Conservation Genetics **4:** 337–352, 2003.

Conservation of genetic diversity in the endangered plant *Eriogonum ovalifolium* var. *vineum* (Polygonaceae)

Maile C. Neel* & Norman C. Ellstrand

*Department of Botany and Plant Sciences and Center for Conservation Biology, University of California, Riverside, California 92521-0124 USA (*Author for correspondence: Department of Natural Resources Conservation, Holdsworth Natural Resources Center, University of Massachusetts, Amherst, MA, 01003; e-mail: mailen@forwild.umass.edu; fax: (413) 545-4358)*

Received 19 February 2002; accepted 13 June 2002

Key words: edaphic endemism, endangered species, genetic diversity

Abstract

The purpose of his research was to describe the organization of genetic variation in the federally endangered plant taxon *Eriogonum ovalifolium* var. *vineum* using allozymes. Such information can help prioritize sites and management choices for capturing and maintaining genetic variation and can reduce the number of populations necessary to commit to conservation, thus reducing costs and conflicts with competing land uses. Information on genetic diversity patterns also provides insight into evolutionary and demographic history of a taxon which can provide means to assess future risk of erosion of diversity. Similar to other *Eriogonum ovalifolium* varieties, *E. ovalifolium* var. *vineum* is diverse at both the taxon (proportion of polymorphic loci [P] = 0.55 and alleles per locus [*A*] = 5.45 [SD = 2.5]) and population (P = 0.56 [SD = 0.11] and *A* = 2.68 [SD = 0.35]) levels. Gene diversity (H_e) within populations averaged 0.19 (SD = 0.03). We found some evidence for limited clonal reproduction within populations. Populations were moderately differentiated from one another ($\theta_S = 0.14$) and showed moderate deviations from Hardy-Weinberg equilibrium within populations ($f = 0.14$ and $F = 0.19$). Mean Nei's genetic distance (*D*) among all pairs of populations was 0.02. Populations with high levels of multiple measures of genetic diversity, high levels of differentiation, and low levels of apparent inbreeding are suggested as conservation priorities.

Introduction

A common goal of conservation is to maintain genetic diversity within rare species. For most rare species no data are available on genetic diversity patterns and its conservation is most often attempted by protecting sufficient habitat to support large enough populations to prevent inbreeding and genetic drift rather than directly focusing on genetic diversity itself. It is assumed that if sufficient habitat is maintained to protect against environmental stochasticity, loss of genetic diversity is not an immediate concern (e.g. Templeton et al. 1990; Schemske et al. 1994; Gaines et al. 1997). However, because genetic diversity may contribute to species persistence, its direct measurement can be an important priority. Quantifying the organization of genetic variation over populations of a rare species can help in prioritizing sites and management choices that will capture and maintain that variation. For example, highly diverse or differentiated populations could be targeted for protection while depauperate populations might be targeted for management actions to restore diversity (e.g. Godt et al. 1996; Petit et al. 1998). Neel and Cummings (in press, accepted) have demonstrated that a large proportion of populations are required to capture the genetic diversity in a species if populations are chosen without knowledge of genetic diversity patterns, regardless of the how those populations are chosen. Thus, knowledge of diversity patterns can reduce the number of populations necessary to commit to conservation and thus reduce costs and conflicts with competing land uses.

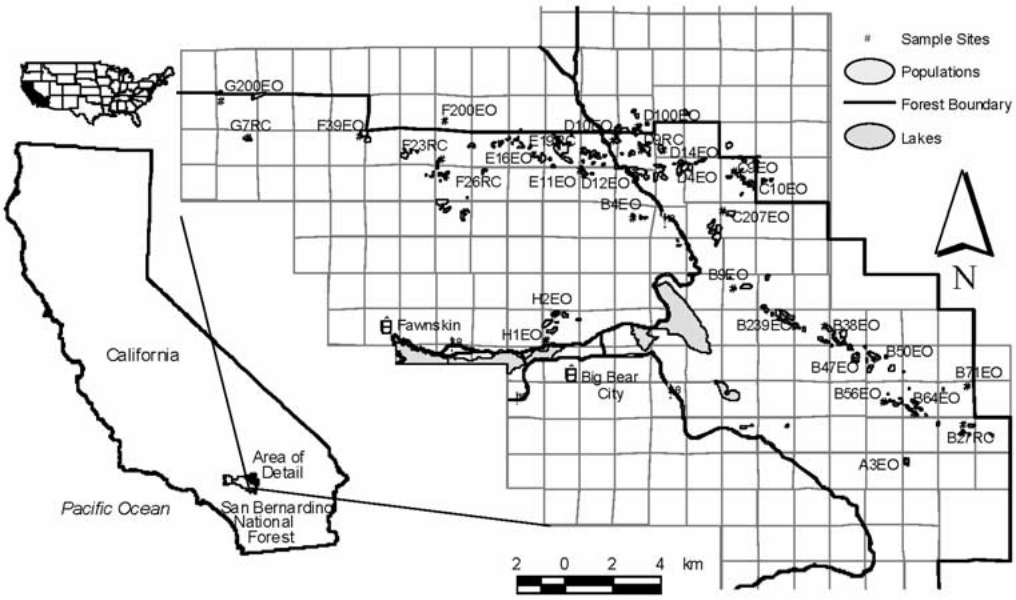

Figure 1. General locations of sampling sites for *Eriogonum ovalifolium* var. *vineum* shown in context of the state of California and the boundary of the San Bernardino National Forest.

Information on genetic diversity patterns also provides insight into evolutionary and demographic history of a taxon (Milligan et al. 1994). Understanding the relative importance of processes that structure diversity within and among populations (specifically inbreeding, gene flow, genetic drift, and selection) can provide both a means to assess future risk of erosion of diversity and a means for designing effective conservation strategies for rare taxa. For example, if genetic diversity is primarily held within populations, fewer populations would need to be conserved to represent the range of variation within a taxon. In such cases genetic diversity might be a less important criterion for selecting particular populations than other criteria. Alternatively, a taxon with most of its variation partitioned among populations would require protection of a larger proportion of existing populations to maintain variation present in the taxon. The structure of genetic diversity within and among populations also has important implications for developing sampling strategies for restoration and reintroductions (e.g. Brown 1989; Ceska et al. 1997). Finally, genetic diversity information can guide future research by focusing attention towards areas of particular potential concern. For example, if genetic diversity is high, and populations do not appear to be at risk of losing that diversity, research and management efforts can be focused on other ecological characteristics related to survival of individuals in relation to threats faced by a taxon.

Because of the insight they provide, we quantified genetic diversity patterns in the federally endangered plant *Eriogonum ovalifolium* var. *vineum* A. Gray. This taxon is endemic to limestone and dolomite substrates that are found in a ~13,200-ha region in the San Bernardino Mountains of southern California (Figure 1) (Hickman 1993; U.S. Fish and Wildlife Service 1994). The taxon grows between ~1400 and ~2400 m elevation (Gonella and Neel 1995; Neel 2000) in limited and often isolated areas totaling ~550 ha within this range (S. Redar, personal communication, San Bernardino National Forest). *E. ovalifolium* var. *vineum* most often occupies slopes and ridge tops with fine, powdery soil with occasional large boulders or rock outcrops (Gonella and Neel 1995; Neel 2000). Environments vary greatly over the elevational range of the taxon, extending from desert to montane conditions. At lower elevations annual precipitation averages 14 cm, and temperature extremes vary from –17 ° to 43 °C. At higher elevations precipitation averages 58 cm/yr, and temperatures range from –28 ° to 32 °C (U.S. Department of Commerce 1999). Habitats in which this taxon occurs vary correspondingly over this environmental gradient (Gonella and Neel 1995; Neel 2000). Lower elevations are dominated by pinyon-Utah juniper/black bush scrub vegetation character-

ized by an open shrub canopy (primarily *Coleogyne ramosissima* [black bush]) with low densities of *Pinus monophylla* (single-needle pinyon pine) and *Juniperus osteosperma* (Utah juniper) in the overstory. Mid-elevations support various phases of single-needle pinyon-Utah Juniper woodland. High-elevation occurrences are in Jeffrey pine-mountain juniper woodlands co-dominated by *Pinus jeffreyi, Juniperus occidentalis* ssp. *australis*, and *P. monophylla*. Despite the substantial ecological variation described above, habitats supporting *E. ovalifolium* var. *vineum* all share the following features: open overstory and shrub canopies, high soil pH, and high percentage of soil calcium (Neel 2000).

Eriogonum ovalifolium var. *vineum* is a long-lived, mound-forming subshrub that grows as high as 35 cm with inflorescences extending 3–6 cm above the vegetative portion of the plant (Hickman 1993). Corolla length measures only 2–7 mm (Hickman 1993); however, hundreds of perfect flowers are clustered into showy, 2–3 cm diameter, head-like inflorescences. Nothing is known of the timing of maturity of male and female parts within flowers, however the large numbers of flowers on a plant are open simultaneously, providing ample opportunities for geitenogamous pollination. Perianths are composed of six tepals, the colors of which range from creamy white through yellowish white, light pink, and burnt orange to magenta. Perianth color varies among plants and also changes on individual plants over a flowering season. As with many *Eriogonum* flowers, there is a dark stripe along the midrib of each tepal that contrasts with the typically lighter-colored tepal margins. Fruits are 2–3 mm long achenes (Hickman 1993) that typically fall from the plant attached to the dried corolla when mature. Pollinators include members of the Order Diptera in the families Bombyliidae, Chloropidae, Muscidae, Tachinidae, and Anthomyiidae as well as the family Halictidae in the Order Hymenoptera (S. Morita, personal communication, University of California, Davis). Prior genetic analysis of open-pollinated progeny arrays indicated that this species is primarily outcrossed (population outcrossing rates averaged 0.80 (SE = 0.03) (Neel et al. 2001).

Where found, *E. ovalifolium* var. *vineum* populations can be quite large, comprising hundreds of individuals, although they are usually smaller. In fact, this taxon has more extant populations and a larger number of total individuals than the typical endangered plant species (Holsinger and Gottlieb 1991; Ellstrand and Elam 1993). Sizes of many populations are above those at which inbreeding and genetic drift are typically of immediate concern (Simberloff 1988; Ellstrand and Elam 1993). Existing populations that are not impacted by human disturbance are not apparently in demographic decline although seedling recruitment is not common. The main concern for persistence of *E. ovalifolium* var. *vineum* is continued habitat destruction and fragmentation, primarily from limestone mining activities (U.S. Fish and Wildlife Service 1994). The limestone deposits on which *E. ovalifolium* var. *vineum* grows provide ore that is extremely valuable in construction, industrial, and pharmaceutical applications (Calzia 1993). Consequently, *E. ovalifolium* var. *vineum* has suffered loss of individuals, loss and reductions of populations, and fragmentation of remaining populations by limestone mining activities (Skinner and Pavlik 1994; U.S. Fish and Wildlife Service 1994). Additionally, most of *E. ovalifolium* var. *vineum*'s range on public land is under valid mining claim and thus at risk from future mining activities. Much of the private land on which this taxon occurs was patented by and is now owned by mining interests. As a result of existing habitat and population losses, continuing threats, and lack of protective mechanisms, the US Fish and Wildlife Service listed this taxon as endangered (U.S. Fish and Wildlife Service 1994). Efforts to conserve this species involve establishing a reserve system that will be protected from mining as well as restoring degraded sites. Due to the high value of the mineral ore, not all existing populations will be maintained. The purpose of our research was to characterize intrapopulation levels of diversity and interpopulation genetic structure of *E. ovalifolium* var. *vineum* to provide guidance in overall reserve design and contribute to ranking the conservation significance of individual populations.

Methods

Sampling

Allozyme variation was analyzed for a total of 929 *Eriogonum ovalifolium* var. *vineum* individuals sampled from thirty-one populations. These populations encompassed much of the ecological and geographic ranges of the species but did not include all known populations. Populations were selected stratified randomly as follows. Where possible,

sampling sites were selected from a subset of plots sampled for vegetation description in a related study (Neel 2000). Each vegetation sampling plot supporting *E. ovalifolium* var. *vineum* was classified according to the vegetation series in which it occurred. Two plots that supported this taxon occurred within the Black Bush Series (BBS), 80 plots occurred within the Singleleaf Pinyon-Utah Juniper Series (PJUOS), 9 occurred within the Singleleaf Pinyon Series (PY) and 10 were found in the Singleleaf Pinyon-Mountain Juniper Series (PJUOC). A provisional classification to the association level (based on understory composition) was developed to guide selecting plots that represented the range of ecological diversity within these series (Neel 2000). Four associations were recognized within the single-leaf pinyon-Utah juniper series (pinyon-Utah juniper/blackbush scrub [PJBBS], pinyon-Utah juniper/mountain mahogany woodland [PJCELE], pinyon-Utah juniper/*Yucca* woodland [PJYUCC], and pinyon-Utah juniper/shrub live oak [PJSLO]). Four associations were also recognized within the singleleaf pinyon series (pinyon/blackbrush scrub [PYBBS], pinyon/*Purshia-Yucca* woodland [PYPUYUCC], pinyon/Great Basin sagebrush [PYARTR], and pinyon-canyon live oak [PCLO]).

Within each vegetation type, populations were randomly selected roughly in proportion to the number of populations within that type. Unfortunately, three associations were not represented in samples (PJSLO, PYBBS, and PCLO). Two populations did not correspond to vegetation sampling plots from Neel (Neel 2000) but were associated with plots sampled by the San Bernardino National Forest using similar methods (USDA Forest Service, unpublished data 1998). These two plots were in the pinyon/*Purshia-Yucca* woodland association of the singleleaf pinyon series. Densities of *E. ovalifolium* var. *vineum* in the 31 sampling locations associated with vegetation sampling plots were crudely estimated using data from those plots.

Up to 200 mg of leaf tissue were collected from 29 or 30 individuals per population, although it was not possible to assess genotypes for all loci for all individuals. Samples were taken from widely spaced individuals from throughout each sampled population; actual distances differed among populations due to variation in number and density of individuals. Leaves were kept on ice during transport to the laboratory, at which time they were refrigerated at 8 °C until extraction.

Approximately 45 mg of leaf tissue were extracted in 10 drops of a 0.1 M, pH 7.5 tris-HCl buffer within four days of collection. Each ml of buffer contained 0.68 mg ethylenediamine tetra-acetic acid (disodium salt), 1.5 mg potassium chloride, 2 μl mercaptoethanol, 4.06 mg magnesium chloride, and 60 mg polyvinyl-pyrrolidine-40 (Mitton et al. 1979). Sterile sand was used to aid in macerating the leaf tissue. The resulting extract was soaked onto four replicate sets of chromatography paper wicks that were frozen at –80 °C until they were run. A large number of replicate wicks were prepared using leaf tissue from a subset of individuals to provide internal controls to calibrate alleles across gels. At least three calibration wicks were run on each gel.

The following enzyme systems were assayed: adenylate kinase (ADK, E.C. 2.7.4.3), aspartate amino transaminase (AAT, E.C. 2.6.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), glucose-6-phosphate isomerase (PGI, E.C. 5.3.1.9), malate dehydrogenase (MDH, E.C. 1.1.1.37), leucyl aminopeptidase (LAP, E.C. 3.4.11.1), phosphoglucomutase (PGM, E.C. 5.4.2.2), triose-phosphate isomerase (TPI, E.C. 5.3.1.1) and uridine diphosphoglucose pyrophosphorylase (UDP, E.C. 2.7.7.9). Acid phosphatase (ACP, E.C. 3.1.3.2), aldolase (ALD, E.C., 4.1.2.13), diaphorase (DIA, E.C. 1.6.4.3), fluorescent esterase (FEST, E.C.3.1.1.1), hexokinase (HEX, E.C.2.7.1.1), mannose-6-phosphate isomerase (M-6PI, E.C., 5.3.1.8) and mallate enzyme (ME, E.C. 1.7.99.5) were examined but could not be resolved. Aconitase (ACO, E.C. 4.2.1.3), 6-phosphogluconate dehydrogenase (6PGD, E.C., 1.1.1.44), and shikimate dehydrogenase (SKDH, E.C., 1.1.1.25) were resolved using fresh material but could not be scored reliably from frozen material and thus were dropped from analyses.

A total of 11 loci were resolved for this taxon as follows: ADK (1), AAT (1), IDH (1), LAP (1), MDH (3), PGI (1), PGM (1), TPI (1), and UDP (1). PGI, PGM, TPI and UDP were resolved on a discontinuous, pH 8.3, lithium hydroxide-borate gel system (Heywood 1980). This gel system was run for approximately five hours; current was kept at 75 mA until voltage reached 200 volts. At that point this voltage was maintained for the duration of the run and current ranged between 25 and 50 mA. AAT, LAP and PGM were resolved on a tris-EDTA-borate gel system run at 50 mA and 200 volts for 5 hours (Heywood 1980). IDH, ADK and MDH were resolved on a continuous

pH 8 morpholine-citrate gel system run for 5 hours at 30 mA and 150 volts (O'Malley et al. 1980).

Data analysis

Levels and Patterns of Diversity – Total number of alleles (TA), proportion of polymorphic loci (P), number of alleles per locus (A), number of alleles per polymorphic locus (A_p), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated for the taxon and for each sampling site using the computer program Genetic Data Analysis (GDA) (Lewis and Zaykin 2001). A locus was considered polymorphic if the frequency of the most common allele was < 0.95. The number of private alleles within sites (that is, alleles detected at only one site) was also counted using GDA.

Hierarchical population genetic structure was assessed following methods of Weir (1996) as implemented by Lewis and Zaykin (2001). The statistic $f(\approx F_{IS})$ represents departures from Hardy-Weinberg equilibrium within individual populations. The statistic $F(\approx F_{IT})$ represents deviations from Hardy-Weinberg equilibrium expectations over all populations. θ_P represents the proportion of genetic differentiation among populations from different vegetation types and was used to examine relationships between genetic diversity and community composition. θ_S represents the proportion of total genetic variation partitioned among sites in relation to the total variation present. θ_S and θ_P are equivalent to F_{ST} for their respective population subdivisions (i.e. by population or habitat type); θ_S and θ_P differ from F_{ST} in that they both loci and sites as samples. Standard deviations of individual estimates of f, F, θ_P, and θ_S were calculated by jackknifing over populations. Bounds of 95% confidence intervals for overall estimates were calculated from 5000 bootstrap replicates across loci. The number of migrants per generation among populations (N_m) was estimated as $N_m = 0.25(1\text{-}\theta_S)/\theta_S$ (Weir 1996) and using the private allele method (Slatkin 1985).

Nei's (1978) unbiased genetic distance was computed for all pairwise combinations of populations. Straight-line geographic distances among these same populations were calculated using ArcView version 3.2 (Environmental Systems Research Institute 1996). The average genetic distance from each site to all other sites was also calculated to determine whether any sites were particularly unique. The strength of the relationship between the genetic and geographic distance matrices was evaluated with a standardized Mantel statistic (Sokal and Rohlf 1995) using PC-ORD (McCune and Mefford 1999). The significance of the Mantel statistic was assessed through a randomization test using 1000 Monte Carlo simulations.

Potential for Clonal Reproduction – After completing all data collection, evidence for clonal reproduction in a closely related taxon came to our attention. Although our field sampling was not designed to detect clonal reproduction by vegetative spread, we felt it necessary to assess whether the patterns we observed were at least consistent with it. To assess potential for clonal reproduction we quantified genotypic diversity and probability of occurrence of particular genotypes within populations following methods of Ellstrand and Roose (1987), Montalvo et al. (1997), Cheliak and Pitel (1984), and Parks and Werth (1993). We excluded 21 individuals that were missing data at one or more loci; thus, this assessment was based on 899 individuals from 31 sites. We described genotype diversity within populations in three ways. First, we simply counted the number of individuals of each multilocus genotype in each population. Second, we used the ratio of the proportion of distinct genotypes (G) to the number of individuals sampled (N) (Ellstrand and Roose 1987). This ratio approaches 0 if all sampled individuals have identical genotypes and equals 1 if each individual has a unique genotype. Third, we used Simpson's Diversity Index (d) corrected for finite population size (Pielou 1969)

$$d = 1 - \sum\{[n_i(n_i - 1)]/[N(N - 1)]\}$$

where n_i is the number of individuals with genotype i and N is the number of individuals in a population. This index estimates the probability that two individuals selected randomly from a population would have different genotypes; small values would indicate high potential for vegetative reproduction (Ellstrand and Roose 1987; Montalvo et al. 1997).

We followed the methods of Parks and Werth (1993) to assess the probability of multiple occurrences of specific multilocus genotypes in a population resulting from sexual reproduction. We first determined the probability of each particular multilocus genotype occurring (P_{gen}) as

$$P_{gen} = (\prod_{i=1}^{L} p_i)2^h$$

where p_i is the frequency of each allele at locus i in a population, h is the number of heterozygous loci in an individual, and L is the number of loci. This relationship assumes linkage equilibrium among loci which was tested and confirmed using GDA (Lewis and Zaykin 2001). Allele frequencies were calculated using each distinct genotype once to reduce effects of the circularity of using genotypes to determine allele frequencies and then using allele frequencies to assess clonality.

For each genotype that occurred more than once in a population, we then calculated the probability of a second occurrence (P_{sec}) among G genotypes as

$$P_{sec} = 1 - (1 - P_{gen})^G.$$

Different ramets with identical genotypes were considered to be potential clones if the probability of a second occurrence was $< 5\%$. Because our sampling was not designed to detect clonal population structure, we do not know the specific locations of the individuals sampled within populations, and thus we do not know if samples with identical genotypes were located adjacent to one another. As a result, our estimates of potential clonal reproduction could be biased upwards if identical multilocus genotypes in fact were spatially distant from one another.

Conservation Implications – Conservation significance of individual populations based on diversity and differentiation was assessed by identifying sites that supported private alleles and had the five highest values of standardized TA, P, H_e, or mean genetic distance to other populations, or the five lowest values for *f*. Tied values resulted in selection of more than five populations for some measures. Populations ranking favorably in multiple categories were considered to be a higher priority for conservation of genetic diversity.

Results

Levels and patterns of diversity

At the species level, 6 (P = 0.55) of the 11 loci examined were polymorphic. A total of 60 alleles were detected at all loci combined, yielding an average of 5.45 (SD = 2.8) alleles per locus and 7.0 (SD = 3.0) alleles per polymorphic locus. Species level H_e was 0.20 (SD = 0.23) and H_o was 0.16 (SD = 0.17).

The proportion of loci that were polymorphic within the 31 sampled populations ranged from 0.36 to 0.73 with a mean of 0.56 across populations (SD = 0.11) (Table 1). The number of alleles per population ranged between 24 and 41 and averaged 29.5 (SD = 3.8). The number of alleles per locus within populations averaged 2.68 (SD = 0.35) and ranged from 2.18 (B9EO) to 3.73 (C9EO). The number of alleles per polymorphic locus within populations ranged from 2.75 to 4.75 with a mean of 3.73 (SD = 0.57). H_e within populations ranged from 0.14 to 0.27 and averaged 0.19 (SD = 0.03) (Table 1). H_o within populations was similar in magnitude, ranging from 0.11 to 0.26 and averaging 0.17 (SD = 0.03) over all populations (Table 1).

A total of nine private alleles was found at seven loci in eight populations (Table 2). Private alleles were always at low frequency in the sampled population, all occurring at a frequency [0.05 (Table 2). There was no obvious geographic pattern to populations with private alleles.

The coancestry coefficient, *f*, averaged 0.14 across all loci and populations and based on confidence intervals derived from bootstrap estimates, was significantly different from zero (Table 3). This result indicates heterozygote deficit in individual populations. *F* averaged 0.19 across all loci in all populations and also was significantly different from zero (Table 3) indicating deviation in heterozygosity in individuals relative to the all populations combined. Population differentiation as assessed using θ_S averaged 0.07 over all loci and all populations and was significantly different from zero (Table 3). Thus, approximately 93% of the diversity in this species was common to all populations. Based on this level of differentiation, the number of migrants among populations per generation was estimated to be 3.3. A much higher level of migration among populations, N_m = 14.7, was estimated using the private allele method. There was even less differentiation among groups of populations grouped by the vegetation type in which they occurred (θ_P= 0.015), although this differentiation was still significantly different from zero (Table 3).

Nei's unbiased genetic distance among all population pairs averaged 0.02 (SD = 0.01) and ranged from 0.00006 between D4EO and B47EO to 0.08 between F39EO and B56EO indicating no strong differentiation among populations. Further, there was not much variation in the average genetic distance of one site to all other sites (Table 1). Geographic distance between all pairs of sample sites averaged 12 km (SD = 7.6) and ranged from 0.8 to 36 km. Distance to a population's nearest neighboring sample population averaged 1.5 km (SD = 0.71) and ranged from 0.8–3.8 km.

Table 1. Sample sizes averaged over loci and genetic diversity statistics for 11 loci in 31 populations of *E. ovalifolium* var. *vineum*. Vegetation types in which each sample site occurred are indicated; abbreviations are as in the text. The five highest ranking values for TA, P, H_e and average genetic distance, the five lowest ranking values for *f*, and all occurrences of private alleles are in boldface type

Pop.	*N*	TA	P	*A*	*A*p	H_e	H_o	*f*	Private Alleles	Average Distance	*G*	*G/N*	*d*	Vegetation Type	Plant Density[1]
A3EO	30.0	25	**0.73**	2.27	2.75	0.14	0.14	**0.04**	0	0.03	24	0.80	0.98	PJUOC	38
B4EO	29.0	27	**0.64**	2.45	3.29	0.17	0.13	0.23	0	0.01	27	0.93	1.00	PYARTR	27
B9EO	30.0	24	0.45	2.18	3.40	0.17	0.13	0.23	0	0.02	28	0.93	0.99	PJCELE	45
B38EO	30.0	**34**	**0.64**	3.09	4.00	0.20	0.19	0.07	0	0.01	28	0.93	1.00	PJCELE	72
B47EO	29.9	32	0.46	2.91	4.20	0.17	0.15	0.08	**1**	0.01	25	0.86	0.98	PJCELE	48
B50EO	30.0	26	**0.64**	2.36	2.86	0.17	0.13	0.22	0	0.02	26	0.87	0.99	PJCELE	38
B56EO	30.0	**35**	**0.64**	3.18	4.43	0.23	0.23	**–0.01**	0	0.03	30	1.00	1.00	PJCELE	61
B64EO	30.0	32	0.54	2.91	4.17	0.16	0.15	0.08	0	0.02	24	0.80	1.00	PJCELE	87
B71EO	30.0	27	0.46	2.45	3.40	0.17	0.14	0.15	0	0.02	28	0.93	1.00	PJCELE	34
B213EO	30.0	29	**0.64**	2.64	3.57	0.21	0.17	0.20	0	0.01	30	1.00	1.00	PJYUCC	nd
B27RC	29.9	29	0.36	2.64	4.75	0.16	0.15	0.08	0	0.01	26	0.90	0.99	PJCELE	0
C9EO	29.5	**41**	**0.64**	3.73	4.71	0.27	0.21	0.22	**1**	0.02	27	1.00	1.00	PJBBS	39
C10EO	29.8	**37**	**0.73**	3.36	4.12	0.26	0.26	**–0.004**	**1**	0.01	28	1.00	1.00	PJBBS	23
C207EO	29.9	29	0.45	2.64	4.00	0.20	0.16	0.21	**1**	0.01	27	0.93	0.99	PJCELE	84
D4EO	29.7	29	**0.64**	2.64	3.14	0.16	0.11	0.29	0	0.01	20	0.74	0.95	PJCELE	33
D10EO	30.0	32	0.55	2.91	4.00	0.19	0.18	**0.04**	0	0.01	29	0.97	1.00	PJBBS	14
D12EO	29.5	26	0.36	2.36	4.00	0.16	0.13	0.19	0	0.01	22	0.88	0.99	PJBBS	15
D14EO	29.6	24	0.45	2.18	3.00	0.15	0.16	**–0.02**	0	0.01	26	0.90	0.99	PJBBS	16
D100EO	29.9	28	**0.64**	2.54	3.29	0.19	0.17	0.09	0	0.03	28	0.97	1.00	PJBBS	19
D9RC	29.7	29	**0.64**	2.64	3.43	0.22	0.18	0.16	0	0.01	29	1.00	0.95	PJCELE	83
E11EO	30.0	30	0.54	2.73	4.00	0.19	0.18	0.08	**1**	0.01	28	0.93	1.00	PJCELE	47
E16EO	30.0	27	0.46	2.45	3.80	0.17	0.14	0.14	0	0.01	25	0.83	0.98	PJBBS	17
E19RC	29.5	**33**	0.64	**3.00**	4.00	0.23	0.20	0.10	**2**	0.01	25	0.89	0.99	PJCELE	0
F39EO	30.0	26	**0.73**	2.36	2.75	0.22	0.21	**0.04**	0	0.04	28	0.93	1.00	PYPUYUCC	12
F200EO	29.9	30	0.54	2.73	3.50	0.18	0.17	0.10	0	0.02	25	0.86	0.98	PYPUYUCC	110
F23RC	29.9	31	0.46	2.82	**4.20**	0.17	0.15	0.09	0	0.01	26	0.90	0.99	PJCELE	19
F26RC	29.8	31	0.45	2.82	**4.20**	0.18	0.14	0.21	**1**	0.01	26	0.93	0.99	PJCELE	22
G200EO	30.0	26	0.55	2.36	3.33	0.22	0.14	0.33	0	0.02	29	0.97	1.00	PYPUYUCC	19
G7RC	29.7	28	**0.64**	2.54	3.29	0.23	0.20	0.15	0	0.02	27	1.00	1.00	PJUOC	17
H1EO	30.0	27	0.36	2.46	**4.75**	0.16	0.12	0.26	**1**	0.04	26	0.87	0.99	PJUOC	28
H2EO	29.8	31	**0.64**	2.82	3.29	0.21	0.15	0.27	0	0.02	27	0.96	1.00	PJUOC	12
Mean	29.8	29.5	0.56	2.68	3.73	0.19	0.17	0.14		0.02	26.6	0.92	0.99		36.0
S.D.		3.8	0.11	0.35	0.57	0.03	0.03	0.09		0.008	2.2	0.07	0.01		28.0

[1]The number of *Eriogonum ovalifolium* var. *vineum* individuals in one, 0.04 ha plot.

The Mantel test indicated that there was a weak but significant correlation between geographic distance and genetic distance among all pairs of sample sites ($r = 0.388$, $P = 0.002$). Thus, there was some apparent geographic pattern to genetic distance among populations.

Potential for clonal reproduction

A total of 597 distinct multilocus genotypes were documented from the 899 individuals with complete genotypes in all populations combined. Of these 597 genotypes, 501 were found in only one individual in the whole sample; 50 were found in 2 individuals, 18 were found in 3 individuals, and 28 were found in > 3 individuals. Within individual populations, on average, 86% (SD = 9.8) of genotypes were restricted to one individual (in a few cases the same genotype was found in other populations) and no genotype was found in more than seven individuals. Population *G*/*N* values were between 0.74 and 1.0 (mean = 0.92, SD = 0.07) (Table 1). In seven populations, *G*/*N* = 1,

Table 2. Distributions and frequencies of private alleles among 11 loci and 31 populations of *E. ovalifolium* var. *vineum*

Locus	Allele	Frequency	Found in
AAT	C	0.017	C207EO
PGI	D	0.033	F26RC
PGI	K	0.017	E11EO
UDP	E	0.017	C9EO
ADK	A	0.017	E19RC
ADK	B	0.017	E19RC
MDH1	A	0.050	C10EO
MDH2	D	0.017	B47EO
MDH3	A	0.017	H1EO

Table 3. Hierarchical F-statistics for 11 loci in 31 populations of *E. ovalifolium* var. *vineum* hierarchically arranged in vegetation types calculated using methods of Weir (1996). Standard deviations for each locus are in parentheses and were calculated by jackknifing over populations. Upper and lower bounds for multilocus estimates were calculated by bootstrapping across loci

Locus	f[1]	F[2]	θ_S[3]	θ_P[4]
AAT	0.25 (0.1)	0.29 (0.11)	0.05 (0.03)	0.0002
LAP	0.28 (0.03)	0.36 (0.04)	0.11 (0.11)	0.03
TPI	–0.02 (0.05)	0.02 (0.03)	0.04 (0.04)	0.02
PGM1	0.10 (0.05)	0.12 (0.05)	0.03 (0.03)	0.007
PGI	0.07 (0.03)	0.14 (0.04)	0.07 (0.07)	0.02
UDP	0.01 (0.03)	0.05 (0.03)	0.04 (0.04)	–0.001
IDH	0.24 (0.05)	0.25 (0.05)	0.01 (0.01)	–0.00002
ADK	–0.03 (0.01)	–0.01 (0.003)	0.02 (0.02)	–0.005
MDH1	0.16 (0.07)	0.18 (0.07)	0.02 (0.02)	0.0002
MDH2	0.07 (0.07)	0.09 (0.06)	0.02 (0.02)	0.006
MDH3	0.03 (0.04)	0.06 (0.04)	0.04 (0.04)	–0.0003
Overall	0.14	0.19	0.07	0.015
Upper	0.23	0.29	0.09	0.022
Lower	0.05	0.08	0.03	0.0008

[1] f represents departures from Hardy-Weinberg equilibrium within individual populations.
[2] F represents deviations from Hardy-Weinberg equilibrium expectations over all populations.
[3] θ_P represents differentiation of populations within vegetation types.
[4] θ_S represents differentiation among all populations.

indicating that all individuals had unique multilocus genotypes. Values of Simpson's diversity index (d) in populations ranged from 0.95 to 1.0 (average = 0.99, SD = 0.01) indicating a high probability that a randomly sampled individual would have a unique genotype (Table 1). The probability of any particular genotype occurring was always < 0.12 (Table 4). In all but one case the probability of a second occurrence of each genotype that occurred more than once in a population was above 0.06, typically the probability was substantially higher (Table 4).

Conservation implications and priorities

Twenty-four sites had private alleles or were among the five highest-ranked values for at least one of the five measures of diversity and the one measure of differentiation, or in the five lowest-ranked values for inbreeding (Table 1). Fourteen sites ranked favorably more than one measure and four sites (B56EO, C9EO, C10EO, and E19RC) ranked for more than two measures (Table 1). The particular measures of diversity for which populations ranked highly in these four sites varied.

Discussion

Levels and patterns of diversity

Given its endemism to a restricted geographic range, *E. ovalifolium* var. *vineum* supports a surprisingly large amount of genetic variation. Values for P, A, and H_e at both the species and population levels were higher than those typical for endemic and narrowly distributed plant taxa (Hamrick and Godt 1989; Loveless and Hamrick 1989; Godt et al. 1996). Species-level P and H_e for *E. ovalifolium* var. *vineum* were comparable to values for widespread species and A exceeded average values for all distributional categories of plant species (Hamrick and Godt 1989). Population-level values also exceeded averages for widespread species. Similarly high levels of diversity were found for the closely related rare taxon *E. ovalifolium* var. *williamsiae* (Archibald et al. 2001) and have recently been noted for a variety of other rare taxa (Prober and Brown 1994; Purdy and Bayer 1995; Young and Brown 1996; Cruzan 2001), including unrelated taxa that occur with or close to *E. ovalifolium* var. *vineum* throughout most of their ranges (*Astragalus albens*, *Erigeron parishii*, and *Oxytheca parishii* var. *goodmaniana*) (Neel 2000; Neel and Ellstrand 2001).

Potential explanations for higher than expected levels of diversity include evolutionary origin, large population sizes, and mating system. *Eriogonum ovalifolium* is a widespread species that is distributed throughout the montane and intermountain regions of western North America comprising a number of

Table 4. Probability of occurrence of each multilocus genotype that occurred more than once in a population and probability of encountering that same genotype a second time

Population	Genotype	No. times genotype occurred	P_{gen}	P_{sec}
A3EO	1	3	0.045	0.670
	3	4	0.113	0.944
	13	2	0.018	0.351
B27RC	3	3	0.039	0.648
	41	2	0.003	0.076
B38EO	1	2	0.012	0.278
	74	2	0.004	0.116
B47EO	3	2	0.051	0.728
	76	4	0.116	0.954
B4EO	29	2	0.006	0.159
	44	2	0.023	0.466
B50EO	3	3	0.116	0.959
	76	3	0.056	0.777
B64EO	3	5	0.072	0.835
	76	2	0.029	0.506
	168	2	0.011	0.230
B71EO	76	2	0.026	0.521
	176	2	0.006	0.157
B9EO	3	3	0.031	0.580
C207EO	3	3	0.019	0.398
D100EO	13	2	0.027	0.532
D10EO	29	2	0.013	0.315
D12EO	3	2	0.032	0.512
	13	2	0.034	0.529
	44	2	0.018	0.323
D14EO	68	2	0.012	0.266
	99	2	0.049	0.733
	183	2	0.106	0.945
D4EO	3	7	0.023	0.374
	76	2	0.093	0.857
E11EO	1	2	0.017	0.377
	29	2	0.019	0.416
E16EO	3	2	0.018	0.360
	29	4	0.022	0.432
	76	2	0.040	0.637
E19RC	76	2	0.011	0.241
	252	3	0.003	0.067
F200EO	76	4	0.025	0.468
	492	2	0.010	0.228
F23RC	3	2	0.055	0.772
	57	2	0.002	0.053
	76	2	0.043	0.685
F26RC	3	3	0.020	0.405
F39EO	232	2	0.007	0.188
	479	2	0.002	0.064
G1EODN	43	2	0.004	0.117
H1EO	589	2	0.000	0.012
	591	2	0.047	0.712
	593	2	0.016	0.349
	602	2	0.004	0.101
H2EO	616	2	0.003	0.070

locally restricted subspecific taxa. It is possible that the ancestor to *E. ovalifolium* var. *vineum* was historically genetically diverse and the current diversity reflects that history. Evidence from Archibald et al. (2001) indicating similarly high levels of diversity in a number of other varieties of *E. ovalifolium* supports this idea. Additionally, high levels of diversity in *E. ovalifolium* var. *williamsiae* were suggested to be due to a hybrid origin from *E. ovalifolium* var. *ovalifolium* and *E. ovalifolium* var. *eximium* (Archibald et al. 2001). There is no evidence of hybrid origin for *E. ovalifolium* var. *vineum.* No other *E. ovalifolium* species occur within the San Bernardino Mountains and the geographically closest relatives are *E. ovalifolium* var. *ovalifolium* and *E. ovalifolium* var. *nivale* in the southern Sierra Nevada no less than 200 km to the northwest. There is no continuous suitable habitat connecting current locations. Two potential origins for *E. ovalifolium* var. *vineum* from ancestral stock include a long-distance dispersal event and subsequent local adaptation, or local adaptation after isolation from a more continuous distribution. The latter alternative is more consistent with the observed levels of diversity since founding by a few individuals via long-distance dispersal would likely cause a significant genetic bottleneck.

Isolation from a more widespread distribution is also consistent with paleobiogeographic vegetation patterns. Vegetation distributions during the Pleistocene likely provided potential for a more continuous distribution of *E. ovalifolium*. Taxa of this species are typical members of pinyon-juniper woodlands and other dry woodland habitats as well as alpine zones (Hickman 1993). Macrofossils from packrat middens indicate that juniper and pinyon-juniper woodlands dominated much of what is currently the Mojave Desert up until ∼7,400–10,000 years before present (Wells and Berger 1967). Assuming *E. ovalifolium*

occupied similar environmental conditions at that time and thus was part of similar vegetation communities as at present, populations of different varieties of the species could have been distributed throughout what is now the Mojave desert. As the climate warmed after the last glacial, these taxa would have receded into refugia in mountain ranges adjacent to the desert and become isolated.

In addition to likely starting with a large amount of genetic diversity, maintaining much of the original genetic diversity would require that populations of *E. ovalifolium* var. *vineum* remain relatively large and interconnected throughout the evolutionary history of the taxon. Compared to many endemic plants, *E. ovalifolium* var. *vineum* current populations can be large and extensive. The large percentage of polymorphic loci and numbers of alleles per locus indicate that *E. ovalifolium* var. *vineum* has no history of severe or long-lasting population bottlenecks (Milligan et al. 1994). It is also possible that the species is not yet in evolutionary equilibrium and that the high levels of variation reflect historical conditions when populations were less isolated. Based on vegetation and climatic history, *E. ovalifolium* var. *vineum* is likely to have been in its current general location for ~10,000 years (Wells and Berger 1967). Populations of other varieties could potentially have been in closer proximity during a time when the environments in which they occur were more widespread, occupying much of the area that is currently the Mojave Desert. Clearly, however, one of the major features of *E. ovalifolium* var. *vineum* habitat is its current edaphic restriction to limestone and dolomite substrates (Gonella and Neel 1995; Neel 2000), and the natural extent of these substrates was not greater than it is at present. Thus, although climatic environments supporting the different varieties could have been in contact, edaphic characteristics could have enforced isolation at an earlier time. Alternatively, *E. ovalifolium* var. *vineum* may have colonized the carbonate substrate as climate or vegetation community distributions changed and the San Bernardino Mountains became habitable and other substrates became unsuitable. Thus, it would seem likely that *E. ovalifolium* var. *vineum* has been isolated from other varieties of the species for between 7,000 and perhaps 15,000 years. Depending on actual generation times and population sizes, such isolation times are potentially not sufficient to reach equilibrium in allozyme allele frequencies (Varvio et al. 1986). Presence of a seed bank would further slow the approach to equilibrium.

The highly outcrossed mating system (Neel et al. 2001) likely also plays a role in maintaining diversity in *E. ovalifolium* var. *vineum*. Excessive inbreeding can increase the rate at which diversity will be lost through indirect effects on effective population size (N_e) (Holtsford and Ellstrand 1989; Young and Brown 1998). Outcrossing will have the effect of increasing N_e for a given census size and this in turn will reduce effects of genetic drift. Mating system is also thought to play a role in maintaining large amounts of diversity in *E. ovalifolium* var. *williamsiae*; it was observed to be gynodioecious which will potentially enforce a large degree of outcrossing (Archibald et al. 2001). Gynodioecy was not detected during our work, although we did not examine large numbers of flowers in bloom and thus the potential for its existence in this taxon warrants investigation.

While *Eriogonum ovalifolium* var. *vineum* is extremely diverse in terms of allele richness and polymorphic loci, we documented relatively large deviations from expected levels of heterozygosity predicted from Hardy-Weinberg equilibrium at certain loci and in ~33% of the populations (Tables 1 and 3). These deviations indicate potential levels of inbreeding that are inconsistent with the mostly outcrossed mating system of this taxon (Neel et al. 2001). They are further inconsistent with the fact that we saw strong selection against homozygotes when comparing seed and adult cohorts within populations in a study of mating patterns in this taxon (Neel et al. 2001). Heterozygote deficiency could result from Wahlund effects if there is substantial substructuring of individuals within populations (e.g. Hartl and Clark 1997). Because our primary focus was to compare diversity among populations, our data are not sufficiently resolved to demonstrate within population structure. In the related outcrossing study we found some evidence to support substructuring as the cause of heterozygote deficiency (Neel et al. 2001). In that study we observed high correlation of outcrossed paternity and consequently small neighborhood sizes (primarily < 3 individuals). Additionally, biparental inbreeding contributed significantly to selfing rates. Both of correlation of outcrossed paternity and biparental inbreeding indicate substructuring of matings within populations.

Eriogonum ovalifolium var. *vineum* populations are naturally patchily distributed; patches vary in size and isolation (Figure 1), and they have been further fragmented by mining activities. Yet θ_S values indicate that populations are not highly differentiated from one

another. Lack of strong differentiation among populations (Table 3) suggests either substantial current gene flow among populations, or that fragmented populations have not yet been isolated long enough for genetic drift or selection to have caused changes in allelic composition (Ellstrand 1992). Unfortunately, it is not possible to separate effects of current gene flow from common history using hierarchical F statistics.

Potential for clonal reproduction

We found no strong evidence for extensive vegetative reproduction in *E. ovalifolium* var. *vineum*, but the data were suggestive of limited clonality in at least some populations (Table 4). For example, probability of finding a second occurrence of a duplicate genotype under the assumption of 100% sexual reproduction was less than 0.05 for genotype 589 in population H1EO and approached 0.05 in five cases: genotype 41 in population B27RC, 252 in E19RC, 57 in F23RC, 479 in F39EO, and 616 in H2EO. While the analytical methods we used have the potential to overestimate clonality, our sampling methods would likely underestimate it or miss it altogether. Because we sought to characterize the genetic diversity of each population, we intentionally sampled widely spaced individuals and avoided sampling closely spaced ones. Archibald et al. (2001) found that most clonal reproduction in *E. ovalifolium* var. *williamsiae* was detectable at distances < 2 m, and our samples were almost always collected from individuals > 2 m apart. Thus, while we cannot rule out clonal reproduction, we can say that it is not a dominant factor in structuring within population variation. It should be noted that even limited amounts of clonal reproduction would contribute to high levels of genetic diversity and low levels of among-population differentiation through extended persistence of individual genotypes and could also play a role in spatial substructuring within populations.

Conservation implications and priorities

Our results indicated that most populations of *E. ovalifolium* var. *vineum* have likely remained large throughout most of their history or have been interconnected at least until the recent past. Because of this, severely reducing population sizes could have detrimental effects. Such reductions could alter mating structure within populations by increasing selfing or mating among relatives, thus increasing total inbreeding and potentially inbreeding depression (Husband and Schemske 1996). Increased selfing could have a large effect on fitness since genetic load (in terms of deleterious recessive alleles) is expected to be high given the highly outcrossed mating system (Neel et al. 2001). Fragmentation isolating previously connected populations could also have deleterious effects by disrupting gene flow among populations and could exacerbate effects of loss of diversity through drift and further increase inbreeding in the remaining fragments (Templeton et al. 1990; Young et al. 1996). Conservation efforts focusing on maintaining large populations of *E. ovalifolium* var. *vineum* as well as connections among those populations should be sufficient to sustain the existing high levels of diversity in this taxon and to prevent the deleterious effects described above. In addition to protecting existing sites, restoration of degraded sites to increase sizes of populations that have been impacted by human activities and to connect anthropogenically fragmented populations could be useful but should be a lower priority than protecting existing sites. There is no evidence that reintroduction is necessary to "genetically rescue" or supplement any population, a measure that was recommended in the draft recovery plan for this taxon (U.S. Fish and Wildlife Service 1997).

Beyond the general guidelines discussed above, patterns of genetic diversity revealed by molecular markers can contribute to setting conservation priorities among populations. While there is general agreement that conserving genetic diversity is important, there is little agreement on the specific measure of diversity that should be targeted. Expected heterozygosity (interpreted as gene diversity) is often targeted because of its direct influence on immediate responses to selection (discussed in Petit et al. 1998). Levels of inbreeding are considered important due to relationships between heterozygosity and fitness (reviewed in Huenneke 1991; Linhart and Grant 1996; Latta and Mitton 1997). Evidence for short-term fitness benefits of heterozygosity is accumulating (Frankel and Soulé 1981; Ellstrand and Elam 1993; Oostermeijer et al. 1994; Raijmann et al. 1994) but is not ubiquitous (e.g. Ritland 1990; Eguiarte et al. 1991; Ouborg and Van Treuren 1995; Lynch 1996). Effects of heterozygosity on fitness are most apparent when comparing selfed and outcrossed offspring (e.g. Waller 1984; Holtsford and Ellstrand 1990) or when sampling across age classes within populations (e.g. Schaal and Levin 1976; Murawski et al. 1994; Allphin et al. 1998).

Petit et al. (1998) strongly advocate using allozyme allelic richness to prioritize populations for conservation as allelic diversity at a locus defines the ultimate limits of evolutionary potential. Maintaining the evolutionary potential of a species is thought to increase the probability of persistence of a taxon (Storfer 1996) especially under changing environmental conditions. Specifically, Petit et al. (1998) suggested evaluating the significance of individual populations based on both levels of allozyme allele richness and distinctiveness. While conserving markers is not the ultimate goal (e.g. Milligan et al. 1994), they do provide an easily and objectively measured index of general levels of diversity. It is worth briefly commenting on relationships between diversity estimates based on allozymes and estimates based on other molecular markers and quantitative traits. Typically diversity will be lower for allozymes than for DNA-based markers used for population level analyses. Specifically, the number of alleles per locus and percent polymorphic loci will be lower for allozymes than for codominantly expressed markers (e.g. microsatellites and restriction fragment length polymorphism [RFLP]) while the percent polymorphic loci will be higher in dominant markers (e.g. amplified frequency length polymorphisms [AFLPs] and randomly amplified polymorphic DNA [RAPD]) (reviewed in Cruzan 1998; Parker et al. 1998). If population bottlenecks have been severe and too recent for sufficient mutation accumulation, diversity estimates based on different molecular markers could be more similar (Butcher et al. 1998). Molecular markers with higher levels of polymorphism (e.g. microsatellites) will also indicate higher levels of population differentiation simply due to resolution differences. Differences in among-population patterns have been attributed to selection at allozyme loci versus neutral behavior at largely non-coding regions sampled by DNA-based markers and to varying evolutionary dynamics of different DNA-based markers (e.g. Zhang et al. 1993). Discordant patterns of population differentiation can also result when loci are sampled from genomes with different inheritance patterns and evolutionary dynamics (e.g. chloroplast, mitochondrial, and nuclear genomes) (Fenster and Ritland 1992; Latta and Mitton 1997). These differences have been useful for quantifying relative contributions of pollen and seed to gene flow among populations (McCauley 1997) but can complicate conservation assessments seeking to prioritize populations based on levels of differentiation.

A larger concern with use of allozymes for setting conservation priorities is the equivocal relationship between (nearly) neutral markers and quantitative traits. There is sometimes strong evidence for relationships between allozyme alleles and environmental characteristics (e.g. Prentice and Cramer 1990; Prentice et al. 1995) and fitness related characters (e.g. Watt et al. 1983; Patarnello and Battaglia 1992; Mitton et al. 1998). Further, Schoen and Brown (1993) and Bataillon et al. (1996) have demonstrated through simulations that marker diversity was indicative of diversity at non-targeted loci. However, evidence for nearly neutral behavior of allozymes and weak relationships between marker-based diversity and differentiation estimates and quantitative trait-based estimates is much more abundant (Lynch 1996; Storfer 1996; Reed and Frankham 2001). Specifically, relative to quantitative traits, markers will typically underestimate overall levels of diversity and differentiation and often indicate different among-population differentiation patterns. Of course different individual quantitative traits that respond to different environmental gradients will also indicate discordant patterns of differentiation. McKay and Latta (2002) argue that rather than conserving patterns based on current quantitative traits we should target variation in alleles at all the genes affecting the phenotypic traits of interest (quantitative trait loci). They provide theoretical evidence that genetic diversity measured using allozyme and DNA markers is correlated with diversity at those loci and thus may in fact provide good estimates of adaptive potential after all. Regardless, if the goal of conservation is to maintain as much genetic diversity as possible, conservation targets and priorities depending solely on markers, or for that matter small numbers of quantitative traits, must be applied cautiously and be interpreted as a low estimate of what needs to be conserved. With these caveats in mind, we make the following suggestions regarding conservation priorities for *E. ovalifolium* var. *vineum* based on allozyme diversity patterns.

Because most populations in this study were relatively diverse in at least one measure of allozyme diversity (Table 1), individual measures provided limited means of prioritizing any particular populations for conservation. For example, 24 (71%) of the populations ranked favorably in at least one measure of diversity, distinctiveness or inbreeding. Therefore, to distinguish among sites we considered those that ranked highly in more than two measures to be of particular conservation priority. Populations that are

high in multiple measures of allozyme-based genetic diversity may also be more likely to be important for other types of genetic diversity. The following populations fell into this category: B56EO, C9EO, C10EO, and E19RC. We can then evaluate how well those four populations do at capturing diversity in this taxon by comparing the levels of diversity captured with the levels captured in the same number of populations selected randomly (using data from Neel and Cummings [accepted]). Together, these four populations have the following levels of diversity: P = 0.91, A = 4.72, TA = 52, H_e = 0.26, H_o = 0.23, and f = 0.11. These values of P, H_e, and H_o were higher than values in 1000 randomly chosen samples of four *E. ovalifolium* var. *vineum* populations; only one of 1000 random samples of four populations had A and (consequently) TA values as large as those from the four high priority populations. In fact, conserving these four populations would capture 52 (∼87%) of the 60 alleles documented from the taxon. The level of inbreeding in these populations was not significantly different than random, but was less than levels in 921 of 1000 random samples of four populations. Thus, selecting populations that are diverse for several measures of genetic diversity appears to be an efficient way to capture genetic diversity and to reduce apparent levels of inbreeding. Even so, 13% of the allelic diversity known from the species would be lost at the outset if only these four populations were conserved. The eight remaining alleles that were not captured were private alleles, each restricted to only one population (Table 1). While the evolutionary value of these specific private alleles is uncertain, they may be indicative of presence of other unique genetic variation in these populations. Conserving only four populations, even four highly diverse ones, also does not reflect the ecological and geographic ranges of the species nor would it serve to maintain gene flow among populations. Specifically, all four populations are in the eastern half of the taxon range (Figure 1) and they do not capture the elevational range of the taxon. Further, only 2 of the 10 vegetation types in which *E. ovalifolium* var. *vineum* is known to occur would be represented. Capturing the ecological and geographic ranges of a taxon is thought to be the most effective way to capture adaptive variation and facilitating gene flow by maintaining connections among populations increases the probability of maintaining diversity over time. Thus, while conserving the most diverse populations is a good start, it is not a sufficient conservation strategy on its own.

In summary, *E. ovalifolium* var. *vineum* currently supports a substantial amount of genetic variation at both the species and population levels. Moderately high apparent levels of inbreeding are potentially of some concern but are likely due to Wahlund effects. Because detrimental effects due to lack of genetic diversity are not of immediate concern for *E. ovalifolium* var. *vineum* and because populations are not highly differentiated, reserve designs based on ecological factors would likely suffice to maintain genetic diversity provided a sufficient number of sites are protected. For example, protecting sites throughout the ecological and geographic range of *E. ovalifolium* var. *vineum* that incorporate the allozyme diversity documented would probably encompass its range of adaptive variation, although that variation was not characterized here. The low levels of among-population differentiation support maintaining a network of interconnected populations to maintain potential for gene flow. Maintaining large populations in such a network would prevent changes in mating system structure and thus limit selfing and biparental inbreeding. Such a reserve system could be established through a combination of protecting existing sites and restoring degraded habitat. The data presented here provide guidance for which populations may be valuable from a genetic perspective and also serve as a valuable baseline for monitoring effectiveness of protected areas and restoration in maintaining genetic diversity, or evaluating consequences of further fragmentation and population loss.

Acknowledgements

The authors thank J. Frie (U.S. Fish and Wildlife Service, permit number PRT 826515), D. Volgarino (USDA Forest Service), and T. Egan (Bureau of Land Management) for facilitating permits for tissue collections; L. Blancas, M. Cummings, S. Morita, and J. Ross-Ibarra for field assistance; and L. Bhattia, L. Blancas, and J. Clegg, for valuable assistance in the lab. This work was partially funded by a Switzer Environmental Fellowship, by the University of California Riverside, and by the US Environmental Protection Agency. Although the research described in this article has been funded in part by the United States Environmental Protection Agency, through grant R826102-01-0 to N.C. Ellstrand, it has not been subjected to the Agency's required peer and

policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

Allphin L, Windham MD, Harper KT (1998) Genetic diversity and gene flow in the endangered dwarf bear poppy, *Arctomecon humilis* (Papaveraceae). *Amer. J. Bot.*, **85**, 1251–1261.

Archibald JK, Wolf PG, Tepedino VJ, Bair J (2001) Genetic relationships and population structure of the endangered steamboat buckwheat *Eriogonum ovalifolium* var. *williamsiae*. *Amer. J. Bot.*, **88**, 608–615.

Bataillon TM, David JL, Schoen DJ (1996) Neutral genetic markers and conservation genetics: Simulated germplasm collection. *Genetics*, **144**, 409–417.

Brown AHD (1989) Genetic characterization of plant mating systems. In: *Plant Population Genetics, Breeding, and Genetic Resources* (eds. Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 145–162. Sinauer, Sunderland, Massachusetts, USA.

Butcher PA, Moran GF, Perkins HD (1998) RFLP diversity in nuclear genome of *Acacia mangium*. *Heredity*, **81**, 205–213.

Calzia JP (1993) *Mineral Resource Assessment of the San Bernardino National Forest, California; Executive Summary and a Nontechnical Summary for Land and Resource Managers*. United States Geological Survey. Open File Report 93-0552. 11 pp.

Ceska JF, Affolter JM, Hamrick JC (1997) Developing a sampling strategy for *Baptisia arachnifera* based on allozyme diversity. *Conserv. Biol.*, **11**, 1133–1139.

Cheliak WM, Pitel JA (1984) Electrophoretic identification of clones in trembling aspen. *Can. J. For. Res.*, **13**, 740–743.

Cruzan MB (1998) Genetic markers in plant evolutionary ecology. *Ecology*, **79**, 400–412.

Cruzan MB (2001) Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). *Evolution*, **55**, 1569–1580.

Eguiarte LE, Perez-Nasser N, Pinero D (1991) Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): Implications for evolution and conservation. *Heredity*, **69**, 217–228.

Ellstrand NC (1992) Gene flow among seed plant populations. *New Forests*, **6**, 241–256.

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: Implications for plant conservation. *Ann. Rev. Ecol. Syst.*, **24**, 217–242.

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. *Amer. J. Bot.*, **74**, 123–131.

Environmental Systems Research Institute (1996) *ArcView 3.2*. Environmental Systems Research Institute. Redlands, CA, USA.

Fenster C, Ritland K (1992) Chloroplast DNA and isozyme diversty in two *Mimulus* species (Scrophulariaceae) with contrasting mating systems. *Amer. J. Bot.*, **79**, 1440–1447.

Frankel OH, Soulé ME (1981) *Conservation and Evolution*. Cambridge University Press, Cambridge, England.

Gaines MS, Diffendorfer JE, Tamarin RH, Whittam TS (1997) The effects of habitat fragmentation on the genetic structure of small mammal populations. *J. Hered.*, **88**, 294–304.

Godt MJW, Johnson BR, Hamrick JL (1996) Genetic diversity and population size in four rare southern Appalachian plant species. *Conserv. Biol.*, **10**, 796–805.

Gonella MP, Neel M (1995) Characterization of rare plant habitat for restoration in the San Bernardino National Forest. In: *Proceedings: Wildland Shrub and Arid Land Restoration Symposium* (eds. Roundy BA, McArther ED, Haley JS, Mann DK), pp. 81–93. General Technical Report INT-GTR-315. USDA Forest Service, Intermountain Research Station, Ogden, Utah.

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: *Plant Population Genetics; Breeding and Genetic Resources* (eds. Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 43–63. Sinauer, Sunderland, Massachusetts, USA.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Sunderland, Massachusetts, USA.

Heywood JS (1980) *Genetic Correlates of Edaphic Differentiation and Endemism in Gaillardia*. Ph.D. dissertation, University of Texas, Austin, Texas, USA.

Hickman J (1993) *The Jepson Manual*. University of California, Berkeley, California, USA.

Holsinger KE, Gottlieb LD (1991) Conservation of rare and endangered plants: Principles and prospects. In: *Genetics and Conservation of Rare Plants* (eds. Falk DA, Holsinger KE), pp. 149–170. Oxford University Press, New York, New York, USA.

Holtsford TP, Ellstrand NC (1989) Variation in outcrossing rate and population genetic structure of *Clarkia tembloriensis* (Onagraceae). *Theor. Appl. Gen.*, **78**, 480–488.

Holtsford TP, Ellstrand NC (1990) Inbreeding effects in *Clarkia tembloriensis* (Onagraceae) populations with different natural outcrossing rates. *Evolution*, **44**, 2031–2046.

Huenneke LF (1991) Ecological implications of genetic variation in plant populations. In: *Genetics and Conservation of Rare Plants* (eds. Falk DA, Holsinger KE), pp. 31–44. Oxford University Press, New York, New York, USA.

Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, **50**, 54–70.

Latta RG, Mitton JB (1997) A comparison of population differentiation across four classes of gene markers in limber pine (Pinus flexilis James). *Genetics*, **146**, 1153–1163.

Lewis PO, Zaykin D (2001) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data. Version 1.0 (d16c)*. Available from http://alleyn.eeb.uconn.edu/gda/.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst.*, **27**, 237–277.

Loveless MD, Hamrick JL (1989) Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Syst.*, **15**, 65–95.

Lynch M (1996) A quantitative-genetic perspective on conservation issues. In: *Conservation Genetics: Case Histories from Nature* (eds. Avise JC, Hamrick JL), pp. 471–482. Chapman and Hall, New York, New York, USA.

McCauley DE (1997) The relative contributions of seed and pollen movement to the local genetic structure of *Silene alba*. *J. Hered.*, **88**, 257–263.

McCune B, Mefford MJ (1999) *PC-ORD. Multivariate Analysis of Ecological Data. Version 4.0*. MJM Software Design, Gleneden Beach, Oregon. USA.

McKay, JK, Latta RG (2002) Adaptive population divergence: markers QTL and traits. *Trends Ecol. Evol.*, **17**, 285–291.

Milligan BG, Leebens-Mack J, Strand AE (1994) Conservation genetics: Beyond the maintenance of marker diversity. *Mol. Ecol.*, **3**, 423–435.

Mitton JB, Linhart YB, Sturgeon KB, Hamrick JL (1979) Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *The Journal of Heredity*, **70**, 86–89.

Mitton JB, Grant MC, Yoshino AM (1998) Variation in allozymes and stomatal size in pinyon (*Pinus edulis*, Pinaceae), associated with soil moisture. *Amer. J. Bot.*, **85**, 1262–1265.

Montalvo AM, Conard SG, Conkle MT, Hodgskiss PD (1997) Population structure, genetic diversity, and clone formation in *Querus chrysolepis* (Fagaceae). *Amer. J. Bot.*, **84**, 1553–1564.

Murawski DA, Nimal Gunatilleke I, Bawa KS (1994) The effects of selective logging on inbreeding in *Shorea megistophylla* (Dipterocarpaceae) from Sri Lanka. *Conserv. Biol.*, **8**, 997–1002.

Neel MC (2000) *The Structure of Diversity: Implications for Reserve Design*. Ph.D. dissertation, University of California, Riverside, California, USA.

Neel MC, Cummings MP (in press) Effectiveness of conservation targets in capturing genetic diversity. *Conserv. Biol.*

Neel MC, Cummings MP (accepted) Genetic consequences of ecological reserve design guidelines. *Conserv. Gen.*

Neel MC, Ellstrand MC (2001) Patterns of allozyme diversity in the threatened *Erigeron parishii* (Asteraceae). *Amer. J. Bot.*, **88**, 810–818.

Neel MC, Ross-Ibarra J, Ellstrand NC (2001) Implications of mating patterns for conservation of the endangered plant *Eriogonum ovalifolium* var. *vineum* (Polygonaceae). *Amer. J. Bot.*, **88**, 1214–1222.

NeiM (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

O'Malley D, Wheeler NC, Guries RP (1980) *A Manual for Starch Gel Electrophoresis. Staff Paper Series 11*. Department of Forestry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin, USA.

Oostermeijer JGB, Van Eijck MW, M Den Nijs JC (1994) Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe* L. *J. Evol. Biol.*, **8**, 739–759.

Ouborg NJ, Van Treuren R (1995) Variation in fitness-related characters among small and large populations of *Salvia pratensis*. *J. of Ecol.*, **83**, 369–380.

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PA (1998) What molecules can tell us about populations: Choosing and using a molecular marker. *Ecology*, **79**, 361–382.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *Amer. J. Bot.*, **80**, 537–544.

Patarnello T, Battaglia B (1992) Glucophosphate isomerase and fitness effects of temperatures on genotype dependent mortality in two species of the genus *Gammarus* (Crustacea: Amphipoda). *Evolution*, **46**, 1568–1573.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, **12**, 844–855.

Pielou EC (1969) *An Introduction to Mathematical Ecology*. Wiley-Interscience, New York, New York.

Prentice HC, Cramer W (1990) The plant community as a niche bioassay: Environmental correlates of local variation in *Gysophila fastigiata*. *J. of Ecol.*, **78**, 313–325.

Prentice HC, Lonn M, Lefkovitch LP, Runyeon H (1995) Associations between allele frequencies in *Festuca ovina* and habitat variation in the alvar grasslands on the Baltic island of Oland. *J. of Ecol.*, **83**, 391–402.

Prober SM, Brown AHD (1994) Conservation of the Grassy White Box Woodlands: Population genetics and fragmentation of *Eucalyptus albens*. *Conserv. Biol.*, **8**, 1003–1013.

Purdy BG, Bayer RJ (1995) Allozyme variation in the Athabasca sand dune endemic *Salix salicicola* and the closely related widespread species *S. alexensis*. *Syst. Bot.*, **20**, 179–190.

Raijmann LEL, Van Leeuwen N, Kersten R, Oostermeijer JGB, Den Nijs HCM, Nemken SBJ (1994) Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conserv. Biol.*, **8**, 1014–1026.

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.

Ritland K (1990) Inferences about inbreeding depression based on changes of the inbreeding coefficient. *Evolution*, **44**, 1230–1241.

Schaal BA, Levin DA (1976) The demographic genetics of *Liatris cylindracea* Michx. *Am. Nat.*, **110**, 191–206.

Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG (1994) Evaluating approaches to the conservation of rare and endangered plants. *Ecology*, **75**, 584–606.

Schoen DJ, Brown AHD (1993) Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. *Proc. Natl. Acad. Sci. USA*, **90**, 10623–10627.

Simberloff D (1988) The contribution of population and community biology to conservation science. *Ann. Rev. Ecol. Syst.*, **19**, 473–511.

Skinner MW, Pavlik BM (1994) *Inventory of Rare and Endangered Vascular Plants of California. California Native Plant Society. Special Publication Number 1*, 5th edn. The California Native Plant Society, Sacramento, California.

Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.

Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. W.H, Freeman, San Francisco, California, USA.

Storfer A (1996) Quantitative genetics: A promising approach for the assessment of genetic variation in endangered species. *Trends Ecol. Evol.*, **11**, 343–348.

Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Ann. Mo. Bot. Gard.*, **77**, 13–27.

U.S. Department of Commerce (1999). *Climate Records for Victorville, California (Station 49325) and Big Bear Lake, California (Station 40741)*. National Climatic Data Center. Available from the World Wide Web (http://wrcc.dri.edu).

U.S. Fish and Wildlife Service (1994) Final Rule. Endangered and threatened wildlife and plants: Five plants from the San Bernardino Mountains in southern California determined to be threatened or endangered. 50 CFR Part 17. *Federal Register*, **59**, 43652–43664.

U.S. Fish and Wildlife Service (1997) *San Bernardino Mountains Carbonate Endemic Plants Draft Recovery Plan*. Region 1, Portland, Oregon. USA.

Varvio SL, Chakraborty R, Nei M (1986) Genetic variation in subdivided populations and conservation genetics. *Heredity*, **57**, 189–198.

Waller DM (1984) Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution*, **38**, 427–440.

Watt WB, Cassin RC, Swan MS (1983) Adaptation at specific loci III. Field behavior and survivorship among *Colias* PGI genotypes are predictable from in vitro biochemistry. *Genetics*, **103**, 725–739.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer, Sunderland, Massachusetts, USA.

Wells PV, Berger R (1967) Late Pleistocene history of coniferous woodland in the Mohave Desert. *Science*, **155**, 1640–1647.

Young AG, Brown AHD (1996) Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Conserv. Biol.*, **10**, 1220–1228.

Young AG, Brown AHD (1998) Comparative analysis of the mating system of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Heredity*, **80**, 374–381.

Young AG, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.*, **11**, 413–418.

Zhang Q, Saghai Maroof MA, Kleinhofs A (1993) Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* ssp *spontaneum*. *Genetics*, **134**, 909–916.