Genetic Variation and the Reintroduction of *Cordylanthus maritimus* ssp. *maritimus* to Sweetwater Marsh, California

Kaius Helenurm[1,2]
Lorraine S. Parsons[3]

Abstract

This study evaluated the genetic consequences of a reintroduction of the endangered annual plant *Cordylanthus maritimus* ssp. *maritimus* to Sweetwater Marsh (San Diego County, California). A survey of 21 enzyme loci in natural populations revealed that genetic diversity is very low and is primarily found as rare alleles at a few loci, making this species especially susceptible to the loss of alleles and heterozygosity through genetic drift. The reintroduction was performed in 1991 and 1992 by sowing seeds (collected from Tijuana Estuary) in numerous small patches of suitable habitat. For this study, leaf tissue was collected from all plants in all patches during flowering in 1995 and surveyed for genotype at the three enzyme loci that are polymorphic at Tijuana Estuary. Rare alleles were absent in 27 out of 30 patches for Pgm-1, in 17 out of 30 patches for Pgm-2, and in 10 out of 11 patches for Mdh-1. In all, half of the patches lacked any rare allele. Rare alleles tended to occur in patches with few individuals. Overall rare allele frequency was lower than in the colonies from which seeds were collected at two of the three loci, and heterozygosity was reduced. The Sweetwater Marsh population is at risk of losing most of its genetic variation at enzyme loci through the extinction of patches with few individuals. Future reintroduction attempts should attempt to create contiguous sets of patches or to periodically reseed existing patches to reduce the loss of genetic variation.

[1]Department of Biology, San Diego State University, San Diego, CA 92182-4614, U.S.A.
[2]Current address: Department of Biology, University of South Dakota, Vermillion, SD 57069, U.S.A.
[3]Sonoma County Water Agency, 2227 Capricorn Way, Suite 105, Santa Rosa, CA 95407, U.S.A.

Introduction

The persistence of species through natural recruitment and survival has been cited as a primary goal of ecological restoration (Jackson et al. 1995). Genetic variation can have a major impact on survival and reproduction. Not only is genetic variation a prerequisite for the continued adaptation of a population to a changing environment (such as global warming and pathogen outbreaks) and therefore essential for long-term survival of populations, but genetic variation has also been shown to affect immediate fitness and hence short-term population growth. Numerous studies of different species have shown that heterozygous individuals enjoy greater rates of survival or reproduction in natural populations (Allendorf & Leary 1986; Lesica & Allendorf 1992). Thus, the maintenance of genetic variation must be considered a major objective in the reintroduction of species to natural or created habitats. Indeed, recognition of this principle guides most captive breeding programs, and an increasing number of reintroduction plans explicitly include genetic considerations (Haig et al. 1990; Pavlik et al. 1993; DeMauro 1994; Russell et al. 1994).

One of the major causes of loss of genetic variation is the genetic drift associated with reduced population size. Genetic drift is the random fluctuation of allele frequencies and can be thought of as a sampling error that occurs in natural populations. It can be caused by mortality that is random with respect to genotype but it is also intrinsic to the process of reproduction, in which the individuals of each new generation represent a sample of the gametes produced by the previous generation. Any sample may not be representative of the population from which it is drawn, and consequently genetic drift can occur in all finite populations. Small samples are much more likely to be unrepresentative, and thus genetic drift is likely to be a potent evolutionary force in small, isolated populations. The ultimate outcome of fluctuating allele frequencies is loss of alleles, because frequencies will at some point fluctuate to 0 or 1, and this will occur more quickly in small compared with large populations. Accompanying effects include reduced heterozygosity and increased inbreeding, as

the alleles at a locus become, on average, more closely related to one another.

An important challenge of ecological restoration is to avoid these random genetic changes during the process of reintroducing organisms to the environment. There are several stages of reintroduction that are susceptible to sampling error and during which drift may occur. These include collection of seeds or other propagules from source populations, *ex situ* maintenance of this material, sampling of maintained material for reintroduction, and the first several generations of mortality and reproduction at the new site, when population sizes may still be small. With sufficient information on the amount and distribution of genetic variation, it is possible to diminish the risk of losing rare alleles and decreasing heterozygosity. However, reintroduction may often need to be initiated before genetic data are available. Consequently, it is of considerable interest to examine reintroductions done without the benefit of genetic data and to evaluate their success in preserving the genetic variation found within natural populations.

This paper investigates genetic variation in an endangered annual plant of coastal wetlands, *Cordylanthus maritimus* Benth. ssp. *maritimus* (Scrophulariaceae; salt marsh bird's beak) and evaluates the genetic consequences of a reintroduction of this species to Sweetwater Marsh, San Diego County, California. The reintroduction was performed in 1991 and 1992 using colonies in Tijuana Estuary as seed sources, before data on genetic variability in natural populations were available. The objectives of this study were: (1) to quantify the amount and distribution of genetic variation in natural populations of *C. m. maritimus*; (2) to determine the fate of rare alleles and heterozygosity at the reintroduction site; and (3) to evaluate the effect of sowing seeds in a number of small patches.

Materials and Methods

Study Species

Cordylanthus maritimus ssp. *maritimus* is an annual, facultatively hemiparasitic species of coastal salt marshes (Vanderwier & Newman 1984). It is bee-pollinated, self-compatible, and weakly autogamous (Lincoln 1985; Parsons & Zedler 1997). Seeds are viable for more than one year, and dispersal occurs by water.

Although once common and widely distributed (Purer 1942), *C. m. maritimus* is currently listed as endangered by both federal and state agencies. Due to substantial habitat loss (USFWS 1984), it is now known from only five salt marshes in southern California (Carpinteria Marsh, Santa Barbara County; Mugu Lagoon, Ventura County; Newport Bay, Orange County; Sweetwater Marsh, San Diego County; and Tijuana Estuary, San Diego County) and one in Mexico (San Quintin Bay; Baja California, Mexico). Populations consist of colonies, groups of plants that remain discrete from one year to another. A reintroduction attempt to Anaheim Bay (Orange County) initiated by the U.S. Fish and Wildlife Service in the early 1980s (using seeds from Newport Bay) persisted for a few years but appears to have ultimately failed (B. Massey, personal communication).

Reintroduction to Sweetwater Marsh

The presence of *C. m. maritimus* in Sweetwater Marsh is presumably entirely because of its reintroduction as part of mitigation for a freeway expansion project in San Diego County (California Department of Transportation Mitigation Agreement, Section 7 consultation); the historic population in this marsh declined and was last seen in 1987 (Parsons & Zedler 1997). The reestablishment of a self-sustaining population was initiated by sowing seeds in 1990 in a high marsh remnant that was made into an island, but this attempt was unsuccessful (Parsons & Zedler 1997). In 1991 and 1992, additional seeds were sown in a larger natural high marsh area southwest of the constructed marsh and east of the historic population (Fink & Zedler 1991; Parsons & Zedler 1997). Although the importance of maintaining genetic variation was recognized, no genetic data were available at that time to guide choice of source colonies. In early 1991, seeds that had been collected the previous summer from at least 100 individuals in each colony at Tijuana Estuary were pooled (B. Fink, personal communication). Equal volumes of seeds (approximately 200–400) were sown and raked into the soil in 30 0.25-m^2 patches separated by at least 3 m. In early 1992, seeds that had been collected the previous summer from only one colony at Tijuana Estuary (Sea Coast) were used, because of low seed production in the other colonies. Seeds were sown as before in 60 patches. Patches were still discrete in 1995 (three to four generations later) even though they had expanded beyond the original 0.25-m^2 area, suggesting that there was not enough movement of seeds among patches to obscure their integrity.

Cordylanthus m. maritimus is now also found in other areas of Sweetwater Marsh, possibly through dispersal of seeds produced at the initial reintroduction site. For this study, however, only the patches sown initially in either 1991 or 1992 were surveyed. The sowing scheme that was used allows evaluation of the effectiveness of using one versus several source colonies for seeds, as well as of the effects of sowing seeds in many small patches.

Sampling and Starch-gel Electrophoresis

Leaf tissue was collected from colonies at all natural populations in the United States in June and July 1995 (Table

Table 1. Study sites and sample sizes for natural populations of *C. maritimus maritimus*.

Population	*Designation*	*Colony*	*N*
Carpinteria Marsh (Santa Barbara County)			
	1	Carpinteria	50
Mugu Lagoon (Ventura County)			
	2	Billboard	40
	3	Corner	50
	4	Cotar	50
	5	GCA	50
	6	Relict	44
Newport Bay (Orange County)			
	7	Big Canyon	41
	8	North Star Beach	48
	9	San Joaquin	50
	10	Shellmaker Island	50
Tijuana Estuary (San Diego County)			
	11	East–West Channel	50
	12	Fifth & Iris	50
	13	Penninsula	50
	14	Sea Coast	50

1). The number of colonies (defined as groups of plants separated by at least 50 m) present in populations ranged from one to five. Up to 50 individuals were sampled in each colony. Genotypes were determined at loci coding for the following enzymes: aspartate aminotransferase (Aat), alcohol dehydrogenase (Adh), aldolase (Ald), catalase (Cat), esterase (Est), glucose-6-phosphate dehydrogenase (G6p), isocitrate dehydrogenase (Idh), malate dehydrogenase (Mdh), malic enzyme (Me), phosphogluconate dehydrogenase (Pgd), phosphoglucomutase (Pgm), and superoxide dismutase (Sod). In total, 673 individuals were surveyed from 14 colonies in four populations.

Leaf tissue was collected from plants at Sweetwater Marsh in August 1995, during the flowering period. All plants that flowered in the extant patches were sampled, and no tissue was collected from seedlings or juveniles that did not survive to flowering and thus did not reproduce. The number of flowering plants per patch ranged from 1 to 50 individuals, with an average of 20.1. Genotypes were determined for the only three loci that were polymorphic at Tijuana Estuary (Mdh-1, Pgm-1, and Pgm-2). In total, 604 individuals were surveyed from 30 patches.

Leaf tissue was stored in a refrigerator until ground in cold phosphate extraction buffer (Mitton et al. 1979), and enzymes were separated in 12.0% Sigma starch gels (Sigma, St. Louis, MO). Aat, Adh, Ald, and Est were resolved using buffer system 7 of Soltis et al. (1983). Cat, G6p, Me, and Sod were resolved using a modification of buffer system 8 of Soltis et al. (1983; electrode buffer composed of 0.030 M lithium hydroxide, 0.190 M boric acid, pH 8.1; gel buffer composed of 0.048 M tris, 0.009 M citric acid, 0.005 M lithium hydroxide, 0.025 M boric acid, pH 8.2). Idh, Mdh, Pgd, and Pgm were resolved using buffer system 9 of Soltis et al. (1983) adjusted to pH 6.2. Staining procedures followed Soltis et al. (1983). When more than one locus was observed for an enzyme, loci were numbered sequentially with the most anodally migrating enzyme designated 1. Alleles were coded alphabetically, with the most anodally migrating allozyme designated a.

Analysis

Allele frequencies, mean number of alleles per locus (A), percent polymorphic loci (P), observed (H_{obs}) and expected (H_{exp}) heterozygosities, F-statistics (Chakraborty 1980), and genetic identities (Rogers 1972; Nei 1978) were calculated for natural colonies and populations using the computer program GeneStrut (Constantine et al. 1994). The reintroduced population at Sweetwater Marsh was evaluated by calculating allele frequencies, observed heterozygosity, and expected heterozygosities for each patch, for the set of patches sown in a particular year, and for the entire population.

Results

Genetic Variation in Natural Populations

A total of 21 loci coding the 12 enzyme systems were scored and analyzed. Two of the four populations, Carpinteria Marsh and Newport Bay, lacked any genetic variation at enzyme loci (Table 2). All colonies at Tijuana Estuary had a rare allele at Mdh-1, Pgm-1, and Pgm-2. Two colonies at Mugu Lagoon had an alternate allele at Mdh-3, and a third colony had a rare allele at Pgm-2. Tijuana Estuary was the most variable population in percent polymorphic loci and mean number of alleles per locus, but Mugu Lagoon was equally variable in heterozygosity (Table 3).

The low genetic variation in *C. m. maritimus* is reflected in low F_{ST} values (Table 4), which indicate little divergence among colonies (F_{ST1}) or populations (F_{ST2}). Likewise, genetic identities among colonies (Table 5) and populations (Table 6) are high.

Average rare allele frequencies at Tijuana Estuary, the source of seeds for the reintroduced population at Sweetwater Marsh, were 0.042, 0.065, and 0.035 at Mdh-1, Pgm-1, and Pgm-2, respectively. Variance in allele frequencies was low among colonies at Tijuana Estuary; rare allele frequencies ranged from 0.02 to 0.06 for all three polymorphic loci except for Pgm-1 at the Fifth & Iris colony (0.14). Expected heterozygosities (calculated as 2pq, where p is the frequency of the common allele, and q is the frequency of the rare allele) were 0.081, 0.122, and 0.068 (mean = 0.090) for the three loci.

Table 2. Allele frequencies at all loci in colonies of natural populations of *C. maritimus maritimus.*

	Colony													
Locus	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Aat-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Aat-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adh-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ald-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cat-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Est-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Est-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G6p-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Idh-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Idh-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.94	0.96	0.96
Mdh-1b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.04	0.04
Mdh-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-3a	1.00	0.58	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-3b	0.00	0.42	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Me-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgd-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgd-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgm-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.86	0.96	0.94
Pgm-1b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.14	0.04	0.06
Pgm-2a	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	0.98	0.94	0.96	0.98
Pgm-2b	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.04	0.02
Sod-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-3a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Genetic Variation in the Reintroduced Population

Two alleles, one common and the other rare, were found at each of the three loci surveyed. In 1995, overall rare allele frequencies at Sweetwater Marsh were 0.011, 0.004, and 0.036 at Mdh-1, Pgm-1, and Pgm-2, respectively (Table 7). Because of low activity for Mdh-1 bands on gels for 19 patches, rare allele frequency is based on 219 individuals from 11 patches. Allele frequencies were significantly lower at Sweetwater Marsh than at Tijuana Estuary for Mdh-1 ($\chi^2 = 7.91$, df = 1, $p = 0.0049$) and Pgm-1 ($\chi^2 = 59.00$, df = 1, $p < 0.0001$), but were not significantly different for Pgm-2 ($\chi^2 < 0.00$, df = 1, $p = 0.9503$). Expected heterozygosities were 0.022, 0.008, and 0.069 (mean = 0.033) for the three loci.

Table 3. Genetic variability at 21 loci in populations and colonies of *C. maritimus maritimus.*

Population/Colony	*Designation*	*A*	*P*	H_{obs}	H_{exp}
Carpinteria		1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Carpinteria	1	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Mugu Lagoon		1.10 (0.09)	9.5	0.01 (0.01)	0.01 (0.00)
Billboard	2	1.05 (0.05)	4.8	0.02 (0.02)	0.04 (0.01)
Corner	3	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Cotar	4	1.05 (0.05)	4.8	0.02 (0.02)	0.02 (0.00)
GCA	5	1.05 (0.05)	4.8	0.01 (0.01)	0.01 (0.00)
Relict	6	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Newport Bay		1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Big Canyon	7	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
North Star Beach	8	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
San Joaquin	9	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Shellmaker Island	10	1.00 (0.00)	0.0	0.00 (0.00)	0.00 (0.00)
Tijuana Estuary		1.14 (0.13)	14.3	0.01 (0.01)	0.01 (0.00)
East–West Channel	11	1.14 (0.13)	14.3	0.01 (0.01)	0.01 (0.00)
Fifth & Iris	12	1.14 (0.13)	14.3	0.02 (0.01)	0.02 (0.01)
Penninsula	13	1.14 (0.13)	14.3	0.01 (0.01)	0.01 (0.00)
Sea Coast	14	1.14 (0.13)	14.3	0.01 (0.01)	0.01 (0.00)

Table 4. Summary of F-statistics at all polymorphic loci of *C. maritimus maritimus*.

Locus	F_{IS}	F_{ST1}	F_{ST2}	F_{IT}
Mdh-1	0.023	0.000	0.001	0.024
Mdh-3	0.062	0.021	0.008	0.091
Pgm-1	0.034	0.001	0.002	0.036
Pgm-2	0.027	0.000	0.000	0.028
Mean	0.037	0.006	0.003	0.045

Table 6. Matrix of Nei's unbiased genetic identity values (above diagonal) and Roger's genetic similarity values (below diagonal) among populations of *C. maritimus maritimus*.

Population	*Carpinteria*	*Mugu Lagoon*	*Newport Beach*	*Tijuana Estuary*
Carpinteria	—	0.999	1.000	1.000
Mugu Lagoon	0.994	—	0.999	0.999
Newport Beach	1.000	0.994	—	1.000
Tijuana Estuary	0.994	0.990	0.994	—

Departure from Hardy-Weinberg equilibrium could not be tested because the low rare allele frequencies lead to very low expected numbers of homozygotes for the rare allele (less than one); combining genotypic classes for chi-square tests does not leave any degrees of freedom for statistical evaluation.

Allele frequencies and expected heterozygosities were higher in the 1991 patches, which were sown using seeds pooled from all Tijuana Estuary colonies, than in the 1992 patches, which were sown using seeds from only the Sea Coast colony at Tijuana Estuary (Table 7). Although the number of plants for Mdh-1 is smaller than for the others, the sample of 69 plants from three 1992 colonies provides less than a 5% chance of omitting an allele present at a frequency of 0.025 or greater. The number of rare alleles was too small to allow statistical evaluation of allele frequencies for Pgm-1 or Mdh-1, but the difference was significant for Pgm-2 ($\chi^2 = 13.24$, df = 1, $p = 0.0003$). Variance in allele frequency among patches sown in 1991 versus patches sown in 1992 did not differ significantly for Pgm-1 (F = 1.24; df = 11, 17; $p = 0.668$; Fig. 1), or for Pgm-2 (F = 1.96; df = 11, 17, $p = 0.207$; Fig. 1).

Most patches (27 out of 30) were missing the rare allele at Pgm-1, and the remainder had this allele at a very low frequency (Table 7, Fig. 1). Patches varied widely in allele frequency at Pgm-2, with only a slight majority of patches (17 out of 30) missing the rare allele (Table 7, Fig. 1). Of the 11 patches for which Mdh-1 data are available, 10 were missing the rare allele and the remaining patch had a rare allele frequency of 0.05. In all, half of the patches (15 out of 30) lacked any rare allele, while only two patches contained rare alleles at two of the three loci (Pgm-1 and Mdh-1 in patch 2, 1991; and Pgm-1 and Pgm-2 in patch 4, 1992).

The number of individuals in a patch (henceforward referred to as patch size) and allele frequency were correlated for Pgm-2, indicating that small patches are more likely to have high rare allele frequencies (Fig. 2; Pearson product-moment correlation coefficient $R = -0.483$, $p = 0.007$, $n = 30$). In addition, copies of the rare allele tend to be found in smaller patches. Of the 43 total copies of the rare allele at Pgm-2, 20 are found in patches with 10 or fewer individuals; given an overall frequency of 0.036 for this allele, the probability that 20 or more copies would be found in the 38 individuals making up these small patches is infinitesimally small ($p < 10^{-11}$). About two-thirds of the copies of the rare allele (28 out of 43) are found in patches with fewer than 20 individuals. Moreover, the variance in allele frequency at this locus is significantly higher among the 15 smallest ($n < 20$) than among the 15 largest ($n > 20$) patches (F = 12.75; df = 14 14; $p < 0.0001$; Sokal & Rohlf 1981).

Table 5. Matrix of Nei's unbiased genetic identity values (above diagonal) and Roger's genetic similarity values (below diagonal) among colonies of *C. maritimus maritimus*.

Colony	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1 Carpinteria	—	0.992	1.000	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
2 Billboard	0.980	—	0.992	0.999	0.992	0.992	0.992	0.992	0.992	0.992	0.992	0.990	0.991	0.991
3 Corner	1.000	0.980	—	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
4 Cotar	0.988	0.980	0.988	—	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.996	0.997	0.997
5 GCA	0.997	0.977	0.997	0.985	—	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
6 Relic	1.000	0.980	1.000	0.988	0.997	—	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
7 Big Canyon	1.000	0.980	1.000	0.988	0.997	1.000	—	1.000	1.000	1.000	1.000	0.999	1.000	1.000
8 North Star Beach	1.000	0.980	1.000	0.988	0.997	1.000	1.000	—	1.000	1.000	1.000	0.999	1.000	1.000
9 San Joaquin	1.000	0.980	1.000	0.988	0.997	1.000	1.000	1.000	—	1.000	1.000	0.999	1.000	1.000
10 Shellmaker Island	1.000	0.980	1.000	0.988	0.997	1.000	1.000	1.000	1.000	—	1.000	0.999	1.000	1.000
11 East–West Channel	0.997	0.977	0.997	0.985	0.996	0.997	0.997	0.997	0.997	0.997	—	0.999	1.000	1.000
12 Fifth & Iris	0.988	0.968	0.988	0.976	0.990	0.988	0.988	0.988	0.988	0.988	0.991	—	1.000	1.000
13 Penninsula	0.994	0.974	0.994	0.982	0.995	0.994	0.994	0.994	0.994	0.994	0.998	0.993	—	1.000
14 Sea Coast	0.994	0.974	0.994	0.982	0.993	0.994	0.994	0.994	0.994	0.994	0.998	0.993	0.998	—

Table 7. Patch size, allele frequency, and observed heterozygosity in patches at Sweetwater Marsh sown in 1991 using seeds from all colonies at Tijuana Estuary and in 1992 using seeds from only one colony (Sea Coast) at Tijuana Estuary.

		Pgm-1		*Pgm-2*		*Mdh-1*	
Patch	*N*	*q*	H_{obs}	*q*	H_{obs}	*q*	H_{obs}
1991							
1	23	0.00	0.00	0.00	0.00	0.00	0.00
2	50	0.02	0.04	0.00	0.00	0.05	0.10
3	24	0.00	0.00	0.00	0.00	0.00	0.00
4	4	0.00	0.00	0.00	0.00	0.00	0.00
5	3	0.00	0.00	0.00	0.00	0.00	0.00
6	3	0.00	0.00	0.00	0.00	0.00	0.00
7	2	0.00	0.00	0.50	1.00	—	—
8	1	0.00	0.00	0.50	1.00	—	—
9	17	0.00	0.00	0.12	0.24	0.00	0.00
10	26	0.00	0.00	0.21	0.42	0.00	0.00
11	2	0.00	0.00	0.00	0.00	—	—
12	8	0.00	0.00	0.25	0.50	—	—
Pooled	163	0.006	0.012	0.067	0.135	0.017	0.033
1992							
1	38	0.00	0.00	0.00	0.00	0.00	0.00
2	42	0.00	0.00	0.00	0.00	—	—
3	24	0.00	0.00	0.04	0.08	0.00	0.00
4	41	0.01	0.02	0.01	0.02	—	—
5	42	0.02	0.05	0.00	0.00	—	—
6	43	0.00	0.00	0.00	0.00	—	—
7	41	0.00	0.00	0.01	0.02	—	—
8	38	0.00	0.00	0.00	0.00	—	—
9	41	0.00	0.00	0.00	0.00	—	—
10	28	0.00	0.00	0.00	0.00	—	—
11	2	0.00	0.00	0.00	0.00	—	—
12	21	0.00	0.00	0.00	0.00	—	—
13	10	0.00	0.00	0.15	0.30	—	—
14	6	0.00	0.00	0.17	0.33	—	—
15	1	0.00	0.00	0.00	0.00	—	—
16	4	0.00	0.00	0.50	1.00	—	—
17	12	0.00	0.00	0.17	0.33	—	—
18	7	0.00	0.00	0.29	0.57	0.00	0.00
Pooled	441	0.003	0.007	0.024	0.048	0.00	0.00
Total pooled	604	0.004	0.008	0.036	0.071	0.011	0.023

Discussion

Genetic Variation in Natural Populations

Although the pattern of quantitative variation may differ, this survey of 21 enzyme loci suggests that genetic variation exists primarily as rare alleles at a small number of loci. Heterozygotes thus occur only with rare alleles. This feature of genetic diversity makes populations of *C. m. maritimus* highly vulnerable to the effects of genetic drift. The loss of rare alleles will automatically decrease heterozygosity, reducing both adaptive potential and the immediate fitness advantages of heterozygosity.

Genetic Drift and Reintroductions

Any reintroduction attempt involves several stages at which genetic drift can occur. The sampling of seeds from source material, mortality of seeds in storage, and the sampling of seeds for sowing may each cause random alteration of allele frequencies. After sowing, the new population may remain small for several generations. Each new generation of individuals represents a sample of the gametes produced by the preceding generation, and thus involves a biological sampling event during which allele frequencies may again be altered. The results presented here represent the accumulated effect of the genetic drift associated with the entire process of reintroduction of *C. m. maritimus* to Sweetwater Marsh.

Random fluctuations of allele frequencies occur in all finite populations, but they are more pronounced in smaller populations, resulting in the loss of all but one of the original alleles at a locus. Associated with this loss of alleles within populations are decreased heterozygosity and increased inbreeding (because of the greater average relatedness of surviving alleles). The probability of fixation is equal to initial allele frequency, and this process takes, on average, $-4N_e[p/(1-p)]\ln(p)$ generations for a neutral allele, where N_e is the effective population size and p is allele frequency (Crow & Kimura 1970). Rare alleles are thus likely to be lost at enzyme loci (at which most alleles are presumably neutral) and rather quickly in small populations. If genetic drift occurs simultaneously in a number of patches derived from a single source, the overall allele frequency across all patches is expected to remain the same, but the variance among patches will increase each generation as allele frequencies fluctuate independently in each patch. This will continue until all patches become fixed for one or another allele. The most immediate consequence of genetic drift is loss of alleles, followed by a reduction in heterozygosity (Allendorf 1986); if genetic drift is of short duration, heterozygosity may be little affected.

Genetic Drift in *C. m. maritimus* at Sweetwater Marsh

Several results of this study are consistent with the expectations of genetic drift. Rare alleles were lost from most patches, and smaller patches had widely varying rare allele frequencies. Although no alleles were lost from the entire reintroduced population, the overall rare allele frequency declined for two of the three loci, and heterozygosity was reduced.

The role of genetic drift, suggested by the loss of alleles from patches and by the accumulated variance in allele frequency among patches, is attributable to various biological attributes of the study species as well as to small patch size. The presence of a seed bank reduces the effect of genetic drift, because some of the germination each year may include seeds that were produced several years ago and that represent earlier generations. There is no direct information on the seed bank in this

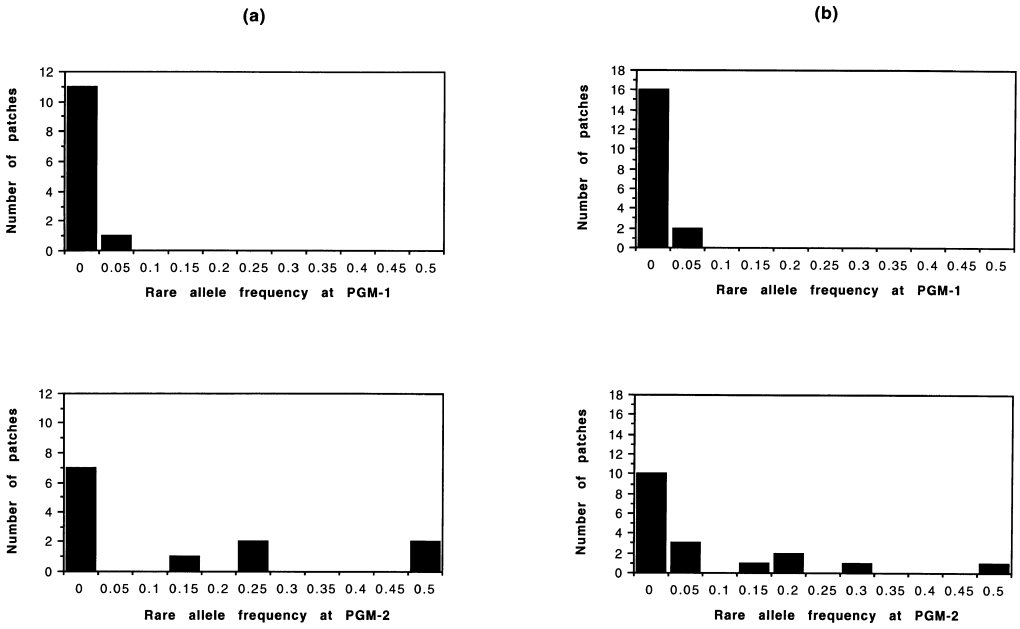

Figure 1. Distribution of rare allele frequencies at Pgm-1 and Pgm-2 for (a) 12 patches sown in 1991 using seeds from all colonies at Tijuana Estuary, and (b) 18 patches sown in 1992 using seeds from one colony (Sea Coast) at Tijuana Estuary. X-axis values are maximum allele frequencies for patches represented by the corresponding bar.

species, but the presence of ephemeral patches in natural populations and the viability of seeds for more than a year when stored in laboratory conditions suggest that a seed bank exists in natural populations. Gene flow among patches counteracts drift by reintroducing rare alleles to patches in which they have been lost. Some gene flow is likely to occur among patches of *C. m. maritimus* through pollen movement by bees. However, high variance in seed production would accentuate the effects of drift in small patches. In 1992–1994, the variance in seed capsule production at Sweetwater Marsh was approximately an order of magnitude greater than the mean (L. S. Parsons, unpublished data); this increases the effect of genetic drift because relatively few individuals produce most of the seeds (Heywood 1986). Predictions regarding genetic drift are based on randomly mating ideal populations that have a Poisson distribution of fecundity in which the mean equals the variance.

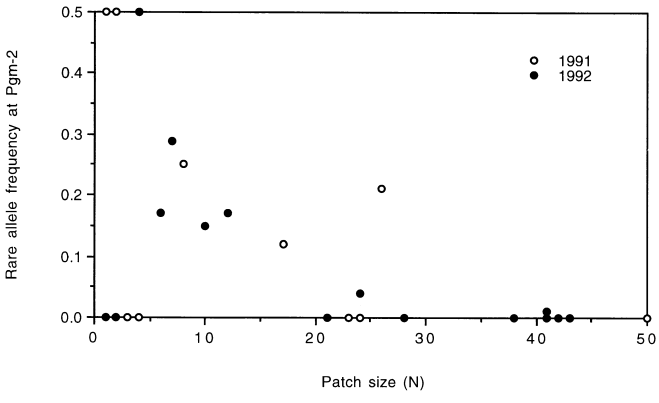

Figure 2. Relationship between rare allele frequency and patch size (number of individuals) at Pgm-2 for 30 patches sown in 1991 and 1992 at Sweetwater Marsh.

The observed variance in allele frequency among patches may be artificially high if there is a substantial seed bank in the soil. With no seed bank, the reported allele frequencies are based on all the living genotypes at each patch, even if this were only one individual. With a seed bank, the reported allele frequencies are based on only the genotypes that germinated and survived to flowering in 1995, and many of the small samples are not likely to provide accurate allele frequency estimates. However, ignoring small patches in the analysis because of possible bias paints an even bleaker picture of the genetic variation maintained at the Sweetwater Marsh reintroduction site. Most rare alleles were found in small patches, and thus their exclusion lowers estimates of overall rare allele frequencies and increases the proportion of patches from which the rare allele has been lost.

Effective Population Size

The effects of drift in ideal (mathematically convenient) populations have been theoretically explored since the 1930s (Wright 1969). Because real populations depart from the assumptions of ideal populations (diploid, sexual populations with nonoverlapping generations, random mating, equal sex ratio, and negligible selection, mutation, and gene flow), their actual size cannot be used to accurately assess drift. The concept of effective population size (N_e) provides a link between the ideal populations for which precise predictions can be made and the real populations of interest to conservation biologists. N_e is defined as the size of a theoretically

ideal population that experiences the same amount of drift as the actual population under consideration. Effective population sizes are typically one-half to one-tenth of census sizes in natural populations (Nunney & Elam 1994; Husband & Barrett 1995), indicating that drift may be considerably more potent than casual observation would suggest.

In principle, N_e can be estimated from the increase in variance in allele frequency among patches since the time of their isolation. Unfortunately, the calculation of N_e from the data reported here is problematic because *C. m. maritimus* is probably not a true annual (in which seeds would not survive more than a year), and because the observed variance is based on some very small sample sizes. However, to give a sense of the relationship between N_e and the variance in allele frequency accumulated among patches, the variance observed in Pgm-2 allele frequency is consistent with an N_e of 16.2 for 1991 patches, and 16.7 for 1992 patches (using $V = p(1-p)[1-(1-1/(2N))^t]$, where V is the variance of allele frequency among patches in which $N > 10$, p is the initial allele frequency estimated as the overall weighted allele frequency at Sweetwater Marsh, and t is the number of generations over which the variance in allele frequency has accumulated (Crow & Kimura 1970). These values are lower than the mean 1995 census sizes of 28.0 and 34.3 for patches sown in 1991 and 1992, respectively, and indicate that over the entire reintroduction period, the amount of drift that has generated allele frequency differences among patches approximates that incurred by theoretically ideal populations of only about 16 individuals.

Recommendations for Future Reintroduction Attempts

Maintaining genetic variation during the process of reintroduction is difficult when natural levels of variability are low. Even minor episodes of genetic drift can cause loss of alleles and simultaneous reduction of heterozygosity in reintroduced populations of *C. m. maritimus*. Preventing this outcome requires higher sample sizes and population sizes than in species with higher levels of variability. Seeds should therefore be collected from the largest possible number of maternal plants at the source population to ensure that rare alleles are included in the sample. The sowing of much larger numbers of seeds in patches would also appear necessary to ensure the maintenance of genetic variability through the process of reintroduction and establishment of a self-sustaining population. It is clear from this study that sowing 200–400 seeds per patch is not enough to ensure maintenance of genetic variability at enzyme loci. Much larger sowing sizes, numbering perhaps at least in the thousands, are needed, as indicated by this study. This will be vital for long-term persistence of reintroduced populations if heterozygosity increases immediate fitness or if genetic variation is required for adaptation to a changing environment.

Unfortunately, recommending the sowing of more seeds per patch is at odds with the ecology of *C. m. maritimus*. Suitable habitat for this hemiparasitic plant generally consists of small patches of open space, which provide both light and nearby individuals of host species. At San Quintin Bay (Baja California, Mexico), cattle grazing has created large areas of poor canopy that are suitable for *C. m. maritimus*, but similar large areas of suitable habitat would have to be created at Sweetwater Marsh (J. Zedler, personal communication). A more practical solution to minimize the loss of genetic variation within patches is to sow seeds in sets of contiguous patches; the close proximity of other patches would increase gene flow, counteracting the effects of genetic drift. Another alternative, if sets of contiguous patches of suitable habitat are not available, is to reseed periodically from natural populations to reintroduce the rare alleles that are easily lost from small patches; however, this method would be labor intensive, and the reintroduced population could hardly be considered self-sustaining.

The question of whether a single large or several smaller patches should be sown at a reintroduction site is analogous to the debate regarding reserve size (Chesser 1991; Nunney & Campbell 1993). A single large patch reduces, but does not eliminate, the effects of genetic drift. Although rare alleles are likely to be eventually lost even in large patches, smaller patches offer even dimmer prospects for the continued presence of rare alleles. Genetic drift is likely to drive the common allele to fixation very quickly at a majority of patches. Although some patches may become fixed for the rare allele (on average, 1 out of 20 for an allele with a frequency of 0.05), the chance extinction of such patches would automatically eliminate the rare allele from the entire population. Extinction of patches already appears to be occurring at Sweetwater Marsh. Heterozygotes are only found in about half the patches and primarily in the very small ones which are least likely to persist. Moreover, any benefits of heterozygosity are lost as patches become fixed for alleles, because heterozygotes will be reduced in frequency. Very large patches, or large sets of contiguous patches, must therefore be considered superior to the planting scheme that was used in 1991 and 1992.

Because nothing is currently known about possible adaptation of colonies or populations of *C. m. maritimus* to their local habitat, it is difficult to make recommendations regarding appropriate source material for reintroductions. However, in the absence of specific information regarding local adaptation and the characteristics of proposed new habitats, the use of seeds collected from a number of colonies at a nearby marsh may best preserve

genetic variation while maintaining adaptation. Patches sown in 1991 with seeds collected from a number of colonies at Tijuana Estuary preserved more genetic variation than patches sown in 1992 with seeds from just one colony. Collecting seeds from several colonies will generally increase genetic variation when allele frequencies vary among the colonies of a population.

Summary

The reintroduction of *C. m. maritimus* to Sweetwater Marsh has to be considered at least a partial success in the maintenance of genetic variation. Although performed without the benefit of genetic data, no rare alleles have yet been lost in the group of reintroduced patches. However, allele frequencies and heterozygosities are reduced in comparison to the source population. Moreover, rare alleles are primarily found in very small patches that are likely to become extinct. Thus, the reintroduced population is in peril of losing its remaining genetic variation. This study illustrates the risk of loss of genetic variation in reintroduction attempts involving small patch sizes in species where natural levels of variation are low.

Acknowledgments

Brian Fink helped collect the plant material for this study (under Federal Fish and Wildlife Regional Blanket Permit PRT-702631, Endangered Species Act Subpermit ZEDLJ-2). Rafi Hanna, Don Koski, Dave Truesdale, and Lori Wilkerson provided assistance with laboratory work. Joy Zedler provided invaluable information about the study species. Wayne Ferren (Carpinteria Marsh), Tom Keeney (Point Mugu), Troy Kelly (Newport Bay), and Mari von Hoffman (Tijuana Estuary) provided access to study populations. Teri McGuire, Abby Powell, and Connie Rutherford provided help with obtaining permits and other administrative matters. This project was supported by a grant from the U.S. Fish and Wildlife Service.

LITERATURE CITED

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology **5:**181–190.

Allendorf, F. W., and R. F. Leary. 1986. Heterozygosity, heterosis, and fitness in natural populations of animals. Pages 57–76 in M. E. Soule, editor. Conservation Biology. Sinauer Associates Inc., Sunderland, Massachusetts.

Chakraborty, R. 1980. Gene diversity analysis in nested, subdivided populations. Genetics **96:**721–726.

Chesser, R. K. 1991. Influence of gene flow and breeding tactics on gene diversity within populations. Genetics **129:**573–583.

Constantine, C. C., R. P. Hobbs, and A. J. Lymbery. 1994. FORTRAN programs for analyzing population structure from multilocus genotypic data. Journal of Heredity **85:**336–337.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York, New York.

DeMauro, M. M. 1994. Development and implementation of a recovery program for the federal threatened Lakeside daisy (*Hymenoxys acaulis* var. *glabra*). Pages 298–321 in M. L. Bowles and C. J. Whelan, editors. Restoration of endangered species. Cambridge University Press, Cambridge, United Kingdom.

Fink, B., and J. B. Zedler. 1991. Salt marsh bird's-beak seed broadcasting and habitat enhancement in San Diego County. California Department of Transportation. San Diego, California.

Haig, S. M., J. D. Ballou, and S. R. Derrickson. 1990. Management options for preserving genetic diversity: reintroduction of Guam Rails to the wild. Conservation Biology **4:**290–300.

Heywood, J. S. 1986. The effect of plant size variation on genetic drift in populations of annuals. American Naturalist **127:** 851–861.

Husband, B. D., and S. C. H. Barrett. 1995. Estimating effective population size: a reply to Nunney. Evolution **49:**392–394.

Jackson, L. L., N. Lopoukhine, and D. Hillyard. 1995. Ecological restoration: a definition and comments. Restoration Ecology **3:**71–75.

Lesica, P., and F. W. Allendorf. 1992. Are small populations of plants worth preserving? Conservation Biology **6:**135–139.

Lincoln, P. G. 1985. Pollinator effectiveness and ecology of seed set in *Cordylanthus maritimus* ssp. *maritimus* at Point Mugu, California. U.S. Fish and Wildlife Service, Endangered Species Office, Sacramento, California.

Mitton, J. B., Y. B. Linhart, K. B. Sturgeon, and J. L. Hamrick. 1979. Allozyme polymorphism detected in mature needle tissue of ponderosa pine. Journal of Heredity **70:**86–89.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:** 583–590.

Nunney, L., and K. A. Campbell. 1993. Assessing minimum viable population size: demography meets population genetics. Trends in Ecology and Evolution **8:**234–239.

Nunney, L., and D. R. Elam. 1994. Estimating the effective population size of conserved populations. Conservation Biology **8:**175–184.

Parsons, L. S., and J. B. Zedler. 1997. Factors affecting reestablishment of an endangered annual plant at a California salt marsh. Ecological Applications **7:**253–267.

Pavlik, B. M., D. L. Nickrent, and A. M. Howald. 1993. The recovery of an endangered plant. I. Creating a new population of *Amsinckia grandiflora*. Conservation Biology **7:**510–526.

Purer, E. 1942. Plant ecology of the coastal salt marshlands of San Diego County. Ecological Monographs **12:**82–111.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Press, Austin, Texas **7213:**145–153.

Russell, W. C., E. T. Thorne, R. Oakleaf, and J. D. Ballou. 1994. The genetic basis of black-footed ferret reintroduction. Conservation Biology **8:**263–266.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Freeman and Company, New York, New York.

Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal **73:**9–27.

USFWS (U.S. Fish and Wildlife Service). 1984. Recovery plan for salt marsh bird's-beak (*Cordylanthus maritimus* subsp. *maritimus*). U.S. Fish and Wildlife Service, Portland, Oregon.

Vanderwier, J. M., and J. C. Newman. 1984. Observations of haustoria and host preference in *Cordylanthus maritimus* ssp. *maritimus* at Mugu Lagoon. Madroño **31:**185–186.

Wright, W. 1969. Evolution and the genetics of populations, vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago, Illinois.