	9	0.010±.010	0
EMLWA	27.6	1.4±.2	2.0	36	0.113±.053	0
EMKPR	26.7	1.4±.2	2.0	36	0.078±.039	0
EMKAW	13.6	1.4±.2	2.0	36	0.048±.023	0
EMKIP	22.0	1.3±.1	2.0	27	0.064±.046	0
mean	23.2	1.3	2.0	30	0.065	
<u>L. remyi</u> subsp. <u>caliginis</u>						
EMKOT	29.2	1.2±.1	2.0	18	0.031±.026	0
EMKOB	9.0	1.1±.1	2.0	9	0.009±.009	0
mean	19.1	1.1	2.0	13	0.020	
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X <u>L. remyi</u> subsp. <u>caliginis</u> (populations of hybrid swarms)						
EMKAE	28.5	1.5±.2	2.0	54	0.127±.055	0
EMWAI	28.2	1.4±.2	2.0	36	0.055±.031	0
mean	28.4	1.5	2.0	54	0.091	
All pops.	21.4	1.5	2.2	40	0.117	
Island endemics (Dejooode and Wendel, 1992)						
		1.32		25	.064	

Mean species values for (A) ranged from 1.1 for L. remyi subsp. caliginis to 1.7 for L. remyi subsp. remyi; (A_p) varied from 2.0 for L. remyi subsp. kipahuluensis to 2.8 for L. glutinosa; (P) varied from 13% for L. remyi subsp. caliginis to 54% for L. remyi subsp. remyi, and L. hillebrandii; and (H) varied from 0.02 for L. remyi subsp. caliginis to 0.21 for L. remyi subsp. subherbacea (Table 3.3).

Graphical presentation (Figure 3.2) of genetic variation within populations emphasizes the fact that there is not necessarily a stepwise decrease in the level of heterozygosity from the oldest through to the youngest island, although populations from East Maui are the least heterozygous. In fact, some populations from West Maui, Oahu and Molokai are as variable, if not more so, than populations from Kauai.

Genetic diversity statistics are presented in Table 3.4. Total diversity was lowest in L. remyi subsp. caliginis ($H_t=0.02$), and highest in L. remyi subsp. subherbacea ($H_t=0.23$); diversity within populations was lowest in L. remyi subsp. caliginis ($H_s=0.02$) and highest in L. remyi subsp. subherbacea ($H_s=0.21$). Variation among populations was highest in L. waianaeensis ($D_{st}=0.031$), L. kalalauensis ($D_{st}=0.014$) and L. remyi subsp. subherbacea ($D_{st}=0.018$) and lowest among populations of L. remyi subsp. kipahuluensis ($D_{st}=0.004$) and L. remyi subsp. caliginis ($D_{st}=0.001$).

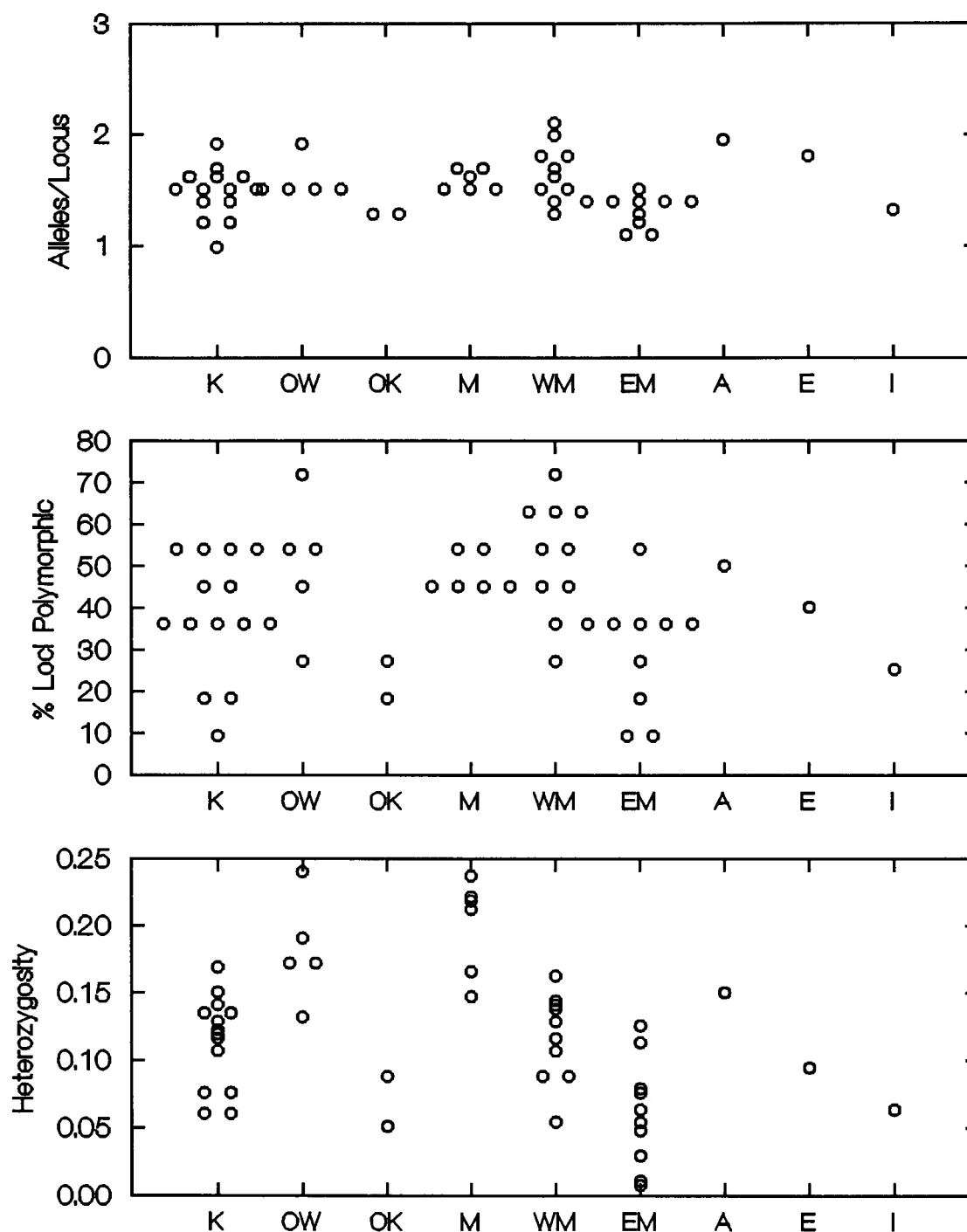


Figure 3.2. Genetic variation by island for populations of endemic Hawaiian *Lysimachia* are compared to average values for species of various geographical distributions. Islands are arranged in decreasing age from left to right: K, Kauai; OW, Oahu-Waianae Mtns.; OK, Oahu-Koolau Mtns.; M, Molokai; WM, West Maui; EM, East Maui. From Hamrick and Godt(1990): A, average of 449 species; E, average of 81 endemic species. From DeJode and Wendel(1992): I, average of 62 insular endemic species.

Table 3.4. Nei's genetic diversity statistics calculated for 11 species and four subspecies of endemic Hawaiian Lysimachia. Values are means calculated from 11 loci. H_t =total diversity; H_s =diversity within populations; D_{st} =variation among populations; G_{st} =proportion of variation among populations.

ISLAND Species	No. of Pops.	H_t	H_s	D_{st}	G_{st}
KAUAI					
<u>L. daphnoides</u>	2	0.129	0.126	0.003	0.022
<u>L. glutinosa</u>	3	0.103	0.096	0.007	0.068
<u>L. kalalauensis</u>	4	0.132	0.117	0.014	0.109
<u>L. ovoidea</u>	2	0.166	0.159	0.008	0.046
<u>L. scopulensis</u>	1	0.135	0.135	-	-
<u>L. pendens</u>	3	0.049	0.047	0.002	0.041
OAHU-WAIAANAE MTNS.					
<u>L. waianaeensis</u>	4	0.255	0.194	0.031	0.140
<u>L. hillebrandii</u>	1	0.132	0.132	-	-
OAHU-KOOLAU MTNS.					
<u>L. filifolia</u>	2	0.076	0.069	0.006	0.083
MOLOKAI					
<u>L. maxima</u>	1	0.146	0.149	-	-
<u>L. remyi</u> subsp. <u>subherbacea</u>	5	0.230	0.212	0.018	0.077
WEST MAUI					
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	1	0.087	0.087	-	-
<u>L. remyi</u> subsp. <u>remyi</u>	9	0.131	0.120	0.011	0.082
EAST MAUI					
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	6	0.069	0.065	0.004	0.059
<u>L. remyi</u> subsp. <u>caliginis</u>	2	0.021	0.020	0.001	0.050
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X <u>L. remyi</u> subsp. <u>caliginis</u>	2	0.102	0.091	0.011	0.109
mean for each taxon		0.119	0.112	0.010	0.073
all populations		0.203	0.118	0.086	0.421
Island Endemics (DeJooode and Wendel, 1992)			0.064		

Compared to the other species, greater geographical distances separate populations of L. kalalauensis, L. waianaeensis and L. remyi subsp. subherbacea. This may have contributed to the higher values of D_{st} for these three species. The percent of the total variation that is partitioned among populations, G_{st} , varied from 4.1% for L. pendens, to 14% for L. waianaeensis.

There is a stepwise decrease in the levels of genetic divergence among populations within a single island from the oldest through to the youngest islands as seen in the values for D_{st} and G_{st} in Table 3.5. In other words, populations on Kauai have diverged the most from each other, followed by Oahu, Molokai and Maui.

3.3.1.2. Allozyme variation within and among species

Mean intra-taxon coefficients of Nei's genetic identity varied from 0.927 among populations of L. waianaeensis, to 0.998 for L. remyi subsp. caliginis (Table 3.6). The range of variation was greatest among populations of L. waianaeensis (0.877-0.958) and least among populations of L. pendens (0.995-0.997). With the exception of populations of L. waianaeensis, all intraspecific values for I were greater than 0.930.

Table 3.5. Genetic diversity statistics of endemic Hawaiian Lysimachia for each island. Values are means calculated from 11 loci. H_t =total diversity; H_s =diversity within populations; D_{st} =variation among populations; G_{st} =proportion of variation among populations.

ISLAND	H_s	H_t	D_{st}	G_{st}
KAUAI	0.107±.03	0.207±.06	0.100	0.482
OAHU-WAIANAE	0.181±.06	0.223±.07	0.041	0.186
OAHU-KOOLAU	0.069±.04	0.076±.04	0.006	0.083
MOLOKAI	0.201±.06	0.229±.07	0.028	0.121
WEST MAUI	0.117±.03	0.128±.04	0.011	0.085
EAST MAUI	0.054±.03	0.061±.03	0.007	0.114

Table 3.6. Intraspecific variation in Nei's Genetic Identities (I) for species of endemic Hawaiian Lysimachia.

Species	No. of Populations	Range of I	Mean I
<u>L. glutinosa</u>	3	0.983-0.998	0.989
<u>L. daphnoides</u>	2	-	0.993
<u>L. kalalauensis</u>	4	0.972-0.988	0.978
<u>L. ovoidea</u>	2	-	0.982
<u>L. scopulensis</u>	1	-	-
<u>L. pendens</u>	3	0.995-0.997	0.996
<u>L. filifolia</u>	2	-	0.989
<u>L. waianaeensis</u>	4	0.877-0.958	0.927
<u>L. hillebrandii</u>	1	-	-
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)	1	-	-
<u>L. remyi</u> subsp. <u>remyi</u>	9	0.930-1.000	0.978
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (East Maui)	5	0.986-0.999	0.995
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X <u>L. remyi</u> subsp. <u>caliginis</u>	2	-	0.976
<u>L. remyi</u> subsp. <u>caliginis</u>	2	-	0.998
<u>L. remyi</u> subsp. <u>subherbacea</u>	5	0.943-0.992	0.972
<u>L. maxima</u>	1	-	-

The lowest mean inter-taxon genetic identity was for the pairwise comparison of L. glutinosa to the two subspecies of L. remyi from East Maui ($I=0.744$ and 0.745) (Table 3.7). The highest pairwise comparisons were for L. kalalauensis and L. remyi subsp. kipahuluensis (WM) ($I=0.986$), for L. filifolia and L. kalalauensis ($I=0.974$), and for L. filifolia and L. remyi subsp. remyi ($I=0.980$).

Among subspecies of L. remyi, I ranged from 0.796 - 1.000 . The lowest value was for L. remyi subsp. subherbacea from Molokai and L. remyi subsp. remyi from West Maui, and the highest for subspecies L. remyi subsp. caliginis and L. remyi subsp. kipahuluensis from East Maui.

Within islands, the greatest range of genetic identities was among populations on Kauai ($I=0.743$ - 0.998). The range of genetic identities within an island is greatest on Kauai and decreases on progressively younger islands, with the least divergence among populations on East Maui ($I=0.965$ - 1.000) (Table 3.8). The greatest divergence among islands is between populations from East Maui and those on Kauai ($I=0.707$ - 0.956). Using the mean genetic identities calculated for each pair of taxa, the mean I among taxa on each island are as follows: Kauai, $I=0.858$; Oahu, $I=0.924$; Molokai, $I=0.933$; Maui, $I=0.941$.

Table 3.7. Genetic identities (I) for species of endemic Hawaiian Lysimachia.

Species	Mean (Minimum-Maximum)				
	1	2	3	4	5
1. <u>L. daphnoides</u>					
2. <u>L. glutinosa</u>	0.926 (0.901-0.948)				
3. <u>L. kalaluaensis</u>	0.888 (0.879-0.901)	0.823 (0.798-0.864)			
4. <u>L. ovoidea</u>	0.829 (0.789-0.865)	0.774 (0.743-0.798)	0.910 (0.855-0.951)		
5. <u>L. scopulensis</u>	0.828 (0.825-0.832)	0.766 (0.752-0.775)	0.949 (0.924-0.986)	0.862 (0.828-0.895)	
6. <u>L. scandens</u>	0.812 (0.797-0.827)	0.847 (0.830-0.870)	0.903 (0.877-0.954)	0.906 (0.888-0.926)	0.853 (0.846-0.858)
7. <u>L. filifolia</u>	0.893 (0.881-0.901)	0.827 (0.811-0.839)	0.974 (0.961-0.987)	0.888 (0.843-0.929)	0.918 (0.906-0.931)
8. <u>L. waianaeensis</u>	0.812 (0.767-0.859)	0.797 (0.763-0.855)	0.901 (0.842-0.966)	0.910 (0.847-0.979)	0.840 (0.805-0.886)
9. <u>L. hillebrandii</u>	0.872 (0.867-0.876)	0.841 (0.830-0.851)	0.963 (0.954-0.982)	0.905 (0.884-0.926)	0.934 (0.934-0.934)
10. <u>L. maxima</u>	0.852 (0.847-0.858)	0.779 (0.770-0.794)	0.928 (0.922-0.933)	0.850 (0.818-0.882)	0.863 (0.863-0.863)
11. <u>L. remyi</u> subsp. <u>subherbacea</u>	0.838 (0.802-0.871)	0.810 (0.772-0.840)	0.921 (0.857-0.973)	0.945 (0.926-0.971)	0.858 (0.820-0.892)
12. <u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)	0.899 (0.898-0.899)	0.853 (0.840-0.862)	0.986 (0.980-0.999)	0.931 (0.910-0.953)	0.938 (0.938-0.938)
13. <u>L. remyi</u> subsp. <u>remyi</u>	0.888 (0.861-0.903)	0.818 (0.764-0.876)	0.976 (0.924-10.000)	0.902 (0.845-0.974)	0.929 (0.873-0.983)

Table 3.7. continued on next page.

Table 3.7. Genetic identities (I) for species of endemic Hawaiian Lysimachia.

Species	Mean (Minimum-Maximum)				
	1	2	3	4	5
14. <u>L. remyi</u> subsp. <u>kipahulensis</u> (East Maui)	0.819 (0.796-0.848)	0.744 (0.707-0.771)	0.910 (0.885-0.950)	0.835 (0.768-0.888)	0.847 (0.838-0.866)
15. <u>L. remyi</u> subsp. <u>caliginis</u>	0.809 (0.804-0.811)	0.745 (0.726-0.759)	0.901 (0.887-0.927)	0.801 (0.769-0.831)	0.839 (0.835-0.842)
16. <u>L. remyi</u> subsp. <u>caliginis</u> X <u>L. remyi</u> subsp. <u>kipahulensis</u>	0.827 (0.800-0.861)	0.755 (0.729-0.775)	0.918 (0.888-0.956)	0.832 (0.760-0.902)	0.854 (0.833-0.875)

Table 3.7. continued on next page.

Table 3.7. continued. Genetic identities (I) for species of endemic Hawaiian Lysimachia.

Species	Mean (Minimum-Maximum)				
	6	7	8	9	10
7. <u>L. filifolia</u>	0.895 (0.886-0.904)				
8. <u>L. waianaensis</u>	0.908 (0.877-0.941)	0.900 (0.854-0.950)			
9. <u>L. hillebrandii</u>	0.944 (0.940-0.947)	0.965 (0.958-0.973)	0.909 (0.877-0.949)		
10. <u>L. maxima</u>	0.853 (0.848-0.861)	0.973 (0.959-0.986)	0.883 (0.842-0.946)	0.918 (0.918-0.918)	
11. <u>L. remyi</u> subsp. subherbacea	0.932 (0.888-0.956)	0.931 (0.892-0.970)	0.919 (0.836-0.961)	0.929 (0.900-0.962)	0.933 (0.905-0.964)
12. <u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)	0.947 (0.945-0.949)	0.985 (0.978-0.993)	0.921 (0.887-0.962)	0.986 (0.986-0.986)	0.942 (0.942-0.942)
13. <u>L. remyi</u> subsp. <u>remyi</u>	0.896 (0.830-0.947)	0.980 (0.923-10.000)	0.901 (0.834-0.967)	0.958 (0.913-0.974)	0.949 (0.909-0.983)
14. <u>L. remyi</u> subsp. <u>kipahuluensis</u> (East Maui)	0.818 (0.786-0.840)	0.908 (0.869-0.938)	0.844 (0.787-0.918)	0.889 (0.871-0.909)	0.909 (0.876-0.937)
15. <u>L. remyi</u> subsp. <u>caliginis</u>	0.813 (0.802-0.826)	0.899 (0.883-0.913)	0.829 (0.785-0.898)	0.883 (0.878-0.889)	0.898 (0.893-0.904)
16. <u>L. remyi</u> subsp. <u>caliginis</u> X <u>L. remyi</u> subsp. <u>kipahuluensis</u>	0.825 (0.807-0.844)	0.915 (0.894-0.942)	0.845 (0.780-0.905)	0.897 (0.881-0.912)	0.910 (0.904-0.915)

Table 3.7. continued on next page.

Table 3.7. continued. Genetic identities (I) for species of endemic Hawaiian Lysimachia.

Species	Mean (Minimum-Maximum)				
	11	12	13	14	15
12. <u>L. remyi</u> subsp. <u>kipahulensis</u> (West Maui)	0.951 (0.921-0.982)				
13. <u>L. remyi</u> subsp. <u>remyi</u>	0.925 (0.845-0.985)	0.981 (0.935-0.998)			
14. <u>L. remyi</u> subsp. <u>kipahulensis</u>	0.873 (0.796-0.947)	0.912 (0.897-0.934)	0.932 (0.884-0.978)		
15. <u>L. remyi</u> subsp. <u>caliginis</u>	0.851 (0.797-0.910)	0.902 (0.898-0.906)	0.925 (0.892-0.979)	0.992 (0.970-1.000)	
16. <u>L. remyi</u> subsp. <u>caliginis</u> X	0.871 (0.792-0.945)	0.919 (0.900-0.938)	0.940 (0.891-0.978)	0.988 (0.965-0.996)	0.989 (0.979-0.997)
<u>L. remyi</u> subsp. <u>kipahulensis</u>					

Table 3.8. Nei's genetic identities (I) within and among islands for endemic Hawaiian species of *Lysimachia*. Islands are arranged from oldest to youngest (left to right, top to bottom).

ISLAND	Mean (Minimum-Maximum)			
	Kauai	Wai'anae (Oahu)	Koolau (Oahu)	Molokai
Kauai	0.880 (0.743-0.998)			
Waianae (Oahu)	0.876 (0.763-0.982)	0.920 (0.877-0.958)		
Koolau (Oahu)	0.903 (0.811-0.987)	0.913 (0.854-0.973)	0.989 (0.989-0.989)	
Molokai	0.884 (0.770-0.973)	0.916 (0.836-0.962)	0.938 (0.892-0.986)	0.959 (0.905-0.992)
Wmaui	0.906 (0.764-1.0)	0.915 (0.834-0.986)	0.981 (0.923-1.0)	0.931 (0.845-0.985)
Emaui	0.830 (0.707-0.950)	0.851 (0.780-0.918)	0.906 (0.869-0.938)	0.928 (0.884-0.979)
				0.992 (0.970-1.)

The phenogram produced using UPGMA (Figure 3.3) delineates two distinct groups: in group one are L. glutinosa and L. daphnoides; in group two are the remaining species. Lysimachia glutinosa and L. daphnoides cluster together and have lower genetic identities in pairwise comparisons with all other species because all individuals of L. glutinosa and most of L. daphnoides are fixed for *Pgi-1a*. Some individuals of L. daphnoides also carry *Pgi-1d*, an allele unique to this species. All other species are fixed for *Pgi-1b* (*Pgi-1c* was a very rare allele in two populations).

3.3.2 Principal Components Analysis

Allele frequencies and mean measurements of floral and vegetative characters were available for 35 populations. The same character codes that were used in Chapter Two were also used here. Fewer populations were included here than in Chapter Two, therefore the results of the PCA are not identical, however the overall pattern remains the same.

3.3.2.1 Principal Components Analysis of floral characters

Component loadings for the PCA of floral characters are presented in Table 3.9. The first component had high loadings for PEDLENM, CORLENM, CORWIDM, CALLENM, CALWIDM, STYLENM, and FILLENM. The second component had high loadings for CALPUBE. The first two axes represent 79% of the variation.

Figure 3.3. UPGMA phenogram derived from Nei's genetic identities of 48 populations of 11 species and four subspecies of endemic Hawaiian Lysimachia. See Table 3.1 for population codes. Cophenetic correlation coefficient is .798.

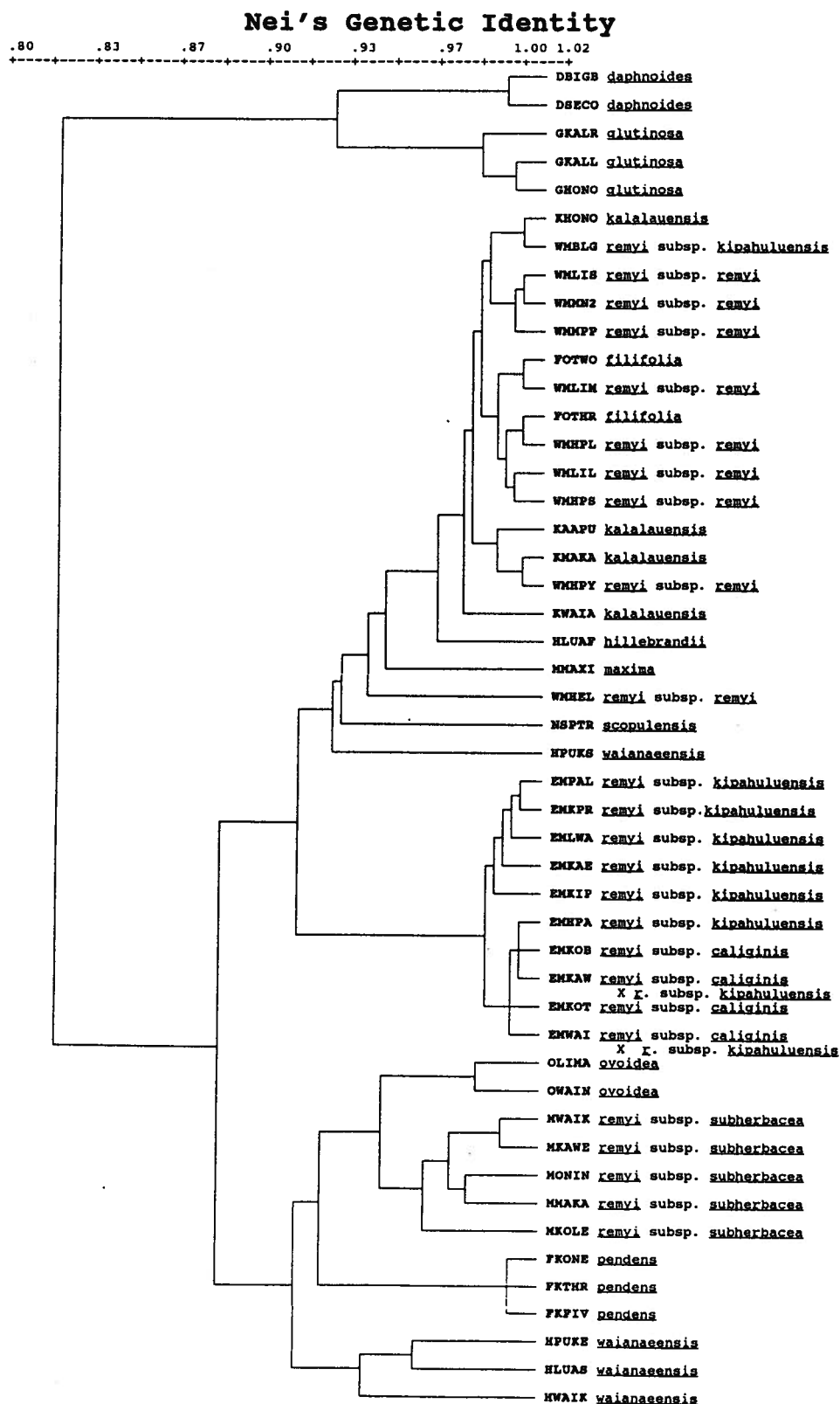


Table 3.9. Principal components analysis of floral characters of endemic Hawaiian Lysimachia averaged over 35 populations.

Variable	Component Loadings	
	1	2
CALPUBE	0.092	-0.971
PEDLENM	0.751	0.032
CORLENM	0.963	0.026
CORWIDM	0.902	0.022
CALLENM	0.881	0.017
CALWIDM	0.852	0.269
STYLENM	0.853	-0.184
FILLENM	0.870	-0.077

Eigenvalues	5.299	1.058
% Variance	66.235	13.226
% Cum. Var.	66.235	79.461

Table 3.10. Principal components analysis of vegetative characters of endemic Hawaiian Lysimachia averaged over 35 populations.

Variable	Component Loadings		
	1	2	3
ULPUBE	0.128	0.664	0.609
LLPUBE	0.060	0.764	0.480
LEAFLM	0.852	-0.275	0.078
LEAFWM	0.890	-0.207	0.100
PETLM	0.805	-0.235	0.288
MINNTRND	0.666	-0.289	0.160
MAXNTRND	0.695	0.509	-0.473
STDNTRND	0.478	0.661	-0.567

Eigenvalues	3.342	1.977	1.271
% Variance	41.777	24.707	15.893
% Cum. Var.	41.777	66.484	82.377

3.3.2.2 Principal Components Analysis of vegetative characters

Component loadings for the PCA of vegetative characters are presented in Table 3.10. The first component had high loadings for LEAFLM, LEAFWM, PETLM. The second component had high loadings for ULPUBE, LLPUBE, and STDNTRND. The first two axes represent 66% of the variation.

3.3.2.3 Principal Components Analysis using allele frequencies

The population frequencies of 33 alleles were included in this analysis. The following alleles had relatively high loadings for the first component: *Idh-1c*, *Idh-1b*, *Mdh-3b*, *Mdh-3c*, *Tpi-2a* and *Tpi-2c* (Table 3.11). Alleles with high loadings for the second component were: *Pgi-1a*, *Pgi-1b*, *Pgm-1c* and *Pgm-1e*. The first two components represent only 27% of variation. The first 11 components have eigenvalues greater than one and represent 82% of the variation.

3.3.2.4 Comparison of morphological and allozyme results

The ordination of populations differs depending on which characters were used in the PCA (Figure 3.4). The Pearson correlation coefficient calculated from the comparison of vegetative characters and allozyme data is 0.489 and the comparison of floral characters to allozyme data is 0.372 (Table 3.12).

Table 3.11. Principal components analysis of endemic Hawaiian *Lysimachia* based on allele frequencies from allozyme analysis of 35 populations.

Variable	Component Loadings				
	1	2	3	4	5
TPI-1A	-0.171	-0.011	-0.012	0.043	-0.360
TPI-1B	0.117	-0.118	0.051	-0.890	0.056
TPI-1C	-0.099	0.119	-0.049	0.885	-0.017
TPI-2A	0.612	0.153	-0.533	0.005	0.052
TPI-2B	0.047	-0.390	-0.041	0.035	0.351
TPI-2C	-0.593	-0.217	0.459	0.135	0.131
TPI-2D	0.048	-0.158	-0.176	0.177	0.052
TPI-2E	0.190	0.257	-0.011	-0.310	-0.308
TPI-3A	0.453	0.245	0.253	-0.367	-0.163
ADH-1A	-0.416	-0.292	0.458	-0.034	-0.099
IDH-1A	0.312	-0.255	0.123	-0.224	-0.308
IDH-1B	0.706	0.240	0.040	-0.057	-0.168
IDH-1C	-0.766	0.112	0.004	0.129	0.273
IDH-1D	0.232	-0.658	-0.157	-0.096	-0.183
IDH-1E	-0.027	0.084	0.592	0.067	0.131
MDH-2A	-0.027	0.084	0.592	0.067	0.131
MDH-2B	-0.676	0.208	-0.147	-0.478	0.121
MDH-2C	0.601	-0.289	0.167	0.460	-0.155
MDH-2D	0.486	0.331	-0.538	0.093	0.064
MDH-3A	0.244	0.038	0.689	-0.050	-0.037
MDH-3B	-0.379	-0.184	-0.732	0.072	-0.225
MDH-3C	0.274	0.221	0.296	-0.049	0.359
SKD-1A	-0.121	-0.146	-0.300	-0.031	0.459
SKD-1B	0.299	0.097	0.339	0.166	-0.464
SKD-1C	-0.326	0.024	-0.170	-0.228	0.174
DIA-1A	-0.073	0.040	-0.023	0.578	0.159
PGM-1C	0.171	-0.641	0.035	0.111	0.382
PGM-1D	-0.459	-0.349	-0.032	-0.056	-0.659
PGM-1E	0.401	0.525	0.000	0.034	0.537
PGI-1A	0.284	-0.898	-0.045	-0.072	0.047
PGI-1B	-0.276	0.901	0.037	0.069	-0.064
PGI-1C	-0.273	0.012	-0.024	-0.042	-0.228
PGI-1D	0.140	-0.544	0.113	0.031	0.389
Eigenvalues	4.637	4.146	3.239	2.851	2.458
% Variance	14.952	12.562	9.814	8.641	7.450
% Cum. Var.	14.952	27.514	37.328	45.969	53.419

Figure 3.4. Comparison of principal components analysis of vegetative, reproductive and allozyme data of endemic Hawaiian *Lysimachia*. Points are of population averages labelled as follows: D=*L. daphnoides*, U=*L. remyi* subsp. *kipahuluensis*, C=*L. remyi* subsp. *caliginis*, P=*L. pendens*, W=*L. filifolia*, G=*L. glutinosa*, H=*L. waianaeensis*, K=*L. kalalauensis*, S=*L. remyi* subsp. *subherbacea*, M=*L. maxima*, O=*L. ovoidea*, R=*L. remyi* subsp. *remyi*, N=*L. scopulensis*, X=*L. remyi* subsp. *kipahuluensis* X *L. r.* subsp. *caliginis* (hybrid swarm).

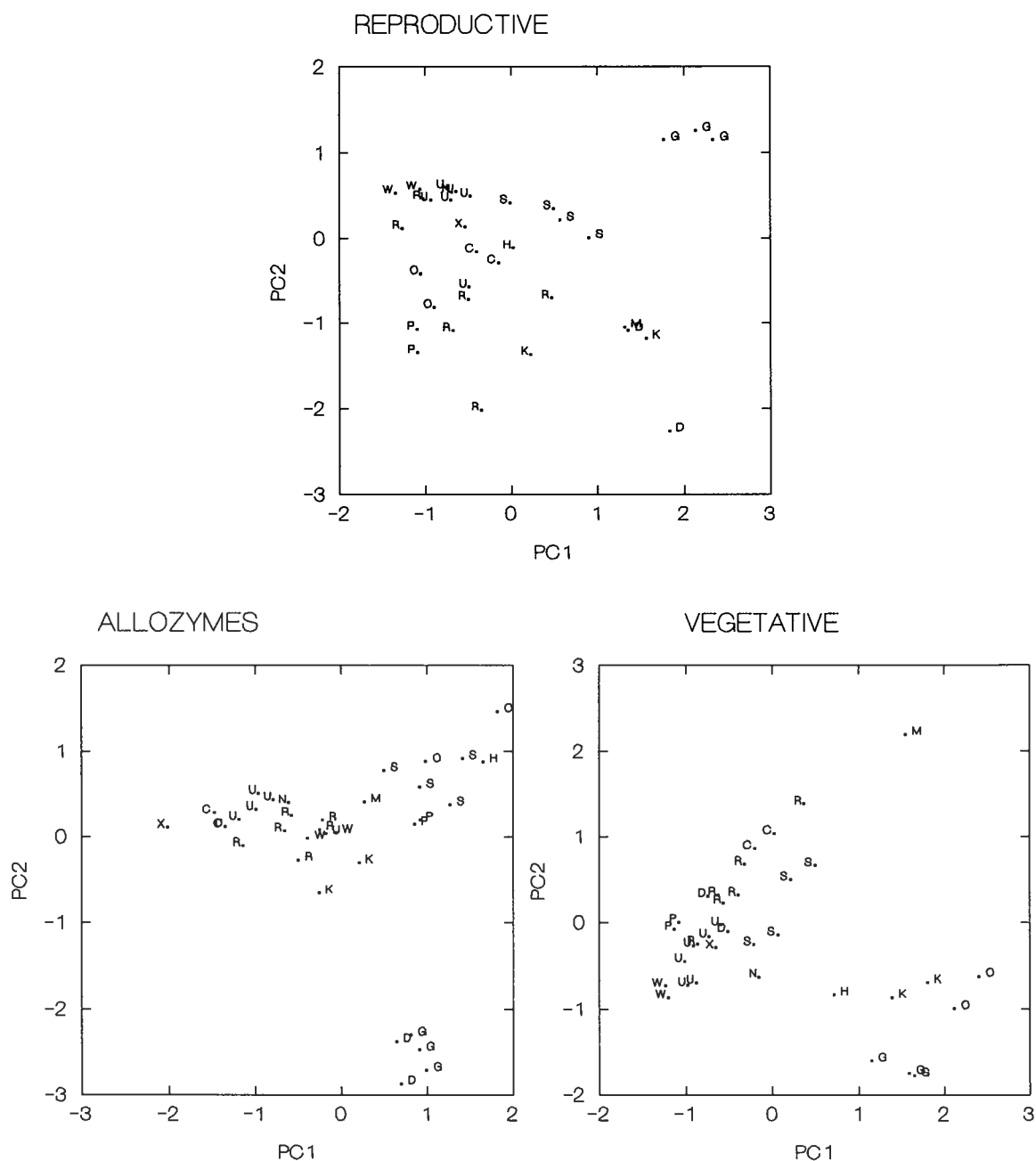


Table 3.12. Summary table of Pearson correlation matrix comparing principal component scores from allozyme, floral, and vegetative data. Allozyme axes are abbreviated as PCISO1 and PCISO2, floral axes as PCFLOW1 and PCFLOW2, and vegetative axes as PCVEG1 and PCVEG2; "1" and "2" refer to the first principal component score and the second score respectively. Values marked with "*" are not significant.

	PCVEG1	PCVEG2	PCFLOW1	PCFLOW2
PCISO1	0.489	-0.272	0.372	-0.128
PCISO2	-0.100	0.355	-0.695*	-0.029

3.4 Discussion

3.4.1 Allozyme variation within species of Hawaiian Lysimachia

Two recent reviews of allozyme variation in plant populations provide a basis for comparison with Hawaiian Lysimachia (Figure 3.2). Hamrick and Godt (1990), calculated average values of genetic diversity within populations, based on a compilation of 449 species in 165 genera. Most of these are continental taxa. For the "average" species the percent of polymorphic loci (P), is 50%, the mean number of alleles per locus (A), is 1.96, and the total diversity (H_t), is 0.149. For 81 endemic species (few were insular endemics), the averages were $P=40\%$, $A=1.80$, and $H_t=0.096$. DeJooe and Wendel (1992), summarized allozyme variation in 62 insular species from 16 genera. The "average" insular endemic species has even less variation than continental endemics, $P=25\%$, $A=1.32$ and $H_t=0.064$. For Hawaiian Lysimachia, these averages are $P=41\%$ (range=14-54%), $A=1.5$ (range=1.1-1.7) and $H_t=0.117$ (range=0.020-0.212) (Table 3.3). Allozyme variation in the "average" Hawaiian Lysimachia is greater than the "average" insular species. In fact, comparing the values for H_t and P , the level of variation in several species of Lysimachia is similar to that found in continental species (Figure 3.2). It should be pointed out however, that P is especially dependant upon the number of loci, and the 11 that were successfully resolved in this study is a relatively small sample.

If genetic bottlenecks follow colonization from the oldest through to the youngest island, a stepwise reduction in genetic variation within populations would be expected. Hawaiian Bidens deviate from this expectation in that the mean values of genetic variation in populations from all islands are nearly equal and are actually slightly higher on the youngest island, Hawaii (Ganders, 1989). Hawaiian Lysimachia do not entirely follow the expected trend either, although there is a marked decrease from Molokai to West Maui and finally to East Maui (Figure 3.2). One explanation for this is that populations of Lysimachia are typically small, consisting of fewer than one hundred individuals. In small populations the loss of genetic variation through random genetic drift is greater in successive generations than it is in large populations. This could explain why populations on the older islands have less variation than would be expected. Two processes are at work here. On the one hand, time is required for the accumulation of novel alleles. On the other hand, the more that time has elapsed, the greater the possibility of loss of variation through random genetic drift especially in small populations.

A serious limitation in this comparison is the lack of samples from two species, L. forbesii and L. hillebrandii, that may now be extinct in the Koolau Mtns. on Oahu. The two populations of L. filifolia cannot be considered to represent the level of genetic variation that may have once been present in species on that volcano. In addition, it cannot always be

assumed that the individuals that found new interisland populations necessarily come from the adjacent, next oldest island. In the Hawaiian Madiinae, it is hypothesized that some species in the Maui complex (includes the islands of Maui, Molokai and Lanai) have evolved from an ancestor from the more distant island of Kauai rather than from the adjacent Oahu (Carr et al., 1989).

From the oldest to the youngest islands genetic variation *within* populations does not decrease in a stepwise manner, however, the level of variation *among* populations (Table 3.5) does decrease in this way. Populations from the younger islands share the same alleles at nearly the same frequencies and few if any unique alleles are present. On Kauai there has been more time for divergence in allele frequencies among populations as well as time for mutations that can result in new alleles. Six of the seven "unique" alleles that were detected are restricted to species on Kauai, the seventh from the oldest part of Oahu, the Waianae Mtns. (Table 3.3). Almost without exception, it is only on Kauai that some species do not share the same highest frequency allele at all loci (Table 3.2).

3.4.2 Allozyme variation among species of Lysimachia

The relatively high genetic identities among species of Hawaiian Lysimachia is a consequence of most species sharing the same highest frequency allele at all or most loci. This supports the hypothesis that they have evolved from a single

ancestor. The decrease in the range of intra-island genetic identities from Kauai to East Maui (Table 3.8) suggests that Kauai was the first extant island to be colonized. This trend parallels the reduction in morphological diversity within islands from the oldest through to the youngest island (Chapter Two).

Gottlieb (1977) calculated that populations of the same species had an average genetic identity of 0.95 ± 0.02 and the average for species belonging to the same genus was 0.67 ± 0.07 . Remarkably, Crawford (1990b) reports that although the range of variation has increased, the averages of Gottlieb (1977) remain essentially the same even with the addition of hundreds more, mostly continental, taxa. The range of genetic identities for populations of Lysimachia, 0.71-1.0, is intermediate to these values. However, the mean of 0.89 indicates that the extent of divergence between most pairs of species is more typical of different *populations* of a continental *species*. By comparison, the mean genetic identity among populations of a single species, Coreopsis integrifolia collected from Florida and Georgia, is 0.925 and the range is 0.786-0.997 (Cosner and Crawford, 1994).

For the following reasons, newly founded populations of insular taxa are probably genetically depauperate compared to those. Because of distance and barriers to dispersal, most insular taxa are probably founded by a few or perhaps a single propagule. In most cases, it is propagules of self-compatible hermaphrodite species that are able to found populations

following long distance dispersal to isolated oceanic islands, in accordance with "Baker's Rule" (Baker, 1967). Such a propagule potentially bears less genetic variation than an obligately outcrossed one. However, Baker and Cox (1984) cite examples and discuss mechanisms whereby propagules of dioecious taxa may establish successful breeding populations, i.e. dioecy does not necessarily always evolve autochthonously, on islands, from hermaphrodite ancestors.

Compared to the few other insular genera (Table 3.13) in which more than one species has been analyzed, species of Lysimachia have diverged from each other somewhat more than have those of Crepidiastrum, Bidens, Metrosideros, Tetramolopium, Wilkesia, or Dubautia (n=13 species), but not as much as those of Dubautia (n=14 species), Wahlenbergia or Robinsonia. In fact, the degree of divergence among species of the latter three genera is similar to that of continental species. One factor that undoubtedly contributes to the differences in the range of genetic identities among the different genera being compared is the fact that they have been in their respective archipelagos for different lengths of time.

Table 3.13. Comparison of Nei's genetic identities among species of island genera.

Taxon (Reference)	No. Taxa	Location	Range	Mean
<u>Metrosideros</u> (Aradhya, 1991)	5	Hawaii	0.79-1.00	0.92
<u>Bidens</u> (Sun and Ganders, 1986)	14	Hawaii	0.92-0.99	0.98
<u>Dubautia</u> (n=14 sp.) (Witter and Carr, 1988)	6	Hawaii	0.43-0.93	0.69
<u>Dubautia</u> (n=13 sp.) (Witter and Carr, 1988)	9	Hawaii	0.73-1.0	0.95
<u>Tetramolopium</u> (Lowrey and Crawford, 1988)	7	Hawaii	0.86-1.0	0.95
<u>Lysimachia</u>	15	Hawaii	0.71-1.0	0.89
<u>Robinsonia</u> (Crawford et al., 1992)	4	Juan Fernandez	0.56-0.71	0.63
<u>Wahlenbergia</u> (Crawford et al., 1990)	3	Juan Fernandez	0.68-0.95	
<u>Crepidiastrum</u> (Ito and Ono, 1990)	3	Bonin Islands	0.75-0.99	0.84
Continental congeneric species				0.67
Continental conspecific populations (Crawford, 1990)				0.95

This is especially important because divergence among insular species is more likely a function of the time necessary for new alleles to appear, rather than divergence through changes in polymorphic gene frequencies (Witter and Carr, 1988). Species of Dubautia are among the first colonists of new lava, often in areas of low rainfall. They have evolved from California tarweeds (Baldwin et al., 1991; Carr, 1985) that are also adapted to dry environments (Hubbell, 1968). This suggests that one of the early arrivals to Hawaii may have been the ancestor of Dubautia and the other members of the "silversword complex" and could explain the relatively lower genetic identities of these species as compared to the others.

Although differences in the mean genetic identities of the genera listed above could be entirely related to differences in the length of time since they arrived in their respective archipelagos, other aspects of their biology may also have contributed to these differences. Several species of the "silversword complex" are self-incompatible (Carr et al., 1986), and species of Robinsonia are dioecious (Crawford et al., 1992). Because the establishment of a population from a single self-incompatible ancestor of Robinsonia or the "silversword complex" is unlikely, Carr et al. (1986) and Crawford et al. (1992) consider it possible that these taxa were founded by more than one propagule. As a consequence, there may have been greater genetic variation in the founding population compared to other genera. Although protogyny

promotes outcrossing in species of Lysimachia, plants often bear many flowers on a single stem, and geitonogamy is possible. The degree to which protogyny succeeds in promoting outcrossing and influences the extent of genetic variation within populations of Lysimachia, is unknown and would require direct measurement of outcrossing rates. Hamrick and Godt (1990) report a mean heterozygosity of 0.074 for populations of 113 species of self-pollinating species; the mean for animal-outcrossed species was 0.124 for populations of 164 species. Levels of heterozygosity in species of Hawaiian Lysimachia ranged from 0.02 to 0.21. Thus some species have levels of heterozygosity that are typical of outcrossing species, while other species are less variable than selfing species. In populations from the younger islands, it is of course difficult to distinguish between reduced genetic variation as a consequence of selfing, or from reduced variation as a consequence of the founder effect.

Obligate outcrossing in Robinsonia, some species of the "silversword complex" and possibly in Lysimachia, may have resulted in greater genetic variation among individuals. Because founders from an outcrossing population contain a smaller proportion of the overall variation present in the ancestral population, it is possible that derivative populations will diverge more from each other more rapidly than they would from populations of selfing species. These explanations do not seem to fit Hawaiian Bidens, however. Although species are self-compatible, the flowers are

protandrous and 9 of the 19 species are gynodioecious. Both of these aspects of their breeding system promote outcrossing. Outcrossing rates in 15 populations ranged from 0.43 to 0.88 (Sun and Ganders, 1988). Despite this, Bidens species nevertheless have high genetic identities.

Genetic identities among species of Lysimachia from Kauai ($I=0.743-0.998$) are lower than for all species of Bidens, Tetramolopium, and Dubautia ($n=13$ only). Divergence among species on Kauai was possibly accelerated by the evolution of three different corolla colors: white, green, and reddish. One hypothesis is that red was the ancestral color and that white and green resulted from mutations at different loci (discussed in more detail in Chapter 4). Reproductive isolation may have been relatively rapid if pollinators distinguished between the different corolla colors at the time each mutant appeared in populations of reddish flowered individuals. The frequency of such a mutation could rapidly increase and become fixed following establishment of a new population from a heterozygous individual or even genetic drift at the margins of existing populations. Bawa (1990) has hypothesized that because the geographical ranges of plants and their pollinators often do not coincide entirely, floral variants of a founder population could become fixed as a result of selection by a different set of pollinators. While corollas of both L. glutinosa and L. scopulensis are open, the petals of L. kalalauensis are often tightly closed around the exerted style. If and when the petals become reflexed the

corolla is still not as widely open as that of L. glutinosa. These differences also suggest a difference in pollinators.

In the upper part of Kalalau valley on the island of Kauai, the white flowered L. glutinosa, green flowered L. kalalauensis and reddish flowered L. scopulensis grow nearly sympatrically. Populations of L. scopulensis and L. glutinosa were sampled from this area. However L. kalalauensis is uncommon here and was not sampled from this location. The mean value for I between populations of L. glutinosa and L. kalalauensis is 0.823; between L. glutinosa and L. scopulensis it is 0.766, and between L. kalalauensis and L. scopulensis it is 0.949. Divergence is not only due to differences in frequencies of shared alleles. Ten alleles were detected at relatively high frequencies in one or two species but not in all three. *Pgi-1a* was homozygous in all individuals of L. glutinosa but was not detected in L. kalalauensis or L. scopulensis. This is strong evidence that L. glutinosa is reproductively isolated from these two species. *Dia-1a* was detected only in L. scopulensis ($f=0.312$) and not in L. glutinosa or L. kalalauensis. This is also evidence of reproductive isolation of L. scopulensis from the other two. Hybrids between L. glutinosa and L. kalalauensis and between L. glutinosa and L. scopulensis do occur in this area. However pollen stainability was very low in two putative natural F_1 's of L. glutinosa and L. scopulensis. Seed collected from a result of a backcross to L. glutinosa of a hybrid between L. glutinosa and L. kalalauensis was grown in

the greenhouse. This plant, putatively resulted from a backcross with L. glutinosa and also had low pollen stainability (see Chapter 4). Other factors may have contributed to reproductive isolation among these three species and possibly to allozyme divergence. These include differences in phenology, flower odor, and pollinator. Unfortunately, little is known about any of these. All are worthy of further research.

3.4.3. Comparison of allozyme and morphological variation

Species of Lysimachia are morphologically and ecologically diverse, yet genetic identities among them are quite high. It is somewhat of a paradox that the estimate of genetic variation, allozyme divergence, suggests few genetic differences, however the morphological differences among species suggests that there has been greater genetic divergence. Continental taxa that have high genetic identities are usually species pairs that are morphologically similar (Crawford et al., 1987b).

The most likely explanation for the occurrence of high genetic identities among morphologically distinct insular species is that divergence has been much more rapid for genes that regulate morphological development and ecological adaptation than for genes that code for enzymes of primary metabolism (Crawford, et al., 1987b). A second possibility is that there is a simple genetic basis for the expression of morphological characters. In Hawaiian Bidens, a number of

characters that are used to distinguish among species are controlled by one or two loci (Ganders, 1989). Characters controlled by one locus are: erect vs. decumbent habit, determinate vs. indeterminate flowering, achene awns smooth vs. barbed and three vs. five to seven leaflets. Other leaflet numbers are controlled by additional duplicated loci. Two loci control the following: achenes straight or curved vs. coiled, achene awns distinct vs. decurrent, achenes winged vs. wingless; leaves pubescent vs. glabrous; disk corolla red vs. yellow; achenes setose vs. glabrous. Gynodioecy (male sterility) is controlled by two loci (Sun, 1987).

The low correlation between morphological divergence and allozyme divergence among populations in Lysimachia (Table 3.12) is similar to that in Bidens (Helenurm and Ganders, 1985). Furthermore, in both studies, scores from the first PCA axis of allele frequencies represent a small proportion of the total variation, 13.3% for Bidens and 14.9% for Lysimachia. Overall, the species composition of morphological clades differ from that of allozyme clades, thus the poor correlation between the two (Table 3.12). This can be seen most clearly by comparing leaf shapes of species in Figure 3.1 to their position in the phenogram of Figure 3.3. In the case of Lysimachia daphnoides and L. glutinosa, allozyme divergence does parallel the possession of a unique morphological character. Nearly all plants of these species also bear the same alternate allele at *Pgi-1* and are differ from the remaining species in having viscid leaves (those of L. iniki

are also viscid, but this species was discovered too late to be included in the present study). Leaves of L. glutinosa are glabrous whereas those of L. daphnoides are viscid-hirtellous and they have diverged from each other in a number of characters including corolla color, habit, habitat and leaf size and shape. Among the remaining species there are no other examples of parallel allozyme and morphological divergence, i.e., the presence in all individuals of a unique allele as well as of a unique morphological character.

3.4.4. Taxonomic implications

Taxonomic conclusions based on the groupings presented here need to be interpreted with caution because the overall genetic identities among populations and species are so high. Allozymes can be useful as taxonomic markers when they occur as mutually exclusive (to one or several taxa among the group of taxa under investigation) unique alleles. However as stated above, the differences between most species pairs were usually differences in allele frequency only. Much of the divergence among species is a function of differences in the frequency of alleles held in common rather than to the presence of unique alleles which occur at a significant frequency. In general, the results presented here support the revisions proposed in Chapter Two. Referring to Figure 3.3, it can be seen that the following changes from the species delineations of Wagner et al. (1990) are in agreement with this electrophoretic study: 1) recognition of L. filifolia and

L. pendens, as distinct species (L. pendens was formerly included in L. filifolia); 2) recognition of subspecies of L. remyi; 3) recognition of L. ovoidea, L. waianaeensis, L. hillebrandii, L. remyi subsp. caliginis and L. remyi subsp. subherbacea as distinct species or subspecies, (these had previously been classified as L. hillebrandii *sensu* Wagner et al. (1990)).

3.4.5. Summary

The pattern of genetic variation in Hawaiian Lysimachia, as estimated from allozyme variation, is similar to that seen in the few other Hawaiian genera that have been investigated to date. There is little divergence among most species as compared with that seen among continental taxa, with the exception of L. glutinosa and L. daphnoides which do form a distinct clade at the lowest branch of the tree. Further, in general there is little correlation between the pattern of allozyme divergence and morphological divergence.

Chapter 4

Fertility of artificial interspecific hybrids and breeding behavior of the endemic Hawaiian Lysimachia

4.1 Introduction

The level of fertility of hybrids can be used as a tool to provide insights into the processes of differentiation between parental taxa, as well as interspecific relationships, especially in conjunction with observations of chromosome pairing at meiosis. There have been no previous attempts to analyze the level of fertility of artificial interspecific hybrids of species of Lysimachia endemic to the Hawaiian Islands. Although interfertility may indicate genetic similarity of the parental genomes, intersterility does not necessarily imply greater genetic divergence unless its cause is known (Davis and Heywood, 1973). From progeny analysis, hypotheses regarding genetic regulation of morphological characters can also be tested. In general, it appears that reproductive isolation between congeneric species of Hawaiian plants is a result of ecological or geographical isolation rather than chromosomal differences such as aneuploidy, polyploidy, or structural rearrangements. This hypothesis is based on the high pollen or seed fertility of interspecific hybrids among species of Bidens (Ganders and Nagata, 1984), Tetramolopium (Lowrey, 1986), Dubautia, Argyroxiphium, and Wilkesia (Carr, 1985a), Vaccinium (Vander Kloet, 1993), Wikstroemia (Mayer, 1991), Schiedea (Weller and Sakai, 1988),

intras sectional species of Lipochaeta (Rabakonandrianina, 1980), and intrasubsectional species of Portulaca (Kim and Carr, 1990a). The results of adaptive radiation in each of these genera have been species whose morphologies and ecological requirements are often remarkably divergent.

Chromosome numbers, available for 18.8% of Hawaiian flowering plant species (Carr 1978, 1985b), are the same for nearly all groups of species that are thought to have evolved from a common ancestor. The only case of aneuploidy is in Dubautia in which a group of $n=13$ species are the result of aneuploid reduction from $n=14$ species (Carr, 1985a). *In situ* polyploidy is unknown in Hawaiian plants except for a single plant of Portulaca that appears to be an allopolyploid between an indigenous species and an introduced one (Kim and Carr, 1990a).

Prior to this study nothing was known about the breeding behavior of Hawaiian Lysimachia, i.e., the type of compatibility system or the existence of mechanisms to promote outcrossing such as dioecy, dichogamy, or heterostyly. Species in Lysimachia section *Seleucia*, restricted to North America, are self-incompatible (Coffey and Jones, 1980). Heterostyly, which is common in at least some genera of the Primulaceae (Richards, 1986) is unknown in Lysimachia, although a single Chinese species, L. crispidens, has long and short-styled flower morphs (Chen and Hu, 1979), but stamen lengths are evidently the same in the two morphs.

The purposes of the present study were to: 1) analyze the fertility of interspecific F_1 hybrids in order to elucidate the nature of reproductive barriers among species; and 2) to arrive at a better understanding of the breeding behavior of Hawaiian Lysimachia. Somewhat ancillary to this study, but also of interest, were observations of the morphology of F_1 hybrids.

4.2 Methods and Materials

4.2.1 Artificial crosses

Seeds and cuttings were collected in the field from 22 populations (Table 4.1) and grown in the greenhouse. This collection consisted of the following taxa: L. daphnoides (A. Gray) Hillebr., L. glutinosa Rock, L. hillebrandii Hook f. ex A. Gray, L. iniki Marr, L. kalalauensis Skottsb., L. maxima (R. Knuth) St. John, L. ovoidea St. John, L. remyi subsp. caliginis (St. John) Marr, L. remyi subsp. kipahuluensis (St. John) Marr, L. remyi subsp. remyi (St. John) Marr, L. remyi subsp. subherbacea (St. John) Marr, L. scopulensis Marr, L. waianaeensis St. John, and L. filifolia C.N. Forbes and Lydgate. All of these flowered except for L. daphnoides and L. scopulensis. Lysimachia iniki and L. ovoidea did not flower in time for the results to be included here.

Table 4.1. Collection localities and numbers for seeds of species of endemic Hawaiian Lysimachia used for artificial hybridizations.

Species Collection locality and voucher number.

L. glutinosa

- Kauai: Kokee, Kalua Puhi Trail, Marr 254.
 Kauai: Kokee, beside road between Kalalau and Puu O Kila Lookouts, Marr 255, 276.
 Kauai: Kokee, below Kalalau Lookout, Marr 1304.

L. kalalauensis

- Kauai: Kokee, Nualolo Valley, Marr 271, Perlman 11287.
 Kauai: Waimea Canyon, Waialae Valley, south of Waialae falls, Perlman 11659.
 Kauai: Kokee, Honopu Valley, Marr 257.
 Kauai: Kokee, Awaawapuhi Trail Marr 273.

L. filifolia

- Oahu: Koolau Mtns., Waiahole Gulch, Perlman 11149.

L. waianaeensis

- Oahu: Waianae Mtns., Makaha Valley, Kamailenunu ridge, Marr 241, 242, 244, 1297.

L. maxima

- Molokai: Pelekunu Valley, north of Ohialele, Marr 1299, 1308.

L. remyi subsp. subherbacea

- Molokai: Kamakou, south slope of Puu Kolekole, Marr 372, 374, 377.
 Molokai: Kamakou, ridge south of Onini Gulch, Marr 380.
 Molokai: Kamakou, Onini Gulch, Marr 386, 387, 388.
 Molokai: Kamakou, rim of Waikolu Valley, ridge SE of Puu Kaeo, Marr 393, 395, 399, 400.
 Molokai: Kamakou, small ridge between Kauanakakai and Kupaia Gulches, Marr 1306.

L. remyi subsp. remyi

- West Maui: near summit of Lihau, Marr 410, 415, 416, 424, 1302, 1303, 829.
 West Maui: ridge between Halepohaku and Ulaula, Marr 935.
 West Maui: Hanaula, Marr 346, 347, 350, 354, 355, 356, 357, 359, 361, 362, 363, 919.

Table 4.1. continued on next page.

Table 4.1. continued. Collection localities and numbers for seeds of species of endemic Hawaiian Lysimachia used for artificial hybridizations.

Species Collection locality and voucher number.

L. remyi subsp. kipahuluensis

West Maui: Iao Valley, upper Nakalaloa stream, Marr 432, 436.

East Maui: Haleakala, notch in ridge above and SE of Paliku Cabin, Marr 281.

East Maui: Kalapawili ridge, rim of Kipahulu Valley, Marr 282, 283, 284.

East Maui: Kalapawili ridge, above Lake Waianapanapa, Marr 285, 290, 291.

L. remyi subsp. caliginis

East Maui: Haleakala, Koolau gap at and below treeline, Marr 336, 337, 338, 343, 344.

Hybrid swarm between L. remyi subsp. kipahuluensis and L. remyi subsp. caliginis

East Maui: Haleakala, Kaupo gap, Marr 296, 308.

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Prior to anther dehiscence, the female parent was emasculated and pollen was transferred from the male parent to the receptive stigma. The manipulations included interspecific crosses and self-pollinations. Two types of self-pollinations were attempted: 1) intrafloral, i.e. pollination within a flower; 2) intraplant, i.e. pollen transferred from an older flower, to a younger emasculated one on the same plant in which the stigma was still receptive. Self-pollinations were attempted in order to determine if plants were self-incompatible.

A total of 605 crosses were attempted among the 6 species and 4 subspecies that reached the flowering stage. Whenever possible, several plants of each species were used for each hybrid combination, however for some species, e.g. L. filifolia, only one plant flowered. The number of crosses attempted per combination varied from 1 to 90 and averaged 16. Forty-five intertaxon hybrid combinations were possible but because species did not flower synchronously, only 38 combinations could be attempted. Reciprocal crosses were attempted for 24 pairs of taxa. A few crosses were attempted using an endemic Hawaiian species as the female parent and as the male parent, an indigenous species, L. mauritiana, and two Asian species, L. litchiangensis and L. barystachys. The endemic Hawaiian species of Lysimachia are believed to be most closely related to those from southeast Asia (Wagner et al., 1990). Crosses with these non-endemic species were performed in this admittedly small sample, to determine if

interfertility might be useful to elucidate the nearest ancestor of the endemic species. Vouchers of hybrids and species are deposited at UBC.

4.2.2 Pollen stainability

The fertility of parental and hybrid plants was estimated from pollen viability. Viability was evaluated by observing pollen grains that had been treated with a cytoplasm stain, cotton blue dissolved in lactophenol. A minimum of 300 pollen grains were examined after they had been treated for at least 24 hours. Those that turned dark blue were scored as viable. Those that were misshapen or that stained lightly were scored as non-viable. Whenever possible, pollen stainability was estimated for at least two flowers per plant. As a control, the fertility of pollen from herbarium specimens of plants from natural populations was also tested in order to evaluate the effect that greenhouse growing conditions might have upon pollen fertility.

Although F_2 and backcrossed plants are currently in cultivation, none have flowered. Seeds were collected on Kauai from a plant whose morphology is the same as artificially produced hybrids between L. glutinosa and L. kalalauensis. The pollen parent of these seeds was probably L. glutinosa because of the proximity of plants of this species to the hybrid. Several plants were grown from these seeds and one has flowered from which a pollen sample was taken. Pollen viability of two wild plants, that based on

morphological intermediacy are putative hybrids between L. glutinosa and L. scopulensis, was tested from herbarium specimens.

4.2.3 Vacuolar flavonoids

In a very preliminary analysis, vacuolar flavonoids were isolated and visualized using thin layer chromatography methods based on Gornall and Bohm (1980).

4.3 Results

4.3.1 Fruit-set in interspecific crosses

Percent fruit-set ranged from 0% to 100% (Table 4.2), however intermediate levels of fruit-set were more typical. Combinations that resulted in either 0% or 100% fruit-set had low sample sizes, generally fewer than four. Crosses involving L. glutinosa failed more often than crosses involving other species. Fruit-set among all other combinations was almost always greater than 50%. Sometimes there were differences between reciprocal crosses, e.g. the success rate of crosses in which L. glutinosa was the male parent was consistently higher than when it was the female parent.

Hybrids grew vigorously in the greenhouse environment, however not all hybrid combinations had flowered at the time of this writing.

4.3.2 Fruit set in self-pollinations

Observations in the greenhouse indicated that flowers were protogynous. Stigmas were receptive 2-4 days prior to anther dehiscence and there was little overlap in the time of stigma receptivity and anther dehiscence. As flowers mature, the filaments bend toward the style and actually clasp it. When the corolla falls from the plant, pollen is easily deposited on the stigma, yet in hundreds of observations of these unmanipulated, selfed flowers, fruit-set was very rare.

Table 4.2. Percent fruit-set of artificial crosses among species of endemic Hawaiian Lysimachia. Upper values of a pair are male parent (row) X female parent (column), lower values are the reciprocal cross.

		Percent Fruit-Set (number of cross attempted)						
		Species						
		2	7	8	9	11	13	15
1.	<u>L. glutinosa</u>	100(3) 85(11)	38(13) 18(17)	** 67(6)	45(11) 17(6)	33(3) 33(3)	71(38) 45(18)	71(14) 33(3)
2.	<u>L. kalalauensis</u>		83(6) **	** **	100(4) **	** **	54(24) 50(2)	60(10) **
7.	<u>L. filifolia</u>			** 0(3)	50(4) 100(1)	** 100(1)	69(23) 100(2)	66(4) 100(2)
8.	<u>L. waianaeensis</u>				** **	** **	0(2) **	100(1) **
9.	<u>L. remyi</u> subsp. <u>kipahuluensis</u> (WM)					100(2) 100(11)	64(11) 80(4)	66(3) **
11.	<u>L. remyi</u> subsp. <u>remyi</u>						50(18) 100(4)	33(3) **
13.	<u>L. remyi</u> subsp. <u>kipahuluensis</u> (EM)							70(10) 70(10)
15.	<u>L. remyi</u> subsp. <u>caliginis</u> (EM)							
16.	<u>L. remyi</u> subsp. <u>subherbacea</u>							
17.	<u>L. maxima</u>							

** cross not attempted

Table 4.2 continued on next page.

Table 4.2. continued. Percent fruit-set of artificial crosses among species of endemic Hawaiian Lysimachia. Upper values of a pair are male parent (row) X female parent (column), lower values are the reciprocal cross.

		Percent Fruit-Set (number of cross attempted)	
		Species	
		16	17
1.	<u>L. glutinosa</u>	79(43) 35(17)	100(1) 50(2)
2.	<u>L. kalalauensis</u>	89(18) 100(1)	100(1) **
7.	<u>L. filifolia</u>	79(19) 57(7)	0(1) 100(1)
8.	<u>L. waianaeensis</u>	70(10) **	** **
9.	<u>L. remyi</u> subsp. <u>kipahuluensis</u> (WM)	87(8) 82(11)	** **
11.	<u>L. remyi</u> subsp. <u>remyi</u>	86(14) 100(9)	100(1) 100(1)
13.	<u>L. remyi</u> subsp. <u>kipahuluensis</u> (EM)	74(34) 55(56)	** 0(1)
15.	<u>L. remyi</u> subsp. <u>caliginis</u> (EM)	76(13) 66(24)	** **
16.	<u>L. remyi</u> subsp. <u>subherbacea</u>		** 100(3)
17.	<u>L. maxima</u>		

** cross not attempted

In the 597 intrafloral self-pollinations that were attempted, fruit-set for all species ranged from 0% to 40% (Table 4.3). However, in the 40 intraplant attempts at self-pollination the percentage of fruit-set was markedly higher.

4.3.3 Morphology of hybrids

Hybrid plants were morphologically intermediate in characters such as the size and shape of calyx lobes, corolla lobes and leaves, the degree of pubescence and growth habit. Progeny of the same cross closely resembled each other in the characters mentioned above, with the exception of corolla pigmentation. Crosses involving species with red corollas e.g. L. filifolia or subspecies of L. remyi, and either L. glutinosa (white corolla) or L. kalalauensis (green corolla), generally produced progeny with red corollas (Figures 4.1 and 4.2), with two exceptions (Table 4.4). One of the progeny of L. glutinosa X L. remyi subsp. remyi had a white corolla, while a sibling had a red corolla (Figure 4.3). The other exception was two progeny from the cross L. remyi subsp. kipahuluensis X L. kalalauensis, both of which had greenish corollas.

Table 4.3. Percent fruit-set of self-pollinations of species of endemic Hawaiian Lysimachia, intrafloral selfs and intraplant selfs. Self-pollination that were not attempted are marked by "na".

Species	Percent Fruit-Set (nu	
	Intrafloral	Intraplant

<u>L. glutinosa</u>	19(32)	0(2)
<u>L. kalalauensis</u>	8(12)	na
<u>L. filifolia</u>	35(14)	50(2)
<u>L. waianaeensis</u>	0(5)	na
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (WM)	40(5)	100(1)
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (EM)	19(122)	75(12)
<u>L. remyi</u> subsp. <u>remyi</u>	13(184)	80(10)
<u>L. remyi</u> subsp. <u>caliginis</u>	25(24)	100(1)
<u>L. remyi</u> subsp. <u>subherbacea</u>	25(199)	75(12)



Figure 4.1. Leaves and flowers of parents and hybrid of L. filifolia (#11149.1) (female parent, left) X L. glutinosa (#255.1). Pollen stainabilities from left to right: 88%, 89%, 63%.



Figure 4.2. Leaves and flowers of the cross L. remyi subsp. remyi (#410.101) (female parent, right) X L. kalalauensis (#271.1). Pollen stainabilities from left to right: 85%, 86%, 77%.

Table 4.4. Corolla pigmentation of F₁ hybrids between species of of endemic Hawaiian Lysimachia. Only those combinations in which the parents have different colored corollas are included here.

Female parent No.	Male parent No.	Sibling No.	Corolla pigmentation
<u>L. glutinosa</u> X <u>L. kalalauensis</u>			
255.2	257.1	1	light yellow above; red base
255.2	257.1	2	light red above; red base
255.2	257.1	3	light green above; red base
<u>L. kalalauensis</u> X <u>L. glutinosa</u>			
271.1	255.2	1	mostly white with red veins
271.1	255.2	2	mostly white with red veins
271.1	255.17	1,2	red
<u>L. glutinosa</u> X <u>L. filifolia</u>			
1304.1	11149.1	1	red
<u>L. filifolia</u> X <u>L. glutinosa</u>			
11149.1	255.1	1	red
<u>L. glutinosa</u> X <u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)			
1304.1	432.1	1	red
<u>L. remyi</u> subsp. <u>kipahuluensis</u> X <u>L. glutinosa</u> (West Maui)			
436.107	255.2	1	red
<u>L. glutinosa</u> X <u>L. remyi</u> subsp. <u>kipahuluensis</u> (East Maui)			
255.2	281.7	1	red
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (East Maui) X <u>L. glutinosa</u>			
281.2	255.2	1,2	red
285.2	255.2	1,3,4,5, 6,7,8	red
<u>L. glutinosa</u> X <u>L. remyi</u> subsp. <u>remyi</u>			
255.8	359.1	1	red
255.8	359.1	2	white with red streaks
<u>L. remyi</u> subsp. <u>remyi</u> X <u>L. glutinosa</u>			
355.103	255.2	1	red
356.201	255.2	1,2	red
361.2	255.2	1,2	red

Table 4.4. continued on next page

Table 4.4. continued. Corolla pigmentation of F₁ hybrids
 between species of endemic Hawaiian Lysimachia.

Female parent No.	Male parent No.	Sibling No.	Corolla pigmentation
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L. glutinosa X L. remyi subsp. remyi

255.1	424.1	1,2	red
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254.4	1301.1	1	red
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L. remyi subsp. remyi X L. glutinosa

410.101	255.2	1,2	red
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L. glutinosa X L. remyi subsp. subherbacea

255.8	372.201	1	red
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255.2	393.5	1,2	red
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255.16	395.205	1	red
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L. remyi subsp. subherbacea X L. glutinosa

372.1	255.2	1	red
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387.202	255.2	1	red
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393.3	255.2	1,2,3,4	red
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399.4	255.2	1	red
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L. kalalauensis X L. remyi subsp. subherbacea

271.1	387.202	1	red
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L. remyi subsp. subherbacea X L. kalalauensis

400.1	271.1	1	red
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L. filifolia X L. kalalauensis

11149.1	271.1	1	red
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L. remyi subsp. subherbacea X L. kalalauensis

410.101	271.1	1,2	red
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L. remyi subsp. kipahuluensis (East Maui) X L. kalalauensis

282.4	257.1	1,2	green outside, red on inside
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282.13	257.1	1	green outside, red on inside
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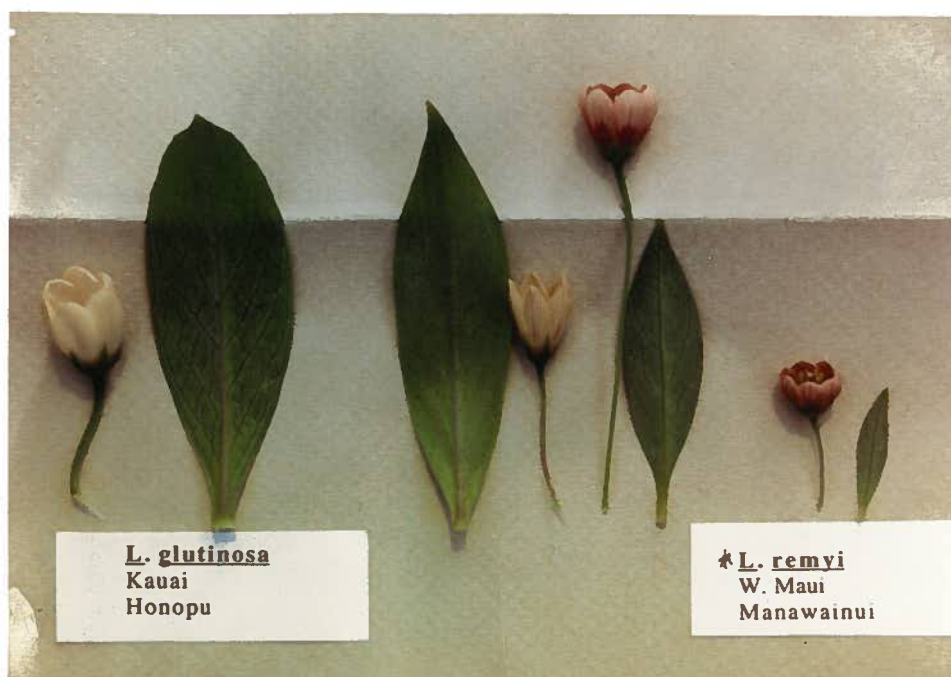


Figure 4.3. Leaves and flowers of two siblings of the cross *L. glutinosa* (#255.8) (female parent, left) X *L. remyi* subsp. *remyi* (#359.1) (formerly classified as *L. remyi*, thus the label). Pollen stainabilities from left to right: not counted, 42%, 78%, 75%.



Figure 4.4. Leaves and flowers of three siblings of the cross *L. glutinosa* (#255.2) (female parent, left) X *L. kalalauensis* (#257.1). Pollen fertilities from left to right: 78%, 75%, 83%, 66%, 73%.

Hybrids between L. glutinosa and L. kalalauensis had corollas that were neither white nor green, but instead, were either red or nearly white, though not as white as that of L. glutinosa (Table 4.4). In this small sample, it was clear that the pigmentation of the hybrid corollas differed among siblings of the same cross (Figure 4.4) as well as among progeny derived from different parents (Table 4.4).

A second character that is not expressed in an intermediate manner is "viscidness". Entire plants of L. glutinosa are very viscid, however progeny of crosses involving this species and non-viscid species, were not viscid.

Leaf size and shape of a hybrid between L. remyi subsp. caliginis from Maui and L. glutinosa from Kauai was remarkably similar to that of L. maxima from Molokai. Although it is unlikely that L. maxima is a hybrid of these species, this observation suggests that relatively minor genetic differences separate L. glutinosa, L. remyi subsp. caliginis and L. maxima, at least in terms of genes that control leaf shape.

4.3.4 Pollen stainability of species

Pollen stainability of plants grown in the greenhouse varied from 36% to 99%. The mean for each species ranged from 57-86% (Table 4.5). Data are presented for each plant in Appendix 1, Table A4.1. At least one plant from each species had pollen stainability greater than 75%. Pollen stainability

of herbarium specimens also varied. No species had consistently high or consistently low pollen stainability.

4.3.5. Pollen stainability of hybrids

Progeny of hybrids involving L. glutinosa, L. kalalauensis, L. filifolia and the three subspecies of L. remyi flowered. Pollen stainability of hybrids varied from 0-98% (Table 4.6). Data for individual hybrids and the parents involved in the cross are presented in Appendix 1, Table A4.2. Mean pollen stainabilities of crosses involving either L. glutinosa or L. kalalauensis ranged from 41-85%, however, the overall range was from 0-97%. Mean pollen stainability was generally higher (72-93%) in hybrids among L. filifolia and the subspecies of L. remyi, yet here as well, the overall range was from 9-98%. Some plants of hybrid combinations that included L. glutinosa had pollen stainabilities of less than 25%.

The progeny of the natural hybrid between L. glutinosa and L. kalalauensis, putatively backcrossed to L. glutinosa, had 5% pollen stainability. The two natural hybrids between L. glutinosa and L. scopulensis, had 6% and 17% pollen stainability.

Table 4.5. Pollen stainability in cotton blue of species of endemic Hawaiian Lysimachia.

Species	Habitat	% Pollen Stainability							
		Mean	N	Flowers	N	Plants	SD	Min	Max
<u>L. glutinosa</u>	greenhouse	77		16		9	13	55	95
<u>L. kalalauensis</u>	greenhouse	76		11		6	10	51	87
	wild	70		2		2	5	66	74
<u>L. maxima</u>	greenhouse	57		4		2	25	30	78
<u>L. ovoidea</u>	wild	68		3		3	32	32	88
<u>L. remyi</u> subsp. <u>caliginis</u>	greenhouse	73		13		9	18	37	97
	wild	64		3		3	10	52	71
<u>L. remyi</u> subsp. <u>kipahuluensis</u>	greenhouse	78		60		35	18	27	98
	wild	47		10		10	21	5	78
<u>L. remyi</u> subsp. <u>remyi</u>	greenhouse	86		86		47	11	53	98
<u>L. remyi</u> subsp. <u>subherbacea</u>	greenhouse	80		53		28	19	28	98
	wild	80		2		2	15	70	91
<u>L. filifolia</u>	greenhouse	86		6		3	6	75	94
<u>L. waianaeensis</u>	greenhouse	77		6		4	34	9	99
	wild	54		1		1	-	-	-

Table 4.6. Pollen stainability in cotton blue of F₁ hybrids between species of endemic Hawaiian Lysimachia, and between endemic species and non-endemic/non-Hawaiian species of Lysimachia.

Cross	% Pollen Stainability				
	Mean	N	Flowers	Plants	N
					SD
					Min
					Max
<u>L. glutinosa</u> / <u>L. kalalauensis</u>	56	18	7	31	0
<u>L. glutinosa</u> / <u>L. filifolia</u>	67	3	2	38	23
<u>L. glutinosa</u> / <u>L. r. subsp. caliginis</u>	62	10	3	19	23
<u>L. glutinosa</u> / <u>L. r. subsp. kipahuluensis</u>	43	21	12	18	13
<u>L. glutinosa</u> / <u>L. r. subsp. remyi</u>	52	24	12	22	18
<u>L. glutinosa</u> / <u>L. r. subsp. suberbacea</u>	42	26	12	16	20
<u>L. kalalauensis</u> / <u>L. r. subsp. kipahuluensis</u>	68	4	3	35	17
<u>L. kalalauensis</u> / <u>L. r. subsp. remyi</u>	85	4	2	10	71
<u>L. kalalauensis</u> / <u>L. r. subsp. suberbacea</u>	79	4	2	8	71
<u>L. kalalauensis</u> / <u>L. filifolia</u>	41	1	2	-	-
<u>L. r. subsp. caliginis</u> / <u>L. r. subsp. suberbacea</u>	64	23	10	21	33
<u>L. r. subsp. kipahuluensis</u> / <u>L. r. subsp. suberbacea</u>	78	35	16	15	9
<u>L. r. subsp. kipahuluensis</u> / <u>L. filifolia</u>	82	13	8	11	56
<u>L. r. subsp. remyi</u> / <u>L. r. subsp. caliginis</u>	89	3	2	2	85
<u>L. r. subsp. remyi</u> / <u>L. r. subsp. suberbacea</u>	70	34	16	18	31
<u>L. r. subsp. remyi</u> / <u>L. filifolia</u>	72	11	6	16	45
<u>L. r. subsp. suberbacea</u> / <u>L. mauritiana</u> *	48	1	3	18	28

* This must be regarded as a putative hybrid because morphologically it resembles their endemic Hawaiian parent; see text for further discussion.

The pollen stainability of putative hybrids between endemic Hawaiian species and indigenous or non-Hawaiian species was high (Table 4.6), given the differences in morphology and chromosome number (L. mauritiana has $n=10$ (Carr, 1978), L. barystachys has $n=24$ (Ko et al., 1986), no chromosomes numbers are available for L. litchiangensis. The morphology of the hybrids resembles that of the endemic Hawaiian parent. Preliminary two dimensional thin layer chromatography of foliar flavonoids indicated that L. litchiangensis and L. barystachys have similar flavonoid profiles. This profile differs from both L. mauritiana and L. remyi subsp. subherbacea. Flavonoid profiles of L. mauritiana and L. remyi subsp. subherbacea are similar to each other. Based on these results, it would appear that putative hybrids between the endemic Hawaiian species and L. litchiangensis and L. barystachys resulted either from contamination (pollen from another endemic Hawaiian species) or self pollination. Flavonoid data are equivocal concerning the veracity of the putative hybrid between L. remyi subsp. subherbacea and L. mauritiana because both species have similar profiles, therefore it is not possible to detect in the hybrid the complementary pattern that would be exhibited by a true hybrid. The wide range of pollen sizes and stainability (Figure 4.5) of the hybrid between L. remyi subsp. subherbacea and L. mauritiana was much greater than was seen in any other cross, and strengthens the case for this plant being a hybrid, not a self.

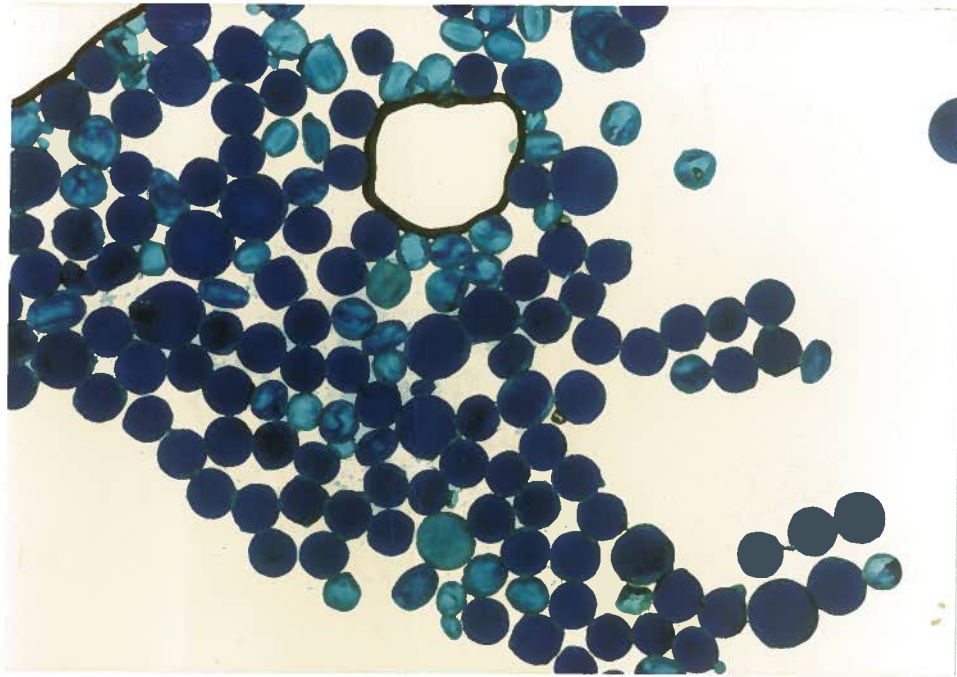


Figure 4.5. Pollen from the cross L. remyi subsp. remyi (female parent) X L. mauritiana.

4.4. Discussion

Interpretation of results presented here are tentative pending further study. It is difficult to draw conclusions from the reduced fertilities of some hybrids of Hawaiian Lysimachia because the species themselves, both in the greenhouse as well as in the natural environment, have such a wide range of fertility.

Species of Hawaiian Lysimachia had a greater range of pollen stainability as well as minimum values lower than those of the few other species that have been measured. Pollen stainabilities from other genera are as follows: nearly 100% in Bidens (Ganders and Nagata, 1984); 85-95% in Portulaca (Kim and Carr, 1990b); 77-99% in Wikstroemia (Mayer, 1991); greater than 90% in Tetramolopium (Lowrey, 1986). There are several possible reasons for reduced pollen fertility in species of Lysimachia. One could be the fact that the species, which by the high chromosome number must be polyploid, may not have fully "diploidized" genomes. A result of this could be the formation of multivalents and unequal segregation of chromosomes during meiosis. Additional possibilities include male sterility or inbreeding depression. The latter would seem to be a logical explanation because many populations are small and isolated, and although protogyny increases the likelihood of outcrossing, within plant pollination can occur. Biparental inbreeding depression is possible and in fact would seem to be likely in a species with a dry dehiscent fruit that inevitably deposits many seeds close to the maternal parent.

The values for genetic variation that were calculated from allozyme analysis (Chapter 3), indicated that many populations have levels of heterozygosity that are typical of selfing species. However, the levels of heterozygosity of other populations are typical of outcrossing species.

A somewhat surprising result, that is not expressed clearly in Tables 4.5 and 4.6, but is evident from careful study of Tables A4.1 and A4.2 in Appendix 1, is that many hybrids were more fertile than their parents. Restoration of fertility in the hybrids could be a manifestation of heterosis. This could be especially pronounced when populations of one or both parental species are experiencing significant inbreeding depression.

Mean pollen stainability is not consistent in hybrids among the four species and three subspecies tested here. Although some hybrids did have very low pollen stainability, in all combinations there was at least one plant that produced nearly 100% fertile pollen. Even in hybrids between such morphologically divergent species as L. glutinosa and L. filifolia (Figure 4.1), post-zygotic barriers to crossability are lacking. Furthermore, it is impossible to distinguish between the possible effect of chromosomal divergence between the two parents as the cause for reduced pollen fertility in the hybrid, versus inheritance in the hybrid of the factors that contributed to the reduced fertility of the parents themselves. The range of mean hybrid F_1 pollen stainability, 43-93%, is similar to that published for hybrids among species

of several other Hawaiian genera as follows: close to 100% in Bidens (Ganders and Nagata, 1984), 75-100% in Tetramolopium (Lowrey, 1986), 66-100% in Lipochaeta (Rabakonanadrianina, 1980), 70-99% in Wikstroemia (Mayer, 1991), and 44-97% for Portulaca (Kim and Carr, 1990a). Pollen stainability of hybrids among Wilkesia, Dubautia and Argyroxiphium (genera of the monophyletic "silversword alliance") ranges from 9-99% (Carr, 1985a). Wherever interspecific hybrids had reduced pollen stainability, cytogenetic evidence implicated aneuploidy and/or reciprocal translocations as the cause of disruption of meiosis leading to reduced pollen stainability in these plants (Carr, 1985a).

The relatively high fertility of the hybrid between an endemic Hawaiian species and L. mauritiana and the similarity of their flavonoid profiles in the preliminary analysis could indicate a closer relationship between these species than was anticipated. Plants of a backcross between the F₁ of L. remyi subsp. remyi X L. mauritiana crossed with the L. remyi subsp. remyi parent, have been produced. Lysimachia mauritiana could theoretically have been the ancestor of the endemic species, however it is markedly different in a number of morphological characters as well as chromosome number. Analysis of DNA could provide further insights into the possible relationships between the endemic species and L. mauritiana as well as with Asian species.

The reduced fruit-set of intrafloral pollinations appears to be the result of protogyny, rather than genetically

controlled self-incompatibility. However, more intraplant crosses need to be attempted to verify this for several species. If the ancestor of the Hawaiian species could be identified, it might be possible to determine whether or not protogyny evolved *in situ* and was selected for as a means of promoting outcrossing.

It is interesting to consider the genetics of corolla pigmentation. All but one cross between L. glutinosa and the red flowered species, produced red corollas (Table 4.4), suggesting that red is dominant to white. The first plant to bloom of the cross L. glutinosa (white corolla) X L. kalalauensis (green), had a red corolla. This lead to the hypothesis that red was the ancestral color at least of the extant Hawaiian species and that at two different points in the biosynthetic pathway for the production of red pigments, there were mutations resulting in the green flowers in one lineage and white ones in the other lineage. Although possible outgroups for the Hawaiian species have white or yellow corollas (some of the yellow flowered species have red at the base of the corolla), from a biosynthetic standpoint, it is more parsimonious to hypothesize that the ancestor of the Hawaiian species arrived with the ability to biosynthesize anthocyanins, rather than *de novo* evolution of the necessary enzymes once the lineage became established in Hawaii. If one assumes that white and green corollas result from fixation as homozygous recessives, but at different loci, then when the two are crossed, the hybrid is heterozygous at each locus and

red is restored. Complicating these hypotheses somewhat is the fact that later crosses between L. glutinosa and L. kalalauensis produced light green, almost white corollas, though not as white as L. glutinosa. This suggests that epistasis may be involved. In addition, there are the red and the white flowered siblings of the cross of L. glutinosa X L. remyi subsp. remyi to consider. Plants with white flowers have not been collected from populations of red-flowered species, suggesting that the "allele" for white is missing. If white is expressed only as a homozygous recessive, and red is dominant to white, then progeny between L. glutinosa and red-flowered species should always be red. It is possible that the "allele" for white is present, but very rare in populations of red-flowered species. It would appear that the inheritance of corolla color in Lysimachia is complex, requiring further, and extensive studies to dissect its various components. Progeny analysis of F₂'s and backcrosses could provide necessary information to interpret the genetic control of corolla pigmentation.

4.4.2. Summary

Although not all species could be included in the crossing program, it appears that speciation in Hawaiian Lysimachia has not been accompanied by pre-zygotic or post-zygotic reproduction isolation. Geographical separation alone appears to be the only means of pre-zygotic reproductive isolation as even morphologically distinct species from

different islands and different ecosystems could be crossed to produce fertile seed. Interspecific hybrid fertility suggests that post-zygotic reproductive isolation is also absent. Observations of meiosis in hybrids, the fertility of F_2 hybrids and the inclusion of more species into the crossing program are needed to test these hypotheses further.

Chapter 5

Summary

5.1 Dissertation findings

The objectives of this dissertation were to produce a taxonomic revision, to estimate the degree of genetic divergence that has accompanied adaptive radiation of the endemic Hawaiian Lysimachia, and to determine if species are isolated by post- or pre-zygotic reproductive barriers. The 16 species and 4 subspecies of Lysimachia recognized in the taxonomic treatment places the genus within the 20 most speciose groups in Hawaii. The discovery of three previously unknown species, since 1987, suggests that more species remain to be found in areas previously unvisited by botanists.

The extensive morphological variation of Lysimachia, with little, if any, allozyme divergence among species, is similar to that seen in other studies of Hawaiian congeners. As is the fact that geographical isolation alone appears to be an adequate isolating mechanism, because pre-zygotic and post-zygotic isolating mechanisms appear to be lacking.

Interisland and intransisland founder events perhaps beginning on Kauai, or on an older island, to progressively younger islands, has resulted in taxa that are morphologically distinct, are usually restricted to a single island and occupy narrow ecological niches. Following each dispersal event, ecological and morphological divergence from the ancestral population is potentially relatively rapid because in small populations the effects of random genetic drift will be

maximized and there is a greater potential for novel genetic recombinations to become fixed.

A combination of factors may have contributed to the fact that morphological divergence among species is greater than measures of genetic divergence (i.e. genetic identities and hybrid fertility), tend to indicate. Among these are : 1) the low genetic diversity of the initial founding population in Hawaii; 2) random genetic drift in populations that are small; 3) the relatively slow rate of accumulation of mutations at allozyme loci in comparison to loci that control morphological characters.

The present study did not attempt to identify the likely progenitor of the endemic Hawaiian Lysimachia. Preliminary investigation of flavonoid composition indicated that L. mauritiana has a similar suite of compounds to the endemic species and there is little if any overlap in the flavonoid profiles of the endemic Hawaiian species and two Asian species.

5.2 Areas for future research

With a thorough taxonomic revision now in place, a number of interesting questions can now be addressed. Among these are the following:

- 1) Use tools such as flavonoids or DNA sequences to attempt to identify the nearest relative of the endemic Hawaiian Lysimachia.

2) Use the greenhouse collection to investigate the types of physiological adaptations that have accompanied speciation and the genetic basis for morphological divergence among species and other aspects of divergence.

3) Chromosome counts of all species, observations of meiosis in hybrids, and estimates of the fertility of backcrosses and F_2 plants would be useful to confirm that post-zygotic isolating mechanisms are lacking.

4) Investigate the degree to which speciation has resulted in divergence in floral fragrances and pollinator specificity.

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Appendix 1

Table A3.1. Table of allele frequencies of Hawaiian *Lysimachia* populations. See Table 3.1 for population codes.

Locus/ allele(N)	Population							
	DBIGB	DSECO	GKALR	GKALL	GHONO	KHONO	KAAPU	KMAKA
TPI-1 (29)	(10)	(20)	(17)	(30)	(29)	(29)	(10)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.950	0.793	0.550
c	0.000	0.000	0.000	0.000	0.000	0.050	0.207	0.450
TPI-2 (25)	(9)	(20)	(17)	(30)	(28)	(29)	(10)	
a	0.120	0.000	0.000	0.235	0.133	0.000	0.000	0.000
b	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.800	1.000	1.000	0.676	0.833	0.946	0.828	1.000
d	0.040	0.000	0.000	0.088	0.033	0.054	0.172	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-3 (25)	(9)	(20)	(17)	(30)	(28)	(29)	(10)	
a	0.140	0.167	0.000	0.000	0.133	0.000	0.000	0.000
b	0.860	0.833	1.000	1.000	0.867	1.000	1.000	1.000
ADH-1 (22)	(9)	(20)	(19)	(30)	(16)	(13)	(9)	
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (16)	(7)	(20)	(18)	(25)	(29)	(28)	(10)	
a	0.000	0.000	0.375	0.083	0.040	0.000	0.000	0.000
b	0.125	0.000	0.275	0.194	0.160	0.310	0.036	0.000
c	0.875	1.000	0.000	0.028	0.140	0.638	0.962	1.000
d	0.000	0.000	0.350	0.694	0.660	0.052	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (27)	(9)	(19)	(17)	(30)	(30)	(28)	(9)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	1.000	1.000	1.000	1.000	1.000	0.964	1.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
MDH-4 (27)	(9)	(19)	(17)	(30)	(30)	(28)	(9)	
a	0.018	0.056	0.000	0.000	0.000	0.000	0.089	0.000
b	0.981	0.944	1.000	1.000	1.000	1.000	0.911	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKD-1 (20)	(4)	(9)	(17)	(29)	(19)	(16)	(9)	
a	0.000	0.000	0.000	0.088	0.000	0.131	0.000	0.000
b	1.000	1.000	1.000	0.912	1.000	0.868	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian
Lysimachia populations.

Locus/
 allele(N)

		Population						
		DBIGB	DSECO	GKALR	GKALL	GHONO	KHONO	KAAPU
								KMAKA
DIA-1		(25)	(5)	(20)	(15)	(30)	(14)	(24)
a		0.000	0.000	0.000	0.000	0.000	0.000	0.000
b		1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-1		(28)	(8)	(20)	(19)	(21)	(27)	(22)
a		0.053	0.000	0.000	0.000	0.000	0.000	0.000
b		0.053	0.000	0.000	0.000	0.000	0.000	0.000
c		0.172	0.187	0.000	0.079	0.071	0.130	0.182
d		0.689	0.500	1.000	0.921	0.929	0.759	0.545
e		0.000	0.312	0.000	0.000	0.000	0.111	0.273
f		0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGI-1		(27)	(9)	(20)	(19)	(27)	(26)	(29)
a		0.923	0.889	1.000	1.000	1.000	0.000	0.000
b		0.000	0.000	0.000	0.000	0.000	1.000	1.000
c		0.000	0.000	0.000	0.000	0.000	0.000	0.000
d		0.074	0.111	0.000	0.000	0.000	0.000	0.000

Table A3.1. continued on next page

Table A3.1. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/ allele(N)	Population							
	KWAIA	OLIMA	OWAIN	NSPTR	FKONE	FKTHR	FKFIV	FOTWO
TPI-1 (29)	(9)	(47)	(7)	(13)	(7)	(6)	(26)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.948	0.889	1.000	0.214	1.000	1.000	1.000	1.000
c	0.052	0.111	0.000	0.786	0.000	0.000	0.000	0.000
TPI-2 (28)	(8)	(43)	(7)	(12)	(6)	(5)	(26)	
a	0.000	0.250	0.035	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	0.750	0.965	1.000	0.917	1.000	1.000	0.846
d	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
TPI-3 (28)	(8)	(43)	(7)	(12)	(6)	(5)	(26)	
a	0.036	0.250	0.314	0.000	0.083	0.250	0.000	0.019
b	0.964	0.750	0.686	1.000	0.917	0.750	1.000	0.981
ADH-1 (20)	(9)	(32)	(30)	(9)	(6)	(5)	(21)	
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (29)	(9)	(47)	(8)	(12)	(7)	(6)	(27)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.444	0.681	0.125	1.000	1.000	1.000	0.000
c	0.931	0.556	0.319	0.875	0.000	0.000	0.000	1.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
e	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (28)	(9)	(44)	(8)	(13)	(7)	(6)	(26)	
a	0.357	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000
c	0.643	1.000	0.966	1.000	1.000	1.000	1.000	1.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-4 (28)	(9)	(35)	(8)	(13)	(7)	(6)	(26)	
a	0.161	0.000	0.000	0.000	0.115	0.143	0.000	0.000
b	0.839	1.000	0.714	1.000	0.885	0.857	1.000	1.000
c	0.000	0.000	0.286	0.000	0.000	0.000	0.000	0.000
SKD-1 (15)	(9)	(2)	(7)	(10)	(6)	(3)	(6)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian
Lysimachia populations.

Locus/
 allele(N)

		Population							
		KWAIA	OLIMA	OWAIN	NSPTR	FKONE	FKTHR	FKFIV	FOTWO
DIA-1	(10)	(7)	(31)	(8)	(14)	(7)	(5)	(16)	
a	0.000	0.000	0.000	0.312	0.000	0.000	0.000	0.000	
b	1.000	1.000	1.000	0.688	1.000	1.000	1.000	1.000	
PGM-1	(22)	(8)	(45)	(4)	(13)	(6)	(3)	(27)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
d	0.750	0.187	0.000	0.750	0.808	1.000	1.000	0.870	
e	0.250	0.813	0.878	0.250	0.192	0.000	0.000	0.130	
f	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.000	
PGI-1	(10)	(7)	(45)	(8)	(13)	(7)	(6)	(13)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table A3.1. continued on next page

Table A3.1. Table of allele frequencies of Hawaiian *Lysimachia* populations.

Locus/ allele(N)	Population							
	FOTHR	HPUKE	HPUKS	HWAIK	HLUAF	HLUAS	WMBLG	WMLIM
TPI-1 (23)	(30)	(6)	(24)	(28)	(12)	(13)	(11)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091
b	1.000	0.900	1.000	0.958	1.000	1.000	0.961	0.909
c	0.000	0.100	0.000	0.042	0.000	0.000	0.038	0.000
TPI-2 (23)	(29)	(6)	(25)	(17)	(11)	(11)	(12)	
a	0.000	0.569	0.000	0.700	0.000	0.364	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.609	0.293	0.583	0.300	0.912	0.454	1.000	0.792
d	0.130	0.017	0.000	0.000	0.088	0.182	0.000	0.000
e	0.261	0.121	0.417	0.000	0.000	0.000	0.000	0.208
TPI-3 (23)	(29)	(6)	(25)	(17)	(11)	(11)	(12)	
a	0.283	0.172	0.250	0.060	0.029	0.000	0.091	0.042
b	0.717	0.828	0.750	0.940	0.971	1.000	0.909	0.958
ADH-1 (23)	(30)	(6)	(23)	(15)	(12)	(12)	(6)	
a	1.000	0.883	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.117	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (23)	(30)	(6)	(24)	(22)	(11)	(13)	(12)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.783	0.417	0.646	0.367	0.318	0.269	0.000
c	1.000	0.217	0.583	0.334	0.636	0.409	0.731	1.000
d	0.000	0.000	0.000	0.000	0.000	0.273	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (22)	(30)	(6)	(24)	(27)	(11)	(13)	(11)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	0.600	0.334	0.750	0.963	1.000	1.000	1.000
d	0.000	0.400	0.667	0.250	0.037	0.000	0.000	0.000
MDH-4 (22)	(30)	(6)	(24)	(27)	(11)	(13)	(11)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKD-1 (20)	(22)	(6)	(24)	(26)	(12)	(7)	(17)	
a	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	0.977	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/
 allele(N)

		Population							
		FOTHR	HPUKE	HPUKS	HWAIK	HLUAF	HLUAS	WMBLG	WMLIM
DIA-1	(12)	(21)	(5)	(18)	(19)	(12)	(9)	(10)	
a	0.000	0.000	0.000	0.000	0.389	0.000	0.000	0.000	
b	1.000	1.000	1.000	1.000	0.611	1.000	1.000	1.000	
PGM-1	(21)	(28)	(6)	(20)	(27)	(7)	(9)	(9)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
d	1.000	0.393	0.917	0.725	0.889	0.500	0.833	1.000	
e	0.000	0.607	0.083	0.275	0.111	0.500	0.167	0.000	
f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
PGI-1	(23)	(30)	(5)	(24)	(28)	(12)	(11)	(12)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table A3.1. continued on next page

Table A3.1. Table of allele frequencies of Hawaiian *Lysimachia* populations.

Locus/ allele(N)	Population							
	WMMN2	WMMPP	WMHPS	WMHPL	WMHPY	WMLIS	WMLIL	WMHEL
TPI-1 (29)	(30)	(26)	(40)	(13)	(61)	(18)	(50)	
a	0.000	0.000	0.057	0.000	0.000	0.000	0.000	0.000
b	0.893	0.933	0.904	0.950	0.577	0.926	0.972	0.990
c	0.107	0.067	0.038	0.050	0.423	0.074	0.028	0.010
TPI-2 (27)	(29)	(24)	(38)	(10)	(60)	(18)	(33)	
a	0.018	0.000	0.000	0.000	0.000	0.017	0.056	0.030
b	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000
c	0.870	0.931	0.938	0.881	0.950	0.883	0.722	0.818
d	0.018	0.000	0.062	0.026	0.050	0.008	0.028	0.182
e	0.092	0.069	0.000	0.066	0.000	0.092	0.194	0.000
TPI-3 (27)	(29)	(24)	(38)	(10)	(60)	(18)	(33)	
a	0.074	0.172	0.125	0.013	0.050	0.108	0.194	0.015
b	0.926	0.828	0.875	0.987	0.950	0.892	0.806	0.985
ADH-1 (27)	(24)	(15)	(29)	(4)	(53)	(11)	(21)	
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (29)	(30)	(29)	(40)	(11)	(59)	(18)	(50)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.034	0.217	0.000	0.000	0.000	0.025	0.139	0.010
c	0.948	0.783	1.000	0.988	1.000	0.975	0.861	0.990
d	0.017	0.000	0.000	0.012	0.000	0.000	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (28)	(30)	(26)	(28)	(11)	(55)	(17)	(48)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.232	0.067	0.231	0.125	0.000	0.127	0.294	0.396
c	0.750	0.917	0.769	0.875	0.954	0.836	0.706	0.604
d	0.018	0.017	0.000	0.000	0.045	0.036	0.000	0.000
MDH-4 (30)	(30)	(26)	(28)	(11)	(55)	(17)	(48)	
a	0.000	0.000	0.000	0.000	0.000	0.036	0.029	0.000
b	1.000	1.000	1.000	1.000	1.000	0.964	0.971	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKD-1 (27)	(26)	(24)	(9)	(11)	(44)	(8)	(39)	
a	0.092	0.000	0.000	0.111	0.000	0.079	0.000	0.051
b	0.870	1.000	1.000	0.889	1.000	0.920	1.000	0.923
c	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.026

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/
 allele(N)

		Population							
		WMMN2	WMMPP	WMHPS	WMHPL	WMHPY	WMLIS	WMLIL	WMHEL
DIA-1	(30)	(21)	(30)	(26)	(6)	(57)	(16)	(40)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
PGM-1	(25)	(22)	(24)	(23)	(9)	(46)	(16)	(43)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
c	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	
d	0.620	0.636	1.000	0.652	0.778	0.663	1.000	0.698	
e	0.380	0.364	0.000	0.348	0.222	0.283	0.000	0.302	
f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
PGI-1	(30)	(28)	(23)	(21)	(13)	(50)	(20)	(50)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	1.000	1.000	0.956	1.000	1.000	1.000	1.000	1.000	
c	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table A3.1. continued on next page

Table A3.1. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/ allele(N)	Population							
	EMPAL	EMHPA	EMLWA	EMKPR	EMKAW	EMKAE	EMWAI	EMKOT
TPI-1 (28)	(28)	(31)	(30)	(17)	(30)	(30)	(32)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-2 (27)	(17)	(30)	(30)	(16)	(26)	(30)	(32)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.944	1.000	0.950	0.967	0.969	0.981	0.950	1.000
d	0.000	0.000	0.000	0.033	0.031	0.019	0.000	0.000
e	0.056	0.000	0.050	0.000	0.000	0.000	0.050	0.000
TPI-3 (27)	(17)	(30)	(30)	(16)	(26)	(30)	(32)	
a	0.111	0.000	0.233	0.083	0.125	0.058	0.033	0.000
b	0.889	1.000	0.767	0.917	0.875	0.942	0.967	1.000
ADH-1 (21)	(16)	(21)	(24)	(11)	(24)	(27)	(24)	
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (26)	(29)	(27)	(29)	(17)	(30)	(27)	(29)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (28)	(28)	(29)	(29)	(17)	(30)	(30)	(31)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.964	0.946	0.759	0.845	0.941	0.700	0.950	1.000
c	0.054	0.036	0.241	0.155	0.059	0.300	0.050	0.000
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-4 (28)	(28)	(29)	(29)	(17)	(30)	(30)	(31)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKD-1 (21)	(20)	(25)	(25)	(7)	(29)	(19)	(28)	
a	0.000	0.000	0.000	0.000	0.000	0.155	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.845	0.789	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.000

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/
 allele(N)

		Population							
		EMPAL	EMHPA	EMLWA	EMKPR	EMKAW	EMKAE	EMWAI	EMKOT
DIA-1	(29)	(18)	(30)	(9)	(7)	(29)	(27)	(20)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
PGM-1	(26)	(24)	(22)	(29)	(16)	(30)	(30)	(30)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
d	0.673	1.000	0.727	0.759	0.938	0.600	1.000	0.833	
e	0.327	0.000	0.273	0.241	0.062	0.400	0.000	0.167	
f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
PGI-1	(28)	(28)	(30)	(30)	(9)	(29)	(30)	(32)	
a	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	
b	1.000	1.000	1.000	1.000	1.000	0.966	1.000	0.969	
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table A3.1. continued on next page

Table A3.1. Table of allele frequencies of Hawaiian Lysimachia populations.

Locus/ allele(N)	Population							
	EMKOB	EMKIP	MWAIK	MKOLE	MONIN	MMAKA	MMAXI	MKAWE
TPI-1 (8)	(23)	(29)	(27)	(27)	(30)	(30)	(20)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-2 (8)	(22)	(29)	(26)	(26)	(24)	(30)	(20)	
a	0.000	0.000	0.069	0.000	0.000	0.125	0.033	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	1.000	0.724	0.904	0.692	0.479	0.433	0.725
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
e	0.000	0.000	0.207	0.096	0.308	0.396	0.533	0.275
TPI-3 (8)	(22)	(29)	(26)	(26)	(24)	(30)	(20)	
a	0.000	0.023	0.362	0.769	0.250	0.583	0.467	0.450
b	1.000	0.977	0.638	0.231	0.750	0.417	0.533	0.550
ADH-1 (7)	(19)	(20)	(3)	(24)	(9)	(19)	(20)	
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1 (10)	(23)	(28)	(27)	(26)	(35)	(29)	(22)	
a	0.000	0.000	0.071	0.370	0.019	0.100	0.000	0.364
b	0.000	0.000	0.393	0.315	0.519	0.671	0.000	0.273
c	1.000	1.000	0.536	0.315	0.461	0.228	1.000	0.364
d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3 (10)	(24)	(25)	(27)	(24)	(26)	(28)	(23)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.950	0.917	0.180	0.000	0.375	0.154	0.250	0.000
c	0.050	0.083	0.820	1.000	0.625	0.827	0.750	0.913
d	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.087
MDH-4 (10)	(19)	(25)	(27)	(24)	(26)	(27)	(23)	
a	0.000	0.000	0.180	0.056	0.000	0.000	0.056	0.283
b	1.000	1.000	0.820	0.944	1.000	1.000	0.944	0.717
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKD-1 (10)	(25)	(30)	(13)	(6)	(24)	(27)	(15)	
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table A3.1. continued on next page

Table A3.1. continued. Table of allele frequencies of Hawaiian *Lysimachia* populations.

[illegible]

Table A4.1. Pollen stainability in cotton blue of Hawaiian Lysimachia species grown in the greenhouse. See Table 4.1 for collection localities. Plant numbers are Marr collection numbers unless denoted by "#", these are S. Perlman collections. Number to left of decimal of plant number is the collection number, number to right is sibling number.

Species	Plant No.	Mean	N	SD	% Pollen Stainability	Min	Max

<u>L. glutinosa</u>							
	254.1	82	1				
	255.1	63	3	10		54	75
	255.2	78	3	6		72	86
	255.7	85	2	7		79	90
	255.18	56	1				
	276.1	94	2	1		93	94
	1304.1	78	1				
	1304.2	87	1				
	1304.3	83	1				
<u>L. kalalauensis</u>							
	11287.1#	71	2	1		70	72
	11659.1#	87	1				
	257.1	73	4	3		70	79
	271.1	85	1				
	271.6	84	2	3		81	86
	273.1	50	1				
<u>L. filifolia</u>							
	11149.1#	88	3	4		86	93
	11149.2#	74	1				
	11149.4#	87	2	3		85	90
<u>L. waianaeensis</u>							
	241.1	77	1				
	242.1	99	1				
	244.2	9	1				
	1297.1	87	2	1		86	88
<u>L. maxima</u>							
	1299.1	35	2	7		30	40
	1308.1	77	1				
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)							
	432.1	85	3	12		72	96
	436.102	84	1				
	436.104	85	2	3		83	87
	436.107	55	1				
	436.108	89	2	0		89	90
	436.201	84	2	1		83	85

Table A4.1. continued on next page.

Table A4.1. continued. Pollen stainability in cotton blue of
 Hawaiian Lysimachia species.

Species	% Pollen Stainability				
	Plant No.	Mean	N	SD	Min Max

<u>L. remyi</u> subsp. <u>subherbacea</u>					
	372.201	55	1		
	374.2	66	1		
	377.2	46	1		
	377.3	38	2	9	31 44
	380.2	59	2	3	57 62
	386.2	97	1		
	386.3	97	2	0	97 98
	387.201	97	2	0	97 97
	387.202	95	2	2	94 97
	387.203	86	1		
	388.2	76	4	11	62 87
	388.3	81	1		
	388.4	89	2	8	83 95
	393.4	97	1		
	393.5	94	2	4	91 97
	393.6	85	2	8	79 92
	393.7	55	1		
	395.106	53	1		
	395.201	95	1		
	395.202	90	2	6	85 94
	395.205	96	2	0	95 97
	399.2	92	1		
	399.3	88	2	1	87 89
	399.4	93	1		
	399.8	92	2	5	89 96
	400.1	94	2	2	92 95
	1306.4	79	2	7	74 85
	1306.7	94	1		

Table A4.1. continued on next page.

Table A4.1. continued. Pollen stainability in cotton blue of
 Hawaiian Lysimachia species.

Species Plant No.	% Pollen Stainability				
	Mean	N	SD	Min	Max

<u>L. remyi</u> subsp. <u>remyi</u>					
346.1	82	2	5	78	86
346.2	77	1			
346.101	78	2	18	65	91
346.102	91	1			
346.105	93	2	4	89	96
347.1	81	2	17	69	94
347.4	87	2	4	84	90
363.1	82	1			
350.1	94	2	0	93	94
350.2	72	4	10	64	88
350.3	93	1			
354.1	95	1			
354.5	94	2	1	93	95
355.103	78	3	14	68	95
355.201	96	2	1	95	97
356.201	85	2	3	83	88
356.202	67	1			
357.1	83	1			
357.2	91	1			
357.3	89	1			
359.1	75	3	5	69	81
361.2	68	4	5	65	75
361.6	69	1			
362.1	86	1			
362.2	91	2	0	91	92
362.3	80	2	17	68	93
362.5	90	2	1	89	91
362.6	95	2	2	93	97
362.7	76	2	8	70	82
362.8	89	2	1	88	90
410.101	86	1			
411.3	96	1			
411.4	96	3	0	96	97
411.5	94	1			
411.20	88	1			
413.2	74	3	18	53	89
415.2	90	1			
416.1	97	1			
416.2	94	2	3	92	96
424.1	97	1			
829.1	90	1			
888.1	95	1			
919.1	92	1			

Table A4.1. continued on next page.

Table A4.1. continued. Pollen stainability in cotton blue of
 Hawaiian Lysimachia species.

Species	% Pollen Stainability				
	Plant No.	Mean	N	SD	Min Max

<u>L. remyi</u> subsp. <u>remyi</u> (continued)					
	935.1	98	1		
	935.2	87	1		
	1302.1	80	1		
	1303.1	88	1		
<u>L. remyi</u> subsp. <u>caliginis</u>					
	336.1	36	1		
	337.2	92	2	1	91 93
	338.1	57	2	9	50 64
	338.5	80	3	13	72 96
	343.29	95	1		
	343.31	80	1		
	344.2	57	1		
	344.4	70	1		
	344.6	67	1		
<u>L. remyi</u> subsp. <u>kipahuluensis</u> (East Maui)					
	281.7	57	2	23	41 73
	281.9	83	3	4	79 87
	281.15	97	1		
	281.23	92	2	2	90 93
	282.1	46	1		
	282.3	96	1		
	282.4	84	2	4	81 87
	282.5	88	3	5	85 94
	282.6	93	1		
	282.7	53	1		
	282.9	90	1		
	282.10	97	1		
	282.11	84	1		
	282.12	96	1		
	283.3	88	2	3	86 91
	283.7	81	1		
	284.4	57	3	4	52 61
	284.6	27	1		
	285.1	96	1		
	285.2	71	4	9	62 82
	285.3	77	3	4	72 82
	285.4	78	1		
	285.5	84	1		
	290.4	82	1		
	291.1	88	3	3	84 91
	291.3	34	3	3	30 37
	291.4	89	1		
	291.201	91	2	1	90 93

Table A4.1. continued on next page.

Table A4.1. continued. Pollen stainability in cotton blue of
 Hawaiian Lysimachia species.

Species	% Pollen Stainability				
	Plant No.	Mean	N	SD	Min Max

Plants from hybrid swarm between <u>L. remyi</u> subsp. <u>kipahuluensis</u> and <u>L. remyi</u> subsp. <u>caliginis</u>					
	296.2	90	2	2	89 92
	308.5	75	2	0	74 75

Table A4.2. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability				
			Mean	N	SD	Min	Max
<u>L. glutinosa</u> X <u>L. kalalauensis</u>							
255.2	257.1	1	75	4	11	59	85
255.2	257.1	2	83	3	9	62	97
255.2	257.1	3	66	2	10	58	73
<u>L. kalalauensis</u> X <u>L. glutinosa</u>							
271.1	255.2	1	0	1			
271.1	255.2	3	18	2	5	7	29
271.1	255.17	1	29	4	2	27	32
271.1	255.17	2	90	2	3	87	92
<u>L. glutinosa</u> X <u>L. filifolia</u>							
1304.1	11149.1	1	23	1			
<u>L. filifolia</u> X <u>L. glutinosa</u>							
11149.1	255.1	1	89	2	1	88	90
<u>L. glutinosa</u> X <u>L. remyi</u> subsp. <u>kipahuluensis</u> (West Maui)							
1304.1	432.1	1	20	1			
<u>L. r.</u> subsp. <u>kipahuluensis</u> (West Maui) X <u>L. glutinosa</u>							
436.107	255.2	1	13	1			
<u>L. glutinosa</u> X <u>L. r.</u> subsp. <u>kipahuluensis</u> (East Maui)							
255.2	281.7	1	48	2	18	35	60
<u>L. r.</u> subsp. <u>kipahuluensis</u> (East Maui) X <u>L. glutinosa</u>							
281.2	255.2	1	29	1			
281.2	255.2	2	72	1			
285.2	255.2	1	73	2	5	69	77
285.2	255.2	3	38	1			
285.2	255.2	4	39	3	8	34	48
285.2	255.2	5	45	1			
285.2	255.2	6	54	2	19	41	67
285.2	255.2	7	32	2	12	24	41
285.2	255.2	8	44	3	8	35	49

Table A4.2. continued on next page.

Table A4.2. continued. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability				
			Mean	N	SD	Min	Max
<u>L. glutinosa</u> X <u>L. r. subsp. remyi</u>							
255.8	359.1	1	78	3	10	68	88
255.8	359.1	2	42	3	4	37	44
255.1	424.1	1	64	4	34	28	97
255.1	424.1	2	38	1			
254.4	1301.1	1	57	3	22	32	75
<u>L. r. subsp. remyi</u> X <u>L. glutinosa</u>							
355.103	255.2	1	70	1			
356.201	255.2	1	19	2	3	18	21
356.201	255.2	2	37	1			
361.2	255.2	1	24	1			
361.2	255.2	2	61	2	7	56	66
410.101	255.2	1	51	1			
410.101	255.2	2	46	2	-	46	46
<u>L. glutinosa</u> X <u>L. r. subsp. subherbacea</u>							
255.8	372.201	1	78	1			
255.2	393.5	1	42	3	3	40	45
255.2	393.5	2	38	3	5	35	43
255.16	395.205	1	24	2	1	24	25
<u>L. r. subsp. subherbacea</u> X <u>L. glutinosa</u>							
372.1	255.2	1	26	1			
387.202	255.2	1	66	3	20	53	89
393.3	255.2	1	29	4	7	20	37
393.3	255.2	2	51	3	9	41	57
393.3	255.2	3	42	3	2	41	44
393.3	255.2	4	46	1			
393.5	255.2	4	38	1			
399.4	255.2	1	37	1			
<u>L. r. subsp. caliginis</u> X <u>L. glutinosa</u>							
338.1	255.2	1	71	2	4	68	73
343.17	255.2	1	71	4	20	48	93
343.17	255.2	2	57	3	7	51	64
<u>L. kalalauensis</u> X <u>L. r. subsp. subherbacea</u>							
271.1	387.202	1	82	3	7	78	89
<u>L. r. subsp. subherbacea</u> X <u>L. kalalauensis</u>							
400.1	271.1	2	71	1			

Table A4.2. continued on next page.

Table A4.2. continued. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability				
			Mean	N	SD	Min	Max

<u>L. filifolia</u> X <u>L. kalalauensis</u>							
11149.1	271.1	1	41	1			
<u>L. filifolia</u> X <u>L. r. subsp. kiahuluensis</u> (East Maui)							
11149.1	290.4	1	56	1			
11149.1	290.4	2	81	2	10	74	89
<u>L. r. subsp. kiahuluensis</u> (East Maui) X <u>L. filifolia</u>							
285.2	11149.1	1	77	3	5	72	82
285.2	11149.1	2	77	1			
<u>L. r. subsp. kiahuluensis</u> (West Maui) X <u>L. filifolia</u>							
436.108	11149.1	1	92	2	5	88	95
436.108	11149.1	2	87	2	2	85	89
436.201	11149.1	1	95	1			
<u>L. r. subsp. kiahuluensis</u> (West Maui) X <u>L. r. subsp. remyi</u>							
436.101	362.1	1	83	1			
<u>L. r. subsp. remyi</u> X <u>L. r. subsp. kiahuluensis</u> (West Maui)							
362.1	436.201	1	73	2	11	65	81
<u>L. r. subsp. kiahuluensis</u> (West Maui) X <u>L. r. subsp. subherbacea</u>							
436.101	399.6	1	63	2	15	53	74
<u>L. r. subsp. remyi</u> X <u>L. kalalauensis</u>							
410.101	271.1	1	77	2	9	71	84
410.101	271.1	2	93	2	2	91	94
<u>L. r. subsp. remyi</u> X <u>L. filifolia</u>							
355.103	11149.1	1	58	3	13	45	70
355.103	11149.1	2	94	1			
355.201	11149.1	1	57	1			
355.1	11149.1	1	79	1			
361.2	11149.1	1	78	4	15	63	93
361.2	11149.1	3	67	1			

Table A4.2. continued on next page.

Table A4.2. continued. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability			
			Mean	N	SD	Min Max

<u>L. r. subsp. kipaaluensis</u> (East Maui) X <u>L. kalalauensis</u>						
282.4	257.1	1	94	1		
282.4	257.1	2	80	2	11	72 87
282.13	257.1	1	17	1		

<u>L. r. subsp. remyi</u> X <u>L. r. subsp. subherbacea</u>						
362.1	377.1	1	84	2	2	83 85
362.1	377.1	2	58	1		
362.7	377.1	1	31	1		
362.8	387.202	1	78	1		
361.2	395.201	1	53	2	11	45 61
361.2	395.202	1	44	2	16	32 55
1302.1	393.5		79	1		

<u>L. r. subsp. kipaaluensis</u> (East Maui) X <u>L. r. subsp. remyi</u>						
284.6	350.2	1	85	1		
282.4	359.1	1	82	1		
282.4	362.1	1	85	4	7	74 91
282.4	362.1	2	96	1		
285.2	362.1	1	72	1		
285.2	415.1	3	39	1		
285.2	415.2	1	70	2	8	65 76
285.2	415.2	2	78	1		
285.2	415.2	3	93	3	4	89 96
285.2	415.2	4	89	1		

<u>L. r. subsp. remyi</u> X <u>L. r. subsp. kipaaluensis</u> (East Maui)						
346.101	282.5	1	85	1		
346.101	290.1	1	81	1		
361.2	282.1		57	2	11	49 65
362.1	282.4	1	91	1		
362.3	290.4	1	64	2	0	64 64
362.4	290.4	2	95	1		

Table A4.2. continued on next page.

Table A4.2. continued. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability			
			Mean	N	SD	Min Max

<u>L. r. subsp. kipahuluensis</u> (East Maui) X <u>L. r. subsp. subherbacea</u>						
285.2	372.201	1	82	1		
290.4	372.201	1	9	1		
281.25	377.1	2	77	1		
282.1	377.1	1	77	2	9	71 83
282.1	377.1	2	62	2	3	60 65
281.25	393.8	1	84	3	2	82 85
281.25	393.8	2	85	3	2	84 87
281.25	393.8	3	84	5	7	75 90
281.25	393.8	4	83	1		
281.25	393.8	5	81	1		
281.25	393.8	6	83	2	2	81 84
<u>L. r. subsp. subherbacea</u> X <u>L. r. subsp. kipahuluensis</u> (East Maui)						
372.201	282.5	1	83	2	5	79 87
393.4	282.10	1	87	2	1	86 87
393.4	282.10	2	71	2	5	68 75
395.201	282.1	1	93	2	1	92 93
372.201	285.2	1	83	1		
<u>L. r. subsp. caliginis</u> X <u>L. filifolia</u>						
337.3	11149.1		93	1		
338.1	11149.1	5	95	1		
338.5	11149.1	2	95	3	4	91 98
338.5	11149.1	3	90	2	4	87 92
<u>L. r. subsp. caliginis</u> X <u>L. r. subsp. remyi</u>						
338.1	354.5	1	85	2	1	85 86
340.1	354.5	1	90	1		

Table A4.2. continued on next page.

Table A4.2. continued. Pollen stainability of artificial hybrids between species of endemic Hawaiian Lysimachia.

Female Parent	Male Parent	Sibling Number	% Pollen Stainability			
			Mean	N	SD	Min Max

<u>L. r. subsp. caliginis</u> X <u>L. r. subsp. subherbacea</u>						
338.1	377.1	2	40	1		
338.1	386.2	1	82	2	5	78 85
338.1	386.2	2	87	1		
336.1	387.202	1	45	2	6	41 49
336.1	387.202	2	35	2	1	34 36
340.1	387.202	1	64	3	26	33 82
<u>L. r. subsp. subherbacea</u> X <u>L. r. subsp. caliginis</u>						
377.1	338.1	1	64	4	20	43 84
377.1	338.1	2	73	1		
377.1	338.1	3	78	1		
377.1	338.1	4	45	1		
<u>L. r. subsp. remyi</u> X <u>L. r. subsp. subherbacea</u>						
362.1	377.1	1	84	2	2	83 85
362.1	377.1	2	58	1		
362.7	377.1	1	31	1		
362.8	387.202	1	78	1		
361.2	395.201	1	53	2	11	45 61
361.2	395.202	1	44	2	16	32 55
1302.1	393.5		79	1		
<u>L. r. subsp. subherbacea</u> X <u>L. r. subsp. remyi</u>						
393.8	350.2	1	83	2	9	76 89
393.8	350.2	2	85	2	9	78 91
393.8	350.2	3	82	1		
393.8	350.2	5	86	1		
393.8	350.2	6	67	1		
393.4	362.1	1	89	2	1	88 89
393.4	362.1	2	91	2	1	90 92
377.1	1302.1	1	73	2	1	73 74
377.1	1302.1	2	38	2	6	35 42
<u>L. r. subsp. subherbacea</u> X <u>L. filifolia</u>						
377.1	11149.1		98	1		
400.1	11149.1	1	83	1		
400.1	11149.1	2	79	2	8	73 84