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Restoration genetics of the vernal pool endemic *Lasthenia conjugens* (Asteraceae)

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Abstract

Restoration of habitat for endangered species often involves translocation of seeds or individuals from source populations to an area targeted for revegetation. Long-term persistence of a species is dependent on the maintenance of sufficient genetic variation within and among populations. Thus, knowledge and maintenance of genetic variability within rare or endangered species is essential for developing effective conservation and restoration strategies. Genetic monitoring of both natural and restored populations can provide an assessment of restoration protocol success in establishing populations that maintain levels of genetic diversity similar to those in natural populations. California's vernal pools are home to many endangered plants, thus conservation and restoration are large components of their management. *Lasthenia conjugens* (Asteraceae) is a federally endangered self-incompatible vernal pool annual with gravity-dispersed seeds. Using the molecular technique of intersimple sequence repeats (ISSRs), this study assessed levels and patterns of genetic variability present within natural and restored populations of *L. conjugens*. At Travis Air Force Base near Fairfield, California, a vernal pool restoration project is underway. Genetic success of the ecologically based seeding protocol was examined through genetic monitoring of natural and restored populations over a three-year period. Genetic diversity remained constant across the three sampled generations. Diversity was also widely distributed across all populations. We conclude that the protocol used to establish restored populations was successful in capturing similar levels and patterns of genetic diversity to those seen within natural pools. This study also demonstrates how genetic markers can be used to inform conservation and restoration decisions.

Introduction

The primary aim of restoration ecology is to return ecosystems that have been damaged, degraded, or destroyed to their historical trajectories (Society for Ecological Restoration Working Group 2002). In doing so, it is often necessary to take individuals, or propagules, from one or more source populations to create a restored population. In most cases, selection of source populations is based solely on the locality and availability of source populations rather than on knowledge of the levels and distribution of genetic variation in potential sources.

Population genetic theory and empirical evidence have shown that genetic variability is positively correlated with short- and long-term population viability (Fisher 1930; Frankham 1995; Newman and Pilson 1997; Wise et al. 2002; Fischer

et al. 2003). Thus, assuring that restored populations maintain similar levels of genetic variability to those present in source or reference populations is essential for the survival of restored populations. Data on the amount and structure of genetic variation found in source and restored populations provide researchers and managers with the critical information required to assess the success of restoration protocols. It also provides an empirical framework for future research and management.

Despite the widespread application of molecular tools in studies of rare plant populations (see Falk and Holsinger 1991 and references therein; Gemmill et al. 1998; Fleishman et al. 2001; Lopez-Pujol et al. 2003, among others) genetic data have only recently been incorporated into restoration efforts. For example, Pavlik et al. (1993) used estimates of genetic variability within potential source populations as the main criterion in choosing source populations for the restoration of *Amsinckia grandiflora* (Boraginaceae) in grasslands of northern California. A second study, in Australia, incorporated genetic data to assess the impact of mining on genetic variability in *Hemigenia exilis* (Lamiaceae), and to guide restoration once mining has ceased (Mattner et al. 2002). Recently, genetic studies have been used to evaluate success of restoration efforts. Allozyme analysis has been used to assess genetic variability of restored or transplanted populations of *Cordylanthus maritimus* ssp. *maritimus* (Scrophulariaceae; Helenurm and Parsons 1997), *Spartina alterniflora* (Poaceae; Travis et al. 2002), and *Zostera marina* (Zosteraceae; Williams and Davis 1996; Williams and Orth 1998). For several species, DNA-level markers were used to compare levels of genetic variation in restored populations with the source populations from which the restored populations originated. The technique of intersimple sequence repeat analysis (ISSR) was applied to the coastal species *Abronia umbellata* subsp. *breviflora* (Nyctaginaceae; McGlaughlin et al. 2002). Random amplified polymorphic DNA (RAPD) analysis was applied to the prairie species *Dalea purpurea* (Fabaceae; Gustafson et al. 2002), *Andropogon gerardii* (Poaceae), and *Sorghastrum nutans* (Poaceae; Gustafson et al. 2004), as well as the Chinese gymnosperm *Metasequoia glyptostroboides* (Taxodiaceae; Li et al. 2005).

DNA fragment analyses provide inexpensive, alternative molecular techniques for estimating population variability and structure in restored species (e.g., ISSRs, RAPDs, and amplified fragment length polymorphisms (AFLPs)). These types of marker data are capable of generating a large number of conservation-informative, polymorphic loci without the use of prior sequence knowledge (Lynch and Milligan 1994; Godwin et al. 1997; Wolfe and Liston 1998). These techniques survey the genetic variation across the genome of an organism, fragment the DNA, and then use variation in fragment size to generate estimates of population variation and structure. ISSR analysis uses a single primer in the PCR process to amplify regions of DNA that lie between two identical microsatellite (repeat) regions within the genome (Zietkiewicz et al. 1994). Through the use of ISSRs, population levels of genetic differentiation have been successfully examined in the following plant taxa: *Saxifraga rivularis* L. (Hollingsworth et al. 1998), *Viola pubescens* (Culley and Wolfe 2001), *Oryza granulata* (Qian et al. 2001), and *Hyobanche* spp. (Wolfe and Randle 2001; see also Jain et al. 1999; Lai et al. 2001 among others).

In this study, we employed ISSRs to evaluate the effectiveness of the seeding protocol used in a vernal pool restoration project in capturing the genetic diversity present within source populations. We also demonstrate the use of genetic markers to inform future restoration decisions.

California's vernal pools are a highly threatened ecosystem within which restoration and habitat creation are broadly used as means to offset habitat destruction (see Black and Zedler 1998; and Ferren et al. 1998). Vernal pools are characterized as seasonally flooded depressions in the landscape with an impermeable soil layer underneath, often claypan or hardpan (Stone 1990). They are ephemeral wetlands that exhibit both terrestrial and aquatic ecosystem traits, and display a patchy, spatially discrete, island-like distribution within a grassland matrix (Jain 1976; Zedler 1990). The Central Valley of California has a Mediterranean climate that once supported an extensive array of vernal pools, it has been estimated that only about 10% of original vernal pool habitat remains due to destruction and land conversion (Baskin 1994).

We examined the genetic effectiveness of the seeding protocol used to restore the vernal pool endemic species *Lasthenia conjugens* E. Greene (Asteraceae) at Travis Air Force Base (TAFB) near Fairfield, California. Both natural and

restored populations were sampled in three consecutive years to evaluate if and how genetic diversity changed over three generations. The specific questions addressed were: (1) what are the levels and patterns of genetic diversity within natural (source) populations of *L. conjugens* at TAFB, (2a) what are the levels and patterns of genetic diversity within restored populations of *L. conjugens* at TAFB, (2b) does genetic diversity within and among restored populations differ as a function of distance from source populations, (2c) does genetic diversity within and among restored populations vary as a function of seeding treatment, and (3) does the genetic diversity in natural and/or restored populations change over three generations? General conclusions and future restoration recommendations are made based on the results.

Materials and methods

Study species

The federally endangered annual herb *Lasthenia conjugens* (section *Ornduffia*, tribe Heliantheae; Chan 2001; commonly called Contra Costa Goldfields); is one of more than 60 species of plants endemic to vernal pools (Ornduff 1966; Zedler 1990). There are 17 species in *Lasthenia*, 10 are endemic to the California Floristic Province and nine are found in or around vernal pools (Thorp 1976; Desrochers and Dodge 2003). *Lasthenia conjugens* is self-incompatible and relies on animal-mediated pollination for seed set (Ornduff 1966). In this species, gene flow among populations has been reported to occur primarily via the movement of pollen carried by solitary bees rather than the dispersal of seeds, because the seeds are gravity dispersed (Ornduff 1966).

Historically, *L. conjugens* populations occurred in seven counties in California including Alameda, Contra Costa, Mendocino, Napa, Santa Barbara, Santa Clara, and Solano (U.S. Fish and Wildlife Service 1997). At the time of listing under the U.S. Endangered Species Act in 1997, 13 populations were known from four California counties. Currently, populations are known to exist in 6 counties (U.S. Fish and Wildlife Service 2003; Ramp 2004) but the species remains threatened due to continued habitat loss. As a result of its rarity, *L. conjugens* is the focus of a restoration project at Travis AFB, Solano County.

Study site and sampling strategy

The present study investigated the genetic variation found within and among populations of *L. conjugens* in vernal pools at Travis AFB, near Fairfield, California (Solano County, 38.266 N, 121.972 W). The site historically supported an extensive vernal pool complex, within which pools were altered or destroyed by agriculture and construction of a small runway in 1952 (Biosystems Analysis 1994). Today, there are 82 semi-natural vernal pools surrounding the runway, taxiway, and buildings (the Aeroclub). These pools were heavily disturbed and inadvertently reconfigured during the construction of the Aeroclub and are hereafter termed 'natural pools'. In addition, there are also 256 constructed pools (constructed in November 1999, termed 'restored pools') forming a grid to the North and the South of the airstrip (Figure 1). For a complete description of the study site see Gerhardt and Collinge (2003).

In December 1999, 192 of the restored pools were each seeded with 100 *L. conjugens* seeds. The ecologically based seeding protocol was designed and completed before the development of this genetic study, and was done with no prior knowledge of genetic variability within the species at or outside of Travis AFB. Three seeding treat-

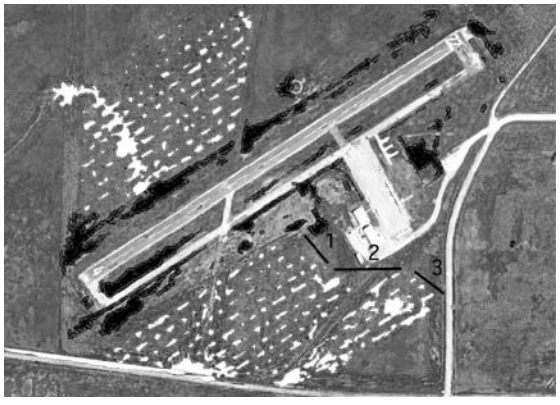

Figure 1. Aerial image of the Travis AFB Aeroclub. Natural pools are represented in black immediately surrounding the runway and buildings. White areas represent the restored pools in a grid to the North and South of the runway. Distance classes are labeled.

ments were used as part of a larger ecological study examining community assembly (S. K. Collinge et al. unpublished). Each treatment consisted of placing 100 seeds (10 seeds from each of 10 unique maternal plants on site) from each seeded species into a 0.5 m^2 seed plot established within each restored pool. One treatment consisted of seeding only *L. conjugens* in 1999. A second treatment consisted of seeding three species (*L. conjugens*, *Eryngium vaseii* J. Coult. and Rose (Apiaceae), and *Deschampsia danthonioides* Munro (Poaceae)) in 1999, and *L. conjugens* along with *Layia chrysanthemoides* (DC ex Lindl.) A. Gray (Asteraceae), and *Plagiobothrys stipitatus* I.M. Johnst. (Scrophulariaceae) in 2000. The third treatment consisted of seeding the two groups of species in the reverse order in 1999 and 2000. Sampling for this study was done from all treatment groups to determine if genotype composition within a population was affected by species composition at time of seeding.

Three distance classes away from the natural pools were delineated within the restored pools on either side of the runway. In a mark-and-recapture study, the primary pollinators, members of the bee subgenera *Diandrena* and *Hesperandrena* (Andrenidae) (Thorp 1976), were not captured more than 20 m from the original site of marking (Thorp 1990). Thus, distance between vernal pools may play an important role in gene flow among pools. The grid of restored pools consists of 11 rows on either side of the runway. We designated the first four rows (10–40 m) as the first distance class, the second four rows (50–80 m) as the second distance class, and the remaining three rows (90–110 m) as the third distance class. Sampling from all three distance classes allowed for a comparison of genetic diversity within and among groups of restored pools that may or may not receive more gene flow than others.

The sampling strategy of this study was designed to examine genetic variability within several different groups based on population type (natural or restored), seeding treatment, and distance class. In the spring of 2001, restored pools were chosen for sampling based on seeding treatment, distance class, and the total number of *Lasthenia conjugens* plants per pool. For the purposes of this study each pool was considered to be a distinct population. The aim was to collect leaves from 10 plants from each of 10 populations within each treatment group and distance class. Often, fewer than 10 individuals were present within a restored pool and samples were collected from all individuals. Samples were collected from populations on both sides of the runway. Natural pools were chosen initially based on populations that were still flowering in early May 2001, when collections occurred. From the natural populations, 20 individuals were collected from each pool sampled.

In 2001, 140 individuals from 7 natural, and 652 individuals from 79 restored pools were collected (Table 1). Because we were able to arrive at the field site early in the flowering seasons in 2002 and 2003, we were able to sample from more populations. For collections made in 2002 and 2003, three natural, and six restored pools were added. Several restored pools were dropped from the study in 2002 due to a lack of *L. conjugens*. In 2002, 198 individuals were collected from 10 natural, and 706 individuals were collected from 76 restored pools (Table 1). In 2003, 200 individuals were collected from 10 natural, and 709 individuals were collected from 74 restored pools (Table 1).

DNA extraction and ISSR amplification

Leaf tissue was stored in silica gel. From all 2001 samples, and all but 200 of the 2002 samples, genomic DNA was extracted from leaf tissue following the CTAB protocol of Doyle and Doyle (1987) modified by adding 3% PVP-40 and 5 mM ascorbic acid. Extracted DNA was standardized to a concentration of 10 ng/μL with the aid of a minifluorometer. The remaining 2002 samples and all of the 2003 samples were extracted at the National Center for Genetic Resources Preservation, in Fort Collins, CO, using Qiagen DNeasy® 96 plant kits.

PCR amplification reactions initially contained 10× PCR Buffer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 5% 0.01 g/ml concentration BSA in the total reaction volume, 0.68 U of Taq Polymerase, 0.4 μM primer, and 1 μL of DNA in 17 μL reactions. PCR conditions were 94 °C (90 s), followed by 34 cycles of 94 °C (40 s), 45 °C (45 s), and 72 °C (90 s), followed by 94 °C (45 s), and 45 °C (45 s) ending with 5 min at 72 °C after cycling was completed. There were slight modifications of the reaction and amplification protocols for each primer in order to obtain the best possible amplification. Forty-eight primers were screened from the

Table 1. Individual samples collected by year, pool type, restored distance class and seeding treatment

Year/Pool type	Seeding treatment used	Number of pools	Number of individuals
2001			
Natural	None	7	137
Restored	Distance 1	25	228
	Distance 2	27	223
	Distance 3	27	201
	Lasthenia conjugens only	19	145
	Group 1 ('99), Group 2 ('00)	29	265
	Group 2 ('99), Group 1 ('00)	31	242
	Total of Restored populations	79	652
Total	86	789	
2002			
Natural	None	10	198
Restored	Distance 1	25	237
	Distance 2	28	244
	Distance 3	23	225
	Lasthenia conjugens only	18	164
	Group 1 ('99), Group 2 ('00)	30	288
	Group 2 ('99), Group 1 ('00)	28	254
	Total of restored populations	76	706
	Total	86	904
2003			
Natural	None	10	200
Restored	Distance 1	25	236
	Distance 2	27	259
	Distance 3	22	214
	Lasthenia conjugens only	17	160
	Group 1 ('99), Group 2 ('00)	30	295
	Group 2 ('99), Group 1 ('00)	27	254
	Total of restored populations	74	709
	Total	84	909

Note that restored pools fall within both a seeding treatment and distance class category even though they are listed separately in the table.

University of British Columbia Primer Kit #9. Three primers showing bright, reproducible bands were chosen for amplification of all individuals. The primers employed were: 811: $(GA)_8$-C; 845: $(CT)_8$-RG; and 850: $(GT)_8$-YA. Fragments generated by amplification were separated by size in 1.5% agarose gels run in TBE buffer, stained with ethidium bromide, and visualized by illumination with UV light. Digital images were captured for each gel.

The reproducibility of amplification was tested for each primer prior to data collection. Positive and negative controls were included on each gel to control for contamination and amplification. Kodak 1D image analysis software (Eastman Kodak) was used to estimate the number of base-pairs represented by each amplified fragment. Fragments (bands) with the same molecular weight and mobility were estimated based on 1-Kb ladder size standards (Invitrogen). Fragment sizes were assigned to loci for each primer (Table 2). Loci were scored as diallelic (1 = band present, 0 = band absent).

Analyses

Genetic diversity

Genetic diversity was estimated within and among the sampled populations with Shannon's Diversity Index (I) (Lewontin 1972), and Nei's (1973) gene diversity (h). The Shannon's Diversity and Nei's gene diversity indices were calculated in POPGENE v1.31 (Yeh et al. 1999). Shannon's Diversity Index is calculated as $I = -\Sigma\, p_i \log_2 p_i$, where p_i is the frequency of the i^{th} ISSR band. This index is

Table 2. Primer sequences and band statistics for the three primers used

Primer	Total bands	Size (bp) Min–Max	Polymorphic bands	Percent polymorphism
811 – GAGAGAGAGAGAGAGA-C	30	515–2675	30	100
845 – CTCTCTCTCTCTCTCT-RG	30	310–2450	30	100
850 – GTGTGTGTGTGTGTGT-YA	24	400–1785	24	100
Total	84		84	100

appropriate for qualitative data and is relatively insensitive to the dominant nature of ISSR data (i.e., the inability to detect heterozygotes) (Dawson et al. 1995). Nei's gene diversity is calculated as $h = 1 - \Sigma_k x^2_k$ where the frequency of the kth allele in the population is represented by x_k. This index yields fairly accurate estimates of diversity for selfing plants in which heterozygotes are infrequent. However, with mainly outcrossing species, Hardy–Weinberg equilibrium must be assumed for each locus, possibly leading to a bias in the estimate (Nybom and Bartish 2000). Lynch and Milligan (1994) suggest including only bands present with an observed frequency of less than 1−(3/N) (where N represents the sample size) to reduce a potential bias from using dominant markers. In this study, all 84 bands were present in a frequency of less than 1−(3/N), so they were all included in the diversity estimates.

Differences in diversity statistics among and within population types and years were examined. Kolmogorov–Smirnov tests were used to test for normal distribution of each data set. All data were non-normally distributed so non-parametric statistics were applied. To compare Shannon's Diversity estimates of natural and restored populations within years, Mann–Whitney U tests were used. Differences among years were examined for all populations combined, and for each population type independently, with Kruskal–Wallis tests. The analyses were repeated to examine differences in Nei's gene diversity estimates within and among years and population types. When differences existed among years for a diversity estimate, pairwise Mann–Whitney U tests were conducted with a Holm's sequential Bonferroni correction for multiple tests to examine specific differences between years.

Genetic structure

Hierarchical genetic structure was examined through an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) as implemented in Arlequin 2.0 (Schneider et al. 2000). AMOVA describes how genetic variation is partitioned within and among populations, and tests for significance against the null hypothesis of no population structure (Stewart and Excoffier 1996). The AMOVA approach also computes ϕ_{ST}, a statistic analogous to F_{ST}. We conducted several AMOVA analyses to examine hierarchical genetic structure. Partitioning of genetic variation among natural and restored populations was examined for each year independently. Partitioning of genetic variation among the three years of sampled natural populations was also analyzed. It was not possible to analyze all three years of restored populations together due to the large size of the data set. Within each year diversity was also partitioned among distance classes and seeding treatments.

Results

Descriptive statistics from ISSR analyses

A total of 84 loci were scored across all three primers for all individuals with 24–30 bands per primer (Table 2). No loci were monomorphic across all populations for all years. Each year there were 82 (97.62%) polymorphic loci present across all individuals. For both 2001 and 2002, one unique locus was sampled for each year. The number of bands present per population, the number of polymorphic loci per population, and the percentage of polymorphic loci per population are presented in Appendix A.

Genetic diversity

Genetic diversity estimates for natural, restored, and all populations combined for each year are presented in Table 3. The average I values for natural and restored populations across the three

Table 3. Diversity estimates for both natural and restored populations for all three years

Year	Population type	Shannon's Diversity (*I*)	Nei's gene diversity (*h*)	# Polymorphic Loci (%)
2001	Natural	0.374 (0.230)[a,b]	0.239 (0.171)[a,b]	77 (91.67)
	Restored	0.396 (0.207)[c]	0.252 (0.156)[c]	82 (97.62)
	Both	0.397 (0.208)	0.252 (0.156)[d]	82 (97.62)
2002	Natural	0.399 (0.209)[a]	0.254 (0.158)[a]	82 (97.62)
	Restored	0.404 (0.207)[c]	0.257 (0.158)[c]	82 (97.62)
	Both	0.405 (0.204)	0.258 (0.156)[d]	82 (97.62)
2003	Natural	0.413 (0.219)[a,b]	0.267 (0.166)[a,b]	81 (96.43)
	Restored	0.388 (0.223)[c]	0.248 (0.169)[c]	82 (97.62)
	Both	0.396 (0.220)	0.254 (0.168)[d]	82 (97.62)

Values presented are the total values for the population type and all populations combined. Standard deviations are in parentheses. Statistically significant comparisons are indicated. For diversity estimates of individual populations see Appendix A.

[a] Statistical comparisons among natural populations of all three years significant at $P < 0.05$ for both Shannon's Diversity and Nei's gene diversity.

[b] Statistical comparison of natural populations from 2001 with natural populations from 2003 significant at $P < 0.01$ for Shannon's Diversity and Nei's gene diversity.

[c] Statistical comparisons between natural and restored populations within a year significant at $P < 0.01$ for both Shannon's Diversity and Nei's gene diversity.

[d] Statistical comparisons of all populations combined within a year among the three years significant at $P < 0.05$ for Nei's gene diversity only.

years were 0.395 (±0.219), and 0.396 (±0.212), respectively. The average values of *h* for natural and restored populations across the three years were 0.253 (±0.165) and 0.252 (±0.161), respectively. The average diversity estimates for all populations combined (natural and restored) across the three years were $I = 0.399$ (±0.211) and $h = 0.255$ (±0.160). Diversity estimates for each population for each year are displayed in Appendix A.

In 2001 and 2002, estimates of *I* were significantly greater in restored populations ($I = 0.396$ and 0.404, respectively) than in natural populations ($I = 0.374$ and 0.399, respectively; 2001: $P = 0.017$; 2002: $P = 0.001$). By contrast, in 2003 *I* was significantly greater in natural populations ($I = 0.413$) than in restored populations ($I = 0.388$; $P < 0.001$). Similarly, in 2001 and 2002, estimates of *h* were also significantly greater in restored populations ($h = 0.252$ and 0.257, respectively) than in natural populations ($h = 0.239$ and 0.254, respectively; 2001: $P = 0.048$; 2002: $P = 0.003$) and, in 2003, *h* was significantly greater in natural populations ($h = 0.267$) than in restored populations ($h = 0.248$, $P < 0.001$).

Values of *I* from all populations combined among the three years sampled did not differ across the three years ($\chi^2_{(2,N=258)} = 5.42$, $P = 0.066$). By contrast, values of Nei's gene diversity index across the three years sampled were significantly different ($\chi^2_{(2,N=258)} = 6.04$, $P = 0.049$), however, there were no significant differences between pairs of years (data not shown).

Shannon's Diversity Index values estimated for natural populations differed significantly among the three years ($\chi^2_{(2,=27)} = 6.65$, $P = 0.036$). Values of *I* differed significantly between 2001 and 2003 ($P = 0.005$ (corrected α value = 0.017)), however, the other pairwise comparisons were not significant (data not shown). Nei's gene diversity index values estimated for natural populations were significantly different among the three years sampled ($\chi^2_{(2,N=27)} = 6.18$, $P = 0.045$). The comparison of Nei's gene diversity between 2001 and 2003 was significant ($P = 0.007$ (corrected α value = 0.017)), however, the other pairwise comparisons were not significant (data not shown).

Values of Shannon's Diversity Index estimated for restored populations among the three years were not significantly different ($\chi^2_{(2,N=231)} = 3.64$, $P = 0.162$), nor were the values of Nei's gene diversity index ($\chi^2_{(2,N=231)} = 4.16$, $P = 0.125$).

Genetic structure

Genetic structure within sampled natural and restored populations was examined independently for each year. Genetic structure among the three years of natural population data was also examined. There was very little genetic structure

defining natural versus restored populations in any of the three years (Table 4). On average, only 1.45% of the variation was partitioned between natural and restored populations across the three years. Genetic variation partitioned among populations within population type was higher with an average of 9.33%. The majority (an average of 89.22%) of the genetic variation was partitioned within populations in each year. When examining the three years of natural population samples together, 4.40% of the variation was distributed across the years. The variation partitioned among populations within each year was 5.44% (Table 5).

When partitioning the variation among restored population distance classes, an average of 1.11% of the variation could be attributed to distance class within each year. An average of 9.80% of the variation was partitioned among populations within distance classes (Table 6). Seeding treatment groups within restored populations did not significantly affect the structure of genetic variation in any of the three years sampled (Table 7). In all three years, when partitioning diversity either across distance classes or seeding treatment groups of restored populations, the majority of the variation was found within populations.

Discussion

Genetic diversity estimates generated through the use of ISSRs

The results of this study show that the ecologically based seeding protocol used to establish populations of *Lasthenia conjugens* at Travis AFB was successful in capturing the majority of the genetic variation present within natural populations and distributing it to the restored populations. There was a high level of genetic variability found at Travis AFB with all 84 loci examined being polymorphic across all years of the study. There was high polymorphism within each year as well, with 82 polymorphic loci per year.

Both Shannon's Diversity and Nei's gene diversity indices yielded moderate to high levels of

Table 4. AMOVA results examining genetic partitioning between natural and restored populations

Source of variation	df	SSD	CV	% Total	ϕ_{ST}
2001					
Among population type	1	70.88	0.2159	2.37***	0.1084
Among populations within population type	84	1266.90	0.7719	8.47***	
Within populations	701	5695.64	8.1250	89.16***	
2002					
Among population type	1	53.72	0.0876	0.86**	0.1065
Among populations within population type	83	1623.58	1.0036	9.80***	
Within populations	805	7369.43	9.1546	89.35***	
2003					
Among population type	1	66.73	0.1231	1.13***	0.1086
Among populations within population type	82	1718.09	1.0538	9.72***	
Within populations	824	7964.04	9.6651	89.15***	

All three years are presented. Statistics include: df – degrees of freedom, SSD – sum of squares, CV – variance component estimates, % Total – percentage of the total variance contributed by each component, and ϕ_{ST}.
$^{**}P < 0.01$, $^{***}P < 0.001$.

Table 5. AMOVA results examining genetic partitioning among natural populations for the three years combined

Source of variation	df	SSD	CV	% Total	ϕ_{ST}
Among year	2	215.84	0.4886	4.40^{***}	0.0569
Among populations within year	24	527.03	0.6033	5.44^{***}	
Within populations	508	5082.36	10.0047	90.16^{***}	

Statistics include: df – degrees of freedom, SSD – sum of squares, CV – variance component estimates, % Total – percentage of the total variance contributed by each component, and ϕ_{ST}.
$^{***}P < 0.001$.

Table 6. AMOVA results examining genetic partitioning among the three restored population distance classes

Source of variation	df	SSD	CV	% Total	ϕ_{ST}
2001					
Among distance classes	2	78.83	0.1110	1.27***	0.0964
Among populations within distance class	76	1053.70	0.7319	8.37***	
Within populations	571	4511.50	7.9011	90.36***	
2002					
Among distance classes	2	103.53	0.1433	1.41***	0.1152
Among populations within distance class	72	1325.32	1.0245	10.11***	
Within populations	617	5532.86	8.9674	88.48***	
2003					
Among distance classes	2	73.46	0.0699	0.66***	0.1157
Among populations within distance class	70	1421.05	1.1493	10.91***	
Within populations	625	5824.49	9.3192	88.43***	

All three years are presented. Statistics include: df – degrees of freedom, SSD – sum of squares, CV – variance component estimates, % Total – percentage of the total variance contributed by each component, and ϕ_{ST}.
*** $P < 0.001$.

Table 7. AMOVA results examining genetic partitioning among seeding treatment

Source of variation	df	SSD	CV	% Total	ϕ_{ST}
2001					
Among seeding treatment	2	38.35	0.0128	0.15 ns	0.0940
Among populations within seeding treatment	75	1090.69	0.8068	9.25***	
Within populations	571	4511.50	7.9011	90.60***	
2002					
Among Seeding Treatment	2	49.27	0.0230	0.23 ns	0.1118
Among populations within seeding treatment	72	1379.58	1.1062	10.96***	
Within populations	617	5532.86	8.9674	88.82***	
2003					
Among seeding treatment	2	51.67	0.0218	0.21 ns	0.1151
Among populations within seeding treatment	71	1468.50	1.1895	11.31***	
Within populations	634	5902.39	9.3098	88.49***	

All three years are presented. Statistics include: df – degrees of freedom, SSD – sum of squares, CV – variance component estimates, %Total – percentage of the total variance contributed by each component, and ϕ_{ST}.
ns = Not Significant at $P < 0.05$, ***$P < 0.001$.

genetic diversity for the sampled populations. The estimates are similar to those calculated for other outcrossing plant species using dominant markers (see reviews in Bussell 1999; and Nybom 2004). The dominant nature of ISSR markers does not allow for the calculation of heterozygosity in the sampled populations. For Nei's gene diversity, it is assumed that each locus in the sampled populations is in Hardy–Weinberg equilibrium. If equilibrium does not exist, it is possible that the calculated estimate is biased (Nybom and Bartish 2000). However, the results obtained here are similar to, or higher than, those found in outcrossing species where levels of inbreeding are known (Nybom and Bartish 2000).

Diversity estimates for the natural populations across the three years were significantly Different from one another. Pairwise comparisons of both Shannon's Diversity estimate and Nei's gene diversity estimate showed significant differences between 2001 and 2003, with 2003 having higher estimates. Estimates of diversity for restored populations did not vary significantly across years. The restored populations had higher levels of diversity than did the natural populations in 2001 and 2002, but the opposite pattern was seen in 2003. The populations are too young at this point to speculate whether or not this trend reversal indicates a real decrease in diversity in the restored pools.

Evaluation of genetic structure in natural and restored populations over three years

Genetic variability was not highly structured between population type (natural or restored) in any of the sampled years, nor did the populations become more genetically distinct over the three generations sampled. There were also no detectable effects of distance class or seeding treatment on the partitioning of genetic diversity among restored populations. The results from the distance class analyses indicate that genetic diversity did not immediately decrease with increased distance from the natural populations. The seeding treatment results indicate that certain genotypes did not have higher survival rates than others depending on which species were initially seeded together. Again, the populations are too young to draw any definitive conclusions about gene flow or genetic drift, but this study provides a solid baseline for further investigations.

Overall success of vernal pool restoration at Travis AFB

Beyond genetics, the restored pools have been monitored each year to measure success of establishment (SK Collinge unpublished). Of the 192 restored pools that were originally seeded in 1999 and 2000, 182 of them have been monitored for five consecutive years (S. K. Collinge unpublished). Although several pools originally seeded do not have established populations of *L. conjugens*, other pools are doing quite well. In 2000, the first spring after initial seeding, 149 pools had *L. conjugens* individuals present with an average of 11 plants per pool (range: 1–39). By 2004, 113 pools contained *L. conjugens* individuals with an average of 224 plants per pool (range: 1–3000). In conjunction with this genetic study, pollinator observations and measurements of seed set were conducted within natural and restored populations. Seed set did not significantly vary between population type indicating that individuals in restored populations are not pollen limited (Ramp 2004). Complete demographic analyses of restoration success have not yet been performed, but the preliminary data indicate that the restored pools at Travis AFB, although small, support sustained populations of *L. conjugens* from one year to the next.

The use of genetic data to assess restoration success

Although genetic considerations have been applied to the planning of restoration projects, there are only a few examples where the success of a restoration, reintroduction, or translocation has been assessed using genetic markers. Enzyme markers detected a loss of genetic variation in reintroduced populations of the coastal annual *Cordylanthus maritimus* ssp. *maritimus* (Helenurm and Parsons 1997). Within transplanted populations of eelgrass (*Zostera marina*), a reduction in allozyme diversity was seen in San Diego County, California, as compared to natural populations (Williams and Davis 1996). However, transplanted populations of the same species in Chesapeake Bay, Virginia, showed little to no loss of diversity as compared to source populations (Williams and Orth 1998). By understanding how diversity levels in transplanted populations compare to source populations of *Z. marina*, appropriate management actions to either increase or maintain the genetic diversity may be applied. Genetic monitoring of restored populations of smooth cord grass (*Spartina alterniflora*) found similar to slightly higher levels of genetic diversity in re-established populations as compared to a natural population (Travis et al. 2002). These results indicate that restored populations of clonal species can maintain genetic variability over time, and may even show greater levels of diversity than those seen in natural populations. All of the above results indicate that monitoring the genetic diversity of species in different restoration projects is essential for proper management.

Studies examining DNA-level genetic variation of restored plant populations found results similar to those from the present study. Restored populations of pink sand verbena (*Abronia umbellata* ssp. *breviflora*) showed similar levels of diversity to those seen in the source population, as well as other natural populations (McGlaughlin et al. 2002). Prairie restoration involving seeding of species from multiple sites has been successful at maintaining genetic diversity across remnant and restored sites of the purple prairie clover (*Dalea purpurea*), big bluestem (*Andropogon gerardii*), and Indian grass (*Sorghastrum nutans*) (Gustafson et al. 2002, 2004). In the Chinese gymnosperm *Metasequoia glyptostroboides* species-level genetic variation was found to be lower than that found in other gymnosperms (Li et al. 2005). Artificial

populations contained slightly less genetic variation than the wild populations leading to the suggestion that *ex situ* conservation efforts be designed to capture all the remaining variation present within the species across both types of populations (Li et al. 2005). In all of the above examples, specific management and restoration implications became evident from the results.

The overall implications of this study are that the seeding protocols used to establish restored populations of *L. conjugens* at Travis AFB were successful in terms of genetics. The natural populations contain a large amount of genetic diversity with an average of 95% of the loci being polymorphic each year. This diversity was broadly distributed into the restored populations in such a way that there was no genetic structure to the established populations. The fact that the genetic diversity did not change over the three years of the study is not surprising considering the high levels of diversity found within the natural populations.

We conducted a thorough examination of the genetic variability present within source and restored populations of the endangered vernal pool endemic *L. conjugens*. High levels of genetic variability were detected with low levels of genetic structure. The seeding treatments applied at Travis AFB were successful in capturing and distributing genetic variability across populations. We recommend that other restoration projects of *L. conjugens* or other vernal pool outcrossing species be seeded in a similar manner when possible.

This study demonstrates that DNA-level genetic information can quickly and effectively be collected for a large number of individuals from both natural and restored populations. The methodology applied in this study can be widely applied to other species (both plant and animal) to gain an understanding of the levels and distribution of genetic diversity within and among species of conservation or restoration priority. The use of ISSR markers in restoration will provide researchers and managers with inexpensive, robust markers that are capable of providing a large amount of informative data in a short period of time.

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Appendix

Appendix 1. Genetic diversity estimates and band statistics for all populations collected from Travis AFB in 2001, 2002, and 2003

	Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
2001	Natural	1			20	0.335 (0.276)	0.222 (0.193)	57	55	65.48
	Natural	27			20	0.344 (0.266)	0.225 (0.189)	61	60	72.62
	Natural	36			20	0.380 (.251)	0.248 (0.183)	68	68	81
	Natural	37			20	0.311 (0.255)	0.200 (0.179)	60	59	70.24
	Natural	39			20	0.340 (0.257)	0.221 (0.182)	62	61	72.62
	Natural	41			17	0.314 (0.251)	0.201 (0.176)	60	59	70.24
	Natural	76			20	0.277 (0.242)	0.174 (0.168)	57	57	67.86

Appendix 1. Continued

	Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
2001	Total natural				137	0.374 (0.223)	0.239 (0.171)	77	77	91.67
	Restored	314	1	1	2	0.038 (0.159)	0.027 (0.115)	7	3	3.57
	Restored	429	1	1	15	0.286 (0.260)	0.184 (0.179)	53	52	61.9
	Restored	433	1	1	15	0.254 (0.256)	0.163 (0.175)	48	47	55.95
	Restored	446	1	1	5	0.115 (0.252)	0.081 (0.179)	19	15	17.86
	Restored	463	1	1	2	0.114 (0.259)	0.082 (0.187)	26	14	16.67
	Restored	304	1	2	17	0.295 (0.248)	0.188 (0.169)	56	55	65.48
	Restored	305	1	2	16	0.289 (0.273)	0.189 (0.192)	51	51	60.71
	Restored	317	1	2	10	0.315 (0.275)	0.208 (0.193)	54	53	63.1
	Restored	341	1	2	10	0.278 (0.292)	0.187 (0.203)	45	43	51.19
	Restored	436	1	2	10	0.252 (0.285)	0.168 (0.197)	41	40	47.62
	Restored	444	1	2	10	0.292 (0.244)	0.185 (0.165)	55	54	64.23
	Restored	458	1	2	9	0.264 (0.269)	0.173 (0.182)	45	44	52.38
	Restored	468	1	2	11	0.334 (0.279)	0.222 (0.196)	55	54	64.23
	Restored	471	1	2	6	0.213 (0.274)	0.141 (0.186)	35	33	39.29
	Restored	302	1	3	3	0.210 (0.301)	0.146 (0.210)	32	28	33.33
	Restored	303	1	3	4	0.301 (0.310)	0.207 (0.215)	43	42	50
	Restored	307	1	3	5	0.246 (0.288)	0.166 (0.197)	42	37	44.05
	Restored	326	1	3	11	0.267 (0.276)	0.176 (0.189)	45	44	52.38
	Restored	329	1	3	10	0.332 (0.272)	0.219 (0.190)	56	55	65.46
	Restored	339	1	3	8	0.307 (0.286)	0.205 (0.199)	49	48	57.14
	Restored	435	1	3	9	0.185 (0.281)	0.127 (0.197)	31	27	32.14
	Restored	447	1	3	2	0.147 (0.285)	0.106 (0.206)	24	18	21.43
	Restored	459	1	3	10	0.249 (0.27)	0.163 (0.184)	43	42	50
	Restored	460	1	3	10	0.270 (0.275)	0.177 (0.190)	46	45	53.57
	Restored	462	1	3	18	0.356 (0.245)	0.230 (0.173)	64	64	76.19
	Restored	364	2	1	1	0	0	12	0	0
	Restored	472	2	1	14	0.261 (0.269)	0.170 (0.185)	46	45	53.57
	Restored	474	2	1	8	0.260 (0.282)	0.173 (0.195)	44	42	50
	Restored	484	2	1	1	0	0	5	0	0
	Restored	493	2	1	14	0.294 (0.276)	0.193 (0.193)	51	50	59.52
	Restored	509	2	1	12	0.304 (0.264)	0.197 (0.182)	54	53	63.1
	Restored	349	2	2	11	0.290 (0.278)	0.192 (0.193)	49	48	57.14
	Restored	373	2	2	10	0.300 (0.261)	0.104 (0.180)	53	53	63.1
	Restored	374	2	2	10	0.292 (0.254)	0.187 (0.174)	53	53	63.1
	Restored	384	2	2	10	0.281 (0.287)	0.188 (0.198)	44	44	52.38
	Restored	385	2	2	5	0.288 (0.299)	0.196 (0.207)	45	42	50
	Restored	473	2	2	9	0.334 (0.261)	0.218 (0.182)	58	57	67.86
	Restored	478	2	2	11	0.297 (0.252)	0.190 (0.173)	54	54	64.29
	Restored	492	2	2	9	0.311 (0.284)	0.207 (0.197)	51	49	58.33
	Restored	494	2	2	8	0.336 (0.280)	0.224 (0.195)	55	53	63.1
	Restored	508	2	2	10	0.381 (0.272)	0.255 (0.192)	62	60	71.43
	Restored	355	2	3	5	0.225 (0.289)	0.153 (0.199)	33	33	39.29
	Restored	362	2	3	5	0.262 (0.292)	0.177 (0.201)	41	39	46.43
	Restored	367	2	3	2	0.188 (0.310)	0.135 (0.224)	28	23	27.38
	Restored	380	2	3	11	0.323 (0.266)	0.212 (0.185)	56	55	65.48
	Restored	386	2	3	10	0.389 (0.273)	0.261 (0.194)	62	61	72.62
	Restored	388	2	3	7	0.367 (0.286)	0.248 (0.199)	56	55	65.48
	Restored	483	2	3	10	0.280 (0.289)	0.188 (0.201)	45	44	52.38
	Restored	496	2	3	10	0.328 (0.289)	0.220 (0.203)	52	51	60.71

Appendix 1. Continued

	Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
	Restored	499	2	3	7	0.266 (0.294)	0.180 (0.204)	42	40	47.62
	Restored	503	2	3	3	0.235 (0.312)	0.165 (0.219)	35	31	36.9
	Restored	505	2	3	10	0.339 (0.290)	0.228 (0.204)	53	52	61.9
	Restored	400	3	1	1	0	0	15	0	0
	Restored	401	3	1	2	0.131 (0.273)	0.094 (0.197)	29	16	19.05
	Restored	409	3	1	1	0	0	10	0	0
	Restored	419	3	1	6	0.347 (0.290)	0.235 (0.202)	53	52	61.9
	Restored	518	3	1	19	0.346 (0.254)	0.224 (0.183)	65	64	76.19
	Restored	524	3	1	10	0.292 (0.283)	0.194 (0.196)	49	47	55.95
	Restored	542	3	1	8	0.198 (0.280)	0.134 (0.194)	32	30	35.71
	Restored	549	3	1	9	0.281 (0.276)	0.185 (0.189)	48	46	54.76
	Restored	399	3	2	3	0.202 (0.298)	0.141 (0.208)	29	27	32.14
	Restored	402	3	2	6	0.333 (0.284)	0.223 (0.196)	52	51	60.71
	Restored	403	3	2	10	0.292 (0.290)	0.196 (0.200)	46	45	53.57
	Restored	413	3	2	1	0	0	20	0	0
	Restored	416	3	2	3	0.240 (0.310)	0.167 (0.217)	36	32	38.1
	Restored	516	3	2	10	0.305 (0.286)	0.204 (0.200)	52	49	58.33
	Restored	529	3	2	10	0.326 (0.269)	0.214 (0.186)	56	54	64.29
	Restored	539	3	2	10	0.324 (0.276)	0.215 (0.193)	56	53	63.1
	Restored	544	3	2	10	0.364 (0.275)	0.243 (0.194)	59	58	69.05
	Restored	553	3	2	10	0.309 (0.281)	0.205 (0.196)	52	50	59.52
	Restored	397	3	3	2	0.073 (0.215)	0.053 (0.155)	23	9	10.71
	Restored	404	3	3	6	0.277 (0.290)	0.186 (0.199)	45	42	50
	Restored	411	3	3	5	0.284 (0.302)	0.194 (0.210)	43	41	48.81
	Restored	412	3	3	10	0.324 (0.291)	0.218 (0.204)	51	50	59.52
	Restored	520	3	3	10	0.312 (0.276)	0.206 (0.193)	53	52	61.9
	Restored	523	3	3	9	0.341 (0.273)	0.226 (0.190)	56	55	65.48
	Restored	526	3	3	10	0.330 (0.286)	0.221 (0.201)	53	52	61.9
	Restored	541	3	3	10	0.299 (0.295)	0.202 (0.207)	49	46	54.76
	Restored	555	3	3	10	0.276 (0.269)	0.179 (0.186)	50	48	57.14
	Total restored				652	0.396 (0.207)	0.252 (0.156)	82	82	97.62
	Total 2001				789	0.397 (0.208)	0.252 (0.156)		82	97.62
2002										
	Natural	1			20	0.331 (0.270)	0.217 (0.192)	59	58	69.05
	Natural	13			20	0.391 (0.252)	0.258 (0.181)	69	67	79.76
	Natural	27			20	0.396 (0.227)	0.256 (0.166)	72	72	85.71
	Natural	33			20	0.376 (0.249)	0.246 (0.179)	68	67	79.76
	Natural	36			20	0.374 (0.240)	0.242 (0.174)	69	69	82.14
	Natural	37			19	0.313 (0.262)	0.203 (0.185)	58	57	67.86
	Natural	39			19	0.371 (0.247)	0.241 (0.179)	67	67	79.76
	Natural	41			20	0.313 (0.232)	0.195 (0.166)	66	66	78.57
	Natural	51			20	0.340 (0.252)	0.219 (0.181)	64	64	76.19
	Natural	76			20	0.324 (0.245)	0.206 (0.175)	65	64	76.19
2002	Total natural				198	0.399 (0.209)	0.254 (0.158)	82	82	100
	Restored	314	1	1	3	0.225 (0.306)	0.157 (0.214)	36	30	35.71
	Restored	429	1	1	10	0.369 (0.264)	0.244 (0.185)	62	60	71.43
	Restored	433	1	1	10	0.337 (0.271)	0.222 (0.192)	59	57	67.86
	Restored	463	1	1	10	0.319 (0.284)	0.213 (0.197)	52	50	59.52
	Restored	304	1	2	10	0.353 (0.277)	0.235 (0.197)	59	57	67.86
	Restored	305	1	2	10	0.351 (0.277)	0.234 (0.195)	59	56	66.67

Appendix 1. Continued

Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
Restored	313	1	2	10	0.326 (0.262)	0.212 (0.183)	57	56	66.67
Restored	317	1	2	10	0.355 (0.280)	0.238 (0.198)	57	56	66.67
Restored	341	1	2	10	0.282 (0.282)	0.187 (0.194)	48	45	53.57
Restored	436	1	2	10	0.349 (0.272)	0.231 (0.191)	58	57	67.86
Restored	444	1	2	10	0.353 (0.267)	0.232 (0.189)	61	59	70.24
Restored	458	1	2	10	0.340 (0.288)	0.229 (0.201)	53	52	61.9
Restored	468	1	2	10	0.272 (0.266)	0.177 (0.181)	50	47	55.95
Restored	471	1	2	10	0.315 (0.272)	0.207 (0.190)	55	53	63.1
Restored	302	1	3	5	0.312 (0.302)	0.213 (0.210)	49	45	53.57
Restored	307	1	3	10	0.374 (0.263)	0.247 (0.185)	62	61	72.62
Restored	326	1	3	10	0.346 (0.258)	0.226 (0.180)	61	59	70.24
Restored	329	1	3	10	0.332 (0.254)	0.215 (0.176)	58	58	69.05
Restored	339	1	3	10	0.310 (0.280)	0.206 (0.194)	52	50	59.52
Restored	435	1	3	10	0.354 (0.248)	0.229 (0.174)	64	62	73.81
Restored	447	1	3	10	0.297 (0.279)	0.196 (0.194)	51	49	58.33
Restored	459	1	3	10	0.277 (0.264)	0.179 (0.182)	50	49	58.33
Restored	460	1	3	9	0.285 (0.270)	0.187 (0.185)	50	48	57.14
Restored	462	1	3	10	0.357 (0.276)	0.238 (0.195)	59	57	67.86
Restored	358	2	1	10	0.289 (0.281)	0.192 (0.194)	49	47	55.95
Restored	364	2	1	4	0.184 (0.289)	0.128 (0.202)	27	25	29.76
Restored	472	2	1	10	0.283 (0.275)	0.185 (0.189)	50	47	55.95
Restored	474	2	1	10	0.237 (0.256)	0.152 (0.173)	45	43	51.19
Restored	484	2	1	10	0.251 (0.264)	0.163 (0.180)	49	44	52.38
Restored	487	2	1	8	0.325 (0.269)	0.214 (0.187)	56	54	64.29
Restored	493	2	1	10	0.268 (0.262)	0.173 (0.178)	49	47	55.95
Restored	509	2	1	10	0.328 (0.284)	0.219 (0.199)	55	52	61.9
Restored	349	2	2	10	0.353 (0.275)	0.235 (0.194)	57	57	67.86
Restored	373	2	2	9	0.242 (0.283)	0.162 (0.194)	40	38	45.24
Restored	374	2	2	10	0.319 (0.271)	0.210 (0.190)	54	54	64.29
Restored	384	2	2	10	0.332 (0.280)	0.221 (0.195)	56	53	63.1
Restored	385	2	2	10	0.329 (0.275)	0.218 (0.193)	56	54	64.29
Restored	473	2	2	9	0.259 (0.286)	0.173 (0.198)	42	41	48.81
Restored	478	2	2	5	0.168 (0.273)	0.115 (0.190)	31	24	28.57
Restored	492	2	2	9	0.345 (0.280)	0.231 (0.195)	55	54	64.29
Restored	494	2	2	9	0.254 (0.287)	0.170 (0.197)	40	39	46.43
Restored	508	2	2	10	0.215 (0.275)	0.143 (0.187)	36	34	40.48
Restored	355	2	3	5	0.120 (0.296)	0.139 (0.208)	29	27	32.14
Restored	362	2	3	5	0.284 (0.295)	0.192 (0.203)	44	42	50
Restored	367	2	3	10	0.314 (0.277)	0.208 (0.192)	53	51	60.71
Restored	380	2	3	10	0.288 (0.278)	0.190 (0.191)	49	47	55.95
Restored	386	2	3	6	0.279 (0.286)	0.186 (0.196)	45	43	51.19
Restored	388	2	3	5	0.272 (0.309)	0.188 (0.215)	39	38	45.24
Restored	483	2	3	10	0.192 (0.256)	0.125 (0.174)	36	33	39.29
Restored	496	2	3	10	0.247 (0.271)	0.162 (0.185)	42	41	48.81
Restored	499	2	3	10	0.202 (0.277)	0.135 (0.190)	36	31	36.9
Restored	505	2	3	10	0.295 (0.291)	0.198 (0.203)	48	46	54.76
Restored	419	3	1	10	0.282 (0.272)	0.185 (0.188)	50	48	57.14
Restored	518	3	1	10	0.272 (0.260)	0.175 (0.179)	49	49	58.33
Restored	524	3	1	10	0.310 (0.273)	0.204 (0.190)	54	52	61.9
Restored	542	3	1	10	0.256 (0.284)	0.171 (0.196)	42	41	48.81

Appendix 1. Continued

	Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
	Restored	549	3	1	9	0.276 (0.284)	0.184 (0.196)	46	44	52.38
	Restored	402	3	2	10	0.268 (0.282)	0.178 (0.194)	44	43	51.19
	Restored	403	3	2	9	0.261 (0.275)	0.172 (0.188)	44	43	51.19
	Restored	416	3	2	9	0.287 (0.279)	0.189 (0.194)	50	47	55.95
	Restored	516	3	2	10	0.325 (0.285)	0.217 (0.200)	55	52	61.9
	Restored	529	3	2	10	0.296 (0.282)	0.197 (0.196)	52	48	57.14
	Restored	535	3	2	10	0.332 (0.268)	0.218 (0.188)	58	56	66.67
	Restored	539	3	2	10	0.352 (0.265)	0.232 (0.188)	59	59	70.24
	Restored	540	3	2	10	0.278 (0.268)	0.181 (0.185)	51	48	57.14
	Restored	544	3	2	10	0.288 (0.280)	0.190 (0.194)	50	47	55.95
	Restored	553	3	2	9	0.310 (0.288)	0.207 (0.202)	49	49	58.33
	Restored	404	3	3	10	0.346 (0.280)	0.231 (0.196)	56	55	65.48
	Restored	412	3	3	10	0.302 (0.280)	0.200 (0.196)	52	50	59.52
	Restored	520	3	3	10	0.345 (0.271)	0.229 (0.189)	58	56	66.67
	Restored	523	3	3	10	0.358 (0.263)	0.235 (0.186)	60	60	71.43
	Restored	526	3	3	10	0.322 (0.277)	0.213 (0.194)	54	53	63.1
	Restored	533	3	3	10	0.360 (0.270)	0.239 (0.191)	60	59	70.24
	Restored	541	3	3	9	0.302 (0.276)	0.199 (0.192)	53	50	59.52
	Restored	555	3	3	10	0.295 (0.275)	0.194 (0.190)	50	49	58.33
	Total restored				706	0.404 (0.207)	0.257 (0.158)	82	82	100
	Total 2002				904	0.405 (0.204)	0.258 (0.156)	82	82	100
2003										
	Natural	1			20	0.369 (0.230)	0.236 (0.167)	71	71	84.53
	Natural	13			20	0.360 (0.269)	0.238 (0.193)	64	62	73.81
	Natural	27			20	0.376 (0.245)	0.242 (0.176)	69	67	79.76
	Natural	33			20	0.391 (0.239)	0.254 (0.174)	71	70	83.33
	Natural	36			20	0.387 (0.247)	0.254 (0.180)	69	68	80.95
	Natural	37			20	0.380 (0.248)	0.248 (0.180)	69	68	80.95
	Natural	39			20	0.388 (0.252)	0.255 (0.183)	69	68	80.95
	Natural	41			20	0.383 (0.243)	0.249 (0.179)	72	70	83.33
	Natural	51			20	0.338 (0.266)	0.221 (0.190)	62	60	71.43
	Natural	76			20	0.361 (0.262)	0.238 (0.185)	62	61	72.62
2003	Natural total				200	0.413 (0.219)	0.267 (0.166)	82	81	96.43
	Restored	314	1	1	4	0.184 (0.281)	0.127 (0.194)	30	26	30.95
	Restored	429	1	1	10	0.305 (0.297)	0.207 (0.207)	49	46	54.76
	Restored	433	1	1	9	0.201 (0.287)	0.224 (0.333)	52	52	61.9
	Restored	446	1	1	9	0.299 (0.280)	0.198 (0.193)	50	48	57.14
	Restored	463	1	1	9	0.295 (0.268)	0.192 (0.184)	54	50	59.52
	Restored	304	1	2	9	0.268 (0.294)	0.181 (0.205)	44	41	48.81
	Restored	305	1	2	10	0.309 (0.288)	0.207 (0.202)	51	49	58.33
	Restored	313	1	2	9	0.276 (0.269)	0.180 (0.182)	47	46	54.76
	Restored	317	1	2	9	0.362 (0.263)	0.238 (0.185)	62	60	71.43
	Restored	341	1	2	9	0.325 (0.284)	0.217 (0.198)	54	51	60.71
	Restored	436	1	2	10	0.317 (0.269)	0.208 (0.188)	56	54	64.29
	Restored	444	1	2	10	0.288 (0.277)	0.190 (0.192)	50	48	57.14
	Restored	458	1	2	10	0.293 (0.270)	0.191 (0.187)	54	50	59.52
	Restored	468	1	2	10	0.323 (0.273)	0.213 (0.192)	55	54	64.29
	Restored	471	1	2	10	0.301 (0.268)	0.197 (0.185)	54	51	60.71
	Restored	303	1	3	10	0.284 (0.267)	0.185 (0.184)	50	49	58.33
	Restored	307	1	3	10	0.249 (0.262)	0.161 (0.178)	46	44	52.38

Appendix 1. Continued

Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
Restored	326	1	3	10	0.286 (0.277)	0.188 (0.193)	49	48	57.14
Restored	329	1	3	10	0.326 (0.276)	0.216 (0.195)	55	54	64.29
Restored	339	1	3	10	0.284 (0.292)	0.191 (0.203)	46	44	52.38
Restored	435	1	3	10	0.297 (0.269)	0.194 (0.187)	51	51	60.71
Restored	447	1	3	10	0.269 (0.269)	0.176 (0.184)	48	46	54.76
Restored	459	1	3	10	0.333 (0.275)	0.220 (0.193)	57	55	65.48
Restored	460	1	3	10	0.287 (0.275)	0.189 (0.191)	50	48	57.14
Restored	462	1	3	9	0.307 (0.281)	0.203 (0.197)	51	50	59.52
Restored	358	2	1	9	0.301 (0.287)	0.201 (0.201)	49	48	57.14
Restored	364	2	1	10	0.270 (0.286)	0.181 (0.199)	45	43	51.19
Restored	472	2	1	10	0.323 (0.273)	0.213 (0.191)	55	54	64.29
Restored	474	2	1	10	0.305 (0.289)	0.205 (0.204)	51	49	58.33
Restored	484	2	1	10	0.265 (0.279)	0.175 (0.193)	47	44	52.38
Restored	487	2	1	10	0.341 (0.268)	0.225 (0.188)	58	57	67.86
Restored	493	2	1	10	0.300 (0.282)	0.199 (0.195)	50	48	57.14
Restored	509	2	1	10	0.282 (0.279)	0.186 (0.195)	50	47	55.95
Restored	349	2	2	10	0.285 (0.269)	0.186 (0.186)	51	49	58.33
Restored	373	2	2	10	0.332 (0.272)	0.219 (0.190)	56	55	65.48
Restored	374	2	2	10	0.285 (0.264)	0.185 (0.182)	52	50	59.52
Restored	384	2	2	10	0.300 (0.287)	0.200 (0.201)	51	48	57.14
Restored	385	2	2	10	0.310 (0.279)	0.205 (0.196)	53	51	60.71
Restored	473	2	2	10	0.270 (0.269)	0.176 (0.184)	48	46	54.76
Restored	478	2	2	10	0.262 (0.279)	0.173 (0.193)	47	43	51.19
Restored	492	2	2	10	0.270 (0.254)	0.173 (0.171)	52	49	58.33
Restored	494	2	2	10	0.306 (0.277)	0.202 (0.194)	54	51	60.71
Restored	508	2	2	10	0.306 (0.284)	0.204 (0.198)	50	49	58.33
Restored	355	2	3	5	0.276 (0.294)	0.186 (0.202)	48	41	48.81
Restored	362	2	3	10	0.296 (0.276)	0.195 (0.191)	51	49	58.33
Restored	380	2	3	10	0.324 (0.276)	0.214 (0.193)	55	53	63.1
Restored	386	2	3	10	0.290 (0.283)	0.193 (0.197)	51	47	55.95
Restored	388	2	3	5	0.282 (0.300)	0.192 (0.208)	46	41	48.81
Restored	483	2	3	10	0.311 (0.259)	0.201 (0.178)	56	54	64.29
Restored	496	2	3	10	0.343 (0.247)	0.221 (0.173)	62	61	72.62
Restored	499	2	3	10	0.352 (0.275)	0.234 (0.196)	60	58	69.05
Restored	505	2	3	10	0.275 (0.285)	0.183 (0.198)	47	44	52.38
Restored	518	3	1	10	0.296 (0.274)	0.195 (0.189)	53	49	58.33
Restored	524	3	1	10	0.305 (0.294)	0.205 (0.206)	50	47	55.95
Restored	542	3	1	10	0.324 (0.263)	0.211 (0.182)	56	55	65.48
Restored	549	3	1	10	0.277 (0.266)	0.180 (0.183)	50	48	57.14
Restored	402	3	2	10	0.284 (0.275)	0.187 (0.191)	51	48	57.14
Restored	403	3	2	9	0.280 (0.282)	0.186 (0.194)	48	45	53.57
Restored	416	3	2	10	0.330 (0.278)	0.219 (0.196)	56	54	64.29
Restored	516	3	2	10	0.295 (0.284)	0.196 (0.196)	48	47	55.95
Restored	529	3	2	10	0.309 (0.285)	0.206 (0.199)	53	49	58.33
Restored	535	3	2	10	0.309 (0.266)	0.202 (0.184)	56	53	63.1
Restored	539	3	2	10	0.356 (0.268)	0.236 (0.189)	61	59	70.24
Restored	540	3	2	10	0.320 (0.290)	0.215 (0.203)	52	50	59.52
Restored	544	3	2	10	0.316 (0.275)	0.209 (0.190)	54	52	61.9
Restored	553	3	2	10	0.316 (0.282)	0.210 (0.198)	53	51	60.71
Restored	404	3	3	10	0.215 (0.268)	0.141 (0.183)	38	36	42.86

Appendix 1. Continued

Population Type	Population ID	Distance Class	Seeding Treatment	# Individual	Shannon's Diversity	Nei's gene diversity	# Bands present	# Polymorphic Loci	Percent Polymorphic
Restored	412	3	3	10	0.282 (0.263)	0.182 (0.181)	52	50	59.52
Restored	520	3	3	10	0.309 (0.261)	0.200 (0.181)	56	54	64.29
Restored	523	3	3	10	0.307 (0.261)	0.199 (0.181)	56	54	64.29
Restored	526	3	3	10	0.297 (0.285)	0.197 (0.199)	50	48	57.14
Restored	533	3	3	5	0.215 (0.283)	0.145 (0.194)	37	32	38.1
Restored	541	3	3	10	0.267 (0.272)	0.175 (0.187)	48	45	53.57
Restored	555	3	3	10	0.306 (0.285)	0.204 (0.199)	53	49	58.33
Total restored				709	0.388 (0.223)	0.248 (0.169)	82	82	97.62
Total 2003				909	0.396 (0.220)	0.254 (0.168)	82	82	97.62

Statistics include: Pop. ID: population identification number; Distance Class: restored distance class for pollination and genetic studies, represented as distance away from natural pools, distance 1 (10–40 m), distance 2 (50–80 m), distance 3 (90–110 m); Seeding Treatment: seeding treatment used to establish restored populations (1: *L. conjugens* only, 2: group 1 ('99), group 2 ('00), 3: group 2('99), group 1('00)) (see text for group identities); Shannon's Diversity and Nei's Gene Diversity: values calculated for all 84 loci with standard deviations in parentheses; # Bands Present: the total number of ISSR bands sampled in the population; # Loci Poly.: the number of polymorphic bands sampled in the population; Percent Poly.: the percentage of total bands that are polymorphic in the population.

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