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HAS HERBIVORY NEGATIVELY IMPACTED GENETIC VARIABILITY IN THE FLORA OF THE CALIFORNIA CHANNEL ISLANDS? INSIGHTS FROM *CROSSOSOMA CALIFORNICUM* (CROSSOSOMATACEAE)

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Alien species have profound ecological and evolutionary impacts on native species. In this study, we used microsatellite loci to evaluate the impact of grazing on *Crossosoma californicum* on the California Channel Islands to predict future threats to this species and to assess the origin of a mainland population. San Clemente Island supports populations of one to seven individuals, whereas Santa Catalina Island supports populations of 30–50 individuals. We hypothesize that these demographic differences are due to variable grazing pressures, and as a result, the islands will differ in genetic structure. We found a strong correlation between population size and genetic variation, with Santa Catalina populations more variable ($p = 85.9$, $A = 3.2$) than San Clemente populations ($p = 49.7$, $A = 1.7$). We also found evidence for ancient but not recent bottlenecks, indicating that *C. californicum* may have historically maintained small populations. The islands are similar in total genetic variation, suggesting that while grazing reduced the number of plants, it did not deplete genetic variation. Simulation studies predict substantial declines in allelic diversity within the next 50 yr. Thus, conservation measures should seek to establish sustainable populations, especially on San Clemente. The mainland population of *C. californicum* was equally divergent from island populations and likely represents insular colonization to the mainland.

Keywords: conservation, genetic variation, insular endemic, microsatellite, population size.

Introduction

Assessments of population genetic structure in rare species are valuable for making biologically sound conservation plans because genetic diversity is a primary component of adaptive evolution and thus an important indicator of long-term survival of populations (Frankel and Soule 1981). Rare species are expected to have levels genetic variation lower than those of widespread congeners. However, species may be considered rare for a variety of reasons, such as narrow geographic range, small population size, or high habitat specificity (Rabinowitz 1981), making it difficult to interpret differences in levels of genetic variation in terms of specific causes. Nevertheless, reviews of allozyme studies have supported this theoretical prediction by demonstrating that rare species generally have lower levels of genetic variation in comparison to those of related widespread species (Gitzendanner and Soltis 2000; Cole 2003), although some rare species have surprisingly high levels of genetic variation (Helenurm 2001).

Population size is a central concept in conservation genetics (Ellstrand and Elam 1993) because it is predictive of the detrimental effects of genetic drift (Nei et al. 1975; Watterson 1984; Lynch et al. 1995). Populations that have recently been reduced in size are especially important foci for management because they are more vulnerable to extinction as a result of catastrophic demographic and genetic factors (Ellstrand and Elam 1993). Genetic drift causes loss of genetic variation, limiting the ability of populations to adapt to changing environments in the long term and potentially reducing their fitness in the short term in spatially or temporally heterogeneous environments by increased inbreeding and other mechanisms (Huenneke 1991). Genetic drift also causes populations to become more differentiated from one another as a secondary consequence of losing variation. Therefore, the degree to which genetic variation is actually lost in diminishing populations of rare and endangered species is of great significance to their long-term persistence. In this study, we examined population genetic structure in *Crossosoma californicum* Nuttall (Crossosomataceae), a rare species found on the Channel Islands of California and Mexico, to assess how historical factors have impacted genetic diversity and to predict future changes in population genetic structure.

Crossosoma californicum is a shrubby species that occurs on Santa Catalina Island and San Clemente Island off the coast of California and Guadalupe Island off the coast of Mexico. A very small population (two plants) exists on the Palos Verdes Peninsula of mainland California (Henrickson 1979), which is roughly 34 km from Santa Catalina and 80 km from San Clemente. It is unclear whether the population at Palos Verdes is natural or introduced. The distribution of *C. californicum* is very different on the two California Channel Islands. On Santa Catalina Island, *C. californicum* is a prominent, large shrub in the chaparral (Kellogg and Kellogg 1994) and is known from several large populations (i.e., 30 to

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~50 individuals). By contrast, *C. californicum* is rare on San Clemente Island and exhibits a scattered, relictual distribution across the entire length of the island. Fifty-eight individuals are known from San Clemente Island. On Guadalupe Island, *C. californicum* is decreasing in number and is threatened with extinction (Moran 1996). Only a few individuals have been seen recently (S. Junak, Santa Barbara Botanic Garden, personal communication), and these occur on inaccessible rock faces.

The difference in the size and distribution of populations on the California Channel Islands may reflect different grazing histories related to the intensity of grazing, preference of grazing species for *C. californicum*, and length of grazing pressure. Santa Catalina has always been under private ownership, and although sheep and cattle ranching occurred on this island, ranching was secondary to recreation and tourism (O'Malley 1994). The scattered distribution of *C. californicum* along the length of San Clemente (fig. 1) and the presence of seemingly suitable habitat suggest that it also was once much more common. Populations on San Clemente may have been more severely grazed than those on Santa Catalina, given that the former island has never been heavily visited or used for recreation (Halvorson 1994). Additionally, heavy concentrations of sheep, cattle, pigs, and goats were present on San Clemente from the mid-1800s until the early 1930s, when the U.S. Navy began to use the island for military training. Since the removal of all feral goats from the island in 1992, considerable vegetative recovery has been observed (Kellogg and Kellogg 1994).

Endemic plants of the Channel Islands evolved as tender flora in the absence of intense grazing. Given that more than 30,000 grazing animals existed on Santa Catalina in 1863

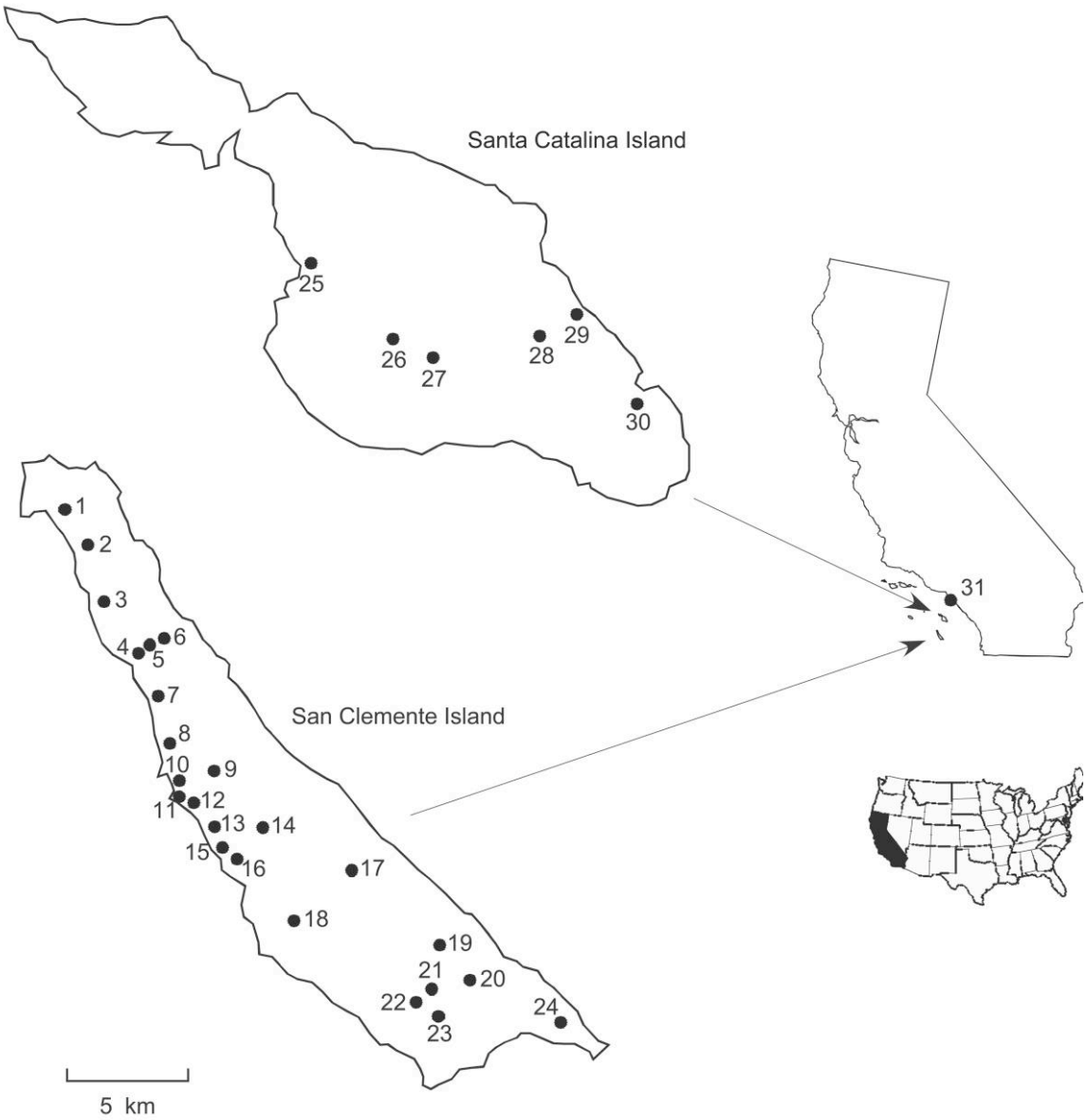

Fig. 1 Locations of sampled populations of *Crossosoma californicum* on the Channel Islands and mainland California. Population numbers correspond to those in table 1. Population 31 is on the Palos Verdes Peninsula. Scale bar is accurate for the inset map only.

(O'Malley 1994) and that San Clemente supported populations of 12,000 sheep and 29,000 goats from the late nineteenth century until 1989 (Keegan et al. 1994; O'Malley 1994), grazing has undoubtedly affected population sizes on both of these islands and perhaps has caused changes in the structure of genetic variation in this species. Here, we report on contrasting levels of genetic variation and structure for populations on San Clemente and Santa Catalina islands using 13 polymorphic microsatellite loci. This study was initiated as part of an investigation of the conservation genetics of rare and endangered plant species of San Clemente Island, California. The objectives of this study are (1) to compare the amount and distribution of genetic variation in populations on San Clemente and Santa Catalina islands, (2) to evaluate the influence of historical demographic factors on the distribution of genetic variation in populations, (3) to evaluate the origin and genetic relationships of the recently discovered population on Palos Verdes Peninsula, and (4) to make recommendations for the conservation of *C. californicum*. We expect that gene flow between islands is very low, resulting in substantial structure by island. Additionally, we expect that intense herbivory over the past century has caused genetic bottlenecks.

Material and Methods

Leaf samples were collected from 31–40 individuals in each of six populations on Santa Catalina Island, all known individuals ($n = 58$) on San Clemente Island, and the two individuals of the Palos Verdes Peninsula population (fig. 1). DNA was extracted from frozen leaf tissue using the CTAB extraction protocol (Doyle and Doyle 1987). Thirteen polymorphic microsatellite loci were amplified in all individuals using primers developed specifically for *Crossosoma californicum*; primer sequences and polymerase chain reaction protocols were reported previously (Wallace and Helenurm 2005). All fragments were genotyped using an Avant 3100 Genetic Analyzer; fragments were sized using GeneMapper software (Applied Biosystems, Foster City, CA).

Although individuals occur in discrete areas on San Clemente, the small size of many of the populations (i.e., one to two individuals) precludes calculation of many traditional population genetic parameters (e.g., expected heterozygosity and inbreeding coefficient) that rely on a model of population structure. Nevertheless, there may still be substantial genetic structure among fragmented populations. Therefore, we used an approach that combined analyses of genetic structure among individuals with traditional estimators of population-level diversity. For all populations, each of the islands, and the species, we determined the percentage of polymorphic loci (p), mean number of alleles per locus (A), effective number of alleles per locus (A_e), and observed heterozygosity (H_O) using POPGENE, version 1.31 (Yeh et al. 1999). For groupings consisting of four or more samples, covering populations, islands, and the species, we also calculated expected heterozygosity (H_E) and the inbreeding coefficient (F_{IS}) over all loci. The F_{IS} values for individual loci were tested for deviation from Hardy-Weinberg equilibrium using probability tests implemented in FSTAT, version 2.9.3.2 (Goudet 1995); we applied a Bonferroni correction for multiple comparisons to determine statistical significance.

We used analyses of molecular variance (AMOVA) based on F_{ST} values between groups, implemented in ARLEQUIN, version 2.0 (Schneider et al. 2000), to evaluate the hierarchical structure of genetic variation between the islands and across populations on Santa Catalina (Excoffier et al. 1992). The significance of variance components was tested through 10,000 permutations of the data. We also used the software STRUCTURE, version 2 (Pritchard et al. 2000), to test for genetic structure within each island. We followed the recommendations of the authors when running the analyses, including the implementation of an admixture ancestral model and a correlated allele frequency model. 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 500,000 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 $K = 1$–7 (i.e., number of populations + 1) for Santa Catalina Island and $K = 1$–25 for San Clemente Island. Eleven independent runs were completed for each K to test for convergence of the Markov chain. We considered both $\ln p(D)$ and ΔK, which represents the rate of change in log probabilities of the data between successive K values and was found by Evanno et al. (1995) to more accurately estimate the true K than $\ln p(D)$ (Pritchard et al. 2000), in the estimation of K.

Because we expected that grazing history likely had a profound influence on the demographic history of *C. californicum* populations, we were also interested in whether recent bottlenecks could be detected in these data. We used BOTTLENECK (Cornuet and Luikart 1996; Piry et al. 1999) to test for an excess of heterozygosity in all populations on Santa Catalina. Because the number of alleles is reduced faster than H_E after a bottleneck (Maruyama and Fuerst 1985), H_E should be higher than the equilibrium heterozygosity predicted in a stable population from the observed number of alleles. 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. Loci that exhibited significant deviations from Hardy-Weinberg equilibrium for each population were not considered in the analysis. Wilcoxon's sign rank test, which combines the results for each locus in a global test, was used to detect a significant excess of heterozygosity within each population (Luikart and Cornuet 1998). We also assessed historical bottlenecks using the M-ratio test (Garza and Williamson 2001), which is based on the ratio of the number of alleles to the range in allele size. 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 90% and the average size of multistep mutations to 3.5. Because little is known about population sizes of *C. californicum* before grazers were introduced to the islands, we tested the inference of a population bottleneck across $\theta = 1$, 5, and 10, representing very small to large prebottleneck populations. We did not test the populations on San Clemente Island for evidence of bottlenecks because of their small population sizes.

The computer program BottleSim, version 2.6 (Kuo and Jansen 2003), was used to predict future rates of decline in genetic variation on the two islands. BottleSim is specifically designed to simulate genetic consequences of bottlenecks and postbottleneck population growth for long-lived species, allowing for an overlapping-generation model. The initial conditions of the simulation were based on the current allele frequencies on each of the islands. Assuming historical panmixia on each of the islands, bottlenecks were modeled for 58 individuals on San Clemente and 208 individuals on Santa Catalina based on the current number of individuals known from each of the islands. The prebottleneck population size was set to 500 individuals for both islands based on Bayesian estimates of θ resulting from an analysis with the software Migrate, version 2.0 (Beerli and Felsenstein 1999, 2001), and 1000 iterations per simulation were performed. Because we also do not know the longevity of this species, we used values of 10, 30, and 50 yr. Likewise, the time to maturation was tested for 2 and 5 yr.

The relatedness of the two individuals from the Palos Verdes Peninsula was evaluated by examining their placement in an unweighted pair group method analysis (UPGMA) based on Nei's (1978) unbiased genetic identity. The UPGMA was performed in TFPGA, version 1.3 (Miller 1997), and support for the phenogram was assessed using 10,000 bootstrap replicates. We conducted an assignment test to determine the most likely population of origin for each of the Palos Verdes individuals. Bayesian analyses of assignment based on multilocus genotypes were performed using the program GENECLASS (Cornuet et al. 1999). The probability of assignment was assessed through the simulation of 10,000 individuals following the method of Paetkau et al. (2004) and using an α value of 0.01.

Results

Genetic Variation and Structure

Seventy-one alleles were observed at 13 microsatellite loci in 268 individuals. The average number of alleles per locus was 5.5 (table 1), but many alleles occurred at very low frequencies. For example, the mean number of alleles per locus averaged 3.2 among the Santa Catalina populations, whereas the mean effective number of alleles per locus across populations on this island was only 1.7 (table 1). Six alleles were unique to San Clemente Island, 10 to Santa Catalina Island, and only one to Palos Verdes Peninsula; 20 alleles were found in all three areas.

Although the islands had many alleles in common, the structure of genetic variation differed between them and largely reflected differences in population size (fig. 2). For example, p, A, and A_E are each highly correlated with population size (Spearman's $\rho = 0.740$, 0.772, and 0.569, respectively; $P < 0.001$ for all comparisons). Additionally, levels of genetic variation varied more among populations on San Clemente Island than among populations on Santa Catalina Island as indicated by the wider range of values for p, A, A_E, and H_O (table 1). Finally, genetic variation within populations was significantly higher for Santa Catalina Island than for San Clemente Island as measured by p (ANOVA, $F_{1,28} = 36.1$, $P < 0.0001$), A ($F_{1,28} = 102.1$, $P < 0.0001$), and A_E ($F_{1,28} = 5.0$, $P = 0.033$). In spite of the small size of Palos Verdes Peninsula, all measures of genetic variability there were similar to or greater than the values in many of the larger populations on Santa Catalina Island (table 1).

Fixation indices were significantly higher on Santa Catalina Island than on San Clemente Island (mean $F_{IS} = 0.07$ vs. -0.135; ANOVA $F_{1,11} = 13.749$, $P = 0.003$). Whereas six of the seven populations on San Clemente Island for which F_{IS} could be calculated exhibited a slight excess of heterozygotes, all populations on Santa Catalina, except Little Harbor, exhibited a slight excess of homozygotes (table 1). However, only three loci in two populations were found to exhibit significant deviations ($P < 0.05$) from Hardy-Weinberg equilibrium (two loci in Garage and one locus in Avalon Canyon) after applying a Bonferroni correction for multiple comparisons.

An AMOVA indicated that only 7.72% of the variation observed in *C. californicum* was distributed between San Clemente and Santa Catalina islands (table 2). Though this is a small percentage, it was statistically significant ($P < 0.0001$). Similarly, 78% and 95% of the variation observed on San Clemente and Santa Catalina islands, respectively, was found to reside within populations. There was some variation in the value of $\ln p(D)$ across STRUCTURE runs even though the MCMC chains converged. The variance in estimates of the likelihood value increased with the number of inferred clusters, a finding that is consistent with the work of Evanno et al. (2005). The mean natural logarithm value across 11 independent runs predicted the same number of clusters as did ΔK for both islands, but the difference in ΔK was considerably greater than the difference in $\ln p(D)$ across K such that a single value of K was easily chosen. On the basis of these results, we recognized four genetic clusters and a great deal of admixture among the six populations on Santa Catalina Island. Only in the Little Harbor population did we find that more than 50% of the individuals were assigned to cluster II (fig. 3). Few individuals from the other populations (3%–16%) were assigned to this cluster. While there was a strong signature of admixture for all of these populations, the four clusters identified roughly correspond to the geographic placement of populations on Santa Catalina. Little Harbor and Skull Canyon represent the southern and northern clusters, respectively. For Skull Canyon, 45% of the genotypes were assigned to cluster III. Avalon Canyon and Toyon Bay make up a central-eastern cluster with 40% of the individuals in each of these populations assigned to cluster I, and Middle Ranch Canyon makes up a central-western cluster with 46% of the genotypes assigned to cluster IV. The Garage population may serve as a stepping-stone between the Skull Canyon and the Middle Ranch Canyon clusters since individuals were assigned in nearly equal proportions (33%) to clusters III and IV (fig. 3). San Clemente Island is recognized to have 24 unique, although extremely small, populations, but only two clusters were inferred for this data set. Most individuals were strongly assigned to a single cluster, and more individuals were assigned to cluster II than to cluster I (fig. 3). Unlike populations on Santa Catalina Island, there was no geographic structure to the clusters on San Clemente Island.

Genetic Signatures of Population Bottlenecks

No evidence of a genetic bottleneck was detected in any of the Santa Catalina populations as measured by an excess of heterozygosity (table 3). All analyses of historical bottlenecks

Table 1

Summary of Genetic Variation at 13 Polymorphic Microsatellite Loci in *Crossosoma californicum*

Population	n	p	A	A_e	H_O	H_E	F_{IS}
San Clemente Island:							
1. West Cove	2	69.2	1.6	1.8	.538	NA	NA
2. Dunes	4	38.5	1.7	1.4	.211	.192	.043
3. Flasher	2	38.5	1.4	1.4	.385	NA	NA
4. Abalone	2	69.2	1.8	1.6	.461	NA	NA
5. Tank	4	61.5	2.4	2.1	.461	.353	−.171
6. Northwest Tank	1	30.8	1.3	1.3	.308	NA	NA
7. Exclosure Cage	2	38.5	1.4	1.3	.346	NA	NA
8. Weather Station	2	38.5	1.4	1.4	.385	NA	NA
9. Eel Cove Canyon	1	30.8	1.3	1.3	.308	NA	NA
10. Seal Cove	4	46.1	1.8	1.6	.346	.245	−.286
11. South Seal Cove	1	53.8	1.5	1.5	.538	NA	NA
12. Lost Point Trail	4	69.2	2.3	1.8	.385	.312	−.091
13. Pappy	2	69.2	2.1	1.9	.500	NA	NA
14. Wall Rock Point	1	46.1	1.5	1.5	.461	NA	NA
15. Wall Rock Canyon	6	69.2	2.5	1.9	.449	.392	−.054
16. Mail Point	1	38.5	1.4	1.4	.385	NA	NA
17. Box Canyon	1	61.5	1.6	1.6	.615	NA	NA
18. Horse Canyon	1	30.8	1.3	1.3	.308	NA	NA
19. China Gully	2	46.1	1.5	1.5	.461	NA	NA
20. China Canyon	2	61.5	1.9	1.8	.461	NA	NA
21. Horse Beach Canyon	1	38.5	1.4	1.4	.385	NA	NA
22. Chenetti Canyon	7	46.1	1.5	1.1	.132	.098	−.274
23. Sibara Ridge	4	61.5	2.1	1.7	.385	.308	−.111
24. China Confluence	1	38.5	1.4	1.4	.385	NA	NA
Mean (SE)	2.4 (.3)	49.7 (2.8)	1.7 (.1)	1.5 (.0)	.400 (.021)	.272 (.038)	−.135 (.045)
All San Clemente	58	84.6	4.2	2.1	.371	.406	.093
Santa Catalina Island:							
25. Little Harbor	31	100.0	3.5	1.8	.392	.367	−.051
26. Garage	35	84.6	3.1	1.8	.309	.348	.109
27. Skull Canyon	35	76.9	3.0	1.7	.279	.306	.102
28. Middle Ranch Canyon	35	76.9	3.1	1.7	.283	.323	.138
29. Toyon Bay	32	84.6	3.1	1.7	.313	.321	.026
30. Avalon Canyon	40	92.3	3.7	1.8	.308	.336	.097
Mean (SE)	34.7 (1.3)	85.9 (3.7)	3.2 (.1)	1.8 (.0)	.314 (.017)	.334 (.009)	.070 (.029)
All Santa Catalina	208	100.0	4.8	1.8	.312	.352	.108
Palos Verdes	2	84.6	2.1	1.9	.423	NA	NA
Species	268	100.0	5.5	1.9	.326	.378	.135

Note. n, sample size; p, percent polymorphic loci; A, mean number of alleles per locus; A_e, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; NA, statistic could not be calculated because of small sample size.

based on heterozygosity excess were quite robust to changes in the mutation model used, yielding P values in excess of 0.8. By contrast, the M-ratio test detected significant evidence of a bottleneck for several Santa Catalina populations, but the level of significance varied strongly across different values of θ (table 3). At $\theta = 1$, the M-ratio was significantly smaller than expected by chance for four of the six Santa Catalina populations ($P < 0.05$), but the probability was significant only for Avalon Canyon and Garage at $\theta = 5$ and only for Garage at $\theta = 10$. The M-ratio values were generally high across all populations and clusters, ranging from 0.70 in Garage to 0.85 in Skull Canyon.

Simulation projections of reductions in the observed number of alleles and expected heterozygosity over the next 200-yr period are summarized in figure 4. The observed number of alleles declines at a faster rate than expected heterozygosity, as predicted by theoretical studies (Nei et al. 1975; Allendorf 1986; England et al. 2003). Increased longevity and a longer time to maturation resulted in the retention of higher levels of genetic variation at the end of the 200-yr period and a slower rate of decline in the first 50 yr, although longevity had a greater impact than maturation time (fig. 4). On the basis of actual frequencies and the current number of individuals on Santa Catalina Island, *C. californicum* is predicted to lose 14%–30% of the number of alleles observed across these 13 microsatellite loci, depending on longevity and maturation time. A maximum of 4% of expected heterozygosity is predicted to disappear in the next 50 yr, and as much as 13% could be lost within 200 yr (fig. 4). The fate of genetic variation in *C. californicum* on San Clemente Island is predicted to be similar for allelic diversity, but a greater amount of heterozygosity will be lost relative to Santa Catalina. On San Clemente, between 16% and 34% of allelic diversity is predicted to be lost in the first 50 yr, and ~56% could be lost within 200 yr (fig. 4). Expected heterozygosity will be re-

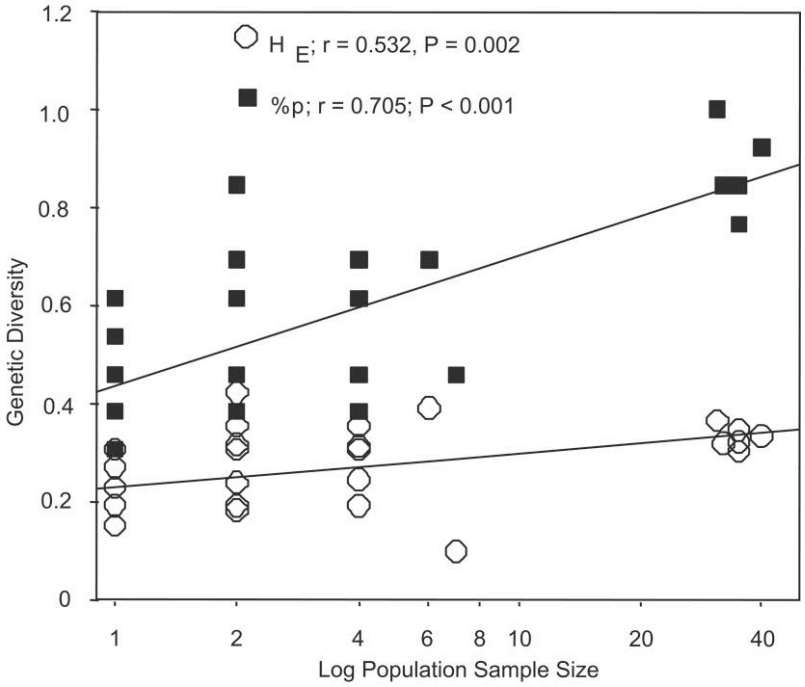

Fig. 2 Relationship between the percent of polymorphic loci (p) and expected heterozygosity (H_E) with log population size in *Crossosoma californicum* populations.

duced by a maximum of 12% in the next 50 yr and by as much as 38% in the next 200 yr, although the predicted decline in expected heterozygosity was strongly dependent on the value of longevity used (fig. 4).

Origin of the Palos Verdes Peninsula Population

Individuals on San Clemente and Santa Catalina exhibited high genetic identities with the two plants on the Palos Verdes Peninsula as indicated in the UPGMA (fig. 5). The mean genetic identity was 0.820 (range = 0.796–0.855) for Santa Catalina and Palos Verdes and 0.797 (range = 0.618–1.0) for San Clemente and Palos Verdes. In contrast, Bayesian assignment tests suggested a low probability of ancestry from the island populations for the Palos Verdes Peninsula individuals. For one plant, the probability of ancestry from the islands was 0, while the probability of ancestry for the other plant was 0.015 from Santa Catalina and 0.131 from San Clemente.

Table 2

Hierarchical Structure of Genetic Variation in *Crossosoma californicum* on Santa Catalina and San Clemente Islands Based on Analyses of Molecular Variance

Source of variation	df	Variance component	Variation (%)
Interisland comparison:			
Among islands	1	.201	7.72
Within islands	530	2.400	92.28
Total	531	2.601	100.00
San Clemente populations:			
Among populations	23	.604	22.12
Within populations	92	2.124	77.88
Total	115	2.728	100.00
Santa Catalina populations:			
Among populations	5	.112	4.79
Within populations	410	2.224	95.21
Total	415	2.336	100.00

Note. No population subdivisions were made for either of the islands in the interisland comparison. Variation residing among the islands (interisland comparison) and among populations on San Clemente is significant ($P < 0.001$).

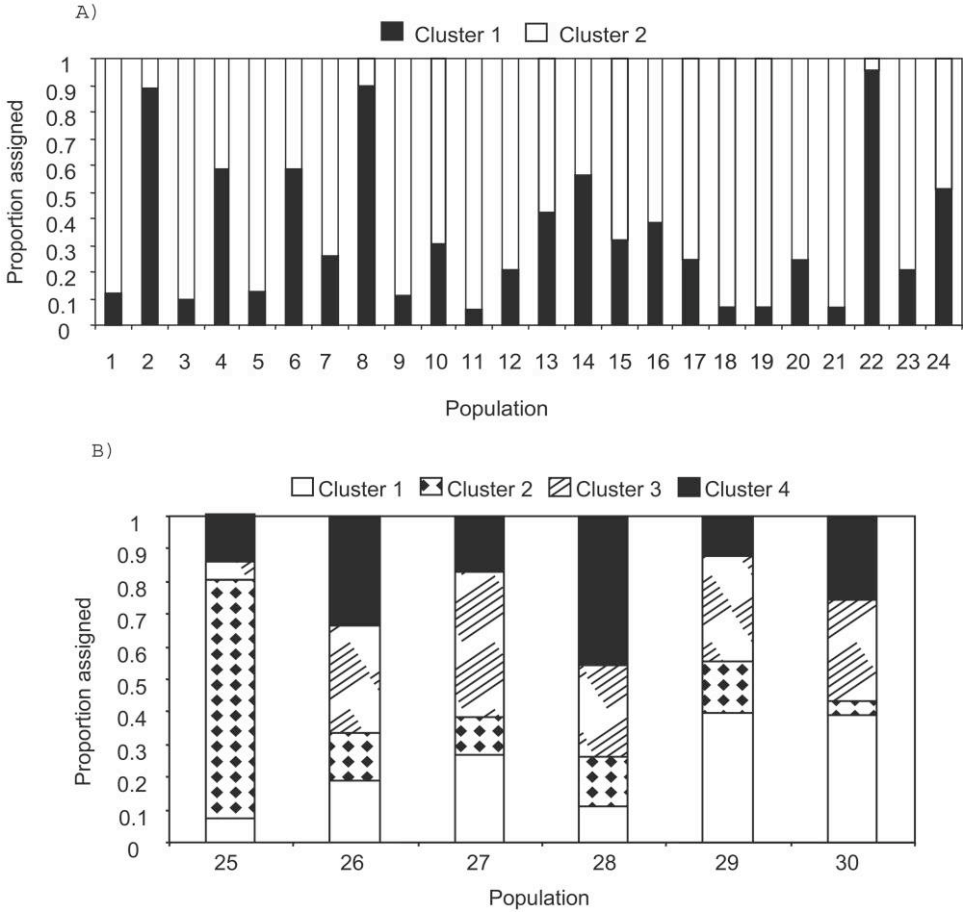

Fig. 3 Proportion of individuals of *Crossosoma californicum* assigned to each of the clusters identified by the program STUCTURE for populations on San Clemente (*A*) and Santa Catalina (*B*). Population numbers correspond to those listed in table 1 and fig. 1.

Discussion

Genetic Variability and Structure

Genetic variation within populations of *Crossosoma californicum* is considerably lower than reports of microsatellite variation in other plants (Nybom 2004), but variation at these microsatellite loci is much higher than a previous survey of genetic variation in *C. californicum* on San Clemente Island on the basis of allozyme variation (K. Helenurm, unpublished data). Importantly, similar levels of genetic variation were found on San Clemente and Santa Catalina islands (table 1) despite a large difference in the number of individuals found on the two islands. Populations of *C. californicum* exhibited a three- to fourfold range in A, p, and H_E, but even the least variable populations were far from lacking genetic variation (e.g., $p = 46.1$, $A = 1.5$, and $H_E = 0.098$ for Chenetti Canyon, $n = 7$). This is remarkable, considering the small population sizes: 18 of the 31 populations consist of only one or two individuals, seven populations have four to seven individuals, and the remaining six populations have 30–50 individuals. The large size and distinctive color of this shrub, combined with intensive searching for this species by a number of biologists over the past 10 yr, make it unlikely that many individuals on San Clemente have escaped detection. Thus, the finding that some of the small populations have surprisingly high levels of genetic variation is an important characteristic of the current species distribution on San Clemente and may reflect selection for high heterozygosity (Baker-Brosh 1996) since six of the seven San Clemente populations, for which F_{IS} could be determined, have an excess of heterozygous genotypes (mean $F_{IS} = -0.135$). This is especially unusual for microsatellite studies (Nybom 2004), which often report heterozygote deficiencies due in part to the presence of null alleles at these loci (Morand et al. 2002).

As is the case for many plant species, the majority of genetic variation occurs within populations (92.3%; table 3), but significant differentiation of *C. californicum* on San Clemente and Santa Catalina islands was detected. AMOVA indicated that a significant portion of genetic variation (7.7%; $P < 0.0001$) occurred among the islands. Additionally, populations on Santa Catalina form a group with high genetic identities to the exclusion of individuals on San Clemente (fig. 5). Last, unique alleles occurred at nine of the 13 loci studied: six alleles were unique to San Clemente Island (ranging in frequency from 0.009 to 0.121) and 10 alleles were unique to Santa Catalina Island (ranging in frequency from 0.002 to 0.096).

Table 3

Identification of Bottlenecks in *Crossosoma californicum* Populations on Santa Catalina Based on the Heterozygosity Excess Test and the *M*-Ratio Test

Population	Het excess	*M*-ratio	*P* value $\theta = 1$	*P* value $\theta = 5$	*P* value $\theta = 10$
Avalon Canyon	.99	.72	.03*	.04*	.10
Garage	.90	.70	.01*	.02*	.05*
Little Harbor	.99	.79	.04*	.37	.60
Skull Canyon	.95	.85	.25	.81	.94
Toyon Bay	.95	.75	.01*	.18	.36
Middle Ranch Canyon	.86	.83	.14	.66	.85

Note. *P* values are presented for the heterozygosity excess test (Het excess) and the *M*-ratio test in addition to the value of the *M*-ratio for each population.

* *P* values ≤ 0.05; indicative of a population bottleneck.

In contrast to the number of geographically recognized populations, only four clusters were identified on Santa Catalina with STRUCTURE. These four clusters roughly correspond to the four geographic areas where populations occur on Santa Catalina (i.e., northern, southern, central-eastern, and central-western; fig. 1). These results suggest that populations on Santa Catalina have been connected for quite some time through long-distance dispersal among widely spaced populations or through a denser collection of populations in the past. The same may hold true for San Clemente Island. Among the 24

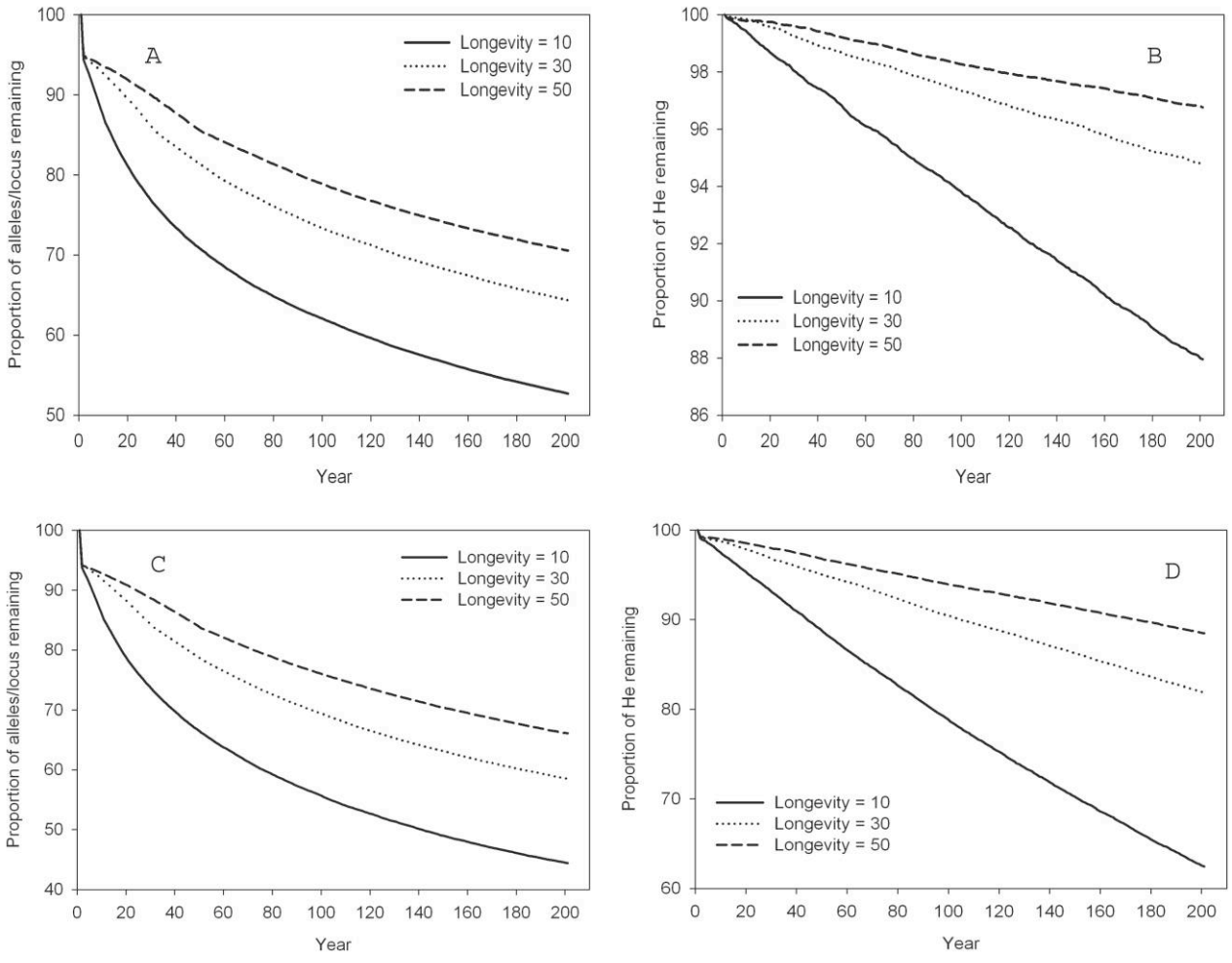

Fig. 4 Proportion of the number of alleles per locus and observed heterozygosity (H_O) expected to be lost in *Crossosoma californicum* over a 200-yr period based on computer simulations of recent reductions in population size on Santa Catalina Island (*A*, *B*) and San Clemente Island (*C*, *D*) expressed as a function of longevity. Time to maturation was set to 2 yr in the simulations.

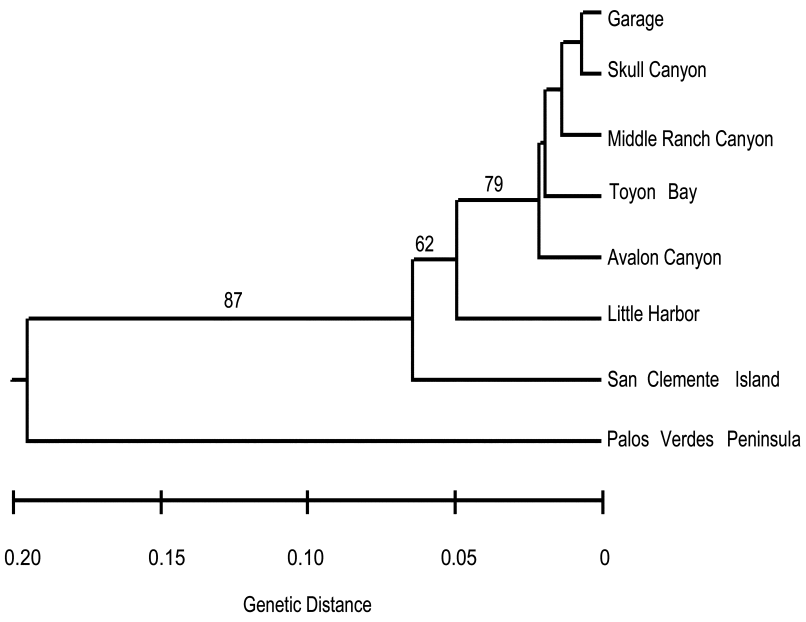

Fig. 5 Midpoint-rooted phenogram based on unweighted pair group method analysis of Nei's (1978) unbiased genetic distance between populations of *Crossosoma californicum* on the California Channel Islands and mainland California. Values above branches indicate bootstrap support greater than 50%. Populations from San Clemente Island were grouped together due to small sample sizes.

discrete populations, STRUCTURE recognized only two genetic clusters on San Clemente, and the placement of individual genotypes into each of the two clusters is nearly random. These results are in contrast to other studies of spatial genetic structure in *C. californicum* on San Clemente, which indicated significant clustering of alleles at ~9-km intervals (K. Helenurm et al., unpublished data). This distance is approximately the distance between successive groups of populations (1–7, 8–18, 19–24). Because STRUCTURE did not identify these three groupings, we suggest that while gene flow may occur maximally at these distances, populations in the three regions are not necessarily in Hardy-Weinberg equilibrium. Given the lack of significant deviations from Hardy-Weinberg equilibrium in the larger populations on Santa Catalina, we expect that *C. californicum* is a primarily outcrossing species. Experimental studies have demonstrated that pollinators are required for seed production, and although the species is self-compatible, seed set is doubled with outcrossed pollen (Scogin and Tatsuno 1982). Gene flow in *C. californicum* is most likely to occur through pollen dispersal rather than seed dispersal because seed dispersal is passive, with seeds falling from follicles and remaining beneath the maternal plant (Junak and Wilken 1998).

Influence of Historical Demographic Factors on Genetic Variation

The similarity in overall levels of genetic variation on San Clemente and Santa Catalina islands is convenient for comparing the effects of population size on the distribution of genetic variation. If genetic drift or inbreeding strongly influences genetic structure in *C. californicum*, the smaller populations on San Clemente Island should contain less variation and exhibit higher levels of interpopulation differentiation than the larger populations on Santa Catalina Island (Ellstrand and Elam 1993). This prediction is borne out in this study; populations on Santa Catalina have, on average, nearly twice as many polymorphic loci ($p = 85.9\%$ vs. 49.7%) and alleles per locus ($A = 3.2$ vs. 1.7) compared with the San Clemente populations. Genetic variation is strongly positively correlated with population size for these two measures (fig. 2), and populations on San Clemente exhibit a higher level of differentiation than those on Santa Catalina ($F_{ST} = 0.22$ vs. 0.05, respectively; table 3).

The levels and distribution of microsatellite variation that we observed on San Clemente suggest that these populations are currently experiencing early stages of drift in which some alleles have been lost in small populations (resulting in lower A and p values), heterozygosity is high even in the smallest populations, and there has been little overall loss of genetic variation on the island. *Crossosoma californicum* is a long-lived shrub. The current populations on San Clemente Island range in size from one to seven individuals and likely represent the remaining members of originally larger populations culled by grazers. No seedling establishment has been observed on San Clemente Island over the past decade despite abundant seed production (K. Helenurm, personal observation), indicating that recruitment is if not generally rare at least highly periodic. Continued drift, then, should cause all of the small populations on San Clemente Island to lose genetic variation at variable rates.

The populations on Santa Catalina, in contrast, may be more typical of this species. The inability to detect significant bottlenecks in these populations using the heterozygosity excess test suggests that *C. californicum* may be characterized by populations of relatively small effective size for a long time (table 3). Although it may be surprising to find no evi-

dence of recent bottlenecks in the Santa Catalina populations, given the presence of grazers on this island from the mid-1800s to the 1950s, other species expected to have experienced bottlenecks have not exhibited losses of genetic diversity consistent with theoretical expectations either (Vernesi et al. 2003; Martinez-Cruz et al. 2004), including an additional endemic species of San Clemente, *Lithophragma maximum* (Furches et al. 2008). Several factors may account for this finding in *C. californicum*. Grazing intensity was probably not as severe on Santa Catalina Island as it was on San Clemente Island (O'Malley 1994), and thus the bottleneck may not have been severe or long lasting. Additionally, if *C. californicum* populations have been present on Santa Catalina Island for a long time and they have always been small (i.e., fewer than 100 individuals), they have probably reached equilibrium (Garza and Williamson 2001) and may be less susceptible to the erosive effects of bottlenecks. Only three loci in two populations deviated significantly from Hardy-Weinberg equilibrium, suggesting that these populations are indeed at equilibrium.

The *M*-ratio test, however, indicated that four populations on Santa Catalina experienced bottlenecks under small historical effective population sizes ($\theta = 1$), which is a reasonable assumption for this species, given its life history. The apparent discrepancy between the heterozygosity excess test and the *M*-ratio test in identifying bottlenecks is likely due to the difference in the age of bottlenecks that each test detects. The *M*-ratio test is best suited for detecting older bottlenecks (Garza and Williamson 2001), perhaps 125–500 generations ago, whereas heterozygosity requires a shorter time to reach equilibrium and therefore detects recent reductions in population size. The older bottlenecks we detected with the *M*-ratio test may have been associated with the original founding of populations on Santa Catalina, as insular colonization generally involves few individuals and thus represents an extreme genetic bottleneck (Abdelkrim et al. 2005). Additionally, populations may have remained relatively small because of the slow-growing nature of this species and the restricted distribution on Santa Catalina Island in chaparral and less frequently in coastal scrub communities (Junak and Wilken 1998; E. Kellogg, personal communication). If populations started out small and remained small, substantial loss of genetic variation after subsequent bottlenecks is not necessarily expected. Finally, Garza and Williamson (2001) suggested that high allelic diversity and a high *M*-ratio, which we found for several populations, are indicative of populations that have been small for a long time.

Origin of the Palos Verdes Peninsula Population

Discovery of *C. californicum* on the Palos Verdes Peninsula raises several interesting possibilities regarding the evolution and dispersal of this taxon. Populations of several other endemic taxa of California's Channel Islands are also found on the peninsula (S. Junak, personal communication). The Palos Verdes Peninsula was originally an island (Vedder and Howell 1980) that is estimated to have uplifted from the Pacific Ocean some 650,000 yr BP. It is younger than San Clemente Island at 4.4 Myr BP (Muhs et al. 2002; Adler 2003) and Santa Catalina Island at 3.2–5.0 Myr BP (T. Rockwell, San Diego State University, personal communication), and the peninsula has only recently become connected to the mainland as a result of dropping sea levels (~80,000 yr BP).

The likelihood that *C. californicum* evolved on the mainland and subsequently dispersed to the Channel Islands seems low given that no other localities have ever been found on mainland California. If *C. californicum* is assumed to have evolved within the Channel Island archipelago and subsequently dispersed to its current distribution, then the Palos Verdes population could be a relictual population from Palos Verdes when it was an island or a recent introduction to mainland California from San Clemente or Santa Catalina. These data clearly do not support a recent origin of the Palos Verdes population(s) from either of the islands. Average genetic identities are similar between Palos Verdes Peninsula and San Clemente Island ($I = 0.797$) and Santa Catalina Island ($I = 0.820$), and the UPGMA based on these identities shows the Palos Verdes population as equally distant from San Clemente and Santa Catalina islands (fig. 5). Assignment tests based on multilocus genotypes link one Palos Verdes individual more strongly to individuals on San Clemente Island, while the other individual is not linked to either of the islands. Shared alleles are similarly discordant: five alleles are shared by Palos Verdes Peninsula and Santa Catalina Island, and one allele is shared by Palos Verdes Peninsula and San Clemente Island. However, all six of these alleles are rare on their islands (ranging in frequency from 0.005 to 0.05), suggesting that they could originally have been present on both islands but were either unsampled (Santa Catalina Island) or lost by genetic drift (either island). Thus, while the distinctiveness of the Palos Verdes population suggests a historically wider distribution for *C. californicum*, these data are not useful for evaluating whether this species had a much wider historical distribution on mainland California. Additional data that would allow us to date the time of divergence of the Palos Verdes population relative to the island populations or to reconstruct possible range boundaries during the Pleistocene would be useful for evaluating alternative phylogeographic hypotheses regarding this population.

Conservation Implications

A primary concern for the preservation of *C. californicum* on the Channel Islands is population size and the effect that a loss of individuals will continue to have on levels of genetic diversity and evolutionary adaptability. While these data show lower levels of genetic variation in *C. californicum* compared with other plant species (Nybom 2004), *C. californicum* is not genetically depauperate. Nevertheless, there are important differences in the structure of genetic variation on each of the islands that support the recognition of these populations as genetically independent units within *C. californicum*. On Santa Catalina, we recommend the preservation of genetic variation in large populations from each of the four clusters identified by STRUCTURE, as these may represent historical centers of genetic variation.

The combination of extremely small population sizes and substantial differences in allelic composition and heterozygosity among populations on San Clemente Island renders them highly vulnerable to extinction due to stochastic factors. Be-

cause the San Clemente populations are believed to be in the early stages of genetic drift, it is a critical time when management should be strongly focused on increasing population sizes to maintain current levels of genetic variation. Continued drift due to small population size on San Clemente Island will further erode the current levels of genetic variation as adult individuals die, and there is little recruitment into these populations. Simulation studies suggest that the populations on San Clemente will suffer substantial losses of allelic diversity within the next 50 yr if census sizes remain as they currently are. Unfortunately, no seed germination or seedling establishment has been observed on San Clemente for over a decade, despite annual fruit production by most plants, even isolated ones. An ex situ program of germinating seeds and transplanting seedlings into current populations may reduce the effects of genetic drift and the time to extinction for extant populations (Lande 1988). High levels of historical gene flow combined with weak genetic differentiation among populations suggest that mixing genotypes from different populations could act to augment or at least sustain current levels of genetic diversity in the populations on San Clemente. Even occasional seedling establishment may go a long way to restore genetic variation in small populations if seeds are fathered by pollen from other populations.

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