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GENETIC DIVERSITY IN THE RARE, INSULAR ENDEMIC *SIBARA FILIFOLIA* (BRASSICACEAE)

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ABSTRACT

This study investigates genetic variation in the rare insular endemic *Sibara filifolia,* a species consisting of few, small, narrowly-distributed populations. Electrophoretic data for 29 allozyme loci were obtained for individuals collected as seed from the three known populations on San Clemente Island. Overall levels of genetic variation are low. Only two polymorphic loci were observed, resulting in low number of alleles per locus (1.01), average observed heterozygosity (0.006) and average expected heterozygosity (0.009) for populations. Interestingly, all polymorphism occurred in just one of the three populations, in spite of their close proximity. Most variation on San Clemente Island is thus found within rather than among populations (G_{ST} = 0.144), and there is significant differentiation among populations (F_{ST} = 0.145). Gene flow is estimated as Nm = 0.41 based on private alleles and Nm = 1.49 based on F_{ST}. The differentiation of populations and low level of gene flow suggests that genetic drift is a potent force in these small populations and may further reduce genetic variation. RAPD and quantitative genetic studies are recommended to further evaluate these populations and the long-term prospects for this species.

Key Words: Allozymes, conservation, *Sibara,* endangered species, endemic, genetic diversity, Brassicaceae, San Clemente Island.

The genetic characteristics of rare species have commanded considerable attention in the last few decades. Genetic variation is understood to be important to the continued survival of organisms, both in the long-term (through ability to adapt to a changing environment; Holsinger and Gottlieb 1991; Ellstrand and Elam 1993) and in the short-term (through increased fitness in spatially or temporally heterogeneous environments, by reducing inbreeding, and by other mechanisms; Huenneke 1991). In general, rare species are assumed to be more vulnerable to extinction because of reduced genetic variation, in addition to habitat and demographic factors.

Rabinowitz (1981) pointed out that rarity can be subdivided into three separate components: a species can be considered rare due to small geographic range, high habitat specificity, or small population size (or combinations of these factors). Reduced genetic variation is associated with all three of these factors. Narrow-ranging species generally contain less genetic diversity than wide-ranging species, both at the population and species levels (Hamrick and Godt 1989; Gitzendanner and Soltis 2000), presumably because of isolation of populations or diversifying selective forces across the species range (Baskauf et al. 1994). Strong directional selection leading to genetic uniformity in a limited array of environments and genetic drift in small populations are expected to cause depleted levels of genetic variation (Wright 1931; Babbel and Selander 1974; Nei et al. 1975; Franklin 1980; Barrett and Kohn 1991). A species exhibiting rarity in all three of these components would be predicted to have very low levels of genetic variation.

This study investigates the genetic variation in the rare insular endemic *Sibara filifolia,* a species consisting of few, small, narrowly-distributed populations, and appearing to demonstrate habitat specificity. The specific goals were to (1) assess the genetic diversity remaining in this geographically highly restricted species, (2) evaluate the distinctness of the populations, and (3) provide management guidelines based on population genetic data. This study was initiated as part of an investigation of the conservation genetics of rare and endangered plant species of San Clemente Island.

MATERIALS AND METHODS

Study Species

Sibara filifolia (E. Greene) E. Greene (Brassicaceae), the Santa Cruz Island Rockcress, is a diminutive annual apparently restricted to rocky outcrops. First described from Santa Cruz Island in 1886 (Greene 1887), it was last seen there in 1936 and was not relocated during a survey of the island in 1985. Trask reported it from two locations on Santa Catalina Island in 1901 (Thorne 1967); it was last seen on that island in 1973 and was not relocated during a survey in 1996. The species was presumed extinct until two individuals were located on a sea terrace at the southern end of San Clemente Island in 1986, growing on volcanic rock scree (Beauchamp 1987); it was given federal endangered status in November 1997. Until June 2001, *S. filifolia* was known only from populations on three adjacent ridges within 150 m of each other on San Clemente Island and from occasional scattered individuals nearby; a few individuals have

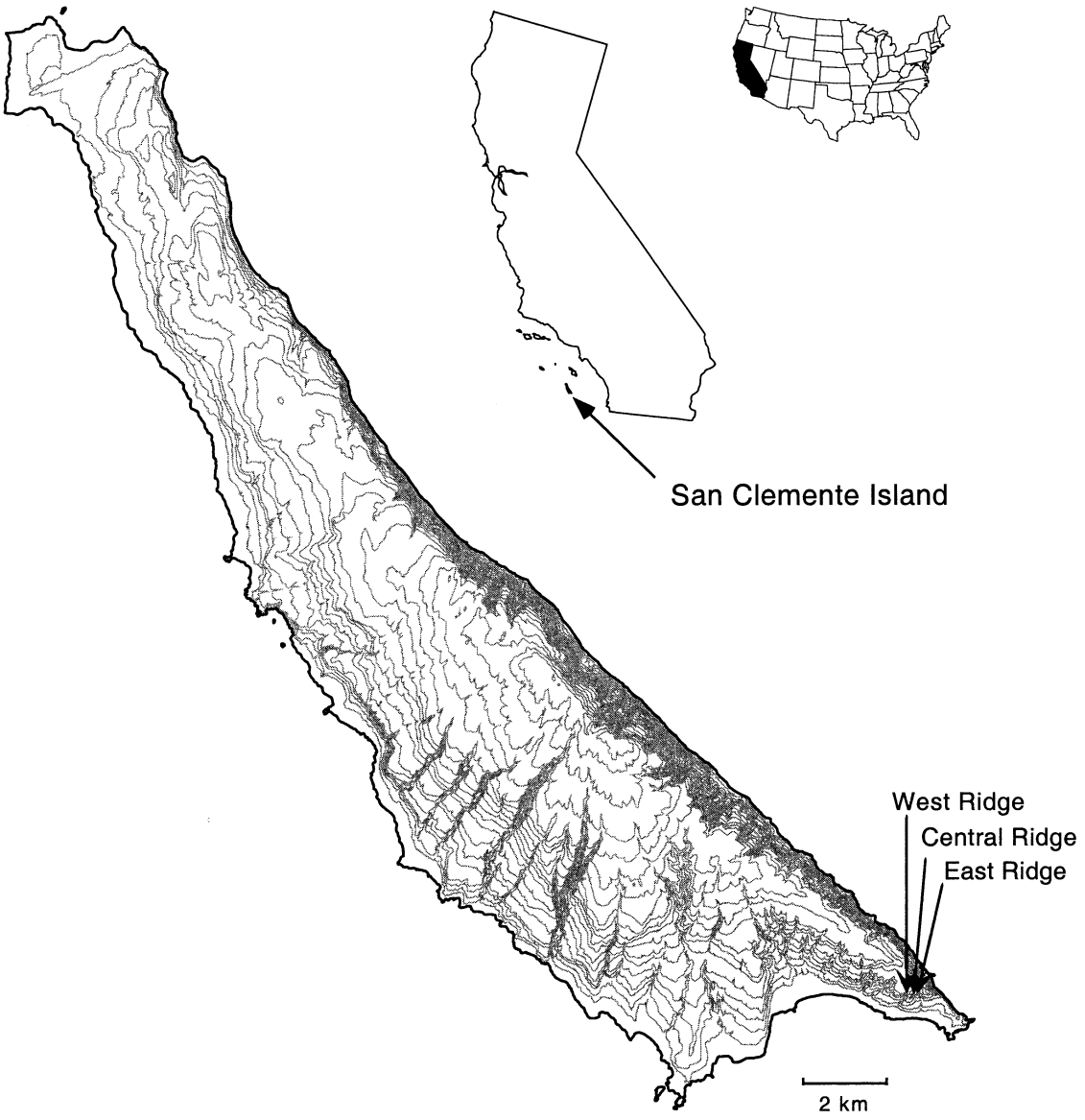

FIG. 1. Sampled populations of *Sibara filifolia* on San Clemente Island.

now been relocated on Santa Catalina Island (G. Wallace, USFWS, personal communication).

Sampling

A single fruit was collected from each individual bearing more than three fruits from the three known locations in June 1996 (Fig. 1). Seeds were sown in the greenhouse and leaf tissue was collected from one progeny from each parent. Sample sizes were 16 (West Ridge), 13 (Central Ridge), and 22 (East Ridge), for a total of 51 individuals; sample size was limited by availability of larger plants and by seed viability and germination.

Electrophoresis

Electrophoretic methods followed Soltis et al. (1983). Leaf tissue was crushed in phosphate extraction buffer (Conkle et al. 1982) and stored at −80°C until electrophoresis was conducted.

Three buffer systems were used to resolve loci coding for 18 enzymes. Acid phosphatase (Acp), isocitrate dehydrogenase (Idh), and phosphogluconate dehydrogenase (Pgd) were resolved using a tris-citrate, pH 6.3/6.7 buffer system (Selander et al. 1971) with 11.5% starch gels. Aldolase (Ald), diaphorase (Dia), fructose-1,6-diphosphatase (Fdp), glucose-6-phosphate dehydrogenase (G6p), leucine

aminopeptidase (Lap), malate dehydrogenase (Mdh), malic enzyme (Me), menadione reductase (Mr), phosphoglucoisomerase (Pgi), and superoxide dismutase (Sod) were resolved using a morpholine citrate, pH 6.1 buffer system (Clayton and Tretiak 1972) with 13% starch gels. Glutamate dehydrogenase (Gdh), glyceraldehyde-3-phosphate dehydrogenase (G3p), phosphoglucomutase (Pgm), shikimate dehydrogenase (Skd), and triose-phosphate isomerase (Tpi) were resolved with using a tris-borate-EDTA, pH 8.6 buffer system (Soltis et al. 1983) with 11% starch gels.

Staining recipes for all enzymes followed Soltis et al. (1983), except for Dia and Sod (Murphy et al. 1990). Loci were numbered sequentially with the most anodally migrating enzyme designated "1".

Data Analysis

Data were analyzed using the computer program Genestrut (Constantine et al. 1994). Mean number of alleles per locus (A), effective number of alleles (A_E), percentage of polymorphic loci (P), observed heterozygosity (H_O), and expected heterozygosity (H_E) were calculated. Loci were considered polymorphic if more than one allele was detected. Levels of genetic variation were calculated for individual populations, and also for the entire species on San Clemente Island. Fixation indices (F), reflecting deviations from Hardy-Weinberg equilibrium, were calculated and outcrossing rates (t) were estimated using $t = (1-F)/(1+F)$ (Weir 1990).

The partitioning of genetic diversity within and among all populations was analyzed using F-statistics (Nei 1973). Nei's (1978) unbiased genetic identity (I) was calculated for pairwise comparisons of populations using Genestrut (Constantine et al. 1994).

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 population; Slatkin 1985).

RESULTS

Loci and Alleles Scored

Enzyme electrophoresis resulted in clear and consistent staining for 18 enzymes encoded by 29 putative loci: Acp, Ald, Dia, Fdp-1, Fdp-2, Gdh, G3p-1, G3p-2, G3p-3, G6p-1, G6p-2, Idh, Lap, Mdh-1, Mdh-2, Mdh-3, Me, Mr-1, Mr-2, Pgd, Pgi-1, Pgi-2, Pgm-1, Pgm-2, Skd, Sod-1, Sod-2, Tpi-1, and Tpi-2. All enzymes migrated anodally.

A total of 32 alleles were detected for the 29 loci. All loci except Mdh-2 and Pgi-2 were monomorphic with all individuals from all populations possessing a single enzyme band with identical mobility for each locus. Mdh-2 had two alternative alleles (found at frequencies of 0.03 and 0.22), and Pgi-2 had one variant allele (at a frequency of 0.25); all of these occurred only in West Ridge. Average frequency of alternative alleles is 0.17. Heterozygotes were observed for both alleles at Mdh-2, but no heterozygotes were observed for Pgi-2.

TABLE 1. GENETIC VARIABILITY AT 19 LOCI IN 3 POPULATIONS OF *SIBARA FILIFOLIA*.

Population	A	A_E	P	H_O	H_E
West Ridge	1.10	1.03	6.9	0.017	0.027
Central Ridge	1.00	1.00	0.0	0.000	0.000
East Ridge	1.00	1.00	0.0	0.000	0.000
Mean	1.03	1.01	2.3	0.006	0.009
(SE)	(0.03)	(0.01)	(2.3)	(0.006)	(0.009)
Species	1.10	1.01	6.9	0.005	0.010

Measures of Genetic Variability

At the species level, *S. filifolia* has low levels of genetic variation, with 6.9% polymorphic loci and low heterozygosities (Table 1). Two of the three populations (Central Ridge and East Ridge) are monomorphic at all loci; genetic variation was only observed in West Ridge, and even this population is low in allozyme variation.

Genetic Identity Measures

Genetic identity values (I; Nei 1978) are very high. Central Ridge and East Ridge populations, being monomorphic and identical, have a genetic identity of 1.000. West Ridge has a genetic identity of 0.997 with the other two populations (mean I = 0.999).

Fixation Indices and Outcrossing Rates

Of the two fixation indices (F; available only for Mdh-2 and Pgi-2 in the West Ridge population), one was non-significant (Mdh-2; $F = -0.292$; ns) and the other was significant and positive (Pgi-2; $F = 1.000$; $P < 0.01$), indicating a deficiency of heterozygotes (mean F = 0.754).

The outcrossing rate based on the mean fixation index is $t = 0.457$, indicating a significant level of inbreeding ($t = 0$ for complete self-fertilization and $t = 1$ for random mating).

F-statistics

Mean F_{IS}, representing average deviation from Hardy-Weinberg expectations within populations, is significant for *S. filifolia* on San Clemente Island (mean $F_{IS} = 0.373$, $P < 0.01$). Differentiation of populations is evident and significantly different from 0 (mean $F_{ST} = 0.145$, $P < 0.01$). Of the total gene diversity found on San Clemente Island, 85.6% is found within populations and 14.4% is found among populations (mean $H_S = 0.133$, mean $D_{ST} = 0.022$, mean $H_T = 0.155$, $G_{ST} = 0.144$).

Gene Flow

Gene flow among populations was Nm = 0.41 using Slatkin's (1985) method based on 3 private alleles with an average frequency of 0.167 and corrected for an average population size of 17. Wright's (1951) method yielded an estimate of Nm = 1.49.

Discussion

Genetic Variation

The low level of genetic variation observed in *S. filifolia* conforms to expectations for a narrowly-distributed taxon consisting of few, small populations. These data agree with empirical observations of many other plant species with a restricted range (Hamrick and Godt 1989; Gitzendanner and Soltis 2000). In addition, insular endemic plants generally appear to have lower levels of genetic variation, even within populations, presumably due to a history of founder events and population bottlenecks (DeJoode and Wendel 1992; Frankham 1997), although studies of other Channel Islands' endemics have revealed a range of levels of genetic variation (Helenurm 2001; Dodd and Helenurm 2002). *Sibara filifolia* also possesses other attributes associated with lower levels of genetic variation, such as production of seeds without pollinators (see below) and gravity-dispersed seeds (Hamrick and Godt 1989).

Recent ecological history is also likely to have affected levels of genetic variation in *S. filifolia.* Grazing pressure by introduced livestock (primarily goats) kept populations of most native species small during the last century (Kellogg and Kellogg 1994). Small populations are vulnerable to genetic drift, and especially to the rapid loss of rare alleles. Population sizes of *S. filifolia* are likely to have been extremely small, as it remained undiscovered by botanists until 1986. It is likely that many rare alleles have been lost in populations, resulting in a genetically depauperate species. Even after removal of goats in 1992 (Kellogg and Kellogg 1994), population sizes of this annual plant have often been small and fluctuated widely (Junak and Wilken 1998; K. Helenurm personal observation), reducing effective population size. Moreover, the original range of *S. filifolia* on San Clemente Island is not known, and may have been considerably larger than at present; range restriction may also have resulted in a loss of genetic variation. Although the seed bank may harbor additional genetic variation not observed in this study, the 51 plants surveyed represent a large enough sample to conclude that genetic variation in *S. filifolia* is low in comparison to most plant species.

Genetic Differentiation

A surprising result of this study is the significant differentiation of populations in spite of their close proximity. Two populations (Central Ridge and East Ridge, separated by 50 m) are completely monomorphic at 29 loci, while the third (West Ridge, only 100 m distant from Central Ridge), contains additional alleles at substantial frequencies at two loci. Although one of the Mdh alleles is rare (Mdh-2a, at a frequency of 0.03), the other is more common (Mdh-2c, at a frequency of 0.22) for a combined alternative allele frequency of 0.25; Pgi-2a occurs at a frequency of 0.25. This suggests that very little gene flow occurs in *S. filifolia,* even over short distances; quantitative estimates of gene flow from the electrophoretic data are Nm = 1.49 based on differentiation at polymorphic loci (Wright 1951) and Nm = 0.41 based on private alleles (Slatkin 1985). While the accuracy of these estimates is questionable due to the detection of only two polymorphic loci and three private alleles, they nevertheless summarize the available genetic data, and both indicate little gene flow based on different methods of estimation.

The significant differentiation of populations at allozyme loci and inference of low gene flow is consistent with greenhouse observations of the breeding system of *S. filifolia,* location of populations, and seed morphology. All individuals grown in the greenhouse produced fruits, suggesting that *S. filifolia* is self-compatible and autogamous (or, possibly, apomictic). Although outcrossing may occur in natural populations, pollinators do not appear to be required for successful seed production (and potential pollinators have not been observed visiting flowers). The capacity for autogamous or apomictic seed production limits gene flow through pollen movement. Potential gene flow through seed movement is also limited in this species. Populations are located near the tops of ridges and the small seeds, lacking any special dispersal mechanism, are likely to tumble or be washed downhill.

It is possible that the alleles found exclusively in West Ridge may also exist in the other two populations, but it is unlikely that they are found in equally high frequencies. Although sample sizes were low owing to the rarity of this species, sampling error is insufficient to explain the observed differences between the populations. Assuming random distribution of alleles, the probability of not detecting an allele actually present at a frequency of 0.25 in a sample of 15 individuals is less than 0.0002; even assuming a completely selfing population (consisting exclusively of homozygotes), the probability of not detecting such an allele is less than 0.02. A more significant issue for a cryptic species in which population differentiation can be maintained over very short distances, such as *S. filifolia,* is that undiscovered populations may harbor additional genetic variation. Genetic variation may be more severely underestimated by lack of sampling of additional populations (or reduced by the extirpation of such populations) than by small sample sizes.

Implications for Conservation

The federally endangered *S. filifolia* is endemic to the Channel Islands, and is currently known from three populations clustered at the southern tip of San Clemente Island, and from scattered individuals nearby and on Santa Catalina Island. Populations range in size from a few to several hundred individuals, and usually vary from one year to another. Thus, this taxon has a narrow range, few populations, and usually small populations. In spite of this rarity, *S. filifolia* on San Clemente Island is not completely devoid of genetic variation; the levels of genetic variation we observed, although low, are not atypical of endemic species. However, because of small and fluctuating population size (which reduces effective population size) genetic drift is likely to continue eroding the remaining genetic diversity in these populations. It will be important to keep populations as large as possible because loss of alleles through genetic drift can cause the random loss of even favorable alleles (Hartl 1988). The loss of alleles is unlikely to be counteracted in this species by the reintroduction of alleles through gene flow. The differentiation of populations separated by only 50 or 100 m thus has a significant conservation implication; drift appears to be a more potent evolutionary force than gene flow in these populations.

The three remaining populations of *S. filifolia* on San Clemente Island are not equivalent genetically. Only West Ridge appears to maintain genetic variation at allozyme loci, suggesting that this population is especially valuable as a source of material for reintroductions or for ex situ collections, which are essential as a safeguard against possible future extirpation in narrow-ranging taxa with few populations (Lande 1988; Simberloff 1988). Loss of this population would cause a significant loss of allozyme variation for *S. filifolia.*

The allozyme data clearly demonstrate low genetic variation and the existence of significant differences among nearby populations. However, conclusions regarding the relative genetic value of different populations may be premature. First, only two polymorphic loci were detected. Other molecular markers more likely to detect polymorphism, such as RAPDs, will permit stronger conclusions regarding the relative value of these populations for reintroductions and ex situ collections. Second, there appears to be only a weak correlation between variation in molecular markers, such as allozymes, and quantitative genetic variation (Reed and Frankham 2001). Molecular markers may offer insights into immediate fitness (through inbreeding depression in small populations leading to reduced population survival, for example; e.g., Newman and Pilson 1997), but long-term survival may also depend on quantitative genetic variation allowing adaptation to changing environments. Surveys of quantitative genetic variation will be important to evaluate the long-term prospects for *S. filifolia.*

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