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IMPLICATIONS OF MATING PATTERNS FOR CONSERVATION OF THE ENDANGERED PLANT *ERIOGONUM OVALIFOLIUM* VAR. *VINEUM* (POLYGONACEAE)[1]

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Mating patterns have direct application to conservation because of their influence on structuring genetic diversity within and among populations and on maintaining that diversity over time. We measured population and family outcrossing rates, biparental inbreeding, correlation of outcrossed paternity, and inbreeding coefficients in six populations from throughout the ecological range of the endangered plant *Eriogonum ovalifolium* var. *vineum* using naturally pollinated families. The taxon was primarily outcrossed: population outcrossing rates averaged 0.80 (SE 0.03) and family outcrossing rates averaged 0.88 (SE 0.03); neither rate varied among populations. Five population rates were significantly different from 1 while family rates differed from 1 in only one population. We found high correlated outcrossed paternity and evidence for biparental inbreeding in five populations each. As expected from the predominantly outcrossed mating system, levels of diversity were high and inbreeding coefficients among maternal individuals were low (averaging −0.05, SE 0.12). Differences between inbreeding coefficients of progeny (average 0.21, SE 0.06) and mothers indicated selection against homozygous offspring. These results indicate that it is important to maintain large populations to prevent increases in inbreeding and to maintain pollinator communities to facilitate outcrossing.

Key words: conservation; *Eriogonum*; inbreeding; mating systems; outcrossing rates; San Bernardino Mountains.

An underlying goal of many plant conservation programs is to conserve the genetic variation present in a taxon and minimize the processes that reduce this variation (e.g., Holsinger and Gottlieb, 1991). Conserving many large populations over the ecological and geographic range of the taxon of interest is considered the best way to capture most of the adaptive variation present (e.g., Schemske et al., 1994). Often, however, many large populations do not exist, and, even if they do, it is rarely possible to conserve all desired populations. Understanding how diversity is structured and predicting future changes in that diversity is essential to designing effective reserve networks and assessing consequences of particular tradeoffs. Knowledge of mating systems can aid in this understanding because of their role in structuring genetic diversity, transmitting diversity across generations, and determining rates of loss of that diversity (Hamrick, Linhart, and Mitton, 1979; Brown, 1989; Ritland, 1989).

The importance of mating systems in structuring genetic variation within and among populations is well documented (Hamrick, Linhart, and Mitton, 1979; Loveless and Hamrick, 1989; Hamrick and Godt, 1989). Predominantly outcrossing taxa typically have most of their diversity residing within populations with relatively little differentiation among populations; highly selfing species show just the opposite pattern. This information has bearing on conservation priorities for in situ conservation and on collection (Brown and Briggs, 1991) and breeding strategies (Ritland and El-Kassaby, 1985) for ex situ conservation and reintroductions. Genetic diversity (at least for neutral or nearly neutral loci) in highly outcrossed taxa is thought to be effectively conserved by maintaining a few populations of sufficient size to be protected from drift. Additional populations provide redundancy but are not likely to capture additional diversity (e.g., Brown, 1989). In contrast, capturing the range of genetic diversity present in differentiated populations of a highly selfed taxon requires protection of a larger number of populations, even if these populations are somewhat smaller.

Effects of mating system characteristics on maintenance of diversity are also well documented. The degree of nonrandom matings in a population, particularly self-fertilization, directly affects rates of inbreeding (reviewed in Barrett and Kohn, 1991). The most immediate effect of inbreeding is to decrease the frequency of heterozygotes in a population relative to a randomly mating population with the same allele frequencies. Inbreeding is of particular interest because it can result in inbreeding depression, lowered fitness of inbred individuals relative to individuals from random matings (e.g., Wright, 1965; Charlesworth and Charlesworth, 1987). Selfing can also indirectly increase loss of diversity due to genetic drift by decreasing effective population size. Consequences of such losses are debated and include possibly limiting the evolutionary potential of a taxon (Barrett and Kohn, 1991).

Three aspects of mating system may make major contributions to nonrandom mating in populations and thus have direct application to conservation: selfing rate, amount of biparental inbreeding, and correlation of outcrossed paternity. If matings

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are some combination of selfing and random outcrossing, inbreeding will occur only in the portion of the progeny derived from self-fertilization (Fyfe and Bailey, 1951; Clegg, 1980). Thus, knowing the selfing rate in a population provides information about risks of loss of genetic diversity. However, if outcrossed matings occur more frequently among close relatives than expected under random mating, apparent selfing rates also include results of consanguineous matings (Ellstrand, Torres, and Levin, 1978; Ellstrand and Foster, 1983; Ritland, 1984). Such matings among related individuals, or biparental inbreeding, can result from spatial substructuring of genotypes in a population (Ennos and Clegg, 1982) due to limited dispersal of pollen and seed (Turner, Stephens, and Anderson, 1982), or from temporal variation in synchrony of mating among individuals (Hall, Walker, and Bawa, 1996). Biparental inbreeding is typically less severe than inbreeding from selfing, but may still cause substantial inbreeding depression (Nason and Ellstrand, 1995). Because of different levels of severity, it is important to separate contributions of biparental inbreeding from selfing.

Further deviations from random matings can occur if outcrossed siblings tend to be sired by the same father (Ritland and El-Kassaby, 1985). This type of correlated mating can be estimated as the correlation of outcrossed paternity (Ritland, 1989), which is the proportion of randomly chosen outcrossed progeny pairs from a family that are full sibs. High correlation of outcrossed paternity can yield a more closely related population than indicated by outcrossing rates alone. This correlation can be accentuated in small populations due to low numbers of potential pollen donors, by reduced pollinator visitation (Young and Brown, 1998), or if male fitness is not evenly distributed among plants (e.g., Devlin and Ellstrand, 1990). A consequence of higher genetic relatedness is increased covariance among sibs and variance among sibships, which reduces the effectiveness of natural selection (Ritland, 1989).

Clearly, understanding mating patterns can be helpful in managing rare plant taxa to maintain genetic diversity. The purpose of our research was to estimate mating system parameters in six populations of a federally listed endangered plant taxon, *Eriogonum ovalifolium* var. *vineum* (Small) Jepson (Polygonaceae), using isozyme electrophoresis of naturally pollinated progeny arrays. Specifically, we compared mating-system parameters among populations across the environmental range of the taxon and examined relationships between mating-system parameters and plant density, fruiting success, and number of inflorescences per plant. We then examined the role of these results in conservation of *E. ovalifolium* var. *vineum.*

MATERIALS AND METHODS

The taxon—*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 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. 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).

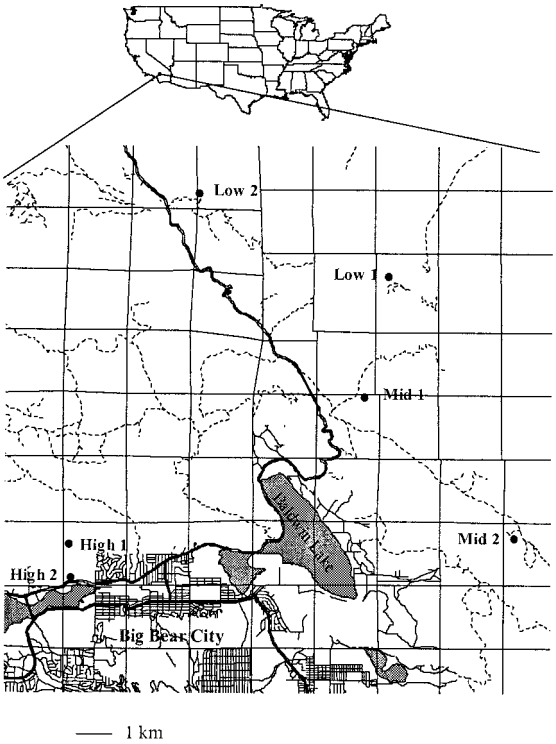

Fig. 1. Locations of six populations of *Eriogonum ovalifolium* var. *vineum* sampled for mating system parameters.

Eriogonum ovalifolium var. *vineum* is endemic to limestone and dolomite substrates that are found in an ~13 000-ha region in the San Bernardino Mountains of southern California (Hickman, 1993; USFWS, 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). Some populations have been lost, and others have been reduced in size due to surface mining of limestone that threatens the taxon throughout its range (USFWS, 1994).

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 characterized 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 codominated by *Pinus jeffreyi, Juniperus occidentalis* ssp. *australis,* and *P. monophylla.* Despite the substantial variation described above, habitats supporting *E. ovalifolium* var. *vineum* all tend to have open overstory and shrub canopies, as well as high soil pH and percentage calcium (Neel, 2000).

Population sampling and tissue extraction—*Mating system*—Mating-system parameters were estimated using naturally pollinated progeny arrays from ten maternal individuals in each of six *E. ovalifolium* var. *vineum* populations (Fig. 1). These populations reflected the elevational range of the taxon in-

cluding two low-, two mid-, and two high-elevation populations. Maternal individuals were selected randomly under the condition that the selected individual had at least five inflorescences with 20 or more flowers with mature fruits per inflorescence to ensure sufficient sample sizes. Fruits were deemed mature if they fell off an inflorescence when shaken or brushed. Leaf tissue was collected from each maternal plant in each population and was stored on ice until reaching the laboratory. From that time until extraction (within 48 h) leaf tissue was stored at ~8°C. Approximately 50 mg of tissue were ground with laboratory-grade quartz sand in ten drops of the extraction buffer of Mitton et al. (1979). Crude extract was soaked onto filter paper wicks and stored at −80°C for up to 2 mo before running.

At the same time leaf tissue was collected, 15 mature achenes were collected from at least five inflorescences on each of the ten mothers in each population. Because achenes are single seeded, each fruit represents a single mating event. Achenes were returned to the laboratory and stored at room temperature until use. Whole achenes were ground in one drop of a 0.2 mol/L tris-HCl buffer containing 1.5 mg/mL dithiothreitol and 120 mg/mL polyvinylpyridinol. Crude extract was absorbed onto filter paper wicks and run fresh. Replicate wicks were frozen at −80°C for confirmatory runs. While embryo tissue was not separated from maternal tissue in the fruit, we are confident that we were interpreting offspring genotypes due to the fact that we knew the maternal genotype, we observed banding patterns that were consistent with diploid tissue, and we readily identified alleles not present in the maternal genotype. If maternal tissue had affected our results we would have expected to see apparent triploid banding patterns in at least some offspring of heterozygous mothers.

Plant density and inflorescence and achene characteristics—Density of *Eriogonum* individuals was estimated by counting the number of individuals at a site and measuring the area occupied. All individuals located within the designated area were marked with pin flags as the area was traversed repeatedly by two observers. The areas in which densities estimated included all of the individuals sampled for mating system characteristics in each population. This method provided a single density estimate for each population; however, each estimate is based on a relatively large area. We chose this method over more numerous small plots due to the large number of empty plots and variation among plots found in preliminary sampling efforts. At sites LOW-2, MID-1, and HIGH-1, all individuals were counted, and the total area occupied was measured. The count from site LOW-1 is considered to be fairly complete, but it is not considered exhaustive because additional searching was limited by steep terrain. Populations at sites MID-2 and HIGH-2 were too large and extensive to obtain complete counts. At these sites we counted the number of individuals within an area measuring ~5000 m^2 (comparable to the area occupied by the first site sampled). Actual area varied due to constraints imposed by steep terrain.

Number of inflorescences was recorded on each plant sampled for mating system parameters. Where possible we counted the number of live achenes produced from 50 flowers on each of 5–6 randomly chosen inflorescences per plant (a total of 299 inflorescences were examined). It was not possible to find 50 flowers on each inflorescence as some flowers had already abscised at the time of sampling. For inflorescences with <50 flowers, all flowers were counted. The actual number counted ranged between 4 and 50 and averaged 37.2 flowers/inflorescence (SD = 12.4). Achenes were considered live if they were plump and colored green or beige to pale rust. These achenes were easily distinguished from dark brown to rust colored, shriveled, achenes that were considered aborted. This method likely overestimates the number of live achenes produced as no germination trials were conducted.

Electrophoresis—Achenes and maternal leaf samples were assayed for four polymorphic allozyme loci using electrophoresis: phosphogluco-isomerase (PGI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 5.4.2.2), leucine amino peptidase (LAP; EC 3.4.11.1), and uridine-5′-diphosphatase (UDP EC 2.7.7.9). Systems were resolved on a discontinuous lithium hydroxide-borate gel system (Ashton and Braden, 1961) on 9% starch gels. Gels were run at 75 mA and 200 V until the borate front reached the end of the gel (~5 h). Internal controls were used to calibrate allele assignment across gels. Staining protocols followed Wendel and Weeden (1989). The Mendelian basis of the banding patterns was inferred from comparison of progeny and maternal zymograms. Banding patterns were explained by codominance of electromorphs.

Only progeny that could be resolved for at least two loci were included in analyses. It was not possible to score complete genotypes for some maternal individuals in four populations (LOW-2, MID-1, MID-2, and HIGH-2). Population LOW-2 proved most problematic, yielding no complete maternal genotypes.

Data analysis—*Allozyme polymorphisms*—Allozyme polymorphisms were described as the number of alleles per population and number of alleles per locus from all individuals in each population.

Population mating system parameters—Multilocus (t_m) and single-locus (t_s) outcrossing rates and correlation of outcrossed paternity (r_p) were estimated for each population using maximum likelihood procedures. These parameters were estimated using the multilocus mating system program MLTR (Multilocus *t*, Revision) (Ritland, 1990, 1996), based on the multilocus mixed-mating model of Ritland and Jain (1981) and on the correlated matings model of Ritland (1989). When maternal genotypes were missing, the most likely genotype was inferred from progeny arrays following Brown and Allard (1970). Likelihood equations were maximized using the Newton-Raphson method as recommended by Ritland (1996). Neighborhood size, i.e., the number of pollen donors contributing to a family, was estimated as the reciprocal of r_p (Ritland, 1989). Standard errors were calculated from 500 bootstrap replicates with resampling among maternal plants within populations. We examined potential effects of missing maternal genotypes on outcrossing estimates by comparing estimates of t_m and t_s calculated based on inferred maternal genotypes with estimates calculated based on known maternal genotypes in each of the five populations with complete or nearly complete maternal data (i.e., all but LOW-2).

Family outcrossing rates—Multilocus, family outcrossing rates (t_{mf}) for individuals within each population were estimated using Newton-Raphson iterations in MLTR. We estimated t_{mf} in two ways: holding pollen allele frequencies (p) constant to the population values and jointly estimating t_{mf} and p (i.e., allowing p to vary among families) (Ritland and El-Kassaby, 1985). The former estimate was compared with population level outcrossing rates; the latter estimate was used in assessing contribution of biparental inbreeding to apparent selfing rates as described below. Standard errors of family estimates were based on 100 bootstraps with resampling of individuals within families. One family estimate in population MID-2 failed to converge and was removed from further family-level analyses.

Biparental inbreeding and apparent selfing rates—While outcrossing rates can be estimated directly, only indirect estimates of contributions of biparental inbreeding to apparent selfing rate are possible. We used three comparisons to assess levels of biparental inbreeding: t_m with t_s ($t_m - t_s$ from MLTR), inbreeding coefficients of progeny (F_p) with inbreeding coefficients expected at equilibrium (F_{eq}), and the two estimates of t_{mf}. In absence of biparental inbreeding, the estimates will be the same in each comparison; with biparental inbreeding the estimates will differ as follows. Single-locus outcrossing rate will be less than t_m (Shaw, Kahler, and Allard, 1981; Ritland, 1990) because outcrossing events that are not detected at single locus due to alleles that are identical by descent have a higher probability of being detected as more loci are examined. Inbreeding coefficients expected at equilibrium will be less than F_p due to higher than expected inbreeding levels (Brown, 1979). Multilocus, family outcrossing rates estimated jointly with p values will be less than t_{mf} estimated holding p constant and equal to the population frequency because when jointly estimated with p, t_{mf} excludes apparent selfs due to consanguineous matings; these mating events are included in t_{mf} when p is held constant to the population value as a result of covariation of pollen allele frequencies and maternal genotypes (Ritland and El-Kassaby, 1985). Most studies have compared inbreeding coefficients of mothers (F_M) to F_{eq} to quantify biparental inbreeding ($\triangle F$ of Brown, 1979). Because F_M can include effects of selection

TABLE 1. Elevation, plant density, sample sizes, and number of alleles per locus (SE in parentheses) in each population. Population mating system parameters including multilocus outcrossing rates (t_m), single locus outcrossing rate (t_s), $t_m - t_s$, and correlation of outcrossed paternity (r_p) (SE from 500 bootstrap replicates is in parentheses). Mean family estimates of t_{mf} estimated with pollen allele frequencies held constant to population values and with variable pollen allele frequencies are also presented (SE from 100 bootstrap replicates in parentheses). Boldface indicates values that are significantly greater than 0 ($t_m - t_s$ and r_p) or significantly less than 1 (t_m, t_s, and t_{mf}).

Characteristic	LOW-1	LOW-2	MID-1	MID-2	HIGH-1	HIGH-2
Elevation	1460	1645	1830	1890	2100	2195
No. plants counted	147	47[1]	263[1]	571	316[1]	695
Plant density/100 m^2	2.48	1.76	28.83	10.52	5.74	12.63
No. families	10	10	10	10	9	10
No. progeny	146	150	147	129	135	147
Alleles per locus	6.0 (1.2)	6.5 (1.3)	6.5 (1.3)	6.3 (1.2)	5.5 (1.3)	5.5 (1.3)
t_m	**0.85 (0.05)**	**0.68 (0.06)**	**0.83 (0.05)**	**0.74 (0.05)**	**0.81 (0.07)**	0.89 (0.06)
t_s	**0.82 (0.05)**	**0.58 (0.05)**	**0.76 (0.06)**	**0.68 (0.05)**	**0.76 (0.07)**	**0.81 (0.06)**
$t_m - t_s$	0.03 (0.03)	**0.10 (0.02)**	0.07 (0.04)	**0.07 (0.01)**	**0.05 (0.02)**	**0.08 (0.02)**
r_p	0.35 (0.18)	0.04 (0.24)	**0.41 (0.20)**	**0.45 (0.12)**	**0.60 (0.22)**	**0.67 (0.12)**
Neighborhood	2.8 (1.42)	24.4 (113.2)	2.5 (5.39)	2.2 (1.16)	1.7 (0.66)	1.5 (0.28)
Mean t_{mf}, constant p	0.88 (0.07)	**0.72 (0.06)**	0.98 (0.11)	0.91 (0.11)	0.92 (0.07)	0.89 (0.06)
Mean t_{mf}, variable p	0.73 (0.07)	**0.64 (0.06)**	0.77 (0.08)	0.67 (0.03)	0.73 (0.08)	0.93 (0.16)

[1] Count represents total population.

between seed and adult life stages, we used F_p as a more direct reflection of mating system effects.

Inbreeding estimates—Inbreeding coefficients for maternal individuals (F_M) were estimated for each population using maximum likelihood procedures in MLTR (Ritland, 1996). The equilibrium value of the inbreeding coefficient (F_{eq}) was calculated as $F_{eq} = (1 - t_m)/(1 + t_m)$ (Fyfe and Bailey, 1951); F_{eq} represents the level of inbreeding expected from selfing alone under the mixed mating model. Progeny inbreeding coefficients (F_P) were calculated from one randomly selected offspring per family using the program GDA (Genetic Data Analysis) (Lewis and Zaykin, 2000). Standard error for each coefficient was calculated from 500 bootstrap replicates.

The three F values were compared within and among populations as described below to analyze patterns of inbreeding. Means differing by more than $\pm 1.96 \times$ SE were considered significantly different. Estimates of both F_M and F_P were compared with the like estimate among populations to determine whether levels of inbreeding varied along the environmental gradient. We compared F_M with F_P in each population to assess generational changes in inbreeding. If F_P was greater than F_M selection against homozygotes between seed and adult populations was indicated, assuming inbreeding levels of adults are at equilibrium (Ritland, 1990). Additionally, we compared F_M with F_{eq} in each population to assess whether levels of inbreeding observed in adults could be explained by mating system alone.

Plant density and inflorescence and achene characteristics—Plant densities in each population were normalized to the number of individuals in a 100-m^2 area. Differences among populations in number of inflorescences per plant were examined through analysis of variance using Statistica (StatSoft, 1997). Variation in proportion of live achenes among inflorescences on plants, among plants in populations, and among populations was examined with nested analysis of variance using SAS (SAS, 1998). Proportion of successful achenes was transformed using an arcsine square-root transformation. Actual proportions are presented in the RESULTS. We examined relationships of mating-system parameters t_m and r_p with plant density, mean number of inflorescences per plant, and the untransformed proportion of flowers producing achenes using Pearson product-moment correlation in Statistica (StatSoft, 1997). Relationships between t_{mf} and number of inflorescences per plant, and the untransformed proportion of successful achenes on all plants were also examined using Pearson product moment correlation. Inbreeding coefficients for maternal individuals and F_P were compared with plant density, mean number of inflorescences per plant, and the untransformed proportion of flowers producing achenes using Pearson product-moment correlation to assess the relationship between inbreeding and ecological factors.

RESULTS

Allozyme polymorphisms—The following numbers of alleles were resolved for each enzyme locus: LAP-8, PGI-9, PGM-3, and UDP-6. LAP and PGI were especially useful for detecting outcrossing events because both loci had several alleles at relatively even frequencies. Number of alleles per population averaged 24.2 (SE = 0.75). The smallest number of alleles (22) was found in the two high-elevation populations, and largest number (26) was in both LOW-2 and MID-1. The number of alleles per locus in each population averaged 6.0 over all populations (SE = 0.19; Table 1).

Population mating-system parameters—Population t_m estimates in all but two populations were above 0.80 (Table 1). Five of the six t_m estimates (HIGH-2 was the exception) were significantly <1 (i.e., $t_m + 1.96 \times \text{SE} < 1$), but none were significantly different from any other. The population with the lowest outcrossing rate was the population from which most maternal genotypes were missing (LOW-2). However, there was no significant difference between population-level outcrossing estimates made with known maternal genotypes ($t_m = 0.83$, SE = 0.05; $t_s = 0.77$, SE = 0.06) and estimates made with maternal genotypes inferred ($t_m = 0.82$, SE = 0.06; $t_s = 0.72$, SE = 0.06) in the five populations for which most maternal genotypes were scored. Thus it is not likely that the missing maternal genotypes reduced detection of outcross events in population LOW-2.

Estimates of correlation of outcrossed paternity averaged 0.42 and were significantly different from 0 in four populations (Table 1). As with other mating-system parameters, r_p did not differ significantly among populations due to large amounts of error in the estimates. Observed values of r_p indicate that few fathers contributed to individual progeny arrays in most populations. In five populations, neighborhood size was less than three individuals (Table 1). In contrast, the neighborhood size in population LOW-2 was 24.4.

Family outcrossing rates—In general, t_{mf} estimated holding p constant to the population value was high and averaged 0.88 (SE = 0.03) across all families ($N = 58$) in all populations (Table 1). However, rates for individual families were highly

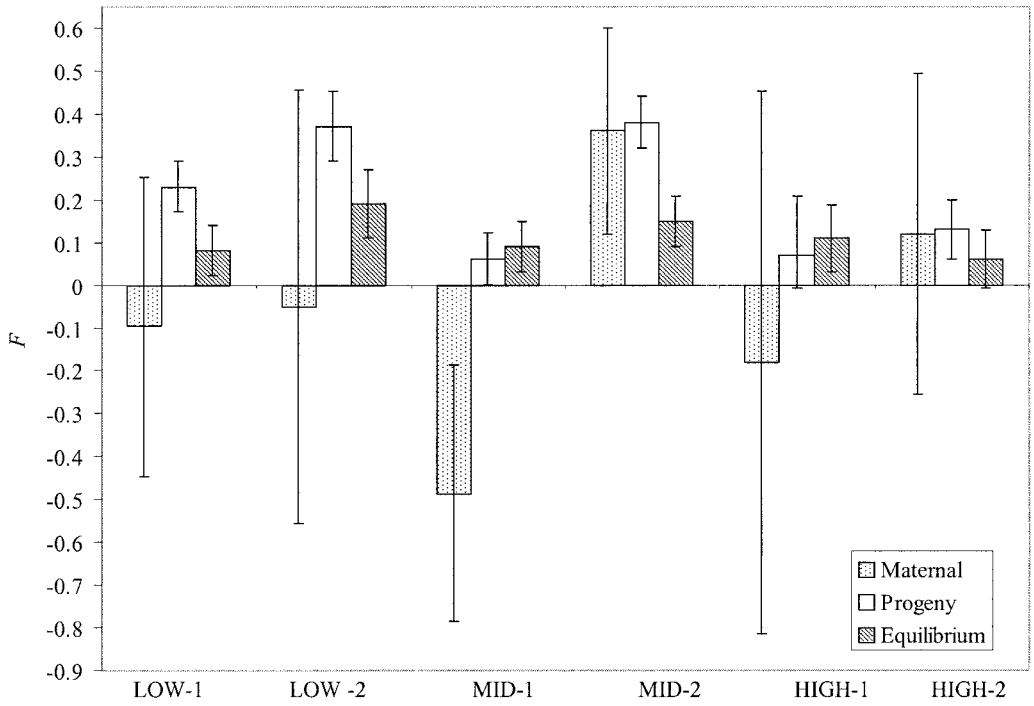

Fig. 2. Observed inbreeding coefficients of progeny (F_P) and maternal individuals (F_M), and inbreeding coefficients expected at equilibrium (F_{eq}) under the mixed-mating model if only selfing is contributing to inbreeding. Error bars represent $\pm$ 1.96 $\times$ SE.

variable. For example, estimates within populations ranged from 0.48 to 1.87 overall, and in one population alone they ranged from 0.65 to 1.87 (MID-2). Estimates of t_{mf} were not significantly different from one in 60–100% of the families in each population. In four populations, outcrossing rates for more than 89% of the families were not significantly different than 1. The population average of t_{mf} differed from 1 only in population LOW-2 (Table 1). Mean values of t_{mf} in each population were generally higher than population estimates of t_m, although the family estimates were each more variable and thus the two estimates did not differ significantly (Table 1). Estimates of t_{mf} made allowing p to vary averaged 0.75 (SE = 0.04) and were smaller than estimates made holding p constant in 93% of the families and in five of six populations (Table 1).

Biparental inbreeding and apparent selfing rates—Two of the three comparisons indicated that biparental inbreeding contributed to the apparent selfing rate in some populations of this taxon. Population t_m values were consistently larger than t_s values and values of $t_m - t_s$ were significantly different from zero in four populations (Table 1). Inbreeding coefficients of progeny were significantly greater than F_{eq} in three populations (Fig. 2), indicating more inbreeding than expected based on the observed outcrossing rates. Comparisons of the two estimates of t_{mf} provided no strong evidence for biparental inbreeding (Table 1). Estimates of t_{mf} made while holding p constant to the population value were higher than estimates calculated jointly with estimates of p in 54 of 58 families examined and in five of the six populations. However, average differences between the two estimates within populations were not significantly different due to high levels of variation within populations (Table 1). Differences approached significance in populations LOW-1, MID-2, and HIGH-1.

Inbreeding estimates—Variation in F_M was not associated with the examined environmental gradient. Inbreeding coefficients of maternal individuals were significantly different from 0 in only the two intermediate populations, and the deviations were in opposite directions (Fig. 2). Inbreeding coefficients of mothers were different from F_{eq} only in population MID-2 (Fig. 2). Progeny inbreeding coefficients were significantly greater than 0 in four of the six populations (Fig. 2), and exceeded F_M in all populations (Fig. 2); the magnitude of differences ranged from 0.045 in HIGH-2 to 0.330 in LOW-2. However, F_M estimates were highly variable and as a result, F_M and F_P were significantly different in only MID-1 (Fig. 2).

Plant densities and inflorescence and achene characteristics—Plant density varied by over 16-fold among populations (Table 1). In general, populations appeared to be larger at higher elevations (Table 1); however, there was no apparent relationship between density and the environmental gradient.

Number of inflorescences per plant differed significantly among populations ($F_{5,58} = 4.10$, $P < 0.05$), but differences were not associated with the elevational gradient (Table 2). Proportion of live achenes varied significantly among plants within populations and among populations, but not among inflorescences within plants (Table 3). There was no relationship between proportion of live achenes and the environmental gradient (Table 2). Additionally, achene set and number of inflorescences produced did not covary ($r^2 = -0.21$, $P = 0.10$, $N = 58$).

There was no significant correlation between t_m and plant density ($r^2 = 0.35$, $P = 0.40$, $N = 6$), number of inflorescences per plant ($r^2 = -0.75$, $P = 0.10$, $N = 6$) or proportion of live achenes ($r^2 = 0.43$, $P = 0.40$, $N = 6$). Correlation of outcrossed paternity was not correlated with plant density (r^2

TABLE 2. Mean number of live achenes, proportion of live achenes, and number of inflorescences counted on each of *N* mother plants in each population (SE in parentheses).

Population	*N*	Mean no. live achenes	Proportion live achenes	Mean no. of inflorescences/plant
LOW-1	10	26.4 (6.74)	0.15 (0.04)	20.2 (3.26)
LOW-2	10	34.9 (4.51)	0.18 (0.03)	41.4 (7.30)
MID-1	10	44.8 (5.15)	0.27 (0.03)	24.1 (4.47)
MID-2	10	40.5 (7.04)	0.22 (0.04)	53.7 (15.15)
HIGH-1	9	70.4 (4.37)	0.32 (0.06)	10.4 (2.76)
HIGH-2	10	35.3 (6.45)	0.21 (0.05)	21.0 (3.84)
Mean	6	42.0 (6.20)	0.23 (0.03)	28.5 (6.51)

$= 0.30$, $P = 0.56$, $N = 6$), number of inflorescences per plant ($r^2 = -0.49$, $P = 0.32$, $N = 6$), or proportion of live achenes ($r^2 = 0.36$, $P = 0.56$, $N = 6$). There was also no correlation between t_{mf} and number of inflorescences ($r^2 = -0.19$, $P = 0.16$, $N = 57$) or t_{mf} and proportion of live achenes ($r^2 = -0.10$, $P = 0.46$, $N = 58$).

There was no significant correlation between plant density and F_M ($r^2 = -0.46$, $P = 0.35$, $N = 6$) or F_p ($r^2 = -0.53$, $P = 0.20$, $N = 6$). In contrast, F_p significantly decreased as the number of inflorescences per plant increased ($r^2 = -0.87$, $P = 0.02$, $N = 6$). There was no such relationship between F_M and number of inflorescences per plant ($r^2 = -0.60$, $P = 0.21$, $N = 6$).

DISCUSSION

Our results indicate that *E. ovalifolium* var. *vineum* is largely outcrossed, with at least 68–89% of matings within populations occurring between two individuals. Estimates of t_m were significantly <1 in 5 populations while average family outcrossing rates were <1 in only one population (Table 1). This difference between population and family estimates is likely due to the large amount of estimation error in family estimates that resulted from relatively small within-family sample sizes. There was no strong relationship between mating system parameters and the environmental gradient or plant density. While the relationship was not significant, there was a suggestion that population outcrossing rates decreased as the average number of inflorescences per plant increased.

Unfortunately, we cannot be certain whether *E. ovalifolium* var. *vineum* is self-compatible with some mechanism to promote outcrossing or whether it is self-incompatible and outcrossing events went undetected due to the small number of loci examined. Results from controlled pollinations in other *Eriogonum* taxa indicate a range from self-compatibility to incompatibility within the genus and thus are not helpful in providing an expected mating system (Moldenke, 1976). Typically, outcrossing rates vary among populations in self-compatible taxa (e.g., Schoen, 1982; Holtsford and Ellstrand, 1989; Liengsiri, Boyle, and Yeh, 1998). In contrast, outcrossing rates did not differ significantly among populations of *E. ovalifolium* var. *vineum* despite large differences in habitat characteristics (Table 1), plant density (Table 1), numbers of inflorescences (Table 2), and proportion of live achenes (Tables 2 and 3). Uniform outcrossing rates over populations support the possibility that *E. ovalifolium* var. *vineum* is self-incompatible. However outcrossing rates do not vary among populations in all self-compatible taxa. For example, Kennington and James (1997) found consistently high outcrossing rates and high rates of seed abortion in the self-compatible rare taxon *Eucalyptus argutifolia.* Geitonogamy is common in *Eucalyptus argutifolia,* and there is no prezygotic self-incompatibility mechanism. Instead, high outcrossing rates are maintained by preferential abortion of seeds that result from self-fertilization. A similar mechanism may be working in *E. ovalifolium* var. *vineum*; however, confirmation of this possibility would require further investigation that is beyond the scope of the present research.

The possibility that *E. ovalifolium* var. *vineum* is self-incompatible is also supported by the low success rate for achene production (~20% over all populations), which is typical for outcrossing taxa (Wiens et al., 1987). It should be noted that self-incompatibility is often a quantitative trait. For example, phenotypic plasticity in self-incompatibility has been documented in *Campanula rapunculoides,* a taxon with gametophytic self-incompatibility (Vogler, Das, and Stephenson, 1998). There is some evidence for variation in self-incompatibility in our taxon based on the variation in t_{mf} estimates within populations. However, such variation can also result from neighborhood structuring within populations. Furthermore, t_{mf} estimates were somewhat unreliable due to small sample sizes and must be interpreted cautiously. It is also possible that *E. ovalifolium* var. *vineum* exhibits cryptic self-incompatibility as defined by Bateman (1956) (i.e., outcrossed pollen has a siring advantage over selfed pollen when both are present).

If the taxon is self-incompatible, the level of selfing observed could be the result of undetected outcrossing events due to the small number of loci examined or of biparental inbreeding. The former is unlikely because, given the allelic polymorphism observed in populations, four loci should have been sufficient to detect most outcross events. Biparental inbreeding has resulted in substantial amounts of apparent selfing. For example, Ellstrand, Torres, and Levin (1978) documented selfing rates as high as 0.66 resulting from biparental inbreeding in the self-incompatible *Helianthus annuus.* Differences between t_m and t_s in *E. ovalifolium* var. *vineum* indicate levels of biparental inbreeding (a minimum of 3–10%) sufficient to account for the observed selfing rates in all but one population (LOW-2). Additionally, the highest level of biparental inbreeding was found in the population with the lowest outcrossing rate (LOW-2).

Thus, it is not clear whether *E. ovalifolium* var. *vineum* is self-incompatible and the observed selfing resulted from bi-

TABLE 3. Nested analysis of variance results comparing proportion of successful achenes among inflorescences within plants, among plants within populations, and among populations.

Component	df	Sum of squares	Mean square	*F*	*P*
Among inflorescence within plant	49	1.043	0.021	0.90	0.6560
Among plant within population	43	4.712	0.109	4.65	0.0001
Among population	5	1.912	0.382	16.21	0.0001
Total	107	9.25	0.086	3.67	0.0010

parental inbreeding or if the taxon selfs but preferentially aborts self-fertilized ovules. Regardless of the specific mechanism responsible for the high outcrossing rates, mating patterns have contributed to maintenance of a great deal of genetic diversity in this taxon. For example, the number of alleles per locus was higher and level of inbreeding at the species level was lower than one would otherwise expect in a narrowly endemic plant taxon (Hamrick, Linhart, and Mitton, 1979; Karron, 1987; Hamrick and Godt, 1989). This high diversity illustrates the importance of mating system in influencing levels and allocation of genetic diversity within and among plant populations. Of course, other factors such as founder effects and evolutionary history may also be important in determining levels of species-wide genetic diversity (e.g., O'Brien, Wildt, and Bush, 1983; McCauley, Raveill, and Antonovics, 1995).

As is typical for a predominantly outcrossed taxon (Brown, 1979), progeny tended to be more inbred than expected based on the observed outcrossing rate and more inbred than adults (Fig. 2). Decreases in the magnitude of inbreeding coefficients between progeny and adults provide evidence for selection against selfed/homozygous offspring although error on F_M estimates was large (Ritland, 1990). Sufficient selection would result in low levels of effective inbreeding. Inbreeding in maternal plants was generally not different than expected at equilibrium given the observed levels of selfing (Fig. 2). Thus, selection may act against selfed individuals during pollination and against homozygotes derived from selfing and consanguineous matings both during and after seed maturation.

While *E. ovalifolium* var. *vineum* is mostly outcrossed, the observed r_p values indicate that outcrossed matings did not occur randomly. In five of the six populations examined, 35–60% of the outcrossed progeny pairs within a family were full sibs, indicating neighborhood sizes of less than three individuals in the paternal mating pool (Table 1). This level of correlated mating is comparable to within-capsule levels observed in *Mimulus guttatus* (Ritland, 1989) and is much higher than was found in *Centaurea solstitialis* individuals (Sun and Ritland, 1998). This correlation indicates that progeny will be more closely related than predicted based on observed outcrossing rates. The correlation of outcrossed paternity could be the result of a combination of repeated independent matings to a limited pollen pool or of nonindependent mating events as a consequence of pollinator behavior (Ritland, 1989). Observations indicate that pollinators spend a great deal of time crawling around within and among inflorescences on a single plant before moving to the next plant and repeating the process (S. Morita, personal communication, University of California Davis). Thus, pollen brought in from one plant by one insect is likely to be deposited on many flowers during a single visit to a plant with little carryover to subsequent plants. However, many individual insects repeat this behavior and could be independently bringing pollen from the same sources such as adjacent plants. Distinguishing between these two explanations would require knowledge of pollinator behavior, which plants were potential fathers (i.e., which plants were in bloom when the flowers were pollinated), as well as genotypes of those plants, all of which we do not have. The observed pollinator behavior also provides opportunities for extensive geitonogamous matings that are not reflected in the progeny arrays. Only one population (LOW-2) had a low r_p and thus a large paternal pollen pool. Among-population variation in r_p is often related to ecological factors such as pollen load competition, fertility variation, pollen carryover, and population substructure (e.g., Barrett, Kohn, and Cruzan, 1992; Sun and Ritland, 1998). The cause of this difference is not clear; however, LOW-2 was the smallest and lowest density population (Table 1). Further, while numbers of inflorescence per plant were not extreme they were higher than average (Table 2).

Implications of our results for conservation of *E. ovalifolium* var. *vineum* include assessment of potential for loss of genetic diversity, guidelines for conserving populations, and also the importance of maintaining pollinators. This taxon appears to have substantial levels of genetic diversity, and its mating system is functioning such that diversity will be maintained as long as populations are not drastically reduced in density or size. Empirical and experimental evidence indicates that density of conspecifics, particularly flowering individuals, has more of an impact on outcrossing rates than does population size. Outcrossing rates tend to be positively correlated with plant density in self-compatible taxa (e.g., Murawski and Hamrick, 1992; Van Treuren et al., 1993, 1994; Karron et al., 1995; Liengsiri, Boyle, and Yeh, 1998) and negatively correlated in obligately outcrossing taxa (Ellstrand, Torres, and Levin, 1978; Young and Brown, 1998). This negative relationship is thought to be due to substructuring of genotypes within higher density populations that result in more opportunities for consanguineous matings (Ellstrand, Torres, and Levin, 1978; Ellstrand and Foster, 1983). Such correlation yields unequal reproductive contributions and results in lower effective population size (N_e) and thus higher rates of drift than would be found in a randomly mating population of the same census size. However, our results indicated few significant mating-system differences over the range of densities and population sizes sampled. It is possible that stronger relationships would have been detected if we had measured densities in the immediate vicinity of maternal plants. Furthermore, although densities varied 16-fold, the highest density of 28 plants/100 m^2 was still very low, potentially below the point at which effects of density differences affect mating-system parameters.

Altering population size or density or degrading and fragmenting associated habitats could also affect pollinator communities and decrease pollinator service (e.g., Rathcke and Jules, 1993). Quantitative reduction in levels of pollinator service could be detrimental because the high observed outcrossing rates indicate that *E. ovalifolium* var. *vineum* is dependent on pollen vectors for successful reproduction and production of fit progeny. Changes in pollinator activities could also affect mating-system characteristics. For example, lower outcrossing rates in self-compatible species at lower plant densities are often attributed to pollinator behavior. At relatively low plant densities pollinators spend more time among flowers on an individual plant than travelling among plants (Van Treuren et al., 1993, 1994; Karron et al., 1995). When insects do travel among plants, they tend to visit nearest neighbors (Schaal, 1978), which tend to be related. This is supported by the highest rate of biparental inbreeding in the lowest density population of *E. ovalifolium* var. *vineum.* Reduced seed set in low-density populations of *Delphinium nuttallianum* and *Aconitum columbianum* documented by Bosch and Waser (1999) was attributed to a greater frequency of selfed and other inbred pollen rather than to differences in numbers of pollinators visiting plants.

Considering the consistently high outcrossing rates we observed, we would not expect to see large increases in rates of selfing if *E. ovalifolium* var. *vineum* populations were reduced

in size or density; rather, we would expect elevated biparental inbreeding, as we see in population LOW-2. Such increases in nonrandom mating could cause substantial inbreeding depression, as typically outcrossed taxa tend to have large genetic loads (Charlesworth and Charlesworth, 1987). Genetic load may already be causing abortion of achenes and the reduction in inbreeding observed between seeds and adults in *E. ovalifolium* var. *vineum* (Fig. 2). Interestingly, despite the low proportion of successful achenes, the actual number of fruits produced by a typical plant was high. If we multiply the number of inflorescences per plant by a conservative estimate of 100 flowers and by the proportion of live achenes observed on each plant, average plants would produce 589 fruits (SE = 77) with a range of 51–2868 fruits per plant. As a result, limited fruit production is not currently a concern for this taxon, at least in the populations examined. We have no information on fitness of these progeny or on mortality at later life history stages. In general, however, managing for ecological security of this taxon will most likely sufficiently address maintenance of genetic diversity within populations. Further, if restoration of currently disturbed sites is undertaken, efforts should include large numbers of genetically unrelated individuals to prevent increased inbreeding in this taxon.

Beyond this specific case, studies of mating patterns are important in conservation genetics. Changes in allele frequencies in populations and in levels of inbreeding or increased differentiation among populations due to degradation, loss, or fragmentation may not be detectable for many generations using population survey data. For long-lived taxa such as *E. ovalifolium* var. *vineum*, this delay may be on the order of centuries. Even when detrimental effects can be detected, it is not possible to separate historical from current processes using *F* statistics (Sork et al., 1999). In contrast, increases in correlated matings and decreases in neighborhood sizes can be detected immediately and can provide an early warning of impending loss of diversity (Aldrich et al., 1998).

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