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Patterns of allozyme diversity in the threatened plant *Erigeron parishii* (Asteraceae)[1]

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Thirty-one occurrences of *Erigeron parishii,* a narrowly endemic plant threatened by mining, were sampled for allozyme diversity. This taxon held considerable genetic variation at the 14 allozyme loci surveyed. Species (e.g., alleles per locus [*A*] = 4.3 and proportion of polymorphic loci [P] = 0.64) and population (e.g., *A* = 2.15 [SD = 0.3] and P = 0.53 [SD = 0.12]) genetic diversity measures were higher than expected for narrowly endemic plant taxa. The proportion of polymorphic loci and numbers of alleles per locus indicated that *E. parishii* has not experienced severe or long-lasting population bottlenecks. Within-population *f* indicated low to moderate levels of inbreeding. Populations were only moderately differentiated (theta-p = 0.12), suggesting either that there is substantial gene flow among populations or that populations have not been isolated long enough to detect effects of genetic drift. There was no significant differentiation among populations in different vegetation types nor was there a relationship between genetic distance and geographic distance among sites. Continued fragmentation by mining activities would isolate populations, disrupting gene flow, exacerbating loss of diversity, and increasing inbreeding in the remaining fragments. Protection of large, interconnected populations throughout the range of the taxon is warranted to maintain processes that have sustained the observed diversity.

Key words: *Erigeron parishii*; genetic diversity; plant conservation; reserve design.

The major cause of extinctions for both plants and animals is loss, degradation, and subsequent fragmentation of once-continuous habitat (Holsinger and Gottlieb, 1991; Falk, 1992; Clegg et al., 1995; Wilcove et al., 1998). Fragmentation, a byproduct of habitat destruction, reduces habitat patch size, increases edge-to-area ratio, increases distance among patches and increases resistance to migration among patches (Opdam et al., 1994). Fragmentation can also rapidly alter interactions among populations within species, among species, and between species and abiotic features of the environment (Cooperrider, 1991; Thompson, 1996). Such changes can increase extinction risks from demographic and genetic stochasticity by decreasing effective population sizes and increasing rates of inbreeding and genetic drift (e.g., Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Young, Boyle, and Brown, 1996). At the same time, rates of gene flow can either increase or decrease, depending on how dispersal patterns are affected by fragmentation (e.g., Ellstrand and Elam, 1993). Most frequently, gene flow is assumed to decrease as distances between remaining populations increase. Under conditions causing loss of genetic variation and decreased gene flow, decreased heterozygosity and increased differentiation among populations are expected. Such patterns are thought to be detrimental and to increase extinction risks. Risks are highest when historically large populations with substantial interpopulation gene flow are reduced in size and isolated from one another (Barrett and Kohn, 1991).

A major objective of conservation biology is to maintain biodiversity by promoting persistence of species in their native ecosystems over time (Harrison, Miller, and McNeely, 1984; Falk, 1992). Because chances of persistence decrease in degraded and fragmented habitat, much attention has focused on protecting areas from destruction to slow habitat loss and fragmentation, and on managing those areas to preserve and enhance biodiversity (e.g., Noss, O'Connell, and Murphy, 1997). Such areas go by many different names; we use the term "reserve" here. Many reserves are designed and established to conserve specific rare taxa, which often occur in small, isolated populations or fragmented populations that have been reduced in size. A common goal of such reserves is to support large enough populations to prevent inbreeding and genetic drift. This goal is most often met by protecting sufficient habitat rather than directly focusing on genetic diversity present within the target species. It is assumed that if sufficient habitat is maintained to protect against environmental stochasticity, loss of genetic diversity is not an immediate concern (Templeton et al., 1990; Schemske et al., 1994; Gaines et al., 1997). However, because genetic diversity contributes to species persistence, its direct measurement can be an important priority.

Quantifying the organization of genetic variation over populations of a sensitive species can help in prioritizing sites and management choices for maintaining 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, Johnson, and Hamrick, 1996; Petit, El Mousadik, and Pons, 1998). Information on genetic diversity patterns also provides insight into evolutionary and demographic history of a taxon (Milligan, Leebens-Mack, and Strand, 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

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Fig. 1. General locations of sampling sites for *Erigeron parishii* shown in context of the state of California and the boundary of the San Bernardino National Forest. The sample site labeled EPBPRR was located at the Burns Pinyon Ridge Reserve and was disjunct from the remaining sites.

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 might need to be conserved to represent the range of variation within a taxon. In such cases genetic diversity might be less important a 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, Affolter, and Hamrick, 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 efforts can be focused on other ecological characteristics related to survival of individuals in relation to threats faced by a taxon.

One example of a plant species that has been subjected to fragmentation for decades is the federally threatened *Erigeron parishii* A. Gray. This taxon is primarily endemic to limestone and dolomite substrates (collectively known as carbonate substrates) in the San Bernardino Mountains. These deposits provide ore that is extremely valuable in construction, industrial, and pharmaceutical applications (Calzia, 1993). Consequently, *E. parishii* has suffered substantial fragmentation by limestone mining activities (Skinner and Pavlik, 1994; USFWS, 1994). Conservation planning efforts for this taxon are focusing on establishing some areas that will be protected from mining while allowing mining at other sites (USFWS, 1997). The purpose of our research was to characterize the intra- and interpopulation genetic structure of *E. parishii.* This information can provide guidance in overall reserve design and contribute to ranking the conservation significance of individual populations.

MATERIALS AND METHODS

Erigeron parishii is a low-growing, perennial herb (Hickman, 1993). Individual plants grow from a taproot and typically reach 1–3 dm in height. The 1–3 cm long, narrow leaves emerge in the spring and become dry by summer's end, at which time the stems remain dormant above ground. Most flowering occurs between May and July, but some individuals bloom into August, especially after abundant summer rains. Lavender ray flowers and yellow disk flowers occur in capitula borne at the ends of leafy stems. Individual plants typically have many capitula and each capitulum produces hundreds of small achenes (2–3 $\times$ 0.5 mm). The pappus on each achene is only 2–3 mm and is unlikely to facilitate long distance dispersal. Nothing is known about *E. parishii*'s mating system; everything from obligate, insect-mediated outcrossing to agamospermy is known from the genus (Moldenke, 1976; Richards, 1999). In addition, many members of the genus *Erigeron* are clonal. Mating system and clonality were not specifically examined in this study; however, some general inferences can be made from our results.

As mentioned above, *E. parishii* is primarily endemic to carbonate substrates in the San Bernardino Mountains (Fig. 1) (Skinner and Pavlik, 1994; USFWS, 1994). These carbonate substrates occur as varying sized outcrops and alluvial deposits totaling ~13 200 ha. *E. parishii* most often occupies

alluvium along ephemeral drainages but is also found on rocky slopes. Occurrences are patchily distributed on appropriate substrate in creosote bush–bursage scrub, black bush scrub, and various phases of pinyon–juniper woodland between ~1200 and 2000 m elevation. Two occurrences on quartz monzonite at the eastern end of the mountain range and one historic location in the Little San Bernardino Mountains are exceptions to strict endemism to carbonate substrates in the San Bernardino Mountains. Known occurrences in July 1999 totaled ~453 ha (S. Redar, personal communication, San Bernardino National Forest).

Where found, *E. parishii* populations can be quite large, comprising thousands of individuals, though 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). Existing populations are not apparently in demographic decline. Sizes of some populations are above those at which inbreeding and genetic drift are typically of immediate concern (Simberloff, 1988; Ellstrand and Elam, 1993). The main concern for persistence of *E. parishii* is continued habitat destruction and fragmentation, primarily from limestone mining activities. Populations of and habitat for *E. parishii* have been lost to mining, and there is imminent threat of continued loss (USFWS, 1994).

Most *E. parishii* populations are threatened because they exist primarily on private land or on public land with no protective status. Additionally, most of *E. parishii*'s range on public land is under valid mining claim and thus at risk from future mining activities. As a result of existing habitat and population losses, continuing threats, and lack of protective mechanisms, the U.S. Fish and Wildlife Service listed this taxon as threatened (USFWS, 1994). Efforts to conserve this species involve designing a reserve system that will be protected from mining as well as restoration of degraded sites. Due to the high value of the mineral ore, not all existing populations will be maintained. Information on patterns of genetic diversity can assist in evaluation of populations for protection.

Sampling—Thirty-one occurrences of *E. parishii* were sampled for allozyme diversity (Fig. 1). Sampling sites were selected stratified randomly to represent the ecological range of the taxon as follows. Twenty-five allozyme sampling sites coincided with plot locations for a related study in which vegetation community composition was described (Neel, 2000). Sixty-one of the 669 0.04-ha vegetation plots in that study included *E. parishii.* Twenty of these plots occurred in black bush scrub (BBS); 27 in singleleaf pinyon–Utah juniper woodlands (PJ); and 14 in singleleaf pinyon woodlands (PY). Allozyme sampling sites were chosen from these plots roughly in proportion to the number of plots within each vegetation type. Six sample sites not associated with vegetation plots were included to represent ecological and geographical variation not included in those plots. One of these sites was a disjunct occurrence of *E. parishii* (EPBPRR) at the Burns Pinyon Ridge Reserve (University of California Natural Reserve System) that represented the easternmost extant populations (Fig. 1). An historic collection further east from the Little San Bernardino Mountains has not been located recently. The Burns Pinyon Ridge Reserve occurrence was found on quartz–monzonite in pinyon–California juniper woodland (PJUCA). The substrate was atypical for this taxon, and the vegetation type did not support *E. parishii* at any other location away from this area. Site EPD135CB was located at the lower elevational limit of the taxon in Creosote Bush–Burrobush Scrub (LATR) vegetation on carbonate alluvium. Site EPBCQ was located near the western edge of the range where populations were not often found in plots. Vegetation composition from the three remaining sites not associated with vegetation plots was subsequently sampled by San Bernardino National Forest botanists (D. Volgarino, personal communication, San Bernardino National Forest). One of these plots was in creosote bush–white bursage scrub and the other two were in the single-leaf pinyon woodland. Densities of *E. parishii* were crudely estimated using counts from the 0.04-ha vegetation plots where available.

Up to 200 mg of leaf tissue were collected from 30 individuals per sample site where possible (a total of 932 individuals were sampled) (Table 1). We were unable to assess genotypes for all loci for all individuals and thus sample sizes averaged over loci are given for each site (Table 1); sample site EPB203 was particularly problematic. Additionally, we were able to obtain allozyme data from only 28 or 29 of 30 individuals at three sites. One other site (EPD135CB) supported only seven individuals, five of which had live leaf material available for sampling. This site was retained in analyses despite the small sample size because the sample represented most of the extant population and the site represented the lower elevational limit of the taxon. Within sites, individuals were chosen from throughout the population within the general vicinity of the vegetation plot. In general, individuals sampled within sites were separated by 5–30 m. Leaves were transported on ice to the laboratory, where they were refrigerated at 8°C.

Allozyme electrophoresis—All samples were initially run from freshly extracted tissue within 7 d of collection. Approximately 30 mg of leaf tissue was ground in five drops of a 0.1 mol/L, pH 7.5 tris-HCl buffer. Each milliliter of buffer contained 0.34 mg ethylenediamine tetracetic acid (disodium salt), 0.75 mg KCl, 1 μl mercaptoethanol, 2.03 mg MgCl, and 30 mg polyvinylpyrrolidine-40. This extract was soaked onto chromatography paper wicks that were loaded directly into 9% starch gels. Extra wicks made from these extractions were frozen at −80°C for use in confirmatory runs.

The following enzyme systems were assayed in all individuals in all populations: aconitase (ACO), aspartate amino transferase (AAT), fluorescent esterase (FEST), leucine amino peptidase (LAP), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), triose phosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UDP). Aconitase, SKDH, and 6PGD were resolved in a pH 7, continuous morpholine-citrate gel system run at 30–35 mA and 150 V for 6 h (O'Malley, Wheeler, and Guries, 1980). Phosphoglucomutase and TPI were resolved on a discontinuous lithium hydroxide–borate gel system at 75 mA and 200 V for 5.5 h (Heywood, 1980). Leucine amino peptidase was resolved on a tris–EDTA–borate gel system run at 50 mA and 200 V for 5.5 h (Heywood, 1980). Aspartate amino transferase and UDP were resolved in both lithium hydroxide and tris–EDTA–borate gel systems.

The genetic basis of allozymes is well known for angiosperms in general and specifically for many species in the family Asteraceae (Weeden and Wendel, 1989). Our interpretations of the banding patterns observed were based on those previous studies and on known quaternary structure of the enzymes studied. Numbers of loci scored for each enzyme system were as follows: ACO (3), AAT (1), FEST (2), LAP (1), 6PGD (2), PGM (1), SKDH (1), TPI (2), UDP (1).

Data analysis—Total number of alleles (TA), TA standardized by sample size, 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 whole species and for each sampling site using the computer program GDA (Lewis and Zaykin, 2000). A locus was considered polymorphic if the most common allele had a frequency <0.95. The number of private alleles within sites (that is, alleles detected at only one site) was also counted using GDA.

One sample (EPD135CB) was much smaller than all others ($N = 5$ vs. 30). The population represented by this sample was also by far the smallest encountered ($N = 7$ vs. hundreds or thousands). Differences in both population and sample size confound direct comparison of genetic diversity in this sample site with other sites. To more directly compare levels of genetic diversity we controlled for differences in sample size by randomly choosing five individuals from each site and calculating all genetic diversity summary statistics on this reduced data set. This comparison allowed us to determine whether the observed levels of diversity in the small population were comparable to those found in equivalent samples from larger populations.

The proportion of individuals with unique genotypes and the number of individuals sharing a genotype in each sample site were calculated to assess the potential for agamospermy and vegetative reproduction (Ellstrand and Roose, 1987). Only individuals with data from at least ten loci were included in this analysis (a total of five individuals were excluded). Furthermore, site EPB203 was not included because of the large number of individuals from which we could score only partial genotypes. Thus this analysis was based on 868 individuals from 30 sites.

Hierarchical population genetic structure was described using f (F_{IS}), F

TABLE 1. Sample sizes averaged over loci (N), proportion of polymorphic loci (P), alleles per locus (A), alleles per polymorphic locus (A_p), expected heterozygosity (H_e), observed heterozygosity (H_o), an estimate of the fixation index over all loci in the population (f), total number of alleles (TA), TA standardized by sample size (Stand. TA), number of private alleles, and mean genetic distance to all other populations for 14 allozyme loci in 31 populations of *Erigeron parishii*. Vegetation types in which each sample site occurred are indicated; sample abbreviations are as in the text (Materials and methods: Sampling). Plant density is the number of individuals in one, 0.04-ha plot. The five highest ranking values for standardized TA, P, H_e, and genetic distance, the five lowest ranking values for f, and all occurrences of private alleles are in boldface type.

Sample	N	P	A	A_p	H_e	H_o	f	TA	Stand. TA	Private alleles	Mean distance	Vegetation type	Plant density
EPB26	28.6	**0.64**	2.4	2.9	**0.20**	0.18	0.12	33	**1.15**	0	0.02	PY	7
EPB31	29.9	**0.71**	2.4	3.0	**0.20**	0.20	0.02	34	1.14	0	**0.04**	PY	93
EPB54	29.9	0.31	1.5	2.5	0.17	0.17	**0.00**	20	.67	0	**0.06**	PJ	17
EPB55	29.9	0.43	1.8	2.8	0.18	0.17	0.04	25	.84	0	0.04	PJ	44
EPB202	29.9	0.36	2.0	3.2	0.15	0.14	0.08	28	.94	0	**0.09**	PY	5
EPB203	50.6	0.57	2.7	3.6	0.15	0.13	0.13	38	.75	**1**	0.02	PY	200
EPC5	28.9	0.50	2.0	2.9	0.17	0.16	0.05	28	.97	0	0.03	BBS	19
EPC13	29.9	0.57	2.4	3.5	**0.23**	0.20	0.11	34	1.14	**2**	0.03	BBS	26
EPC19	29.5	0.36	2.2	3.4	0.13	0.14	**−0.09**	30	1.02	0	**0.04**	PJ	7
EPC102	28.5	0.50	2.2	3.1	0.15	0.14	0.05	31	1.09	0	0.01	LATR	59
EPC104	27.5	0.50	2.1	3.0	0.14	0.13	0.06	29	1.05	0	0.02	BBS	31
EPD5	29.6	0.50	1.9	2.9	0.14	0.14	**−0.01**	27	.91	0	0.02	PJ	61
EPD6	29.0	0.57	2.4	3.0	0.18	0.17	0.09	34	**1.17**	0	0.02	PY	10
EPD12	27.6	**0.71**	2.6	3.1	**0.22**	0.20	0.07	36	**1.30**	**1**	**0.03**	BBS	39
EPD100	28.7	0.29	1.9	3.0	0.14	0.13	0.07	26	.91	0	**0.03**	PY	31
EPD102	29.7	0.36	1.6	2.6	0.10	0.09	0.05	23	.77	0	0.02	BBS	53
EPD104	29.6	0.50	1.8	2.4	0.13	0.09	0.28	25	.84	0	0.02	BBS	16
EPD135CB	4.8	0.38	1.5	2.2	0.15	0.15	**0.00**	19	**3.96**	0	**0.03**	LATR	6
EPD200	29.4	0.57	2.1	2.6	0.18	0.16	0.10	30	1.02	0	**0.03**	LATR	2
EPE18	30.0	0.54	2.5	3.7	0.18	0.18	**0.00**	33	1.10	**2**	0.02	PY	24
EPE24	32.6	0.50	2.2	3.0	0.15	0.14	0.03	31	.95	0	0.02	PJ	27
EPE100	30.0	0.50	2.1	2.9	0.15	0.12	0.23	29	.97	0	0.02	BBS	14
EPF47	29.6	0.64	2.4	3.1	0.19	0.18	0.06	33	1.12	0	0.02	PJ	17
EPF49	30.0	0.57	2.4	3.3	0.15	0.14	0.05	34	1.13	**1**	**0.03**	PY	98
EPFBCQ	30.0	0.50	2.2	2.6	0.14	0.11	0.26	31	1.03	**2**	0.02	PJ	ND[a]
EPF100	29.5	**0.71**	2.2	2.7	0.18	0.19	**−0.07**	31	1.05	0	0.02	PJ	10
RCC8	29.0	**0.64**	2.4	3.1	**0.23**	0.20	0.11	33	1.14	0	0.02	PJ	1
RCD10	28.5	**0.71**	2.4	2.7	**0.23**	0.22	0.08	34	**1.19**	0	0.02	PJ	4
RCD121	29.9	0.50	2.1	3.0	0.17	0.15	0.11	29	.97	0	**0.03**	BBS	1
RCE25	29.3	**0.64**	2.1	2.6	0.19	0.19	**−0.01**	30	1.02	0	0.02	PJ	1
EPBPRR	29.5	**0.64**	2.1	2.7	**0.20**	0.15	0.25	30	1.02	**1**	**0.03**	PJUCA	ND[a]
Mean	29.3	0.53	2.2	2.9	0.17	0.16	0.08	29.9	1.11	0.29	0.03		
SD	5.99	0.12	0.3	0.3	0.03	0.03	0.02	4.35	0.55	0.64	0.01		

[a] No data.

(F_{IT}), theta-p, and theta-s (two measures of F_{ST}) following methods of Weir (1996) as implemented by Lewis and Zaykin (2000). The statistic f, the coancestry coefficient or fixation index, represents departures from Hardy-Weinberg equilibrium expectations within individual sites; F represents such deviations over all sites. Theta-p represents the proportion of genetic differentiation among populations from different vegetation types and was used to examine the relationship between genetic diversity and community composition. Theta-s represents the proportion of total genetic variation partitioned among sites in relation to the total variation present, treating both loci and sites as samples. We calculated 95% confidence intervals for these estimates from 5000 bootstrap replicates across loci. Gene flow, the number of migrants per generation (N_m), was estimated both as $N_m = 0.25(1 - \text{theta-s})/\text{theta-s}$ (Weir, 1996) and by using Slatkin's (1985) private allele method.

Nei's (1978) unbiased genetic distance and geographic distance were computed for all pairwise combinations of sites. 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.

Conservation significance of individual sites 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 sites for some measures. Populations ranking favorably in multiple categories were considered to be a higher priority for conservation of genetic diversity.

RESULTS

Erigeron parishii holds a considerable amount of genetic variation at the allozyme loci surveyed. At the species level, nine of the 14 loci examined were polymorphic (P = 0.64). A total of 61 alleles were detected for all loci with an average of 4.3 alleles per locus (SD = 1.94) and 5.2 alleles per polymorphic locus (SD = 1.39). Species-level H_e was 0.19 (SD = 0.17) and H_o was 0.15 (SD = 0.13).

The proportion of loci polymorphic within populations ranged from 0.29 to 0.71 with a mean of 0.53 (SD = 0.12) (Table 1). The total number of alleles per population averaged 29.9 (SD = 4.4) and ranged from 19 (EPD135CB, sampled from a small, peripheral population) to 38 (EPB203, from a large, centrally located population). The second smallest number of alleles (20) was found at site EPB54, another peripheral

TABLE 2. Distribution of private alleles among 14 loci in 31 populations of *Erigeron parishii*.

Locus	Allele	Frequency	Population
ACO1	C	0.02	EPF49
ACO2	A	0.02	EPFBCQ
ACO3	F	0.18	EPD12
SKDH	D	0.03	EPFBCQ
PGDI	G	0.12	EPE18
PGDI	D	0.02	EPB203
PGM	A	0.07	EPBPRR
PGM	B	0.03	EPE18
TPI1	C	0.02	EPC13
TPI1	B	0.13	EPC13

TABLE 3. *F* statistics for 12 loci (FEST1 and FEST2 were excluded because they were monomorphic) in 31 populations of *Erigeron parishii* hierarchically arranged in vegetation types calculated using methods of Weir (1996). Theta-p represents differentiation of populations within vegetation types; theta-s represents differentiation among all populations. Standard deviations for each locus are in parentheses and were calculated by jackknifing over populations. Upper and lower bounds were calculated by bootstrapping across loci.

Locus	*f*	*F*	Theta-p	Theta-s
ACO1	0.10 (0.16)	0.12 (0.15)	−0.004	0.02 (0.01)
ACO2	−0.02 (0.04)	0.05 (0.04)	−0.004	0.08 (0.02)
ACO3	0.14 (0.05)	0.23 (0.06)	0.001	0.10 (0.03)
SKDH	0.03 (0.06)	0.16 (0.08)	0.012	0.14 (0.05)
PGD1	0.05 (0.04)	0.15 (0.04)	0.006	0.11 (0.03)
PGD3	0.16 (0.09)	0.20 (0.08)	−0.009	0.05 (0.01)
LAP	0.17 (0.04)	0.25 (0.04)	0.025	0.01 (0.02)
PGM	0.13 (0.05)	0.34 (0.12)	0.008	0.24 (0.13)
AAT1	0.03 (0.04)	0.14 (0.04)	0.002	0.11 (0.03)
TPI1	0.12 (0.05)	0.18 (0.05)	−0.012	0.07 (0.02)
TPI2	−0.02 (0.04)	0.02 (0.04)	−0.006	0.04 (0.01)
UDP	−0.02 (0.03)	0.10 (0.04)	−0.006	0.12 (0.04)
Overall	0.08	0.18	0.003	0.12
Upper bound	0.12	0.25	0.013	0.16
Lower bound	0.03	0.13	−0.005	0.09

population. When TA was standardized by sample size, the largest number of alleles was found in EPD135CB (3.96) and the smallest number was in EPB54 (0.67) (Table 1). The number of alleles per locus within populations averaged 2.2 (SD = 0.3), and the number of alleles per polymorphic locus averaged 2.9 (SD = 0.3). H_e within populations ranged from 0.10 to 0.23 and averaged 0.17 (SD = 0.03) (Table 1). H_o within populations was comparable in magnitude ranging from 0.09 to 0.22 and averaging 0.16 (SD = 0.03) over all populations (Table 1). Coancestry coefficients averaged over loci were positive within 26 of the 31 populations, indicating slight-to-moderate nonrandom mating (Table 1). Only four populations (EPE100, EPFBCQ, EPD104, and EPBPRR) exhibited substantial inbreeding (i.e., $f > 0.20$) and 71% of all populations had coancestry coefficients <0.1.

A total of ten private alleles was found in seven populations (Table 2). Three populations (EPC13, EPE18, and EPFBCQ) had two private alleles each (Table 2). When found, these alleles were always at low frequencies; in fact, all but two of these alleles occurred at frequencies <0.07. The remaining private alleles occurred at frequencies of 0.12 and 0.13. The distribution of private alleles did not have an obvious geographic pattern (Table 2, Fig. 1).

Levels of genetic diversity were typically below average in the sample from site EPD135CB, the smallest population and smallest sample (Table 1), and this site had the smallest number of alleles. However, individuals at this site were not inbred ($f = 0.0$). While values of P, *A*, A_p, and TA were below average, when compared with equal sample sizes from other sites, levels of genetic diversity in EPD135CB were not different from those sites. When $N = 5$ in all samples, the average value of TA was 21.4 (SD = 2.1), P was 0.41 (SD = 0.09), *A* was 1.5 (SD = 0.15), A_p was 2.9 (SD = 0.24), H_e was 0.16 (SD = 0.04), H_o was 0.14 (SD = 0.04), and *f* was 0.09 (SD = 0.17). Thus the apparently low levels of diversity at site EPD135CB (Table 1) appear to be the result of a small sample size, not the result of an especially genetically depauperate, small population. In fact, this site had the largest standardized TA. Additionally, it should be noted that no inbreeding was detected at this site. In fact, it ranked among the least inbred sites.

On average, 90% of the multilocus genotypes within sample sites were unique (SD = 7.3). This percentage ranged between 77% (EPD5 and EPD104) and 100% (EPB26, EPB55, EPD12, EPE100, RCC8, and RCD10). The nonunique genotypes were not, however, all identical to one another. A total of 60 individuals out of the 868 analyzed that had nonunique genotypes within their respective sites. These nonunique genotypes were never widespread within sites. For example, 80% of the nonunique genotypes within sites were each shared by only two individuals and 17% were shared by three individuals. At most, four individuals within sample sites shared a given genotype, and this only occurred twice. Further, it is likely that the proportion of unique genotypes was underestimated because many individuals that were indistinguishable were missing data at one or more of the 12 polymorphic loci.

The coancestry coefficient, *f*, averaged 0.08 across all loci and sites; based on confidence intervals derived from bootstrap estimates, *f* was significantly different from zero (Table 3). Coancestry coefficients for individual loci varied from $f = -0.02$ (ACO2, TPI2, and UDP2) to $f = 0.17$ (LAP) (Table 3). These results indicate a slight heterozygote deficit in individual sites due to nonrandom mating. Indicating deviations in heterozygosity in individuals relative to the all sites combined, *F* was also significantly greater than zero (Table 3), This deviation has contributions from both drift and nonrandom mating (Weir, 1996). Overall, population differentiation estimated from samples was moderate and significantly different from zero, with theta-s averaging 0.12 over all loci among all populations (Table 3). In other words, ~88% 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 1.8. The private allele method yielded a similar result, indicating 1.6 migrants per generation. In contrast, there was no differentiation among populations in different vegetation types as theta-p averaged 0.003 and did not differ significantly from zero (Table 3).

Nei's unbiased genetic distance among all pairs of sampling sites averaged 0.028 (SD = 0.023) and ranged from 0.001 between EPC104 and EPB203 to 0.133 between RCD121 and EPD202, indicating little overall differentiation among sampling sites. Further, there was not much variation in the average distance of one site to all other sites (Table 1). Geographic distance between all pairs of sample sites averaged

10.0 km (SD = 9.65) and ranged from 0.4 to 49.2 km. When the disjunct site EPBPRR was not included, the greatest distance between sites was 27.3 km and the average distance was 7.0 km (SD = 5.65). At the same time, the average genetic distance among populations did not change when EPBPRR was excluded (0.028 [SD = 0.02]). The Mantel test indicated that there was no significant correlation between geographic distance and genetic distance among all pairs of sample sites ($r^2 = 0.01$, $P = 0.32$). Thus, there was no apparent geographic pattern to genetic distance among populations.

Twenty-two sites ranked at least once in the top five values for five measures of diversity and one measure of differentiation or the bottom five values for inbreeding or had private alleles (Table 1). Fourteen sites ranked favorably more than once and six sites ranked more than twice (Table 1), although the measures of diversity with high ranks in these five sites varied. Sites EPD12 (4), EPB26 (3), EPB31 (3), EPD135CB (3), RCD10 (3), and EPBPRR (3) all ranked favorably more than twice.

DISCUSSION

Given its endemism and history of fragmentation, *E. parishii* supports a surprisingly large amount of genetic variation. Values for all genetic diversity measures were higher than those typical for narrowly endemic plant taxa (Hamrick and Godt, 1989; Loveless and Hamrick, 1989; Hamrick et al., 1991; Godt, Johnson, and Hamrick, 1996). In fact, all species-level measures except H_e were higher than mean values for 329 dicotyledonous species (reviewed by Hamrick and Godt 1989). Species-level H_e for *E. parishii* was between means reported by Hamrick and Godt (1989) in 81 endemic (H_e = 0.096) and 101 narrowly distributed (H_e = 0.137) taxa. Most of the observed diversity was held within populations and, as such, within-population diversity estimates were also higher than those expected for narrowly endemic plant taxa. For example, P, A, and A_p within populations of *E. parishii* were higher than the average values for dicots as well as widespread taxa in general (Hamrick and Godt, 1989). Within *E. parishii* populations, H_e was more similar to levels found in regionally distributed taxa. One possible explanation for these discrepancies in expected levels of diversity is that, compared to many endemic plants, *E. parishii* populations can be large and extensive. Another explanation is that the species is not yet in evolutionary equilibrium and that the high levels of variation reflect a recent time before fragmentation when populations were less isolated.

The smallest population (EPD135CB) did have the smallest number of alleles and below average proportion of polymorphic loci, however, measures of heterozygosity and inbreeding were not significantly lower than average measures from all populations. Furthermore, when compared with the same sample sizes from larger populations, the total number of alleles in EPD1325CB was not particularly low. Thus, while this population is genetically depauperate, it is most likely due to effects of sample size rather than inbreeding or drift. This result is curious given the large amount of theoretical and empirical evidence predicting severe effects of inbreeding and drift in small populations (reviewed in Barrett and Kohn, 1991; Ellstrand and Elam, 1993). The amount of genetic diversity and lack of inbreeding observed indicate that this population may represent a recent colonization event. The seven individuals at this site were found in an ephemeral wash at the base of Blackhawk Mountain near the lower elevational limit for the taxon. *Erigeron parishii* had not been documented from this site prior to 1995 when five individuals were found in a vegetation sampling plot. A number of populations occur upslope from this location and seeds could have washed downstream during a rainstorm. *Erigeron parishii* typically occupies washes and is thought to potentially disperse in this manner, although no direct evidence regarding dispersal mechanisms exists. The closest potential source population is ~0.8 km from and at an elevation 60–80 m above site EPD135CB. It is possible that individuals do not persist at this site but rather the site is colonized from larger populations during favorable years. It would be worthwhile to follow this population over time to see how long it persists and to determine the frequency and source of newly recruited individuals.

We found no evidence for extensive agamospermy or clonal reproduction in *E. parishii.* If these reproductive modes were common, we would expect a large proportion of individuals within samples to share multilocus genotypes (Ellstrand and Roose, 1987). In fact, on average, at least 90% of the multilocus genotypes in each sample were unique (at least 77% of genotypes within sites were unique). Most (80%) of the non-unique genotypes were shared by only two individuals. In comparison, Noyes and Soltis (1996) found that only 10% of individuals in seven agamospermous populations of *Erigeron compositus* had distinguishable genotypes. In the same study, an average of 60% of the individuals in sexually reproducing populations had unique genotypes. They also found that populations were highly differentiated from one another, which also strongly contrasted with our results. Based on the high diversity as well as low levels of inbreeding and population differentiation we conclude that *E. parishii* likely primarily reproduces sexually through outcrossed matings (Heywood, 1991).

The levels and patterns of diversity documented also allow us to make inferences about some aspects of the demographic history of this taxon (Milligan, Leebens-Mack, and Strand, 1994). The large percentage of polymorphic loci and numbers of alleles per locus indicate that *E. parishii* has no history of sufficiently severe or long-lasting population bottlenecks to cause loss of genetic diversity. Large historical populations are also indicated by the observed low to moderate levels of inbreeding (Table 1). The lack of major differentiation among populations (Table 3) suggests either substantial gene flow among populations or that fragmented populations have not yet been isolated long enough for genetic drift (or selection) to have caused population differentiation (Ellstrand, 1992).

Because most populations of *E. parishii* have likely remained large throughout most of their history or have been interconnected at least until the recent past, severely reducing population sizes could have detrimental effects. Such reductions could alter the mating structure within populations by increasing selfing or mating among relatives and thus increase total inbreeding and potentially inbreeding depression (Husband and Schemske, 1996). Fragmentation isolating previously connected populations could also have deleterious effects by disrupting gene flow among populations and could exacerbate loss of diversity through drift and further increase inbreeding in the remaining fragments (Templeton et al., 1990; Young, Boyle, and Brown, 1996). Conservation efforts focusing on maintaining large populations of *E. parishii* 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 population sizes and to connect fragmented populations could be useful but should be a lower priority than protecting existing sites. However, 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 (USFWS, 1997).

Beyond general guidelines, 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. Heterozygosity and inbreeding are typically targeted because of the direct influence of levels of diversity on 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, Van Eijck, and Den Nijs, 1994; Oostermeijer, Van Leeuwen, and Den Nijs, 1995) but is not ubiquitous (e.g., Ritland, 1990; Eguiarte, Perez-Nasser, and Piñero, 1992; 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, Nimal Gunatilleke, and Bawa, 1994; Allphin, Windam, and Harper, 1998).

Recently, Petit, Mousadik, and Pons (1998) strongly advocated using allelic richness (standardized for different sample sizes using rarefaction) as allelic diversity 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). In addition, Petit, El Mousadik, and Pons (1998) suggested evaluating the significance of individual populations based on both levels of allelic richness and distinctiveness. While conserving markers is not the ultimate goal, they do provide an easily measured index of general levels of diversity. However, because patterns of allozyme diversity are not always concordant with other types of diversity they thus must be applied cautiously (Hamrick, 1989; Lynch, 1996; Storfer, 1996).

Because all populations in this study were relatively diverse and there was relatively little differentiation among sampling sites (Table 1), individual measures of allozyme diversity provided limited means of prioritizing any particular populations for conservation. For example, 71% of the samples ranked favorably in at least one measure of diversity, distinctiveness, or inbreeding. To distinguish among sites we considered those that ranked highly in more than two measures to be of particular conservation priority. These sites included EPD12 (4), EPB26 (3), EPB31 (3), EPD135CB (3), RCD10 (3), and EPBPRR (3). Interestingly, only two of these samples (EPD12 and EPBPRR) had any private alleles, another measure of the contribution of a population to diversity within a taxon. Furthermore, some populations high in some genetic diversity measures were low in others. For example, EPBPRR ranked highly in terms of P, H_e, and genetic distance and supported one private allele, but was one of the most inbred of all sites (Table 1). Clearly, the rank of a given population depends on the measure of genetic diversity examined. We would not advocate conserving only the six populations that ranked highly in multiple measures of diversity as they would not represent the range of *E. parishii* and thus would not likely include the range of adaptive variation in the taxon. Rather, we recommend including populations represented by these samples in a reserve network that included populations from throughout the ecological and geographic range of the taxon. Inclusion of particular populations in a reserve network should also take into account population size and extent, and habitat quality and defensibility.

In summary, *E. parishii* currently supports a substantial amount of allozyme variation at both the species and population levels. Additionally, rates of inbreeding and drift appear to be moderate to low. Because loss of genetic diversity is not of immediate concern for *E. parishii* and because populations were not highly differentiated, reserve designs based on ecological factors would likely suffice to maintain genetic diversity. For example, protecting sites throughout the ecological and geographic range of *E. parishii* that incorporate the allozyme diversity documented would probably encompass its range of adaptive variation, although that variation was not characterized here. The high levels of diversity combined with the low levels of differentiation among populations support maintaining a network of large, interconnected populations. Such a network would prevent changes in mating system structure that would increase selfing and biparental inbreeding and increase rates of drift. Given the low levels of differentiation, it would not be necessary to conserve a large number of populations simply to represent the genetic diversity in terms of including all alleles sampled. However, more numerous, large, interconnected populations would be necessary to maintain the processes that support that diversity. This could be accomplished through a combination of protecting existing sites and restoring degraded habitat. The data presented here provide a valuable baseline for future comparisons of genetic diversity to evaluate effectiveness of protected areas and restoration in maintaining genetic diversity or evaluating consequences of further fragmentation and population loss.

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