

# Genetic diversity following demographic recovery in the insular endemic plant *Galium catalinense* subspecies *acrispum*

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**Abstract** *Galium catalinense* (Rubiaceae) is a perennial shrub consisting of two subspecies endemic to California's Channel Islands: *Galium catalinense* subsp. *catalinense* on Santa Catalina Island, and *G. catalinense* subsp. *acrispum*, a state-endangered taxon on San Clemente Island. A long history of overgrazing by introduced herbivores has contributed to population declines in *G. catalinense* subsp. *acrispum*. We surveyed 12 populations throughout the taxon's range for genetic variation using eight polymorphic microsatellite loci to determine the genetic impact of this demographic bottleneck. At the taxon level, 65 alleles were identified with an average of 8.1 alleles per locus, although many alleles were rare; the effective number of alleles per locus averaged 2.6. Expected heterozygosity was 0.550. Individual populations had between six and eight polymorphic loci, with expected heterozygosities ranging from 0.36 to 0.60, and effective numbers of alleles ranging from 1.8 to 3.5 per locus. Populations fell into three or four genetic clusters, depending on type of analysis, which may represent refugia where the populations persisted during intense herbivory. There is little evidence of genetic bottlenecks or substantial inbreeding within populations. These findings, coupled with indications of recent migration between populations, suggest that *G. catalinense* subsp. *acrispum* is currently unlikely to be endangered by genetic factors, but small population sizes make the taxon vulnerable to future loss of genetic diversity. Management

strategies based on these genetic data, population sizes, and the spatial distribution of populations are discussed.

**Keywords** San Clemente Island · Endemic · Microsatellite · Perennial · Population genetics · *Galium catalinense*

## Introduction

San Clemente Island, the southernmost of the California Channel Islands, has the highest levels of botanical endemism found in the archipelago (Philbrick 1980). Forty-seven of the 272 native plant taxa found there are endemic to the Channel Islands as a group (Junak et al. 1995), and 14 of these are endemic to San Clemente Island alone (Wallace 1985). The current flora has persisted despite a century and a half of intensive grazing by a variety of introduced herbivores (Halvorson 1994; Keegan et al. 1994; Knowlton et al. 2007), decades of naval bombing (Furches et al. 2009; Keegan et al. 1994), frequent fires (Randall 2008) and repeated periods of drought (Erskine-Ogden and Rejmánek 2005; Halvorson 1994). However, many taxa are found in extremely low numbers and some (e.g. *Dissanthelium californicum*) are presumed extirpated (Junak and Wilken 1998). The future persistence of many native species is uncertain.

Goats, the last of the exotic herbivores on San Clemente Island, were finally extirpated in the early 1990s (Keegan et al. 1994). Many California Channel Island native species have been increasing in census size, percent cover, and range since herbivore removal (Corry 2006; Halvorson 1994; Klinger et al. 2002; McEachern et al. 2009; S. Junak pers. comm.). On San Clemente Island, some rare endemic taxa (*Delphinium variegatum*, *Castilleja grisea* and

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*Lupinus guadalupensis*, among others) appear to have recovered without active management, while many (*Crossosoma californicum*, *Lavatera assurgentiflora* and *Sibara filifolia*, among others) have not (Junak and Wilken 1998; Helenurm pers. obs.; Junak pers. comm.). Habitat degradation caused by the exotic herbivores, including widespread erosion, soil compaction, loss of organic matter, and reduced soil water capacity, has been extensive (Halvorson 1994). Heavily disturbed areas have been colonized by invasive exotics, further limiting recovery of native plants (Erskine-Ogden and Rejmánek 2005; Halvorson 1992; Zavaleta et al. 2001).

The extent to which native populations will recover naturally, or can be aided by focused management efforts, is generally unknown. Although initial responses to herbivore removal may seem encouraging, undesirable and unpredictable long-term responses of island floras to the removal of introduced herbivores have been reported (Donlan et al. 2002, 2003; Erskine-Ogden and Rejmánek 2005; Klinger et al. 2002; McEachern et al. 2009; Zavaleta et al. 2001). The potential of native plants to recover may be influenced by life history characteristics; in particular, perennial shrub populations are more likely to recover after herbivore removal because they are less likely to be competitively excluded by invasive annuals (Halvorson 1992; Klinger et al. 2002) and because long-lived organisms have an intrinsically lower annual extinction risk (Pimm et al. 1988). Additionally, long-lived perennial shrubs are less likely to have lost genetic potential than shorter-lived species because the period of low census sizes will have affected fewer generations.

Demographic bottlenecks may have genetic consequences such as loss of alleles and reduced heterozygosity because of the impact of genetic drift in small populations; these may in turn reduce population viability in stochastic environments (Endels et al. 2007), reduce community resilience to environmental perturbations (Reusch et al. 2005), and inhibit adaptation during periods of climate change (Jump et al. 2008; Kramer and Havens 2009). Moreover, the increased homozygosity caused by genetic drift may result in inbreeding depression. These impacts may be especially severe for insular endemics because island populations are expected to have reduced genetic diversity even without additional demographic bottlenecks caused by introduced herbivores (Frankham 1997). At least one San Clemente Island species (*Delphinium variegatum*, with one mainland subspecies and two endemic subspecies on San Clemente Island) fits this expectation (Dodd and Helenurm 2002). However, the range of genetic variation in endemic taxa is broad on San Clemente Island, ranging from barely detectable variation for allozymes in *Sibara filifolia*, *Lavatera assurgentiflora* subsp. *glabra*, *Crossosoma californicum*, and *Lithophragma maximum* (Helenurm

2003; Helenurm unpublished data) to one of the highest levels ever reported for flowering plants in *Jepsonia malvifolia* (20 polymorphic allozyme loci out of a total of 21; Helenurm 2001). Similarly, microsatellite studies have revealed a range of genetic diversity in very rare San Clemente Island endemic plants (Wallace and Helenurm 2006, 2009; Furches et al. 2009; McGlaughlin and Helenurm unpublished data).

*Galium catalinense* A. Gray subsp. *acrispum* Dempster (Rubiaceae), the San Clemente Island bedstraw, is a perennial shrub endemic to San Clemente Island. Currently, this taxon is recognized as state-endangered in California and as a federal species of concern. It occurs primarily in the only habitat left largely intact during intensive grazing: rocky outcrops of steep canyon walls (Junak and Wilken 1998). Raven (1963) listed eight historical locations for *G. catalinense* subsp. *acrispum* from herbarium specimens and reported its presence, from his own visits to San Clemente Island in 1962, as occasional on canyon walls. Beauchamp (1987) counted a total of 215 individuals in the 12 canyons in which he encountered *G. catalinense* subsp. *acrispum* in a survey of rare plants on the island. Following the elimination of goats in the early 1990s, Junak and Wilken (1998) estimated 1,300 individuals in a botanical survey of the entire island; revisiting two-thirds of the locations in 2003–2006 yielded an estimate of approximately 2,100 individuals for the entire island (Junak, pers. comm.). Thus, the number of individuals of *G. catalinense* subsp. *acrispum* seems to have increased by an order of magnitude over the last several decades in response to the elimination of introduced herbivores. Populations also appear to have increased in size and, although they rarely exceed 30 individuals, several discrete patches may be found in the same canyon. Populations are increasingly found in canyon bottoms, open coastal slopes, and the tops of canyons, suggesting that this taxon may have been more broadly distributed in the past and that recovery and recolonization are occurring. The goal of this study was to evaluate the genetic status of *G. catalinense* subsp. *acrispum* following its demographic recovery.

In this study we surveyed polymorphic microsatellite loci in *G. catalinense* subsp. *acrispum* to assess several aspects of the genetic component of its recent demographic recovery. Specifically, our goals were to (1) assess whether populations are genetically depauperate, (2) assess differentiation of populations throughout the range, (3) detect evidence of historical population bottlenecks, and (4) estimate recent gene flow among populations. In addition, we were interested in identifying potential historical refugia for *G. catalinense* subsp. *acrispum* that may warrant more direct protection and management. Finally, we sought to compare *G. catalinense* subsp. *acrispum* to other San Clemente Island endemics to detect general patterns of

neutral genetic diversity, which might inform future island-wide conservation efforts.

**Materials and methods**

**Sampling and microsatellite amplification**

We sampled a total of 269 individuals from 12 populations of *Galium catalinense* subsp. *acrispum* (Fig. 1). Populations were chosen (a) to span the range of this taxon and (b) to generally provide more than 20 individuals for more accurate estimates of allele frequencies. Although precise locations of historical populations mentioned in Raven (1963) or Beauchamp (1987) are difficult to determine from verbal descriptions because of the complex canyon structure of the island, six of our populations seem to be sampled from four canyons corresponding to these previously reported populations (Bryce, Horse, Twin Dams, and Wall Canyons). These canyons represented the largest populations found by Beauchamp (1987) on both the east and west sides of the island, and account for nearly a third of the individuals he recorded. Sample sizes ranged from 3 to 37 individuals per population (Table 1). Fresh leaf tissue was stored at  $-80^{\circ}\text{C}$  prior to extraction. Genomic DNA was extracted from frozen leaf tissue using a modified CTAB protocol (Doyle and Doyle 1987). Eight microsatellite primer pairs developed for *Galium catalinense* subsp. *acrispum* (McGlaughlin et al. 2009) were used to gather

**Table 1** Population diversity statistics for *Galium catalinense* subsp. *acrispum*

	<i>N</i>	<i>P</i>	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>A<sub>E</sub></i>	<i>F<sub>IS</sub></i>	<i>S</i>
1. Nanny Cyn.	33	100.0	0.504	0.514	2.45	0.020	0.00
2. Burns Cyn.	27	87.5	0.522	0.492	2.36	-0.060	0.01
3. Twin Dams Cyn.	30	100.0	0.532	0.541	2.60	0.016	0.08
4. North Wall Cyn.	3	75.0	0.313	0.421	2.11	0.257	0.38
5. Waynuk Cyn.	24	87.5	0.420	0.385	1.87	-0.093	0.00
6. Box Cyn.	37	100.0	0.500	0.517	2.49	0.033	0.18
7. Lower Horse Cyn.	22	100.0	0.619	0.599	3.11	-0.032	0.00
8. Upper Horse Cyn.	4	87.5	0.594	0.545	3.51	-0.090	0.00
9. Lower Bryce Cyn.	23	100.0	0.565	0.566	2.83	0.003	0.13
10. Upper Bryce Cyn.	22	100.0	0.501	0.483	2.15	-0.036	0.00
11. Canchalagua Cyn.	29	87.5	0.335	0.364	1.79	0.082	0.26
12. Matriarch Cyn.	15	100.0	0.465	0.507	2.70	0.083	0.35*
Overall	269	100.0	0.494	0.550	2.58	0.102	

*N* number sampled (all individuals for populations of <24); *P* number of polymorphic loci; *H<sub>O</sub>* observed heterozygosity; *H<sub>E</sub>* expected heterozygosity; *A<sub>E</sub>* average effective number of alleles per locus; *F<sub>IS</sub>* inbreeding coefficient; *S* selfing rate. Population numbers as in Fig. 1  
 \* Significantly larger than 0 at *P* = 0.05



**Fig. 1** Sampled populations of *Galium catalinense* subsp. *acrispum* on San Clemente Island. Population names as in Table 1

data for all individuals. Amplification was carried out in 12  $\mu\text{l}$  reactions on an MJ Research PTC-200, following the protocols in McGlaughlin et al. (2009). Amplification products were diluted with water and combined into two multiplexes of four primers each (GACA32, GACA94, GACA102, and GACA148; and GACA3, GACA178, GACA198, and GACA208). Each multiplex was electrophoresed with the LIZ-500 size standard on an Avant-3100 Genetic Analyzer (Applied Biosystems), following the manufacturers instructions. Fragments were sized with GeneMapper version 3.7 (Applied Biosystems).

**Analysis of genetic diversity**

The presence of null alleles and size scoring errors were assessed for all loci in all populations with Micro-Checker (Van Oosterhout et al. 2004). MSA (Dieringer and Schlotterer 2003) was used to determine polymorphic loci, observed (*H<sub>O</sub>*) and expected (*H<sub>E</sub>*) heterozygosities, effective number of alleles (*A<sub>E</sub>*), and inbreeding coefficients (*F<sub>IS</sub>*). Deviations from Hardy–Weinberg equilibrium (HWE) and the presence of linkage disequilibrium (LD) between loci were assessed with GENEPOP (Raymond and

Rousset 1995). Deviations were considered significant at  $P < 0.01$ , because the test statistics have been shown to underestimate the error rate for both multiallelic data and small sample sizes (Lauretto et al. 2009). Selfing rates were estimated with RMES (David et al. 2007). Both the model based on estimation of the two-locus heterozygosity disequilibrium (1,000 iterations), and the model maximizing the log-likelihood of the multilocus heterozygosity in the population (precision = 0.001,  $k_{\max} = 10$ ) were implemented.

BOTTLENECK (Cornuet and Luikart 1997; Piry et al. 1999) was used to test for an excess of expected heterozygosity relative to the theoretical equilibrium heterozygosities based on the number of observed alleles in a population. The two-phased model (Di Rienzo et al. 1994) was used, following the settings recommended by the authors (Piry et al. 1999), which were 95% single-step mutations and 5% multiple-step mutations with a variance among multiple-step mutations of 12. A significant excess of heterozygosity was assessed using Wilcoxon's sign rank test, which summarizes across all loci within a population (Luikart and Cornuet 1998).

#### Analysis of genetic divergence and population structure

Analysis of molecular variance (AMOVA) and pairwise  $F_{ST}$  were estimated in ARLEQUIN version 3.11 (Excoffier and Schneider 2005). A Mantel test of isolation-by-distance was performed in GENEPOP (Raymond and Rousset 1995), using the natural log of the Euclidean distance in meters as the geographic distance and  $F_{ST}/(1 - F_{ST})$  as the genetic distance. Bayesian estimates of recent migration were calculated in BAYESASS 1.3 (Wilson and Rannala 2003), with 3,000,000 iterations, a sampling frequency of 2,000, a burn-in of 999,999, and all delta values set to 15.

The overall level of historical gene flow was estimated using Wright's (1951) formula  $Nm = (1 - F_{ST})/4F_{ST}$ , with  $F_{ST}$  considered equivalent to  $G_{ST}$  (Nei 1977). A second estimate was based on the frequency of private alleles (alleles found in a single populations; Slatkin 1985).

Bayesian estimation of population membership ( $K = 1-8$ ) based on genetic distribution of samples was implemented in STRUCTURE 3.2 (Hubisz et al. 2009; Falush et al. 2003, 2007; Pritchard et al. 2000) using flat priors under models of admixture with both uncorrelated and correlated allele frequencies. Twenty independent runs of 50,000 steps, after a burn-in of 25,000 steps, were performed for each  $K$  and model. The number of clusters was determined by examining the rate of change of  $K$  following the method of Evanno et al. (2005).

Geographically explicit analyses were employed to determine the spatial boundaries of populations in GENE- LAND (Guillot et al. 2005). Twenty-five independent

analyses were performed with spatial modeling, uncorrelated allele frequencies, and without null alleles. Geographic coordinates were UTM positions of the sampling locations with an uncertainty of 10 m. The number of populations was allowed to vary between 1 and 12 over 100,000 MCMC iterations with thinning every 100th iteration. Post-processing included a spatial realm of  $300 \times 250$  pixels and a burn-in of 250.

## Results

### Data evaluation

All loci amplified successfully in all populations. In tests for scoring errors and the presence of null alleles, one locus, GACA198, had heterozygote deficiencies possibly suggestive of null alleles in three populations (Box, Lower Horse, and Nanny Canyons). Two additional loci had heterozygote deficiencies within a single population (GACA3 in Canchalagua Canyon and GACA208 in Twin Dams Canyon). However, these three loci had high average heterozygosities (0.45, 0.66, and 0.47, respectively) and high amplification success. There was no evidence of large allele dropout at any locus. Subsequent analyses were run without correction for null alleles. The high amplification success and negligible evidence for null alleles, large allele drop-out, or scoring errors jointly suggest that our data allow reliable inference about the population genetics of *Galium catalinense* subsp. *acrispum*.

Only five of 96 locus-by-population comparisons showed significant Hardy–Weinberg disequilibrium (Box and Lower Horse Canyons, locus GACA94; Canchalagua Canyon, locus GACA3; Nanny Canyon, locus GACA198; Twin Dams Canyon, locus GACA208; data not shown), consistent with the Type 1 error of  $P = 0.05$ . Two populations, Canchalagua Canyon and Lower Horse Canyon, deviated from multilocus Hardy–Weinberg expectations (using Fisher's method across all loci,  $P = 0.0023$  and  $0.0025$ , respectively). Additionally, only eight of 270 intra-population locus-by-locus comparisons showed significant linkage disequilibrium (data not shown); there was no evidence of pervasive association between alleles for any pair of loci.

### Genetic variation

For the eight polymorphic loci surveyed, a total of 65 alleles were observed among 269 individuals of *Galium catalinense* subsp. *acrispum*. Four to 13 alleles were found at each locus, with a mean of 8.13 alleles per locus (data available from corresponding author upon request). However, many alleles are rare; 38 (58%) occur at overall frequencies of 0.05 or less. Consequently, the effective

number of alleles per locus ranges from only 1.60 to 4.00 at the taxon level, with a mean of 2.58. Eleven alleles (17%) occur in a single population (nine of the 12 populations had at least one unique allele) and more than half of these private alleles (64%) occur with a frequency less than 0.05. Observed heterozygosity ( $H_O = 0.494$ ) at the taxon level is less than the expected value ( $H_E = 0.550$ ).

The number of polymorphic loci within populations ranged from six to eight (75–100%; Table 1). One to nine alleles were found at each locus, with a mean of 3.86 alleles per locus, but the effective number of alleles per locus ranged from only  $A_E = 1.8$  to 3.5 within populations (mean  $A_E = 2.50$ ). Observed heterozygosities (mean  $H_O = 0.488$ ) were similar to expected heterozygosities (mean  $H_E = 0.493$ ) within populations and inbreeding coefficients were generally low ( $F_{IS} = -0.093$  to 0.083 for populations of more than ten individuals; Table 1); selfing rates based on  $F_{IS}$  (deviation from Hardy–Weinberg equilibrium within populations) ranged from  $s = -0.21$  to 0.15 for these populations (calculated using  $F_{IS} = s/(2 - s)$ ;

Hartl and Clark 1989). Selfing rates calculated from multilocus genotype data using RMES (David et al. 2007) ranged from 0.00 to 0.38, but only one value ( $s = 0.35$  in Matriarch Canyon) was significantly different from zero ( $P = 0.009$ , ML 95% CI 0.045–0.496; Table 1). One population, Lower Horse Canyon, exhibited a significant excess of heterozygosity ( $P = 0.02$ ), indicative of a population bottleneck.

Genetic structure and migration

Population differentiation was relatively low: pairwise  $F_{ST}$  estimates ranged from  $F_{ST} = -0.015$  to 0.216, with a mean of  $F_{ST} = 0.104$  (Table 2). Although marginally non-significant, there was some indication of isolation by distance (Fig. 2;  $P = 0.07$ ). Using  $G_{ST}$  to calculate rates of historic gene flow among populations (Wright 1951) yielded an estimate of  $Nm = 1.57$ . Slatkin’s (1985) private alleles method, based on the average frequency of alleles unique to single populations, yielded a similar value,  $Nm = 2.50$ .

GENELAND consistently recovered four discrete clusters of populations (Fig. 3), trending from north to south. STRUCTURE analyses found evidence for three distinct clusters (Figs. 4, 5). These clusters generally followed those recovered by GENELAND, but lumped the far northern and north-central clusters identified by GENELAND into a single group. Estimates of pairwise  $F_{ST}$  between STRUCTURE-identified clusters were relatively low (0.09–0.21, mean 0.13). BAYESASS estimated recent migration above  $m = 0.05$  in eight of 78 among-population comparisons (Table 4). Half of these were between populations within the same STRUCTURE-identified cluster, and half were between clusters. Inter-cluster migrants were inferred to originate primarily from the Box Canyon population in the central cluster (Table 4).

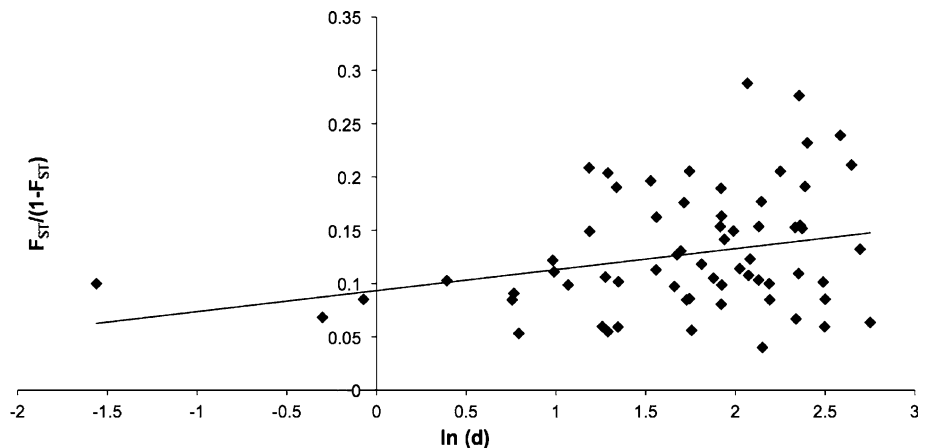
An AMOVA using canyons to group populations indicated that most (89.4%) of the variance in allele frequencies occurs among individuals within populations, and only

**Table 2** Below diagonal shows population pairwise  $F_{ST}$  for 12 sampled *Galium catalinense* subsp. *acrispum* populations; above diagonal shows significant pairwise  $F_{ST}$   $P$ -values ( $P < 0.05$ )

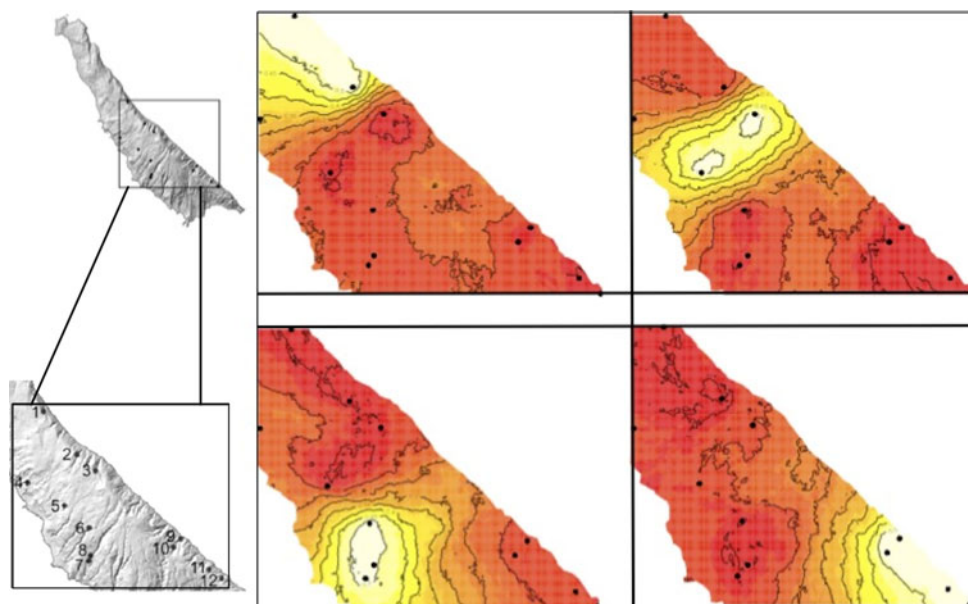
	1	2	3	4	5	6	7	8	9	10	11	12
1.		+	+	+	+	+	+		+	+	+	+
2.	0.06		+	+	+	+	+	+	+	+	+	+
3.	0.09	0.09		+	+	+	+	+	+	+	+	+
4.	0.11	0.14	0.13		+	+	+		+	+	+	+
5.	0.09	0.13	0.08	0.15		+	+	+	+	+	+	+
6.	0.09	0.10	0.10	0.08	0.08		+	+	+	+	+	+
7.	0.13	0.14	0.17	0.13	0.16	0.08		+	+	+	+	+
8.	0.07	0.09	0.05	0.13	0.09	0.04	0.07		+	+		
9.	0.06	0.09	0.07	0.11	0.10	0.08	0.11	-0.01		+	+	+
10.	0.08	0.14	0.12	0.19	0.13	0.12	0.15	0.07	0.06		+	+
11.	0.11	0.19	0.16	0.17	0.13	0.10	0.22	0.10	0.10	0.09		+
12.	0.06	0.09	0.10	0.15	0.16	0.08	0.15	0.04	0.05	0.06	0.07	

Population numbers as in Table 1 and Fig. 1

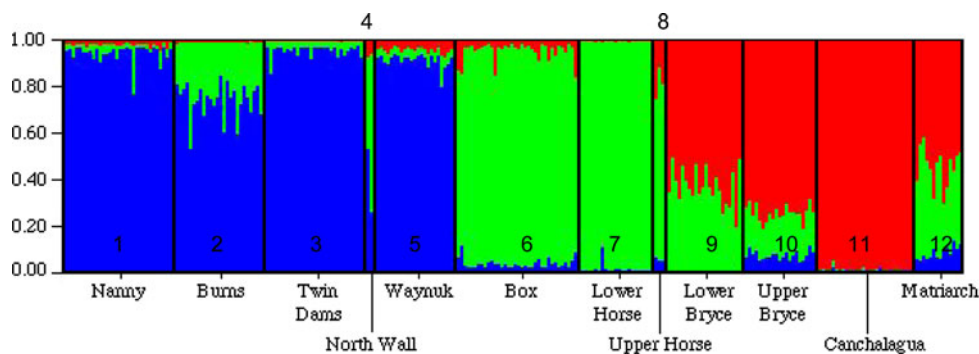
**Fig. 2** Mantel test of isolation-by-distance correlation,  $F_{ST}/(1 - F_{ST}) = 0.020(\ln(\text{geographic distance, km})) + 0.093$ .  $R^2 = 0.06491$ ,  $P = 0.070$



**Fig. 3** *Inset, Left:* San Clemente Island with area covered by GENELAND model (*heavy line*) and sampling locations (*black dots*). Population names as in Table 1. *Right:* Maps of posterior probabilities of membership in each of four *Galium catalinense* subsp. *acrispum* clusters identified by GENELAND trending from *dark* (low) to *light* (high)



**Fig. 4** STRUCTURE cluster assignment of *Galium catalinense* subsp. *acrispum* individuals and populations for  $K = 3$ . Population numbers as in Fig. 1

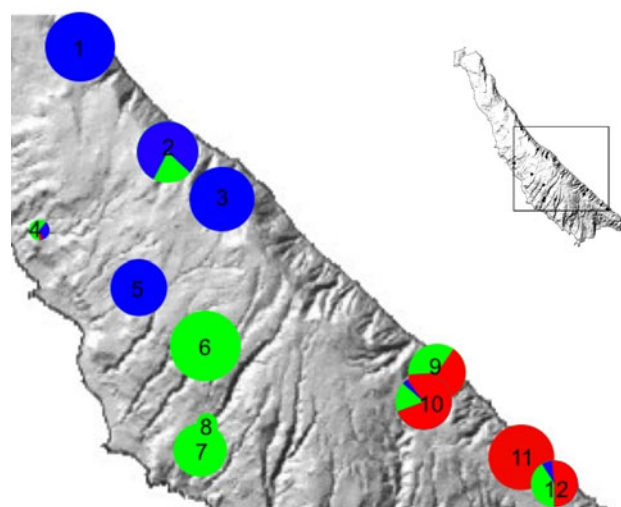


4.07% of the total variation is due to differences among canyons (Table 3). Additional AMOVA's using the GENELAND and STRUCTURE clusters as groups were similar to the canyon results, with the four groups of populations identified by GENELAND accounting for 4.04% of the total variation in the data set, and the three groups identified by STRUCTURE accounting for 3.43% of the total variation (data not shown).

**Discussion**

Genetic variability and structure

In general, *Galium catalinense* subsp. *acrispum* is characterized by relatively high levels of variation at microsatellite loci (Table 1). Allelic diversity is considerably higher than that reported for other San Clemente Island endemic plant species ( $A = 9.1$  vs.  $A = 6.4$  for *Lithophragma maximum*, Furches et al. 2009, and  $A = 4.2$  for *Crossosoma californicum*, Wallace and Helenurm 2009), both at



**Fig. 5** *Inset, Right:* San Clemente Island with area covered by STRUCTURE model (*heavy line*) and sampling locations (*black dots*) *Left:* Map of *Galium catalinense* subsp. *acrispum* population membership in STRUCTURE-identified clusters. Population names as in Table 1. Shading corresponds to Fig. 4. Area of circle is proportional to sample size

**Table 3** AMOVA results testing the genetic structure of *Galium catalinense* subsp. *acrispum* populations between canyons on San Clemente Island

	df	SS	Variance component	Percent variance
Among groups	9	117.7	0.086	4.07
Among populations within groups	2	10.5	0.138	6.55
Within populations	526	988.3	1.879	89.38
Total	537	1116.5	2.102	

Groups are single populations except the Horse Canyon drainage (populations 7 and 8, Lower and Upper Horse Canyons) and the Bryce Canyon drainage (populations 9 and 10, Lower and Upper Bryce Canyons).  $F_{ST}$  and  $F_{SC}$  significance tests,  $P < 0.001$ .  $F_{CT}$  significance test,  $P = 0.074$

the taxon and population level. Expected heterozygosity, while only slightly higher at the taxon level ( $H_E = 0.550$  vs.  $H_E = 0.504$  for *L. maximum* and  $H_E = 0.406$  for *C. californicum*), is approximately twice that reported for the other two species at the population level ( $H_E = 0.493$  vs.  $H_E = 0.229$  for *L. maximum* and  $H_E = 0.272$  for *C. californicum*). Our values also exceed those reported for single-island endemics in other island systems (Friar et al. 2006; Mitsui et al. 2009; Tsuda and Ide 2005).

*Galium catalinense* subsp. *acrispum* has several life history and habitat characteristics that may account for its relatively high levels of genetic variation despite a long history of overgrazing. First, it is a perennial shrub with potentially long, overlapping generations; both of these factors function to retain genetic variability (Hairston et al. 1996; Petit and Hampe 2006). While intensive grazing leading to low population sizes occurred over approximately 150 years, this period of time may represent relatively few generations of *G. catalinense* subsp. *acrispum* for genetic drift to eliminate alleles and reduce heterozygosity.

Second, populations are commonly (and historically) found on rocky outcrops of steep canyon walls, a habitat relatively inaccessible even to goats; this may have allowed larger numbers of individuals to survive the period of heavy grazing than was possible for many other species on San Clemente Island. At least two rare endemic species of San Clemente Island are represented by fewer than 65 individuals even now (Junak and Wilken 1998; Wallace and Helenurm 2009), and several others number only in the several hundreds (Junak and Wilken 1998; Furches et al. 2009; Helenurm 2003). Furthermore, steep slopes are where competition with invasive species is expected to be lowest (Corry 2006).

Third, *G. catalinense* subsp. *acrispum* appears to be primarily outcrossing. Observed and expected heterozygosities are similar within populations, so that selfing rate estimates based on  $F_{IS}$  (deviation from Hardy–Weinberg equilibrium within populations) are low. Selfing rates estimated on the basis of multilocus genotypes are similarly low, with only one population having a selfing rate significantly different from zero. Although self-compatibility has

been reported in other species of the genus *Galium* (Moore 1975), nothing is known of the details of the mating system of *G. catalinense*. The low selfing rates inferred from our microsatellite data using two different methods indicate that this taxon is primarily outcrossing and is, therefore, predicted to maintain relatively large effective population sizes and high genetic diversity (Armbruster and Pfenninger 2003).

Fourth, the rapid increase in numbers of *G. catalinense* subsp. *acrispum* over the last 20 years indicates either an effective seed dispersal system that permits rapid colonization of new habitats, gene flow among populations reducing the effect of genetic drift, a seed bank with long-lived seeds, or all three. Studies of pollen and seed dispersal are lacking for the taxon, but Proctor et al. (1996) report that other *Galium* species attract vagile pollinators such as flies, bees, and moths that would facilitate long distance gene flow. Seed dispersal may be far more limited and may occur primarily via gravity because the fruits, which are initially fleshy, become dry before falling off the maternal plant (Junak and Wilken 1998). Interestingly, two other perennial species that have shown rapid demographic recovery on San Clemente Island since the elimination of introduced herbivores, *Delphinium variegatum* and *Castilleja grisea*, have surprisingly high levels of genetic variation for insular endemic taxa that have undergone striking reductions in population number and size (Dodd and Helenurm 2002; Helenurm et al. 2005). Moderate to high levels of genetic diversity may be correlated with rapid recovery, due in part to factors such as effective dispersal or long-lived seeds.

Highly congruent results from different analyses reveal that the overall level of differentiation among populations is low, albeit significant. Estimates of gene flow based on  $F_{ST}$  ( $Nm = 1.57$ ) and on the mean frequency of private alleles ( $Nm = 2.50$ ) are similar in spite of different theoretical bases, and indicate sufficient past gene flow among populations to prevent substantial differentiation due to genetic drift. Although populations are grouped into three or four genetic clusters by STRUCTURE and GENELAND (Figs. 3, 4 and 5), the differences account for little of the variation in the data (3.43 and 4.04%, respectively).

Additionally, BAYESASS inferred the occurrence of recent migration between populations in the same and in different clusters (Table 4), which is consistent with the low level of evidence for isolation by distance (Fig. 2). Interestingly, not all populations were equivalent; gene flow proceeded mainly from Box Canyon in this analysis. Box Canyon, being relatively large, central in location, and inhabiting a less steep canyon, may well provide a disproportionate share of migrants. However, it is also possible that other nearby and genetically similar populations, unsampled for this study, may be providing migrants that are attributed to Box Canyon in this analysis.

Demographic history and genetic variation

The recorded history of *Galium catalinense* subsp. *acrispum* indicates that this San Clemente Island endemic consisted of few individuals during the period of intense herbivory by goats, but that its numbers have increased from several hundred to several thousand individuals since that time. Fortunately, the demographic bottleneck does not seem to have caused a depletion of genetic variation. The observed levels of neutral genetic diversity are relatively high for an insular endemic plant, individual populations retain many rare alleles, and most populations have not diverged from one another as would be expected with severe genetic drift in independent populations. Tests for the genetic signal of a population bottleneck were significant in only one population (Lower Horse Canyon). This surprising lack of genetic evidence for a bottleneck was also observed for most populations of *Lithophragma maximum*, an endemic of San Clemente Island comprised

only of very few, small populations (Furches et al. 2009). A combination of features, including a relatively inaccessible habitat, a perennial habit, an outcrossing mating system, and a persistent seed bank may have insulated this taxon from the most pernicious genetic effects of the heavy grazing which decimated much of the San Clemente Island flora.

The genetic clusters revealed by GENELAND and STRUCTURE (Figs. 3, 4 and 5) suggest the existence of refugia from which recolonization has occurred over the last several decades. Interestingly, the populations in canyons in which *G. catalinense* subsp. *acrispum* was previously reported (Bryce, Horse, Twin Dams, and Wall Canyons; Beauchamp 1987), fall within the different clusters identified by GENELAND and STRUCTURE. Furthermore, in both analyses each cluster is represented by at least one original population from 1987. Although the clusters may also be the product of natural barriers to gene flow, average pollinator movement distances, or other historical factors that are independent of the impact of grazing, the generally low pairwise  $F_{ST}$ 's and limited evidence for isolation by distance suggest that any fragmentation has been recent, incomplete, or both. Historic confinement to multiple refugia is consistent with the deviations from panmixia detected by GENELAND and STRUCTURE coupled with limited evidence for selfing within populations and low levels of differentiation among populations.

The rapid recovery of *G. catalinense* subsp. *acrispum* after herbivore removal may partially account for the low levels of population differentiation revealed by our genetic data. The recent observations of individuals in canyon

**Table 4** BAYESASS estimates of recent migration between *Galium catalinense* subsp. *acrispum* populations

Source population	Destination population											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Nanny Canyon	<b>0.82</b>	0.01	0.00	0.03	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.01
2. Burns Canyon	0.00	<b>0.68</b>	0.00	0.02	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.01
3. Twin Dams Canyon	0.01	0.28	<b>0.99</b>	0.03	0.00	0.00	0.01	0.07	0.01	0.01	0.00	0.01
4. North Wall Canyon	0.00	0.00	0.00	<b>0.73</b>	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.01
5. Waynuk Canyon	0.00	0.00	0.00	0.02	<b>0.68</b>	0.29	0.01	0.02	0.00	0.00	0.00	0.01
6. Box Canyon	0.14	0.00	0.00	0.02	0.28	<b>0.68</b>	0.27	0.02	0.27	0.00	0.00	0.24
7. Lower Horse Canyon	0.00	0.00	0.00	0.02	0.00	0.00	<b>0.68</b>	0.02	0.01	0.00	0.00	0.01
8. Upper Horse Canyon	0.00	0.00	0.00	0.02	0.00	0.00	0.00	<b>0.72</b>	0.01	0.00	0.00	0.01
9. Lower Bryce Canyon	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	<b>0.68</b>	0.00	0.00	0.01
10. Upper Bryce Canyon	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.02	0.00	<b>0.68</b>	0.00	0.01
11. Canchalagua Canyon	0.01	0.00	0.00	0.06	0.00	0.00	0.01	0.04	0.01	0.27	<b>0.99</b>	0.01
12. Matriarch Canyon	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.02	0.00	0.01	0.00	<b>0.69</b>

Source population on left. STRUCTURE-identified clusters are: Northern (populations 1–5), Central (populations 6–8), and Southern (populations 9–12). Population numbers as in Fig. 1 and Table 1



bottoms and on open coastal slopes may represent recent colonization, which may have disrupted a previous genetic pattern of isolation by distance. Additionally, although BAYESASS estimates of recent migration were generally low, there were several large estimates between distant populations, suggesting that the species does have high migration and colonization potential. The occasional high migration rates, high outcrossing rates, and the many significant, albeit low, pairwise  $F_{ST}$ 's are most congruent with small populations and historic barriers to panmixia.

The rapid rebound of *Galium catalinense* subsp. *acrispum* may be favored by characteristics of San Clemente Island. Research on California's and Mexico's Channel Islands indicate that climate plays an important role in floristic recovery (Corry 2006; Donlan et al. 2002, 2003; McEachern et al. 2009). Studies of vegetation change following herbivore removal indicate that responses of both natives and exotics are mediated by precipitation, soil moisture, or temperature regimes. Donlan et al. (2002) found that drought lengthened recovery time, but that more arid islands had fewer exotics, and potentially greater recovery. Although not addressing aridity, Garcillán et al. (2008) recorded dramatic recovery of the native flora on the highly isolated and arid Guadalupe Island after herbivore removal. In contrast, Corry (2006) found that natives were favored by more mesic sites (e.g. north facing slopes) or higher soil moisture. Similarly, McEachern et al. (2009) reported that the recovery expected after herbivore removal had been masked to some extent by population declines correlated with warmer temperatures. San Clemente Island is among the most arid and isolated of the California Islands. As such, it may, like the San Benito Islands and Guadalupe Island, be more resistant to invasion by exotics than the northern Channel Islands. Conversely, recovery on San Clemente Island may follow the pattern seen by Corry (2006) and McEachern et al. (2009) on the northern islands, where natives are largely excluded from open, upland habitats. In either case, many researchers indicate that long-term recovery is more likely among perennials (Corry 2006; Halvorson 1994; Zavaleta et al. 2001).

### Conservation implications

*Galium catalinense* subsp. *acrispum*, which has undergone recent recruitment and range expansion into more open habitats, has relatively high neutral genetic diversity. Individual populations have moderately high levels of genetic variation, whether measured in terms of polymorphic loci, number of alleles, or heterozygosity. In contrast to many San Clemente Island endemics, our data indicate that continued recovery of *G. catalinense* subsp. *acrispum* and development of long-lasting sustaining populations is unlikely to be inhibited by lack of genetic variation.

However, even in species that are not yet genetically endangered, genetic drift in small populations may steadily erode genetic variability. Effective management will require a number of approaches to maintain genetic variation in this taxon.

Although overall population differentiation is low, most populations contain unique rare alleles. Moreover, three or four geographic clusters of populations were identified on the basis of genetic data. Overall migration rates appear to be high enough to prevent substantial differentiation of populations due to genetic drift, but some populations contribute far more migrants than others. Gene flow is highly important in *G. catalinense* subsp. *acrispum* because the small populations of this taxon are especially susceptible to genetic drift.

Several management actions would enhance the natural recovery of *Galium catalinense* subsp. *acrispum* that has occurred since herbivore removal. First, maximizing available habitat by removing invasive species would help maintain or increase current population sizes and thereby reduce the chance of losing genetic variation through genetic drift in the future; maintaining weed-free corridors between the canyons where most natives remain confined may be especially helpful. Second, ensuring that populations are protected throughout the range of *G. catalinense* subsp. *acrispum* would help maintain the genetic variation in the taxon as a whole because of the geographic structure revealed by the genetic data. Third, ensuring that populations are protected in sufficient density that large gaps in population distribution do not occur would help genetic cohesion of populations. Although there appears to be sufficient gene flow to counteract the effects of genetic drift, elimination of key populations may cause genetic isolation of populations in different areas. The size of populations and the migration rate among them have been shown to be the most important factors affecting fitness in subdivided populations (Theodorou and Couvet 2006).

There are also several populations, or areas, that merit individual management actions to promote the long-term survival of this taxon. In the absence of data on the distribution of adaptive genetic variation in this taxon, management decisions need to be based on the pattern of variation revealed by neutral markers (quantitative genetic variation generally appears to be correlated with neutral variation; Leinonen et al. 2007). Thus, Lower Horse Canyon, for which there is evidence of a recent genetic bottleneck, may require aggressive habitat improvement to increase population size to reduce further loss of diversity. Similarly, Canchalagua Canyon and Twin Dams Canyon, the most diverse populations sampled from the southern and northern regions, respectively, should be actively maintained to preserve the range of diversity currently found in the taxon. However, most populations appear to

contain unique variation. Finally, the Box Canyon area is the apparent source of many migrants to other populations and thus particularly important in maintaining genetic cohesion and diversity. Populations in that area of the island should receive special management attention because of this role.

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