Genetic structure and diversity of a rare Hawaiian endemic, *Lobelia villosa* (Campanulaceae: Lobelioidae)

By Carol T. Tran*, Ania M. Wieczorek, and Clifford W. Morden

Abstract

Microsatellite markers are valuable tools for determining the amount and distribution of genetic diversity and differentiation within and between populations. In this study we examined the level of microsatellite variability within and among five populations of *Lobelia villosa*, a rare Hawaiian endemic lobeliad on the island of Kaua‘i. Populations of *L. villosa* were sampled from two regions on Kaua‘i: two populations at the Kilohana Lookout area and three populations from Alaka‘i Swamp. Nineteen microsatellite DNA primers were developed for *L. villosa*, 12 of which demonstrated polymorphism and were subsequently multiplexed and labeled for genotyping. An overall moderate degree of genetic differentiation was found within and between populations (\(F_{ST} = 0.06; \text{P}=0.001\)). Pairwise \(F_{ST}\) data showed population structure and analysis with Structure software indicated two genetic clusters \((K = 2)\) corresponding to the two sampled geographic regions. Although *L. villosa* exhibits moderate diversity, which exceeds that of other Hawaiian [either use the diacritics everywhere or not at all] endemics with restricted distributions, measurements of \(F_{IS}\) were positive across 10 out of twelve loci suggesting that inbreeding is occurring at the population-level (mean \(F_{IS} = 0.28\)).

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Introduction

For almost five million years, the Hawaiian Islands have provided an isolated environment for the evolution of over one thousand endemic plant species (Ziegler 2002). The Islands are located approximately 3500 km from all other landmasses, and a modest 280 colonization events have turned Hawai‘i into a significant hotspot for botanical biodiversity (Wagner et al. 1990). There is nearly 90% endemism among flowering plant taxa in Hawai‘i (Wagner et al. 1990); unfortunately, 25% of these are currently listed as either threatened or endangered, representing the majority of endangered plant species within the entire United States (Harrington & Ewel 1997), particularly in the families Asteraceae (47%), Campanulaceae (50%), and Malvaceae (67%).

Since the Hawaiian Islands evolved in isolation, they are particularly subjected to the negative impacts of invasive species such as competition, novel pathogens, and predation (Harrington and Ewel 1997, Vitousek et al. 1997, Denslow 2003). Impacts such as these are known to limit or reduce the size of the plant populations (Baillie et al. 2004, Frankham 2003). Rare plants, especially island endemics, are generally adapted to unique and fragile microhabitats further increasing their vulnerability to extinction (Falk and Holsinger 2003, Lesica et al. 2006).

The Hawaiian lobeliad radiation (Campanulaceae: Lobelioidae) comprises 135 species in six genera that originated from a single founding colonist 13 MYA (Givnish et al. 2008) and are all endemic to the Hawaiian archipelago. This assemblage represents 13% of the entire flowering plant flora in Hawai‘i and provides an outstanding example of phylogenetic radiation with striking diversification in habitat, growth form, leaf shape, flower morphology, and modes of seed dispersal (Rock 1919, Carlquist 1965, 1970, Lammers 1990, Givnish et al. 1994, 1995).

*Lobelia villosa* (Rock) St. John & Hosaka, a member of the Hawaiian lobeliad radiation, is known only from the Alaka‘i Swamp and Mount Wai‘ale‘ale on the Island of Kaua‘i (Fig. 1). *Lobelia villosa* is a monocarpic, perennial shrub and only occurs in bogs between 1200 and 1580
meters elevation. The woody stems grow to a height of about two meters, are unbranched, have lanceolate to oblong pubescent leaves, and the inflorescence is terminal on the stem producing yellowish to greenish white flowers (Wagner et al. 1999) (Fig. 2). Small plants are often present surrounding the base of larger plants and are thought to be adventitious from below the soil level, but this has not been documented and they may represent seedlings from the parental plant. Presently, there is very little known about the biology or population genetics of this species. Populations have been monitored in recent years and population sizes appear to be in decline (V. Caraway, Recovery Botanist, USFWS, pers. com.) Because of the uncertainty surrounding the status of *L. villosa*, the USFW Service presently lists it as a species of concern.

Genetic diversity is fundamental to a species evolutionary potential and is an important factor that should be considered by conservation biologists (Neel and Ellstrand 2003, Xiao et al. 2004). Small populations, such as those typical of *L. villosa*, are susceptible to inbreeding and genetic drift, which can lead to a reduction in overall genetic variation and ultimately, an inability to respond to changes within the environment (Huenneke 1991, Cole 2003).

In the present study, we developed species-specific microsatellite DNA markers to investigate genetic variation within *L. villosa* in order to better assess the long-term survival and continued evolution of this species. Microsatellite markers are valuable tools for determining the amount and distribution of genetic diversity and differentiation within and between populations (Avise 2004). Using these theoretically neutral and highly polymorphic genetic markers allowed us to quantify the degree of differentiation and inbreeding within and between populations of *L. villosa*. These factors are critical in the assessment of recovery needs, progress towards recovery, and allocation of funds for conservation relative to other species or populations (Haig 1998).
MATERIALS AND METHODS

Study Sites

A total of 125 individuals from five populations of *L. villosa* were sampled from two regions on Kaua‘i at elevations between 1227 and 1267 meters. Two populations of *L. villosa* were sampled at Kilohana Lookout area (Ki1: n = 8; Ki2: n = 36), and three populations were sampled from the Alaka‘i Swamp (A1: n = 34; A2: n = 22; A3: n = 25) (Figure 1, Table 1). Kilohana Lookout is located northwest of Alaka‘i Swamp and the distance between these sampled regions was approximately 3.65 km. Fresh leaf material was collected for DNA extraction and transported on ice before being stored at -20°C.

<Table 1 about here>

DNA Extraction

DNA from leaf material was isolated using the CTAB extraction protocol of Morden et al. (1996). A sample from the Ki1 population was used in developing the genomic library. Leaf material from all other populations was finely ground in liquid nitrogen in 1.5 ml tubes, and DNA was subsequently extracted with either the phenol-chloroform (Sambrook et al. 1989) or the Qiagen DNeasy Plant Mini Kit (QIAGEN® Industries) methodology. Eluted DNA was diluted to acquire concentrations between 5 and 20 ng/µl for optimal PCR amplification.

Microsatellite Development and Amplification

A genomic library containing microsatellite inserts was constructed using the protocol from Hamilton et al. (1999) with slight modifications. Plasmids from 76 positive clones were sequenced using BIOEDIT® Sequence Alignment Editor (Hall 1999) and primers were designed for 19 of the microsatellite inserts using FAST PCR (Kalendar 2009). The microsatellite primers were individually tested for successful PCR amplification and screened among 12 random individuals to test for microsatellite polymorphism. Individual PCR reactions contained 1x PCR
buffer (New England Biolabs, Ipswich, Massachusetts), 0.2 mM deoxynucleotides (dNTPs),
2.0mM MgCl$_2$, and 0.4 U of Taq polymerase. Individual PCR reactions were performed in a
thermocycler (BIORAD, Hercules, California) PCR cycle: initial denaturation at 94°C for 2
minutes, 35 cycles of denaturation at 94°C for various annealing temperatures for 60 seconds,
elongation at 72°C for 60 s and a final elongation at 72°C for 12 min. Optimized annealing
temperature for each primer pair was determined to be 58.7°C. Through BIOANALYZER
(Agilent Technologies, Inc, Fort Worth, Texas) analysis, 12 of the 19 microsatellite primers
demonstrated polymorphism and were fluorescently labeled at the 5’ end with 6-FAM, NED, or
HEX for genotyping (Table 2). A multiplex reaction was designed using all 12 primer pairs,
resulting in two multiplexes (Multiplex 1: L4, L23, L34, L35, L33, L37, L52, L73, L48, and
L90; Multiplex 2: L56 and L93). The composition for each multiplex PCR was performed in a
total reaction volume of 10 µl (Multiplex 1: 5 µl of 2× Multiplex PCR mix (Qiagen ®), 0.11 µl
of each primer (10 pmole) and 2.5 µl of molecular biology grade water; Multiplex 2: 5 µl of 2×
Multiplex PCR mix (Qiagen ®), 1.25 µl of each primer (10 pmole) and 2.5 µl of molecular
biology grade water). Multiplex PCR reactions were performed in a thermocycler (BIORAD,
Hercules, California) under the following conditions: initial denaturation at 95°C for 15 min, 30
cycles of denaturation at 94°C for 30 sec, annealing at 58.7°C for 90 sec, elongation at 72°C for
60 sec, and final elongation at 60°C for 30 min. In total, 125 individuals were screened at all 12
loci. The fluorescent PCR fragments were analyzed with an Applied Biosystems 3730XL
Genetic Analyzer and characterized using GENEMARKER® version 1.5.

<Table 2 about here>

Statistical Analysis

GENALEX version 6.1 (Peakall and Smouse 2006) and FSTAT version 2.9.3.2 (Goudet
2002) were utilized to calculate the following population diversity indices: the significance of
deviation from Hardy-Weinberg Equilibrium (1000 randomizations at the 5% level); allelic diversity ($A$), allelic richness ($A_R$), private alleles ($A_P$), expected ($H_E$) and observed ($H_O$) heterozygosities (Nei 1987), and Wright’s (1969) inbreeding coefficient ($F_{IS}$). The inbreeding coefficient, $H_O$ and $H_E$ were calculated for all individuals at each locus and among loci.

Estimation of the true number of populations of *L. villosa* among the two different regions on the island of Kaua‘i and evaluating the structure of population based on their genetic composition was conducted using Structure version 2.1 (Pritchard et al. 2000). The number of approximated populations or clusters ($K$) was assessed between one and eight as suggested by Evanno et al. (2005) with ten independent runs of each. Analyses were run using the admixture ancestry model without *a priori* information of the observed population structure. The burn-in period and MCMC were both set at 100,000. Estimated $K$ values were determined through Structure output in addition to an *ad hoc* statistic, $\Delta-K$ (Evanno et al. 2005).

Our data set was not large enough to use the program Bottleneck version 1.2.02 (Cornuet and Luikart 1997; Piry et al. 1999) with confidence. Instead, we used a qualitative graphical method, which calculates the allele frequency class distributions as in Luikart et al. (1998). Using this method, populations with a normal history show a ‘normal L shaped’ distribution of allele frequencies, whereas those that have recently suffered from a bottleneck will be expected to show a ‘shifted-mode’ distribution due to a reduction in rare alleles. The Ki1 population was excluded due to low sample size ($N = 8$). Estimations were based on 1000 replications with 30% and 70% for variance and probability respectively.

Population differentiation was quantified through $F_{ST}$ values (Weir & Cockerham 1984) with probability coefficients ($p = 0.05$) calculated based on 1000 randomizations in Arlequin version 3.11 (Schneider et al. 2000). Pairwise $F_{ST}$ (Weir & Cockerham 1984) were evaluated and p-values tested for significance after Bonferroni correction (Rice 1989) to account for multiple comparisons.
Genetic diversity among populations was investigated under three levels of population structure using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) within the framework of Arlequin version 3.11 (Schneider et al. 2000). AMOVA partitioned genetic variation among groups, among populations within groups, and within populations to allow for greater insight into the degree of genetic variation within populations of *L. villosa* we sampled.

**RESULTS**

*Asexual Reproduction*

Asexual reproduction via adventitious plant development was confirmed. Twelve plantlets surrounding the base of a larger plant in population Ki1 were sampled, and their genotypes compared. Genotypes among all 13 plants were identical for the 12 loci examined in this study. Branching near the base occurs in other Hawaiian *Lobelia* species (C. Morden, personal observation), but in those species it occurs above, rather than below, ground level.

*Genetic variation, deviation from Hardy-Weinberg equilibrium and linkage disequilibrium*

A total of 91 alleles were identified at 12 polymorphic loci (average number of alleles per locus = 7.6) among the 125 individuals of *L. villosa* examined (Table 3). Most loci had several common alleles present, and only one locus had an allele with a frequency greater than 0.90. The mean number of alleles per locus ($A$) among populations ranged from 4.2 at Ki1 to 6.3 at Ki2 (mean across populations was $A = 4.9$). Many of these alleles occurred at low frequency within populations, and Allelic Richness ($A_R$) ranged from a minimum of 3.4 (A2) to 4.1 (Ki1 and A3) ($P = 0.001$; Wilcoxon’s signed rank test). There were a total of 26 private alleles found among the five populations sampled. In most cases, these were in very low frequency (<0.125); only eight private alleles had a frequency within their respective populations greater than 0.05. Genetic distinctiveness was highest in the largest populations of each region (Ki2: $A_P = 0.92$; A1: $A_P = 0.58$) and was lowest in the two other Alaka‘i populations (A2: $A_P = 0$; A3: $A_P = 0.08$); the
smallest population had a relatively moderate number of private alleles (Ki1: $A_p = 0.17$). There were seven instances of alleles present in all populations of their respective region; four alleles restricted to Alaka`i populations and three to the Kilohana populations. Three additional alleles were present in two of the three Alaka`i populations and absent at Kilohana.

Genetic diversity present within and among populations of *L. villosa* was moderate. $H_O$ among populations is considerably lower than $H_E$. $H_O$ ranged from 0.32 (Ki2) to 0.50 (Ki1) and $H_E$ ranged from 0.51 (A1) to 0.59 (Ki1). As would be expected, the average $F_{IS}$ over all loci ($F_{IS} = 0.25$) demonstrates a higher number of homozygotes than heterozygotes within the region and an overall likelihood that inbreeding is occurring more frequently than outcrossing. Inbreeding coefficients among loci ranged from a low level at L35 ($F_{IS} = -0.24$) to a high at L34 ($F_{IS} = 1.0$). Estimates of $F_{IS}$ by population (mean = 0.28) ranged from 0.21 (Ki1) to 0.40 (Ki2).

Of the 60 population-locus combinations tested for Hardy-Weinberg equilibrium, 17 showed significant deviation at the 1% level. In addition, linkage disequilibrium was evident in eight out of 66 loci pairs within the sampled populations. However, after Bonferroni corrections were made to correct for multiple pairwise testing of the data set (Rice 1989), all were found to meet Hardy-Weinberg expectations.

*Population Differentiation*

The 12 polymorphic loci utilized in this study demonstrated an overall low degree of genetic differentiation in the five populations of *L. villosa* on Kaua`i. The overall $F_{ST}$ by population (0.06; $P = 0.001$) and by region (0.05) was indicative of moderate differentiation (Wright 1967) and was supported by pairwise estimates of $F_{ST}$ with all estimated values < 0.09. $F_{ST}$ estimates were highest between Alaka`i Swamp and Kilohana Lookout, and lowest when comparing populations within each specific region. Pairwise $F_{ST}$ values were all significant ($P <$
0.05) except for populations between A2 and A3 (P = 0.05) and highest between more
geographically distant sites A3 and Ki1 (0.09) and A1 and Ki2 (0.07). The lowest values were
calculated between geographically adjacent populations A1 and A2 (0.03), and A2 and A3
(0.02).

The results show that gene flow has been occurring between regions and populations, and
thus supports categorizing *L. villosa* as an outcrossing species. To compare the observed regional
differentiation to the differentiation between Alaka‘i Swamp and Kilohana Lookout, AMOVA
was performed to summarize the percentage of variation among populations within each region.
AMOVA revealed that over 83% of molecular variance was explained by differentiation among
individuals within populations, while only 9% of differentiation occurred among populations,
and 8% occurred among regions (Table 6).

Population Structure

All *L. villosa* individuals of the Alaka‘i and Kilohana populations were checked for their
assignment to the sampled population (Structure 2.1). The results of the analysis showed that the
observed population structure in the field was reflected by the genetic data; however, due to the
high level of admixture between populations, both Structure and the *ad hoc* statistic *Δ-K* (Evanno
et al. 2005) were implemented to determine a *K* that best reflected the overall data set, and
revealed *K* = 2 (FIGURE 3a and 3b). An arbitrary threshold value (0.60) defined populations Ki1
and Ki2 as a single genetic cluster and populations A1, A2, and A3 as the second.
Testing for Bottlenecks

The distribution of allele frequency classes over the 12 loci was analyzed for each population except Ki1. Populations that have not experienced a recent bottleneck retain relatively large numbers of rare alleles across all loci, which result in an “L-shaped” graph. During a bottleneck, rare alleles are lost rapidly resulting in a “mode-shift” pattern. Using the 10 frequency classes suggested in Luikart et al. (1998) revealed that *L. villosa* populations had not experienced a recent genetic bottleneck.

DISCUSSION

We present here the first microsatellite DNA study of any Hawaiian *Lobelia* species. Given that genetic diversity within a population is usually assumed to be indicative of population fitness, an understanding and indirect modeling of community and ecosystem dynamics as they relate to *L. villosa* is fundamental to this species’ persistence. By collecting from multiple populations with varying geographic distances separating them, this study quantifies the genetic diversity within and between populations of *L. villosa*. Previous studies on related and critically endangered Hawaiian lobeliads, *Cyanea asarifolia* and *Delissea undulata* using randomly amplified polymorphic DNA (RAPD) analysis indicated no genetic diversity within geographically isolated populations (Koob 1996). A study of variation among seedlings produced from the last remaining naturally extant individual of *Cyanea superba* similarly had no variation among RAPD markers (C. Morden, unpublished data). Analysis of natural populations of *C. calycina* and *Cyanea grimesiana* did find substantial variation among populations, but little within populations variation (Crooker 2004). Due to the inherent drawbacks of RAPD markers, including dominance of all markers, unknown amplification regions, and the need for greater resolution to better understand these native organisms, more recent studies concerning rare,
endemic species have utilized microsatellites in an effort to better model genetic diversity (Wolfe and Liston 1998).

Our present study demonstrated a moderate degree of genetic diversity in the five geographic populations of *L. villosa* (0.40 observed heterozygosity, 7.6 alleles per locus and 0.35 proportion of private alleles) surpassing that found for other endangered Hawaiian lobeliads (Koob 1996, Crooker 2004). As might be expected, examination of other Hawaiian taxa has found that widespread species generally have greater levels of variation whereas rare species have far less. Populations of the ecosystem dominant species *Metrosideros polymorpha* (Myrtaeae) on Hawai‘i Island have an observed heterozygosity of 0.665 with 11.4 alleles per locus (Crawford et al. 2008), and heterozygosity among populations of *Acacia koa* (Fabaceae) across islands ranges from 0.566 to 0.988 (Fredua-Agyeman et al. 2008). In contrast, the endangered species that have been examined have measures of variation far lower than found in *L. villosa*. The rare Hawaiian endemic *Schiedea adamantis* (Caryophyllidae) had similar, but lower, levels of genetic diversity (0.291 observed heterozygosity and 3.7 alleles per locus) (Culley et al. 2008). Four species of the silversword alliance genus *Dubautia* (Asteraceae) have observed heterozygosity ranging from 0.047 to 0.235 (Friar et al. 2006). The Mauna Loa silversword (*Argyroxyphium kauense*) averaged fewer than 3.0 alleles per locus and 0.307 observed heterozygosity (Friar et al. 2001) whereas the severely bottlenecked population of the Mauna Kea silversword (*A. sandwicense* ssp. *sandwicense*) had by far the least variation with 0.033 observed heterozygosity and 1.33 alleles per locus (Friar et al. 2000).

Although *L. villosa* exhibits moderate diversity, which exceeds that of other Hawaiian endemics with restricted distributions, measurements of $F_{IS}$ were positive across 10 out of twelve loci suggesting that inbreeding is occurring at the population-level (mean $F_{IS} = 0.28$). In addition, differentiation based on estimates of $F_{ST}$ and both Bayesian analyses demonstrated that sites within Alaka‘i Swamp comprise one genetic cluster and those within Kilohana Lookout another,
signifying population structure and genetic drift. Our results suggest that contemporary gene flow may be limited between these plants in these two areas, and possibly attributable to the loss of a long distance pollinator, seed dispersal mechanism, and/or the extinction of other populations in or between each region. Also, clonality, in the form of vegetative shoots from the stoloniferous mother (personal observation, C. Morden), may further explain the current population structure of *L. villosa*. The results obtained from analyzing the mutation-drift equilibrium corroborate this, showing a non-significant level of excess heterozygosity, thus indicating a recent bottleneck event did not occur and other factors are affecting gene flow. Additionally, changes in factors, such as population size, population fitness, and degree of isolation should be monitored as warning signs that populations may be threatened. Furthermore, this study highlights the need for the evaluation of long-term genetic impacts on other rare and endangered plants within the Hawaiian lobeliads. The assessment of recovery needs, progress towards recovery, and allocation of funds for conservation relative to other species or populations should also be a priority.
ACKNOWLEDGEMENTS

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TABLE. Location of *Lobelia villosa* Collection Sites on Kaua‘i Island, Hawai‘i (refer to map)

<table>
<thead>
<tr>
<th>Region</th>
<th>Site Description</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>UTM (E)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>UTM (N)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilohana Lookout (Ki)</td>
<td>Ki1 - Mohihi &amp; Circle Bog</td>
<td>8</td>
<td>0438675</td>
<td>2449995</td>
</tr>
<tr>
<td></td>
<td>Ki2 - North Bog</td>
<td>36</td>
<td>0438355</td>
<td>2450611</td>
</tr>
<tr>
<td>Alaka‘i Swamp (A)</td>
<td>A1 - Circle Bog</td>
<td>34</td>
<td>0440916</td>
<td>2446950</td>
</tr>
<tr>
<td></td>
<td>A2 - North of Circle Bog</td>
<td>22</td>
<td>0440550</td>
<td>2446895</td>
</tr>
<tr>
<td></td>
<td>A3 - <em>Exocarpus</em> Bog</td>
<td>25</td>
<td>0440546</td>
<td>2447070</td>
</tr>
</tbody>
</table>

<sup>a</sup> number of collected individuals in each population. All individuals in geographic area of each population were sampled; <sup>b</sup> GPS coordinates of population.
TABLE 2
Microsatellite Loci Information and Primers for *Lobelia villosa*

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer sequence (5’-3’)</th>
<th>Repeat Motif</th>
<th>Size (bp)</th>
<th>label</th>
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<td>L4</td>
<td>F-ACGTCTAGGGGCACTGCCAAGCCAG</td>
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<td>FAM</td>
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<td>R-TCCAAATGGGAGACTACTGCGAGAAAGG</td>
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<td>334-338</td>
<td>HEX</td>
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<td>R-TGCAAGGATGACGAGGGGGTC</td>
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<tr>
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<td>F-ACAGGGCGCTATGGCGTCCCT</td>
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<td>142-150</td>
<td>FAM</td>
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<td>R-GTTGTATGATCATGAGACCGTC</td>
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<td>L35</td>
<td>F-GCTTACAACAAATTCGCTTC</td>
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<td>F-GGATCACTCAAGGATGAACTCGCAAGG</td>
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<td>L52</td>
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R-CTACTGTGTGAAGTCGGAAAACC

L93

F-TCTAGCAGAAGCCTCACCCCGGA (GT)$_{10}$ 338-346 FAM

R-CACCAGAACTCAAGCAAGGCGAC

$^a$ Size range of amplified product.

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**TABLE 3**

Genetic Diversity Indices for *Lobelia villosa*

<table>
<thead>
<tr>
<th>Site</th>
<th>N$^a$</th>
<th>$A^b$</th>
<th>$A_R^c$</th>
<th>$A_P^d$</th>
<th>$H_O^e$</th>
<th>$H_E^f$</th>
<th>$F_{IS}^g$</th>
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<tbody>
<tr>
<td>Ki1</td>
<td>8</td>
<td>4.2</td>
<td>4.1</td>
<td>0.17</td>
<td>0.50</td>
<td>0.59</td>
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<td>Ki2</td>
<td>36</td>
<td>6.3</td>
<td>3.5</td>
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<td>0.40</td>
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<tr>
<td>A1</td>
<td>34</td>
<td>5.2</td>
<td>3.7</td>
<td>0.58</td>
<td>0.36</td>
<td>0.51</td>
<td>0.30</td>
</tr>
<tr>
<td>A2</td>
<td>22</td>
<td>4.6</td>
<td>3.4</td>
<td>0</td>
<td>0.40</td>
<td>0.53</td>
<td>0.27</td>
</tr>
<tr>
<td>A3</td>
<td>25</td>
<td>4.4</td>
<td>4.1</td>
<td>0.08</td>
<td>0.41</td>
<td>0.51</td>
<td>0.23</td>
</tr>
<tr>
<td>Averages</td>
<td>-</td>
<td>4.9</td>
<td>3.8</td>
<td>0.35</td>
<td>0.40</td>
<td>0.53</td>
<td>0.28</td>
</tr>
</tbody>
</table>

$^a$ Population size, $^b$ Allelic diversity, $^c$ Mean Allelic richness per population, $^d$ proportion of private alleles, $^e$ Observed heterozygosity, $^f$ Expected heterozygosity, $^g$ Inbreeding coefficient.
### TABLE 4

Pairwise Estimates of $F_{ST}$ and their Significance for Populations of *Lobelia villosa*

<table>
<thead>
<tr>
<th></th>
<th>Ki1</th>
<th>Ki2</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki2</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> significant $P < 0.05$

### TABLE 5

Analysis of Molecular Variance (AMOVA) Results for *Lobelia villosa*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df &lt;sup&gt;a&lt;/sup&gt;</th>
<th>SS&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Variance Components</th>
<th>Percentage of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Regions</td>
<td>1</td>
<td>20.45</td>
<td>0.09</td>
<td>8%</td>
</tr>
<tr>
<td>Among Populations</td>
<td>3</td>
<td>25.11</td>
<td>0.10</td>
<td>9%</td>
</tr>
<tr>
<td>Among Individuals</td>
<td>120</td>
<td>491.69</td>
<td>0.94</td>
<td>83%</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>537.25</td>
<td>1.13</td>
<td>100%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Degrees of freedom; <sup>b</sup> Sum of squares.
Figure 1. Distribution of populations of *Lobelia villosa* on Kauaʻi, Hawaiʻi.
Figure 2. Photos of *L. villosa*. (A) Rosette leaf arrangement of *L. villosa*, Alaka‘i Swamp, Kaua‘i, Hawai‘i. Photo by Johannes J. Le Roux. (B) Flowering arrangement of *L. villosa* showing immature fluorescence stalk, Alaka‘i Swamp, Kaua‘i, Hawai‘i. Photo by Clifford W. Morden.


