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RESEARCH ARTICLE

High genetic divergence characterizes populations of the endemic plant *Lithophragma maximum* (Saxifragaceae) on San Clemente Island

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Abstract Narrowly-ranging species frequently harbor less genetic variability relative to widespread relatives and face graver extinction threats due to the heightened impacts of stochastic events on ecological and genetic diversity. In this study, we examined the impact of historical and current threats to the maintenance of genetic variation in *Lithophragma maximum* (Saxifragaceae), a perennial herb endemic to San Clemente Island, California. This species exists as small populations confined to canyons along 4 km of the southeast coastline of the island. In 15 populations analyzed with 10 microsatellite markers, we identified an average of 2.05 alleles per locus and 58.7% polymorphic loci. Significant departures from Hardy–Weinberg equilibrium existed in six populations; five of these exhibited heterozygote deficiency. Bayesian inference of genetic structure indicated a significant amount of structure among populations and canyons and infrequent gene flow even over very short distances. We also identified a significant and positive correlation between genetic and geographic distances, indicative of isolation by distance. There was no evidence of recent bottlenecks in any of the sampled populations, but older bottlenecks were detected in two populations. These results suggest that populations of *L. maximum* have historically been small and isolated, which is likely due to the rugged habitat in which this species occurs and limited pollen and seed dispersal. Given the high degree of structure observed across populations, we suggest that conservation efforts should focus on preserving populations in multiple canyons, maintaining large population sizes to preserve genetic variation, and controlling the spread of invasive species in areas where *L. maximum* occurs.

Keywords Conservation genetics · Microsatellites · *Lithophragma* · Endemic species · Channel Islands

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Introduction

As extinctions of rare and endangered species continue to rise, so does the effort to learn more about the reasons for extinction and how to manage species that are threatened by human-induced changes to the environment. Given the dynamic nature of the extinction process, species decline should be considered over ecological as well as evolutionary time scales and should be informed by studies of biotic and abiotic factors influencing genotypic and phenotypic responses to the environment. The probability of a species' long-term survival is closely linked to the degree of genetic diversity available to respond to natural selection (Frankham 1998; Frankham et al. 2002). Without genetic variability, short-term evolutionary adaptation to a fluctuating environment, microhabitat differentiation, or disease outbreaks cannot occur (Huenneke 1991). Genetic variation is also important over longer periods of time by providing species the ability to respond to changing environments (Ellstrand and Elam 1993; Frankham et al. 2002; Holsinger and Gottlieb 1991). In addition, lack of genetic

variation can lead to inbreeding depression (Frankham et al. 2002). As such, population genetic studies are important for understanding evolutionary and population dynamics of rare species. Additionally, knowledge of population genetic structure can provide important information to aid in the management of rare and endangered species, for example, by identifying populations of greatest evolutionary potential and populations best suited for source material for ex situ preservation or reintroduction.

Island species may be at increased risk of extinction because populations of native species occurring on islands often harbor lower levels of genetic variation than their mainland counterparts (Barrett and Husband 1990; Frankham 1997, 1998; Dodd and Helenurm 2002). Genetic drift resulting from founder events and small population sizes, typical of many island taxa, has been invoked to explain this pattern on isolated, distant islands. Taxa on island systems that are relatively close to continental areas do not always fit these expected patterns, though. For example, studies of endemic plant species on the California Channel Islands have shown a wide variety in levels and patterns of genetic variation despite similarities in life history characteristics, distributions, and habitats and using similar sampling strategies and a variety of molecular markers (Helenurm 2001, 2003; Dodd and Helenurm 2002; Helenurm and Hall 2005; Helenurm et al. 2005). This result demonstrates the need to broaden the framework of island biogeography theory by assessing genetic structure and demographic history of endemic island species. Such empirical studies are necessary to fully understand the evolution of island taxa and to plan for their preservation in the future. In this paper, we report results from a study of all known populations of *Lithophragma maximum* Bacig. (Saxifragaceae), an endangered endemic species of San Clemente Island (California Channel Islands; Fig. 1), based on variation at microsatellite loci.

The Channel Islands comprise a unique archipelago of eight islands situated along the southern coast of California. In spite of their relatively small size and proximity to the mainland, the proportion of native species that are insular endemics to the Channel Islands is greater than 15%, much higher than documented for other islands of similar size and isolation (Davis et al. 1994, 1995). The islands are all volcanic in origin, varying considerably in age, distance from the mainland, area, and level of endemism (Philbrick 1967). San Clemente, the southernmost island and fourth largest (145 km^2), possesses more Channel Island endemic plants (47) than any of the other islands (14–45). Additionally, a higher percentage of its flora (17%) is endemic compared to the floras of the other Channel Islands (9–15.9%) (Junak et al. 1995; Moody 2000; Raven 1967).

Lithophragma maximum is confined to a specialized habitat in an extremely restricted range on San Clemente Island. It occurs in canyons along 4 km of the southeastern coastline of the island. Because it is only found in mesic

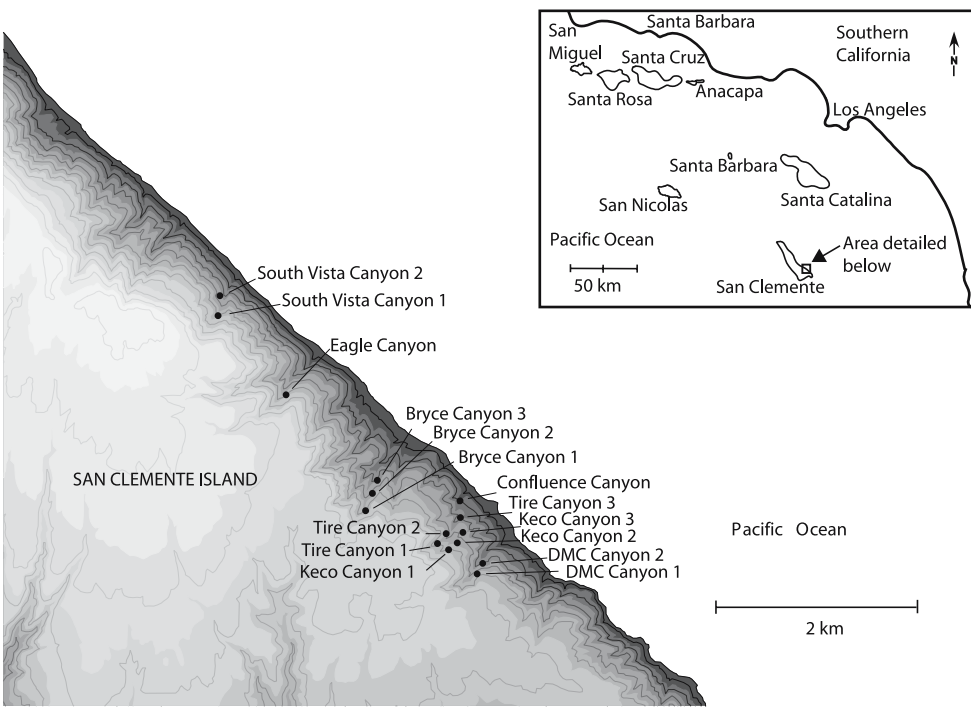

Fig. 1 Location of San Clemente Island, California, including the current range of *Lithophragma maximum* and the locations of populations sampled for this study

pockets at the bottom of north-facing slopes of these canyons and has never been reported from other habitats or other canyons, it is unlikely that it ever occurred beyond its current range. All populations are small, ranging from four to a maximum of approximately 100 individuals. The species has been listed as endangered by the state of California since 1982 and the U.S. Fish and Wildlife Service since 1997. Previous studies of *L. maximum* based on allozyme variation revealed no polymorphism at 23 loci (K. Helenurm, unpubl. data). However, recently evolved or rare species often lack substantial variability at allozyme loci but not at DNA markers (e.g., Wallace 2002; Nybom 2004). Thus, in this study, we surveyed polymorphic microsatellite loci in *L. maximum* to address the following questions: (1) how much variation does this rare, narrowly-ranging species contain compared with other rare and endemic plant species?, (2) is genetic variation retained primarily within or among populations of *L. maximum*?, (3) are canyons significant barriers to gene flow?, and 4) is there evidence of genetic bottlenecks in populations as a result of historical human-induced impacts to San Clemente Island? In addition to discussing the evolutionary and demographic history of *L. maximum*, the results of this study are used to assess current and future conservation and management strategies for *L. maximum*.

Materials and methods

Study species

Lithophragma maximum is a rhizomatous, perennial species that is distinguished from other *Lithophragma* species by its basal trifoliate compound leaves (Elvander 1993; Munz 1974). It typically produces 2–3 flowering stems of 40–60 cm in height in April-June. Each inflorescence produces 20 or more small (ca. 1 cm) white campanulate flowers (Bacigalupi 1963). Pollination of *L. maximum* has not been studied; however, several other *Lithophragma* are pollinated by species of *Greya* moths, which are also parasitic on seeds (Brown et al. 1997; Thompson 1997). The mating system has not been studied, but all other species in this genus are self-incompatible (Taylor 1965). Seeds appear to be gravity-dispersed.

The habitats in which extant populations occur are extremely vulnerable. On at least one occasion, large portions of canyon walls have sloughed off, destroying many endemic plants (Beauchamp and Ferguson 1980). Additionally, the entire range of *L. maximum* lies within the U.S. Navy's Shore Bombardment Area. The introduction of invasive plant species (Junak et al. 1995), severe herbivory by feral goats and domestic sheep, cattle, and pigs (U.S. Fish and Wildlife Service 1997), erosion of topsoil and loss of organic matter, reduced soil nutrient cycling and water holding capacity, formation of deeply incised canyons as a result of overgrazing, and increased fire frequency (Carroll et al. 1993), are also expected to have negatively impacted genetic variation and long-term survival of this species on San Clemente Island.

Plant collection, DNA extraction, and genetic analysis

Leaf tissue was collected from every individual in all known populations ($n = 15$) of *L. maximum* on San Clemente Island (Fig. 1) in 2000. Population sizes ranged from four to 55 individuals with a mean population size of 24.2 (Table 1). Leaf samples were stored at −80°C until DNA was extracted using a DNeasy kit (Qiagen, Valencia, CA). Ten polymorphic microsatellite loci were amplified in all individuals using primers developed for *L. maximum*; primer sequences and polymerase chain reaction protocols are reported elsewhere (Wallace et al. 2006). Individuals were genotyped by electrophoresing fluorescently labeled products through an Avant 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Fragment sizes were determined by comparison to the LIZ500 size standard in the GeneMapper software (Applied Biosystems).

Data analysis

Standard measures of genetic diversity, including percent polymorphic loci (P), alleles per locus (A), effective number of alleles per locus (A_e), observed heterozygosity (H_O), and expected heterozygosity (H_E), were calculated for all populations using Popgene vers. 1.31 (Yeh et al. 1999). Spearman and Pearson tests were performed to test for a correlation between population size and within-population genetic diversity in the program SPSS vers 10.0. We used Weir and Cockerham's (1984) F-statistics, calculated in FSTAT v.2.9.3.2 (Goudet 2001), to evaluate the hierarchical structure of genetic variation among populations. F is a measure of heterozygosity of individuals relative to the total population to which they belong, f is a measure of heterozygosity of individuals relative to the subpopulation to which they belong, and θ is a measure of heterozygosity of a subpopulation relative to the total population to which it belongs. Inbreeding coefficients for individual loci were tested for deviation from Hardy–Weinberg equilibrium using probability tests in FSTAT (Goudet 2001); we applied a sequential Bonferroni correction for multiple comparisons in determining statistical significance at the 5% level.

Several Bayesian methods of analysis were used to detect underlying patterns of genetic structure and recent

Table 1 Measures of genetic variation at ten microsatellite loci in 15 populations of *Lithophragma maximum*

Population	N	P (%)	A (SD)	A_e (SD)	H_e (SD)	H_O (SD)	F_{IS}
S. Vista Canyon 1	35	20.0	1.20 (0.42)	1.19 (0.41)	0.098 (0.207)	0.140 (0.327)	−0.417*
S. Vista Canyon 2	9	40.0	1.50 (0.71)	1.26 (0.44)	0.141 (0.216)	0.172 (0.330)	−0.157
Eagle Canyon	29	60.0	2.50 (1.72)	1.42 (0.46)	0.234 (0.220)	0.200 (0.300)	0.162
Bryce Canyon 1	18	30.0	1.40 (0.70)	1.27 (0.50)	0.136 (0.228)	0.167 (0.292)	−0.197
Bryce Canyon 2	9	60.0	1.80 (0.79)	1.43 (0.41)	0.243 (0.219)	0.289 (0.336)	−0.134*
Bryce Canyon 3	11	90.0	2.40 (0.84)	1.82 (0.66)	0.376 (0.238)	0.219 (0.303)	0.457**
Confluence Canyon	35	90.0	2.50 (1.18)	1.74 (0.78)	0.342 (0.228)	0.219 (0.270)	0.372**
Tire Canyon 1	51	60.0	2.70 (1.57)	1.52 (0.55)	0.256 (0.261)	0.205 (0.292)	0.211**
Tire Canyon 2	6	60.0	1.80 (0.79)	1.31 (0.38)	0.186 (0.197)	0.267 (0.335)	−0.356
Tire Canyon 3	4	70.0	1.80 (0.63)	1.49 (0.46)	0.266 (0.221)	0.225 (0.299)	0.289
Keco Canyon 1	10	60.0	2.00 (0.94)	1.46 (0.56)	0.235 (0.241)	0.270 (0.362)	−0.100
Keco Canyon 2	32	60.0	1.80 (0.79)	1.43 (0.49)	0.233 (0.235)	0.206 (0.312)	0.129
Keco Canyon 3	4	30.0	1.30 (0.48)	1.16 (0.32)	0.094 (0.169)	0.150 (0.316)	−0.500
DMC Canyon 1	55	70.0	3.00 (2.00)	1.65 (0.74)	0.285 (0.278)	0.236 (0.314)	0.182**
DMC Canyon 2	55	80.0	3.00 (1.94)	1.86 (1.05)	0.309 (0.317)	0.257 (0.320)	0.178**
Mean	24.2	58.7	2.05	1.47	0.229	0.215	
SD	18.8	21.0	0.60	0.22	0.084	0.045	
Species	363	100.0	6.40	2.70	0.504	0.214	0.576

P = percent polymorphic loci

A = alleles per locus

A_e = effective number of alleles per locus

H_e = expected heterozygosity

H_o = observed heterozygosity

F_{IS} = inbreeding coefficient calculated over all loci

Standard deviation (SD) is included for A, A_e, H_e, and H_o. Populations exhibiting significant departures from Hardy–Weinberg equilibrium are indicated with an asterisk (*$P < 0.05$; **$P < 0.001$)

migration in *L. maximum*. These included STRUCTURE vers. 2.0 (Pritchard et al. 2000), BAPS (Corander et al. 2003, 2004), and BAYESASS (Wilson and Rannala 2003). For the STRUCTURE analyses, we used an admixture model with correlated allele frequencies. After experimentation with variation in run length and the number of inferred genetic clusters (K), we ran the analyses using a burn-in length of 10^5 MCMC chains and 5 million additional simulations to estimate the value of K with the highest likelihood and to assign individuals to clusters. The number of clusters was tested for $K = 1$–15, and 20 independent runs were conducted for each K to verify convergence of the Markov chain. The resulting likelihood values at each level of K (across 20 runs) were averaged in considering the most likely number of clusters. We examined mean likelihood values of K as well as ΔK (Evanno et al. 2005) in estimating the most likely number of clusters for the dataset. Individuals were then assigned to clusters based on posterior probability estimates of inclusion in each of the groups. Individuals with a posterior probability of 0.8 or greater were considered confidently assigned to a single cluster. Those with split probabilities were assigned to two or more clusters and considered to be migrants. We conducted the BAPS analyses with the incorporation of prior information on the population origin of individuals because most populations were found to be genetically homogeneous, and such information is expected to increase the statistical power of the analysis (Corander and Marttinen 2006). BAPS analyses were conducted using 1 million iterations of the MCMC chain and a burn-in of 500,000 steps before compiling the posterior probability distribution. Individual assignment and migration rates between all pairs of populations were estimated with the software BAYESASS (Wilson and Rannala 2003). In performing these analyses, we used a chain length of 20 million generations and a sampling frequency of 2000, and we discarded the first 2 million cycles as burn-in before computing posterior probabilities. Analyses were repeated seven times, with the most frequent pattern of immigration inferred for each population. The confidence intervals surrounding migration estimates were compared to estimates based on a lack of data in assessing the goodness of fit of the model implemented in BAYESASS.

Genetic distances between populations were computed using the coancestry distance measure of Reynolds et al. (1983) conducted with the software TFPGA (Miller 1997). The coancestry coefficient measures genetic distance based on the drift model, where the forces of mutation and selection are largely ignored. A Mantel test was used to assess a relationship between geographic and genetic distances (measured as the coancestry coefficient) for all pairwise comparisons of populations. This analysis was conducted with FSTAT and used 10,000 permutations of the data to test significance of the relationship between distance matrices. Un-weighted pair group method analysis (UPGMA) of coancestry genetic distances was used to assess relationships among all populations. This analysis was performed with the TFPGA software (Miller 1997), and support for relationships was assessed through 1,000 bootstrap replicates.

Recent (e.g., 2–4N_e generations) reductions in population size were investigated using BOTTLENECK (Cornuet and Luikart 1996; Piry et al. 1999) and historical reductions were assessed through analyses of allele size range with the program M-ratio (Garza and Williamson 2001). Recent bottlenecks are detected by an excess of heterozygosity relative to equilibrium heterozygosity in a stable population (Piry et al. 1999) because populations undergoing a bottleneck experience a loss in allele number faster than expected heterozygosity (Maruyama and Fuerst 1985). Following the recommendations of the authors (Piry et al. 1999), we used the two-phase model (Di Rienzo et al. 1994) with 95% single-step mutations and a variance of 12 among multiple steps. Wilcoxon's sign rank test was used to identify a significant excess of heterozygosity across all loci within each population (Luikart and Cornuet 1998). The M-ratio test (Garza and Williamson 2001) considers the ratio of the number of alleles to the range in allele size in evaluating historical bottlenecks. The mean value of this ratio (M) across loci can be used to detect a bottleneck because a reduction in population size is expected to reduce allele number faster than the range of allele sizes. The M-ratio test was performed using the software M_P_Val (Garza and Williamson 2001) and setting the proportion of single step mutations to 3.5. Because little is known about effective population sizes of *L. maximum* before herbivores were introduced to the islands, we tested the inference of population bottlenecks across $\theta = 1$, 5 and 10, representing very small to large pre-bottleneck populations.

Results

Genetic variation

For the ten polymorphic loci surveyed, a total of 64 alleles were observed among 363 individuals of *L. maximum*. Two to 14 alleles were found at each locus, with a mean of 6.40 alleles per locus (data available from corresponding author upon request). However, 14 alleles (22%) were found in just a single population and more than half of the private alleles (55%) occurred with a frequency less than 0.05. Consequently, the mean number of effective alleles per locus was only 1.47 across the populations and 2.70 for the species (Table 1). Observed heterozygosity ($H_o = 0.214$) at the species level was less than half the expected value ($H_e = 0.504$).

Percent polymorphic loci ranged from as little as 20% in South Vista Canyon 2 to as much as 90% in Confluence Canyon and Bryce Canyon 3. Expected heterozygosities for populations ranged from 0.094 in Keco Canyon 3 to 0.376 in Bryce Canyon 3. Observed heterozygosity was generally much lower, ranging from a low of 0.140 in South Vista Canyon 2 to a high of 0.289 in Bryce Canyon 2. The only diversity measure significantly correlated with population size was the number of alleles per locus (Pearson's $r = 0.690$, $P = 0.004$; Spearman's $r = 0.613$, $P = 0.015$). Significant deviations from Hardy–Weinberg equilibrium were found in six populations (Table 1): South Vista Canyon 1 ($F_{IS} = -0.417$; $P < 0.05$), Bryce Canyon 3 ($F_{IS} = 0.457$, $P < 0.001$), Confluence Canyon ($F_{IS} = 0.372$, $P < 0.001$), Tire Canyon 1 ($F_{IS} = 0.211$; $P < 0.001$), DMC Canyon 1 ($F_{IS} = 0.182$, $P < 0.001$), and DMC Canyon 2 ($F_{IS} = 0.178$, $P < 0.001$). Across all populations, six loci showed significant ($P < 0.05$) deficiencies of heterozygous individuals in at least one population, whereas one locus (Lima 142) showed a significant ($P < 0.05$) excess of heterozygous individuals in seven populations.

Genetic structure and migration

We found considerable divergence among the populations of *L. maximum*, with 52.4% of the observed diversity occurring among and 47.6% within populations (Table 2). Most loci exhibited moderate to strong population differentiation with 95% confidence intervals of θ from 37.2% to 65.3%. Bayesian analyses performed with STRUCTURE also suggested the presence of genetic structure in *L. maximum*, although estimating the actual number of clusters was not straightforward. Considering mean likelihood values, at least 12 clusters are apparent in the data. When ΔK is used to indicate the number of clusters, there is a strong peak at 3 with smaller peaks at 5, 9, and 12 clusters. Re-analysis of the three clusters suggested by the ΔK values, however, suggests the presence of substructure in these clusters (data not shown). Thus, we have selected $K = 12$ as the best fitting model of structure. At $K = 12$, many individuals were assigned to a single cluster with a

Table 2 F-statistics (Weir and Cockerham 1984) across loci

Locus	*F*	θ	*f*
Lima31	0.799	0.682	0.368
Lima47	1.000	0.885	1.000
Lima49	0.900	0.679	0.688
Lima13	0.676	0.506	0.344
Lima132	0.833	0.690	0.461
Lima5	0.609	0.508	0.205
Lima6	0.917	0.682	0.741
Lima134	0.301	0.225	0.098
Lima142	−0.382	0.153	−0.632
Lima77	0.707	0.463	0.455
Mean	0.636	0.547	0.373
All (95% CI)	0.599 (0.274, 0.824)	0.524 (0.372, 0.653)	0.156 (−0.202, 0.508)

F = a measure of the variation observed at the individual level relative to the total amount of variation across all individuals

θ = a measure of the amount of variation residing within subpopulations relative to the total variation observed in the species

f = a measure of the variation in individuals relative to the subpopulation in which the individual resides

high posterior probability (0.86) and non-overlapping confidence intervals (Fig. 2). Averaging across the multiple runs at $K = 12$, the pattern of genetic structure in *L. maximum* is summarized as follows: Cluster 1 = South Vista Canyon 1; Cluster 2 = South Vista Canyon 2; Cluster 3 = Eagle Canyon; Cluster 4 = Bryce Canyon 1; Cluster 5 = Bryce Canyon 2 + 3; Cluster 6 = Bryce Canyon 3; Cluster 7 = Confluence Canyon + Tire Canyon 3; Cluster 8 = Tire Canyon 1 + 2 + 3 + Keco Canyon 2; Cluster 9 = Keco Canyon 1 + 3 + Tire Canyon 2 + 3; Clusters 10–12 − DMC Canyon 1 + 2 (Fig. 2).

Bayesian analyses performed in BAPS also strongly suggested the presence of 12 clusters with a posterior probability of 1.0. Populations Tire Canyon 1 and Keco Canyon 2 were grouped together, Keco Canyon 1 and 3 populations formed a cluster and DMC Canyon 1 and 2 populations formed a cluster. All other populations were considered to represent single population clusters. This analysis was in agreement with Weir and Cockerham's F-statistics in suggesting that most of the variation is distributed among rather than within populations (model-averaged $F_{ST} = 0.57$, SD = 0.011). Bayesian estimation of migration rates in BAYESASS indicated an absence of recent migration between most populations. Posterior probabilities of the proportion of non-migrants ranged from 0.683 to 0.994 with small confidence intervals surrounding the point estimates (Table 3). Four populations (DMC Canyon 2, Keco Canyon 3, Keco Canyon 2 and Tire Canyon 3) exhibited evidence of migration from other populations. The point estimate of non-migrants in DMC Canyon 2 was 0.683 (CI: 0.667, 0.688), with a detectable amount of migration from DMC Canyon 1 (m = 0.295; CI: 0.271, 0.328). For Keco Canyon 2, the point estimate of non-migrants was 0.702 (CI: 0.667, 0.701) and migration was inferred from Tire Canyon 1 (m = 0.263; CI: 0.231, 0.325). In both of these populations, migration rates from all other populations were less than 0.011. Although Keco Canyon 3 and Tire Canyon 3 also exhibited evidence of migration (non-migration rates = 0.722 and 0.724, respectively), migration from a single source population was not apparent. That is, migration rates from all other populations varied between 0.01 and 0.07.

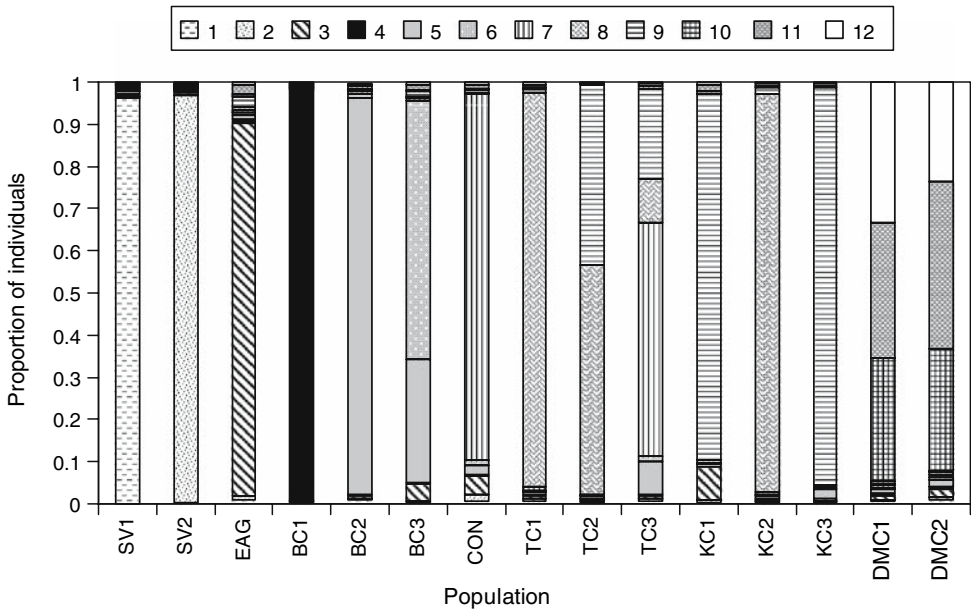

Fig. 2 Classification of individuals from 15 populations of *Lithophragma maximum* into 12 clusters identified by STRUCTURE

Table 3 Means and 95% confidence intervals of the posterior distributions of the proportion of non-migrant and migrant individuals for each population of *Lithophragma maximum* on San Clemente Island

Population	Non-migrants (95% CI)	Migrants (95% CI)
SV1	0.991 (0.968, 0.999)	NA
SV2	0.970 (0.899, 0.999)	NA
EAG	0.989 (0.963, 0.999)	NA
BC1	0.983 (0.939, 0.999)	NA
BC2	0.969 (0.897, 0.999)	NA
BC3	0.974 (0.909, 0.999)	NA
CON	0.991 (0.967, 0.999)	NA
TC1	0.994 (0.978, 0.999)	NA
TC2	0.931 (0.805, 0.997)	NA
TC3	0.724 (0.668, 0.853)	All < 0.022[a]
KC1	0.962 (0.883, 0.999)	NA
KC2	0.702 (0.667, 0.701)	TC1 0.263 (0.231, 0.325)
KC3	0.722 (0.669, 0.837)	All < 0.016[a]
DMC1	0.942 (0.934, 0.955)	NA
DMC2	0.683 (0.667, 0.688)	DMC1 0.295 (0.271, 0.328)

Seven duplicate runs of the Bayesian assignment method of Wilson and Rannala (2003) in BAYESASS were conducted. Migrants are indicated by the source population and the migration rate

Population names are abbreviated as SV1 = S. Vista Canyon 1, SV2 = S. Vista Canyon 2, EAG = Eagle Canyon, BC1 = Bryce Canyon 1, BC2 = Bryce Canyon 2, BC3 = Bryce Canyon 3, CON = Confluence Canyon, TC1 = Tire Canyon 1, TC2 = Tire Canyon 2, TC3 = Tire Canyon 3, KC1 = Keco Canyon 1, KC2 = Keco Canyon 2, KC3 = Keco Canyon 3, DMC1 = DMC Canyon 1, DMC2 = DMC Canyon 2

[a] No single source population could be identified. Migration rates from all populations were below the value indicated

Coancestry-based genetic distances ranged from 1.6972 between Bryce Canyon 1 and Keco Canyon 3 to 0.0157 between DMC Canyon 1 and DMC Canyon 2 (Table 4). The average value across all populations was 0.8637. Geographic and genetic distances in *L. maximum* were significantly positively correlated ($r = 0.45$; $P = 0.01$). The patterns of population similarity indicated in the UPGMA were largely in agreement with the results of the Bayesian analyses in suggesting strong support (i.e., bootstrap values 88–100%) for grouping Tire Canyon 1 and Keco Canyon 2 populations together, Keco Canyons 1 and 3 together, and DMC Canyons 1 and 2 together (Fig. 3).

None of the 15 populations showed genetic evidence of recent reductions in effective population size ($P > 0.05$). Bryce Canyon 1 exhibited a slight heterozygote excess ($P = 0.0625$) that is only marginally non-significant. In contrast, the test of long-term population bottlenecks indicated evidence of reductions in size for Confluence Canyon and DMC Canyon 2, at all three values of theta. The M-ratio value for these populations was 0.66 and 0.69, respectively, with $P < 0.02$ for all values of theta. Additionally, Bryce Canyon 3 and DMC Canyon 1 exhibited evidence of historical bottlenecks at theta = 1 ($P < 0.03$), but not at larger values.

Discussion

Genetic variability and structure

We identified substantial genetic variation in *L. maximum* at microsatellite loci, in contrast to the complete lack of variation observed at 23 allozyme loci (Helenurm, unpubl. data). Although this species possesses many characteristics associated with low levels of genetic diversity, such as moth pollination, gravity-dispersed seeds, and endemism (Loveless and Hamrick 1984), it is not genetically invariant. In fact, estimates of allelic diversity in *L. maximum* ($A = 6.40$) are comparable or higher than reports based on microsatellite markers from some other rare plant species ($A = 1.9$–12.8; (Collevatti et al. 2001; Gao 2005; Gaudeul et al. 2002; Kikuchi and Isagi 2002; Setsuko et al. 2004)). In comparison to *Crossosoma californicum*, another endemic plant on the Channel Islands for which genetic variation has been examined at microsatellite loci (Wallace and Helenurm 2005; Wallace and Helenurm, unpubl. data), *L. maximum* harbors greater allelic diversity ($A = 6.40$ in *L. maximum* compared to $A = 4.2$ in *C. californicum*). Nevertheless, *L. maximum* deviates considerably in terms of heterozygosity, as it exhibits lower heterozygosity than other plant species, regardless of life history (Nybom 2004). Specifically, *L. maximum* exhibits less heterozygosity ($H_e = 0.23$) than short-lived ($H_e = 0.55$) or long-lived ($H_e = 0.68$) perennial species, endemic species ($H_e = 0.42$), species with outcrossing ($H_e = 0.65$) breeding systems or gravity-dispersed seeds ($H_e = 0.47$; Nybom 2004). Only two studies have reported heterozygosity values lower than *L. maximum.* Average expected heterozygosity in the critically endangered Iberian species *Borderea chouardii* was 0.13, possibly due to ant-pollination and poor seed dispersal (Segarra-Moragues et al. 2005). Gao (2005) also reported low expected heterozygosity ($H_e = 0.22$) and even lower observed heterozygosity ($H_o = 0.09$) in wild rice due to inbreeding.

Inbreeding may also be an important determinant of genetic structure in populations of *L. maximum.* Significant inbreeding coefficients were identified across multiple loci for six populations and five of these exhibited deficiencies of heterozygous individuals (Table 1). The strong degree of differentiation among populations ($\theta = 0.52$; Table 2) and the lack of immigrants in most populations (Table 3) may also contribute to the lack of heterozygosity. Although the specific mechanisms involved in the pollination

Table 4 Coancestry distances (above the diagonal) and geographic distances (in km, below diagonal) among 15 populations of *Lithophragma maximum*

	SV1	SV2	EAG	BC1	BC2	BC3	CON	TC1	TC2	TC3	KC1	KC2	KC3	DMC1	DMC2
SV1	****	1.691	1.268	1.600	0.923	1.037	0.887	1.036	1.624	1.310	1.507	1.225	1.651	0.817	0.718
SV2	0.30	****	0.907	1.557	1.131	0.976	0.794	1.018	1.428	1.063	1.210	1.133	1.538	0.931	0.836
EAG	1.10	1.20	****	1.344	0.910	0.814	0.468	0.733	1.100	0.816	0.815	0.838	0.971	0.547	0.458
BC1	2.45	2.55	1.40	****	0.996	0.750	0.903	1.218	1.652	1.315	1.454	1.331	1.697	0.986	0.903
BC2	2.45	2.50	1.35	0.10	****	0.304	0.458	0.810	0.929	0.625	0.905	0.912	0.924	0.661	0.574
BC3	2.40	2.45	1.30	0.20	0.10	****	0.459	0.804	0.716	0.482	0.649	0.881	0.572	0.670	0.605
CON	3.10	3.15	2.05	0.90	0.80	0.85	****	0.5959	0.765	0.395	0.767	0.607	0.780	0.573	0.504
TC1	3.20	3.25	2.10	0.75	0.75	0.80	0.40	****	0.581	0.502	0.708	0.032	0.824	0.660	0.597
TC2	3.20	3.25	2.10	0.80	0.80	0.85	0.30	0.25	****	0.527	0.674	0.542	0.868	0.967	0.880
TC3	3.20	3.25	2.15	0.90	0.90	0.90	0.15	0.10	0.35	****	0.654	0.562	0.795	0.660	0.577
KC1	3.30	3.35	2.10	0.85	0.85	0.90	0.45	0.10	0.15	0.35	****	0.781	0.207	0.709	0.643
KC2	3.30	3.35	2.10	0.85	0.85	0.90	0.45	0.10	0.15	0.35	0.05	****	0.969	0.733	0.661
KC3	3.30	3.35	2.10	0.85	0.85	0.90	0.40	0.15	0.10	0.30	0.10	0.10	****	0.836	0.761
DMC1	3.70	3.75	2.60	1.25	1.25	1.30	0.70	0.50	0.50	0.55	0.45	0.40	0.45	****	0.016
DMC2	3.70	3.75	2.60	1.25	1.25	1.30	0.70	0.50	0.50	0.55	0.45	0.40	0.45	0.03	****

Population names are abbreviated as: SV1 = S. Vista Canyon 1, SV2 = S. Vista Canyon 2, EAG = Eagle Canyon, BC1 = Bryce Canyon 1, BC2 = Bryce Canyon 2, BC3 = Bryce Canyon 3, CON = Confluence Canyon, TC1 = Tire Canyon 1, TC2 = Tire Canyon 2, TC3 = Tire Canyon 3, KC1 = Keco Canyon 1, KC2 = Keco Canyon 2, KC3 = Keco Canyon 3, DMC1 = DMC Canyon 1, DMC2 = DMC Canyon 2

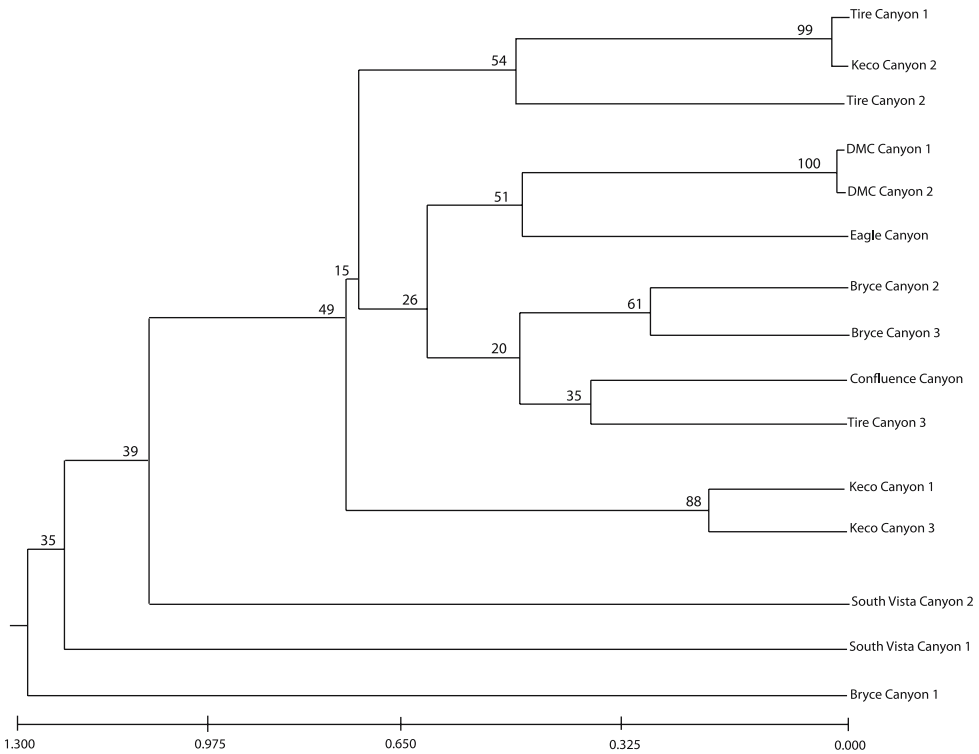

Fig. 3 UPGMA for 15 populations of *Lithophragma maximum* using the coancestry coefficient (Reynolds et al. 1983); bootstrap values greater than 50% are shown on nodes

syndrome of *L. maximum* are not known, most other *Lithophragma* taxa are self-incompatible and pollinated by *Greya* moths (Brown et al. 1997; Pellmyr et al. 1996; Thompson 1997). Inbreeding in *L. maximum* could still result from mating between related individuals. If *L. maximum* is indeed adapted to outcrossing, the frequency and negative impacts of inbreeding are expected to be strengthened by the small size and physical isolation of most populations on San Clemente Island.

The hypothesis that gene flow can be limited in *L. maximum* due to its occurrence in the bottoms of deep canyons is supported by the data presented here. We found a significant positive correlation between genetic and geographic distances, strong genetic differentiation among populations, and a low migration rates for most populations. The average genetic distance between populations was high (0.8637). These data also suggest that gene flow can be limited within canyons since populations separated by less than half a kilometer and in the same canyon had genetic distances of 0.500 or greater (Table 4). For example, the two South Vista Canyon populations are separated by only 300 meters, yet they shared no alleles at five loci and had a genetic distance of 1.691. Additionally, the three populations in Keco Canyon are separated by only 100 meters. Whereas Keco Canyon populations 1 and 3 are genetically similar, Keco Canyon 2 is clearly distinct from the other two populations and instead shows greater similarity to the Tire Canyon 1 population 350 meters away ($D = 0.032$; Table 4).

Although the Bayesian analyses also indicated limited gene flow among most populations, the structure of genetic variation in the DMC, Keco, and Tire canyons is noteworthy. Genetic structure was lacking between the two populations in DMC Canyon as well as between Keco Canyon 2 and Tire Canyon 1. The BAPS analysis also indicated strong similarity between the Keco Canyon 1 and 3 populations, which was in part suggested by STRUCTURE and BAYESASS as well. However, BAYESASS could not identify specific sources of migrants in Keco Canyon 3, suggesting that the information available from just four sampled individuals in each of these populations was not sufficient to accurately identify their source of origin under the model of Wilson and Rannala (2003). Thus, the lack of structure between populations in DMC Canyon suggests that gene flow or seed dispersal can occur across relatively short distances whereas the high similarity between Keco Canyon 2 and Tire Canyon 1 suggests that dispersal and/or gene flow occurs across canyons.

Given the high degree of genetic differentiation observed within such a small geographic range, just 3.7 km of the southeastern coast of San Clemente Island, topographical features of the landscape are likely to be important barriers to gene flow impacting seed dispersal and pollinator activity. Because the seeds of *L. maximum* are gravity-dispersed, there may be little opportunity for long-distance movement if seeds are transported only as far as the immediate area or into suitable habitat directly downhill of the maternal plant. The ruggedness of the

canyons in which *L. maximum* occurs may further isolate populations by limiting pollinator movement among populations in different canyons. Thompson and Cunningham (2002) found a high degree of geographical structure in the abundance of *Greya politella* pollinators in populations of *Lithophragma parviflorum* in the northern Rocky Mountains as well as variation in the frequency that seeds were parasitized, which suggests that coevolutionary relationships with pollinators play a key role in how variation is structured in both species. Similar patterns in *L. maximum* could also explain the high degree of genetic differentiation we observed. Future studies directed at understanding the pollination biology of *L. maximum* would be extremely useful for providing greater understanding of the causes of genetic structure observed in this study as well as reproductive patterns in this species.

Demographic history

The recorded history of the discovery and subsequent rediscovery of *L. maximum* on San Clemente Island suggest that bottlenecks were likely. After a single specimen was collected in 1936, none were found for the following 42 years, in spite of extensive surveys in 1962 (Bacigalupi 1963). At the time of its rediscovery in 1978, only 12 individuals were found. Subsequent field surveys have yielded progressively larger numbers, all at or near the original sites of Bryce and Eagle Canyons (U.S. Fish and Wildlife Service 1997). This apparent recovery coincided with the removal of goats from the island. Shortly after the complete elimination of goats in 1992, census numbers increased to over 200 in 1996 and more than 400 in 2000 (U.S. Fish and Wildlife Service 1997; Helenurm, per. obs.). However, populations of *L. maximum* can be difficult to locate when not in flower. Historical census numbers may not accurately reflect population sizes. The lack of evidence for genetic bottlenecks in most populations may also reflect survival of plants, specifically of rhizomes during heavy herbivory of the above-ground portions of plants by goats. As goat populations were reduced, flower production and reproduction may have resumed, thereby producing the apparent demographic bottleneck and subsequent recovery. Seed banks could also have contributed to the retention of genetic variation during periods of herbivory, given that we did not find any evidence of recent genetic bottlenecks in these data. *Lithophragma maximum* populations almost certainly suffered some reductions in survival and reproduction during intense herbivory. The presence of bottlenecks in the more distant past in as many as four populations may reflect founder events associated with the initial origin and evolution of this species on San Clemente Island.

Conservation implications

Lithophragma maximum is endemic to an extremely small area on southeastern San Clemente Island. One of the greatest threats to its survival has already been eliminated with the final elimination of goats in 1992. The number of individuals located during field surveys has been steadily increasing since the species' rediscovery in 1978. This may be due to a combination of flowers not being eaten by goats as well as an increase in the frequency and intensity of surveys. *Lithophragma maximum* still faces an uncertain future. Theodorou and Couvet (2006) showed that the size of subpopulations and migration rate among subpopulations are the most important factors affecting fitness in subdivided populations. So, while extremely deleterious alleles may be purged, lack of gene flow and small population size, particularly sizes of less than 100 individuals, can decrease fitness due to accumulation of mildly deleterious alleles (Couvet 2002; Frankham et al. 2002; Theodorou and Couvet 2006). Our study indicates that gene flow is highly restricted among most populations of *L. maximum* and no population contains more than approximately 100 individuals. About one-fourth of all known individuals of *Lithophragma maximum* exist in a single canyon (DMC Canyon), which harbors a large amount of the diversity observed in this species (Table 1). A single stochastic event such as a fire or sloughing off of a large section of canyon wall could destroy a large portion of remaining individuals as well as much of the remaining genetic diversity.

Given the overall rarity of *L. maximum* and the fact that a large portion of genetic diversity exists among rather than within populations, great importance should be placed on protecting as many populations as possible to maintain current levels of genetic variability. Populations in three canyons, South Vista Canyon, Keco Canyon, and DMC Canyon, should have highest priority since they contain nearly 77% (49 of 64 alleles) of the microsatellite variation observed in *L. maximum*.

The Fish and Wildlife Service (1997), ostensibly citing Kellogg and Kellogg (1994), stated that *L. maximum* is thought to have existed on the plateau of the island before the introduction of non-native grasses. This statement, however, does not appear in Kellogg and Kellogg (1994) and is not thought to be true (Kellogg, pers. comm.). The microhabitat within the rugged canyon walls of San Clemente Island's southeast coast where *L. maximum* is now found is very unlike the habitat found on the plateau in terms of competitors, water availability, and light. There is no evidence to suggest that *L. maximum* ever existed in a different habitat than it does now, nor in a canyon beyond its current range. *Lithrophragma maximum* is likely to have evolved as an insular habitat specialist and to have always

been rare and restricted to the eastern canyons of San Clemente Island.

Control of invasive species that affect *L. maximum* should also be an integral component of management. Specifically, weed control should be focused on species, such as *Amsinckia intermedia* or *Bromus diandrus*, that are found in the canyons along with *L. maximum* and may compete for suitable habitat or pollinators (U.S. Fish and Wildlife Service 1997). Maximizing available habitat by removing exotic competitors is critical to maintaining current populations and preventing further loss of genetic variation. The current patterns of genetic structure indicate that inbreeding is a potential concern for this species and this may be heightened by the lack of genetic connectivity among most populations. Future studies that focus on ecological and evolutionary causes and consequences of this apparent genetic disconnection and the associated fitness effects would also be useful for management of this species.

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