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Genetic diversity and seed production in Santa Lucia fir (*Abies bracteata*), a relict of the Miocene Broadleaved Evergreen Forest

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Abstract

Santa Lucia fir (*Abies bracteata*), is a unique fir, the sole member of the subgenus *Pseudotorreya*. It is a relict of the Miocene broadleaved evergreen sclerophyll forest, and is now restricted to a highly fragmented range in the Santa Lucia Mountains of central coastal California. Expected heterozygosity for 30 isozyme loci in 18 enzyme systems, averaged over six populations that spanned the species' north–south range, was only 0.036. Despite a fragmented range and isolated populations, differentiation (F_{ST}) was only 0.080 for mature trees, and the number of migrants per generation (Nm) was 2.88 or 3.83, depending on the method of estimation. F_{ST} for embryos was lower, 0.025, and Nm correspondingly higher, 9.75. Nei's genetic distances were small and unrelated to geographic distances between populations. The proportion of full seeds per cone was only 0.082–0.488, depending on population, which suggests a high incidence of selfing followed by embryo abortion. However, the level of accumulated inbreeding, F_{IS}, in mature trees was low, only 0.049. By contrast, F_{IS} for embryos was 0.388, which indicates a high proportion of selfed progeny, in agreement with the low seed yields. The difference in inbreeding coefficients between seed trees and their progeny suggest that most inbreds are eliminated before maturity and, therefore, seed production, already low, overestimates the true potential for regeneration of these populations. These results have implications for conservation.

Introduction

The fate of Santa Lucia fir (*Abies bracteata* D. Don ex Poiteau, synonym *A. venusta* (Dougl.) Koch.) has been the subject of debate. The species is classed as LRcd under guidelines of the International Union for Conservation of Nature and Natural Resources (IUCN), meaning low risk but conservation-dependent and likely to qualify for vulnerable or endangered without protection (Farjon and Page 1999). Early authors thought that: ''Fires...seem destined sooner or later to exterminate it'' (Sargent 1898) or that its survival was endangered by seed parasites (Legg 1953). Based on observations of abundant reproduction in ravines, Talley (1974) doubted these warnings.

Whether or not Santa Lucia fir is a genetically diverse and healthy species or in danger of extinction, its present rarity is not a result of human encroachment but a consequence of climate change resulting from tectonic activity since the Miocene. However, if its decline was accompanied by a loss of genetic diversity, that would limit its capacity to respond to the anthropogenic warming projected over the next 50 years.

Santa Lucia fir is a lustrous dark green, spire-like tree that may reach a maximum 50 m in height (usually 30 m) and 1.3 m dbh, and ages of at least

200 years (Talley 1974; Cowley 2000). It is "the most curious fir tree in the world" because of its long, exserted, awl-like cone bracts that bear resin droplets at their tips, which reflects sunlight like a crystal chandelier (Sudworth 1908). Its long, sharply pointed needles are also unique among firs.

Among the 50 or so species of the genus *Abies*, it is the sole member of the subgenus *Pseudotorreya* (Liu 1971) and considered to have no close living relatives. Although Isoda et al. (2000) found that Santa Lucia fir differed from some other firs of western North America by only a single tandem repeat in a chloroplast DNA spacer region, Suyama et al. (2000) found it quite distinct from 23 Japanese, European, and North American species in its chloroplast DNA *rbc*L sequence. Uniqueness makes it an important target for conservation.

Santa Lucia fir covers a northwest-southeast range of about 95 km from a ridge southeast of Monterey to San Simeon, California, entirely within the Santa Lucia Mountains and never more than 14 km inland from the Pacific Coast (Griffin and Critchfield 1972). Most of the Santa Lucia fir within that narrow strip are confined to the northern half of the range; of an estimated 30 km^2 of Santa Lucia fir forest, only 2.5 km^2 is found in the southern half (Talley 1974). Santa Lucia fir grows from a lower elevational limit of 213 m, beginning just above coast redwood (*Sequoia sempervirens* (D. Don) Endl.) forest, to 1571 m on the summit of Cone Peak (Talley 1974).

Steep topography is the most obvious feature defining Santa Lucia firs' distribution. It is thin-barked and susceptible to fire, so it occurs in relatively open stands on steep, rocky slopes that average 35°–45°, too steep to accumulate deep litter and, therefore, not prone to hot fires (Talley 1974).

Although Santa Lucia fir now has a restricted range in coastal California, a putative ancestor occurred much further east in the middle and late Miocene, 13.5 Myr B.P. (Axelrod 1976). Fossil cone bracts, needles, and seeds in the Purple Mountain and Middlegate floras of Nevada have been attributed to *A. scherrii* Axelrod, which is considered synonymous with Santa Lucia fir. *Abies scherri* was associated with broadleaved evergreen sclerophyll forest, similar to that in which Santa Lucia fir now occurs, but richer (Axelrod 1976). Santa Lucia fir is now almost confined to the canyon live oak (*Quercus chrysolepis* Liebm.) phase of the Mixed Evergreen Forest (Talley 1974), and in the Miocene *A. scherrii* was found in floras dominated by *Q. hannibalii*, the fossil equivalent of canyon live oak (Axelrod 1976).

Over time, the Miocene flora was broken into separate components by increasingly dry climates in the interior West, especially as mountain building accelerated at the close of the epoch. Cool moist forests shrunk toward the coast or higher elevations (Axelrod 1976). Santa Lucia fir retreated to the relatively equable maritime climate of the Santa Lucia Mountains.

Within its present range, Santa Lucia fir occurs singly or in small disjunct populations (Talley 1974), a situation likely to lead to loss of diversity, inbreeding, and population differentiation through genetic drift. Our goal was to assess the level of genetic diversity and genetic structure in Santa Lucia fir and to gauge whether inbreeding was a problem. Areas high in genetic diversity are likely to represent populations with high viability, best able to cope with future environmental change and the best targets for conservation. The structure of diversity should inform decisions on whether few or many reserves must be managed and monitored. The level of inbreeding in conifers is crucial because inbreeding reduces reproductive output as well as exposing deleterious alleles (e.g., Franklin 1970).

Materials and methods

Plant materials

We collected cones from 21 to 50 trees in each of five populations of Santa Lucia fir in mid-September 1982, and, after numerous attempts, from a sixth population, San Simeon, in late August 1995 (Table 1 and Figure 1). The populations were chosen to represent, as closely as possible given the difficult access, the latitudinal and altitudinal ranges of Santa Lucia fir. Trees averaged 30–50 cm dbh, depending on population. Cones were maintained separately by trees within populations.

Once the cones dried and shattered, seeds were separated from the bracts with a Clipper Grain, Seed, and Bean Cleaner. The total mass of seeds from each tree in the 1982 collection was weighed, and the total number of seeds per tree was calculated from the relationship between

Table 1. Santa Lucia fir populations included in isozyme studies and mean number of trees (*N*) sampled

Location	*N*	Latitude (N)	Longitude (W)	Elevation (m)
Miller Canyon, Los Padres National Forest, Monterey County	30	36°19′	121°35′	1370
Ventana Double Cone, Los Padres National Forest, Monterey County	30	36°17′	121°45′	1220
Big Sur, Los Padres National Forest, Monterey County	21	36°13′	121°41′	975
Cone Peak, Los Padres National Forest, Monterey County	50	36°02′	121°30′	1295
Villa Creek, Los Padres National Forest, Monterey County	30	35°51′	121°20′	945
San Simeon, Hearst Ranch, San Luis Obispo County	21	35°42′	121°09′	150

the total weight and the weight of a 100-seed sample that was both weighed and counted. Total seeds divided by number of cones provided a measure of reproductive potential. Because Santa Lucia fir seeds may develop to full size in the absence of fertilization and because they are heavily predated by seed chalcids (*Megastigmus* spp.), full, and presumably viable, seeds were separated from hollow seeds and small, undeveloped seeds by winnowing in the Clipper Mill. As was done for estimates of total numbers of seeds, the total numbers of full seeds from each tree were estimated from the total weight of full seeds and the weight of a 100-full seed sample that was both weighed and counted. When there were fewer than 100 full seeds, all were weighed and counted. For the 1995 collection from San Simeon, only full seeds were weighed and counted.

Seeds were stored under refrigeration until needed for electrophoresis, and then germinated in Petri dishes. When radicles emerged, megagametophytes and embryos were dissected from the seeds, separated, and extracts of each prepared by grinding in buffer solution. The 1982 collections were analyzed in 1983 and the 1995 collection in 1997. Co-electrophoresis of samples was performed in 1999 to ensure that scoring was consistent for the 1982 and 1995 collections.

Electrophoresis

We used techniques of starch gel electrophoresis based on Conkle et al. (1982) to assay enzyme

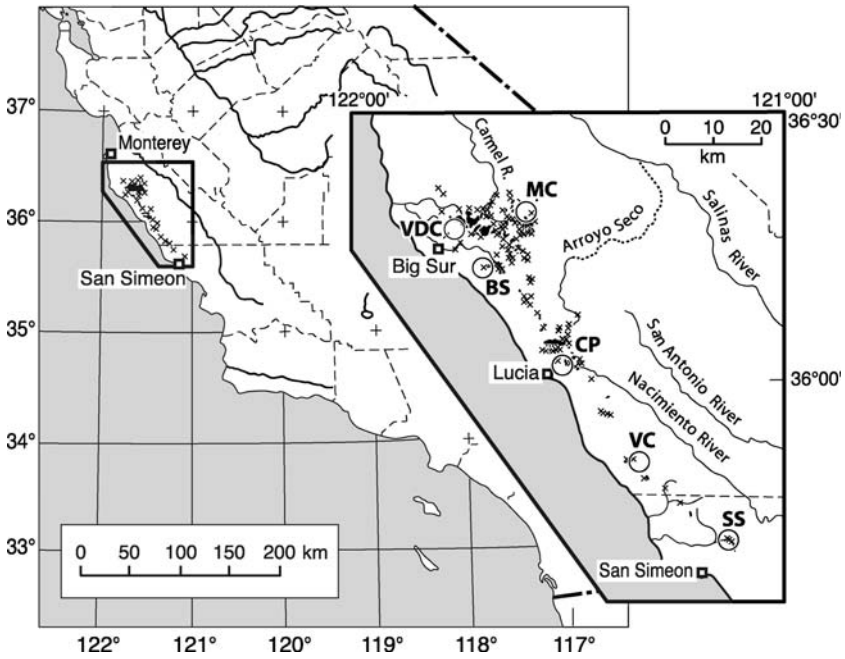

Figure 1. The range of Santa Lucia fir after Griffin and Critchfield (1972) and the general locations of populations (open circles) sampled for isozyme analysis; MC = Miller Canyon; VDC = Ventana Double Cone; BS = Big Sur; CP = Cone Peak; VC = Villa Creek; SS = San Simeon.

systems, and were able to consistently score 30 presumptive loci in 18 enzyme systems in all six populations. We interpreted the number of loci and alleles by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species (Conkle 1981; Ledig et al. 1997, 2000, 2002). Samples of red pine (*Pinus resinosa* Ait.), which are almost invariably homozygous at all loci, were included as standards on each gel to aid interpretation.

The genotype of a seed parent can be determined by analyzing a number of megagametophytes. We assayed at least five megagametophytes per tree. With a sample of five, the probability of misclassifying a heterozygote as a homozygote is 0.0625. That is, the probability that all five megagametophytes in a sample from a heterozygous tree carry the same allele is $2(1/2)^5 = 0.0625$. For estimating allele frequencies, the number of parent trees per population, N, varied from 18 to 49 (or $2N = 36$–98 genomes).

We also assayed two embryos per tree in each of the five 1985 collections. By analysis of two embryos per seed-tree, we effectively doubled the number of genomes sampled; from 32 to 84 pollen genomes per population were scored in addition to the 36 to 98 seed-tree genomes.

Statistical

We estimated percent polymorphic loci, alleles per locus, heterozygosity, and Nei's (1978) unbiased genetic distance with BIOSYS (Swofford and Selander 1981) for tree and embryo genotypes separately. For small samples such as ours, BIOSYS calculates unbiased heterozygosity (Nei 1978). Fixation indices (F) within populations were calculated as the mean deviation of loci from Hardy–Weinberg expectations. We also used BIOSYS to calculate Wright's (1965) F-statistics. BIOSYS calculates F_{IS} and F_{ST}, the fixation indices relative to the population and the meta-population, respectively, as weighted averages across alleles. F_{ST}, the proportion of the total genic diversity among populations, is calculated from the relationship: $1-F_{IT} = (1 - F_{IS})(1 - F_{ST})$. Pairwise F_{ST} were calculated using FSTAT2.9.3.2 (Goudet 2001). By necessity, all inferences apply to the populations of mature, cone-bearing trees or to their offspring.

The degree of genetic isolation among populations was estimated by Nm, the number of migrants per generation. Nm was calculated from Wright (1951): $Nm = (1 - F_{ST})/4F_{ST}$, and from the number and mean frequency of private alleles, the unique alleles found in only one population (Slatkin 1985; Barton and Slatkin 1986).

We used the computer program BOTTLENECK (Cornuet and Luikart 1996) to determine whether effective population numbers had been restricted in the recent past. We employed the infinite allele model (Kimura and Crow 1964) because empirically it tends to fit allozyme data better than alternatives (Luikart and Cornuet 1998). The Wilcoxon sign-rank test was preferred to the sign test because the former has higher power and can be used with as few as four polymorphic loci (Piry et al. 1999). However, we had four or more polymorphic loci only in the samples from Miller Canyon, Big Sur, and Cone Peak.

The association between geographic and genetic distances was investigated using Mantel's (1967) generalized regression procedure with Cavalcanti's (1988) program. Geographic distance between populations was calculated from latitude and longitude using Kindred's (1997) distance calculator. Phylogeographic relationships among the populations were investigated with cluster analysis, using BIOSYS (Swofford and Selander 1981). Statistica 5.1 was used for analyses of variance of seeds per cone, 100-seed weight, percent full seeds, and full seeds per cone, and to calculate correlations among these statistics and between them and the number of heterozygous loci per tree and dbh.

Results

Of the 30 loci consistently scored for the mature trees, nine were polymorphic in at least one population (Table 2), and 21 (*Adh*, *Ala-1*, *Ala-2*, *Cat*, *Fles*, *G6p-1*, *G6p-2*, *Gdh*, *Got-1*, *Got-2*, *Got-3*, *Idh*, *Lap-1*, *Lap-2*, *Mdh-1*, *Mnr-2*, *Mnr-3*, *Mpi-1*, *Pgi-1*, *Pgm*, *6Pg-2*) were monomorphic in all populations. Many of these monomorphic loci are polymorphic in other conifer species and, in fact, are often represented by more than one locus (e.g., *Gdh* and *Idh*). Of the nine loci that were polymorphic, fully half (*Aco*, *Fdp*, *Mnr-1*, *Mpi-2*, and *Pgi-2*) were polymorphic in only one popu-

lation each, usually because of rare variants with frequencies of less than 0.05. Of eight private alleles (those detected in only one population), six were found at Cone Peak and two were found at Big Sur (Table 2). None of the other four populations had any private alleles. Only three loci, *Mdh-2*, *Mdh-5*, and *Skd-1* were polymorphic in all or most populations. A few additional alleles were found in the embryos (at *Adh*, *Ala-2*, and *Mdh-5*), contributed by the pollen.

For most populations, unbiased estimates of expected heterozygosity, H_e, for the 30 loci fell in a narrow range, from 0.020 ± 0.015 (SE) to 0.050 ± 0.026 with a mean of 0.036 ± 0.005 for mature trees (Table 3). Percent polymorphic loci, P, ranged from only 6.7 to 23.3%, and the number of alleles per locus, A, varied from 1.1 to 1.4. Results for the embryos were similar to those for the mature trees; P for embryos ranged from 13.3 to 23.3%, A varied from 1.1 to 1.3, and H_e from 0.022 ± 0.014 to 0.034 ± 0.015 with a mean of 0.028 ± 0.002 in the five populations in which embryos were scored (Table 4).

For mature trees, observed heterozygosity, H_o, was usually only slightly lower than H_e (Table 3). Therefore, means for H_e and H_o over all populations were nearly the same, 0.036 and 0.034, respectively. However, San Simeon, one of the least diverse populations, had excess heterozygosity, $H_o = -0.167$. The unweighted mean of the inbreeding coefficients, 0.071 (Table 3), was low and similar to Wright's F_{IS}, 0.049 (Table 5).

Embryos, in strong contrast, had observed heterozygosity much lower than Hardy–Weinberg expectations, and F_{IS} was 0.388 (Table 5). Embryos were not assayed for the San Simeon population, however, in which mature trees had excess heterozygosity.

Wright's F_{ST} for mature trees was 0.080; i.e., 8% of the total genic diversity was among populations (Table 5). However, much of this variation resulted from the inclusion of Big Sur. The mean of pairwise F_{ST} values between Big Sur and the other five populations was 0.111 ± 0.036 (SE). Pairwise F_{ST} values among the other five populations averaged only 0.054 ± 0.015.

Table 2. Allele frequencies for nine polymorphic loci in mature trees in six populations of Santa Lucia fir

Locus/allele		Population[a] (number of genomes[b])					
		MC (60)	VDC (56)	BS (36)	CP (98)	VC (40)	SS (40)
Aco	1	1.000	1.000	0.889	1.000	1.000	1.000
	2	0.000	0.000	0.111	0.000	0.000	0.000
Fdp	1	1.000	1.000	1.000	0.969	1.000	1.000
	2	0.000	0.000	0.000	0.031	0.000	0.000
Mdh-2	1	0.817	0.982	0.500	0.816	0.750	1.000
	2	0.167	0.018	0.167	0.061	0.000	0.000
	3	0.017	0.000	0.333	0.061	0.250	0.000
	4	0.000	0.000	0.000	0.020	0.000	0.000
	5	0.000	0.000	0.000	0.041	0.000	0.000
Mdh-4	1	0.883	1.000	1.000	0.969	1.000	1.000
	2	0.117	0.000	0.000	0.010	0.000	0.000
	3	0.000	0.000	0.000	0.020	0.000	0.000
Mdh-5	1	0.650	0.929	0.722	0.663	0.700	0.850
	2	0.350	0.071	0.278	0.337	0.300	0.150
Mnr-1	1	1.000	1.000	1.000	0.990	1.000	1.000
	2	0.000	0.000	0.000	0.010	0.000	0.000
Mpi-2	1	1.000	1.000	1.000	0.990	1.000	1.000
	2	0.000	0.000	0.000	0.010	0.000	0.000
Pgi-2	1	1.000	1.000	0.972	1.000	1.000	1.000
	2	0.000	0.000	0.028	0.000	0.000	0.000
Skd1	1	0.800	0.696	0.889	0.745	0.875	0.675
	2	0.200	0.304	0.111	0.255	0.125	0.325

[a]MC = Miller Canyon; VDC = Ventana Double Cone; BS = Big Sur; CP = Cone Peak; VC = Villa Creek; SS = San Simeon.
[b]$2N$, or twice the number of trees.

Table 3. Genic diversity and fixation index in mature trees in six populations of Santa Lucia fir: H_e – expected heterozygosity (unbiased estimate), H_o – observed heterozygosity, P – percent polymorphic loci, P_{95} – percent polymorphic loci at 95% criterion, A – number of alleles per locus, F – fixation index (standard errors in parentheses)

Population	N^a	H_e	H_o	P	P_{95}	A	F
Miller Canyon	29.9	0.044 (0.022)	0.043 (0.021)	13.3	13.3	1.2 (0.1)	0.023
Ventana Double Cone	27.8	0.020 (0.015)	0.017 (0.012)	10.0	6.7	1.1 (0.1)	0.150
Big Sur	17.9	0.050 (0.026)	0.050 (0.025)	16.7	13.3	1.2 (0.1)	0.002
Cone Peak	48.7	0.044 (0.022)	0.038 (0.018)	23.3	10.0	1.4 (0.2)	0.136
Villa Creek	19.9	0.035 (0.020)	0.025 (0.015)	10.0	10.0	1.1 (0.1)	0.286
San Simeon	20.0	0.024 (0.017)	0.028 (0.021)	6.7	6.7	1.1 (0.0)	−0.167
Mean		0.036	0.034	13.3	10.0	1.2	0.071

[a]Mean number of trees sampled per locus at 30 loci.

The number of migrants per generation, *Nm*, estimated from Wright's F_{ST} was 2.88. *Nm* estimated from the mean frequency of private alleles for a sample of 25 and corrected for the actual mean sample size of 27.5 was of the same order, 3.83. The embryos in the five northern populations showed even less differentiation than the mature trees (Table 5); F_{ST} was only 0.025 and *Nm* was 9.75 based on Wright's method.

Nei's unbiased genetic distances (D) among populations were minimal, ranging from 0.000 to 0.009 for the mature trees and even lower, 0.000 to 0.002, for the embryos in the northern populations (data not shown). For mature trees, mean D between Big Sur and the other five populations

Table 4. Genic diversity and fixation index in embryos from five populations of Santa Lucia fir: H_e – expected heterozygosity (unbiased estimate), H_o – observed heterozygosity, P – percent polymorphic loci, P_{95} – percent polymorphic loci at 95% criterion, A – number of alleles per locus, F – fixation index (standard errors in parentheses)

Population	N^a	H_e	H_o	P	P_{95}	A	F
Miller Canyon	56.1	0.028 (0.015)	0.018 (0.010)	16.7	10.0	1.2 (0.1)	0.357
Ventana Double Cone	48.3	0.022 (0.014)	0.012 (0.007)	13.3	6.7	1.1 (0.1)	0.455
Big Sur	34.5	0.034 (0.015)	0.020 (0.009)	16.7	16.7	1.2 (0.1)	0.412
Cone Peak	81.6	0.028 (0.015)	0.018 (0.010)	23.3	6.7	1.3 (0.1)	0.357
Villa Creek	38.6	0.028 (0.015)	0.017 (0.009)	16.7	10.0	1.2 (0.1)	0.393
Mean		0.028	0.017	17.3	10.0	1.2	0.388

[a]Mean number of embryos sampled per locus at 30 loci.

Table 5. Estimates of Wright's (1965) *F*-statistics for polymorphic loci in mature trees (six populations) and embryos (five populations) of Santa Lucia fir

Locus	Mature trees			Embryos		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Aco	−0.125	−0.019	0.094	0.355	0.392	0.056
Adh	–	–	–	0.641	0.648	0.019
Ala-2	–	–	–	0.120	0.124	0.004
Fdp	−0.032	−0.005	0.026	–	–	–
Mdh-2	0.040	0.189	0.155	0.512	0.534	0.045
Mdh-4	−0.108	−0.022	0.077	–	–	–
Mdh-5	0.080	0.132	0.056	0.475	0.486	0.021
Mnr-1	−0.010	−0.002	0.009	1.000	1.000	0.011
Mpi-2	−0.010	−0.002	0.009	0.661	0.665	0.014
Pgi-2	−0.029	−0.005	0.023	–	–	–
Skd-1	0.069	0.105	0.039	0.265	0.284	0.026
Mean	0.049	0.126	0.080	0.388	0.404	0.025

was 0.005 ± 0.003, more than twice as large as the mean among those five populations, 0.002 ± 0.002. Pairwise D values were not related to distance between populations. The Mantel matrix correlations between either D or pairwise F_{ST} and geographic distance were essentially zero, perhaps because the genetic distances between populations were so low. Therefore, cluster analysis failed to reveal any geographically meaningful structure among populations (not shown).

In general, the Wilcoxon sign-rank test for mature trees indicated no greater heterozygosity than that expected for populations at mutation-drift equilibrium in the three populations that had at least four polymorphic loci (Table 6), suggesting that these populations have not been bottlenecked recently. Miller Canyon was, perhaps, an exception, where a probability of 0.094 for the one-tail test for heterozygosity excess was marginal. However, these tests lacked power because so few polymorphic loci were available. Furthermore, the test may be biased because the model assumes neutral alleles, and alleles in the *Mdh-2*, *Mdh-5*, and *Skd-1* loci may possibly be maintained by balancing selection, as suggested by intermediate allele frequencies in nearly every population.

Differences among populations for total numbers of seeds per cone, 100-seed weight, percent full seeds, and full seeds per cone were all very highly significant, according to analyses of variance (Table 7). Differences among populations were more than four-fold for full seeds per cone, from 18.6 to 93.7; the very isolated populations at Villa Creek and San Simeon had much lower viable seed counts per cone than Cone Peak, Miller Canyon, or Ventana Double Cone. Most of the variation in full seeds per cone was the result of differences in the percentage of full seeds, and not due to differences in total seeds per cone. Total seeds per cone among the five populations for which data was recorded varied only from 188 to 262, but the percentage of full seeds varied among populations from 8.2 to 48.8%. Variation among trees in populations was immense; e.g., at Villa Creek, from 0 to 124.1 full seeds (0 to 43.7% of the total) per cone. The greatest number of full seeds per cone was 262.2 for a tree at Big Sur. The number of full seeds per cone was not related to a tree's dbh or to the number of heterozygous loci, which ranged from 0 to 3 among trees.

Discussion

Genic diversity

Genic diversity in Santa Lucia fir was low, perhaps not surprising for such a narrow endemic. Even so, expected heterozygosites, H_e, of 0.036 for mature trees and 0.028 for embryos were less than the mean of 0.056 for 20 other long-lived, woody endemics (Hamrick et al. 1992) and 0.142 for 57

Table 6. Cornuet and Luikart (1996) test for recent bottlenecks in three Santa Lucia fir populations under the infinite allele model of mutation-drift equilibrium for neutral alleles

Locus	Miller Canyon				Big Sur				Cone Peak			
	n[a]	k[b]	H_e[c]	H_{eq}[d]	n	k	H_e	H_{eq}	n	k	H_e	H_{eq}
Aco-1	–	–	–	–	36	2	0.203	0.231	–	–	–	–
Fdp	–	–	–	–	–	–	–	–	98	2	0.060	0.190
Mdh-2	60	3	0.310	0.355	36	3	0.629	0.393	98	5	0.327	0.510
Mdh-4	60	2	0.210	0.209	–	–	–	–	98	3	0.060	0.319
Mdh-5	60	2	0.463	0.210	36	2	0.413	0.233	98	2	0.451	0.175
Mnr-1	–	–	–	–	–	–	–	–	98	2	0.020	0.196
Mpi-1	–	–	–	–	–	–	–	–	98	2	0.040	0.183
Pgi-2	–	–	–	–	36	2	0.056	0.242	–	–	–	–
Skd-1	60	2	0.325	0.199	36	2	0.203	0.241	98	2	0.384	0.188
Prob.[e]			0.094				0.500				0.656	

[a]Number of genomes sampled.
[b]Number of alleles observed.
[c]Expected heterozygosity under Hardy–Weinberg proportions.
[d]Expected heterozygosity under infinite allele model of mutation-drift equilibrium (Kimura and Crow 1964).
[e]Wilcoxon sign-rank test one-tailed probability for heterozygosity excess.

Table 7. Seeds per cone, percentage of full seeds, full seeds per cone, and 100-seed weights (standard errors in parentheses) in six populations of Santa Lucia fir, and *F*-ratio and associated probability for analysis of variance of differences among populations

Population	Seeds/cone	% Full	Full seeds/cone	100-Seed weight (g)
Miller Canyon	255.4 (10.40)	30.2 (1.99)	78.2 (6.71)	3.29 (0.076)
Ventana Double Cone	212.4 (11.64)	32.6 (3.49)	73.1 (8.95)	3.13 (0.074)
Big Sur	262.2 (13.56)	15.5 (4.41)	45.7 (15.13)	3.38 (0.144)
Cone Peak	188.0 (7.26)	48.8 (2.93)	93.7 (7.08)	3.04 (0.072)
Villa Creek	236.4 (23.39)	8.2 (2.01)	18.6 (5.21)	3.47 (0.100)
San Simeon	–	–	22.7 (3.90)	3.64 (0.060)
F-ratio	5.493395	22.42016	8.63775	4.13121
Probability	0.000366	0.000000	0.000003	0.003358

outcrossing endemic plant species (Hamrick and Godt 1996). Only 9 of 30 loci were polymorphic in mature Santa Lucia firs, and the mean percent polymorphic loci for a population was only 13.3%, compared to 26.3% for woody endemics reviewed by Hamrick and Godt (1996).

Many published estimates of genetic diversity in firs have been based on few loci and/or restricted to loci that were highly polymorphic, making extrapolations to the genome level questionable (Table 8). The differences within species between estimates of H_e and P in Table 8 result from differences in the portions of a species' range that were sampled and, in part, on the number of loci surveyed (with few loci, say <20, P is often underestimated and H_e tends to be overestimated). Even with these caveats, several firs seem to have low genetic diversity compared to other conifers. At least some populations of balsam fir (*Abies balsamea* (L.) Mill.), Guatemalan fir (*A. guatemalensis* Rehder), and Maries fir (*A. mariesii* Mast.) all have H_e of 0.085 or lower (Suyama et al. 1997; Aguirre et al. 2000; Shea and Furnier 2002). The situation in balsam fir is confusing; in Iowa and Minnesota, H_e was 0.025 or less (Shea and Furnier 2002) but in the eastern United States, H_e was reported as 0.130 for combined populations of Fraser fir (*Abies fraseri* (Pursch.) Poir.) and balsam fir (Jacobs et al. 1984) and 0.274 along a single elevational transect in New Hampshire (Neale and Adams 1985). The H_e of 0.274 can be discounted because only polymorphic loci were included in the calculation; however, the value of 0.130 cannot be so easily dismissed. Perhaps, populations of balsam fir in the north-central United States (Shea and Furnier 2002) lost diversity through the action of genetic drift in Midwestern glacial refugia or, if they migrated westward from eastern refugia during the present interglacial, lost diversity as a result of founder effects. Small populations and isolation can account for the relatively low H_e in Maries fir (Suyama et al. 1997), which also seems to be the case for the Mexican species, Hickel fir (*A. hickeli*), Guatemalan fir, and sacred fir (*A. religiosa*; Aguirre et al. 2000), as for several other Mexican conifers (Bermejo 1993; Ledig et al. 1997, 2000, 2002).

European firs, such as silver fir (*A. alba* Mill.), Bulgarian fir (*A. borisii regis*, Matt.), Turkish fir (*A. bornmuelleriana* Matt.), and Greek fir (*A. cephalonica* Loud.), seem to have high levels of diversity (Table 8). However, relict populations of silver fir are less diverse; e.g., H_e is 0.079 and P is only 29% in the Białowieża Primeval Forest, Poland (Mejnartowicz 1996). Mediterranean firs with limited distribution and small, isolated populations, also have low levels of diversity like Santa Lucia fir; examples are Sicilian fir (*A. nebrodensis* Lojac. Mattei), based on cpSSRs, and Spanish fir (*A. pinsapo* Boiss.), based on isozymes (Pascual et al. 1993; Parducci et al. 2001; D. Delany pers. comm. 2004)

Genetic structure

Little intra-population structure was detected in the mature Santa Lucia firs. Inbreeding was only moderate; F_{IS} was 0.049. By contrast, many of the firs listed in Table 8 have accumulated high levels of inbreeding: e.g., 0.167 in a population of silver fir, 0.140 in Turkish fir, and 0.229 in Greek fir (Fady and Conkle 1993); 0.235 in Guatemalan fir, 0.121 in Hickel fir, and 0.216 in sacred fir (Aguirre et al. 2000); 0.341 in subalpine fir (*A. lasiocarpa* (Hook.) Nutt.; Shea 1990); and 0.496 in isolated refugial populations of balsam fir (Shea and

Table 8. Estimates of means of population diversity (P and H_e) and inbreeding (F), and differentiation among populations (F_{ST}) in fir species, based on isozyme markers

Species	Loci	P	H_e	F	F_{ST}[a]	Citation
A. alba	4	100.0	≤ 0.397	0.006	0.071	Breitenbach-Dorfer et al. (1992)
A. alba	17	58.8	0.209	0.167	–	Fady and Conkle (1993)
A. alba	16	≤ 87.5	≤ 0.233	–	–	Konnert (1993)
A. alba	12	≤ 38.1	0.102	−0.002	0.110[b]	Vicario et al. (1995)
A. alba	15	45.3	0.136	−0.222	0.015	Matůšová (1995)
A. alba	15	–	0.148	0.040	0.088	Parducci et al. (1996)
A. alba	17	29.4	0.079	−0.557	–	Mejnartowicz (1996)
A. alba	11	≤ 71.6	0.223	0.135	–	Ducci et al. (1999)
A. alba	10	60.4	0.154	−0.034	–	Fady et al. (1999)
A. alba	8	72.5	0.169	–	–	Scaltsoyiannes et al. (1999)
A. alba	13	39.7	0.080	−0.041	0.049	Lewandowski et al. (2001)
A. alba	28	67.9	0.260	−0.017	0.052	Mejnartowicz (2003)
A. alba	28	71.2	0.269	−0.021	0.223	Mejnartowicz (2004)
A. amabilis	22	46.7	0.085	0.089–0.114[c]	–	El-Kassaby et al. (2003)
A. balsamea	8	91.6	0.274	−0.008	–	Neale and Adams (1985)
A. balsamea isolated	22	6.9	0.011	0.496	0.037	Shea and Furnier (2002)
A. balsamea central	22	34.1	0.025	0.007	0.037	Shea and Furnier (2002)
A. balsamea–fraseri	20	42.0	0.130	–	–	Jacobs et al. (1984)
A. bracteata	30	13.3	0.036	0.049	0.080	Current study
A. fraseri	13	30.8	0.085	0.086	0.004	Diebel and Feret (1991)
A. borisii regis	14	87.5	0.279	−0.122	–	Fady and Conkle (1993)
A. borisii regis	8	84.4	0.274	–	–	Scaltsoyiannes et al. (1999)
A. bornmuelleriana	17	58.8	0.201	0.140	–	Fady and Conkle (1993)
A. bornmuelleriana	11	72.7	0.188	0.165	–	Ducci et al. (1999)
A. bornmuelleriana	8	87.5	0.191	–	–	Scaltsoyiannes et al. (1999)
A. cephalonica	17	65.9	0.239	0.229	0.048	Fady and Conkle (1993)
A. cephalonica	11	100.0	0.298	0.114	–	Ducci et al. (1999)
A. cephalonica	8	87.5	0.311	–	–	Scaltsoyiannes et al. (1999)
A. cilicica	8	75.0	0.258	–	–	Scaltsoyiannes et al. (1999)
A. equi-trojana	19	42.7	0.121	0.076	–	Gülbaba et al. (1998)
A. equi-trojana	11	90.9	0.188	0.133	–	Ducci et al. (1999)
A. equi-trojana	8	87.5	0.282	–	–	Scaltsoyiannes et al. (1999)
A. flinckii	16	30.2	0.113	0.074	0.271	Aguirre et al. (2000)
A. guatemalensis	16	20.0	0.069	0.235	0.122	Aguirre et al. (2000)
A. hickeli	16	28.2	0.100	0.121	0.021	Aguirre et al. (2000)
A. kawakamii	7	93.0	0.284	0.884	0.091	Kormutak and Yang (1998)
A. koreana	8	75.0	0.365	–	0.030	Chung and Lee (1985)
A. lasiocarpa	18	43.4	0.124	0.341	0.017	Shea (1990)
A. lasiocarpa	15	50.4	0.129	0.054	0.031	Ettl and Peterson (2001)
A. mariesii	22	40.1	0.054	–	0.144	Suyama et al. (1997)
A. nebrodensis	12	≤ 58.3	0.138	−0.210	–	Vicario et al. (1995)
A. nebrodensis	12	83.3	0.219	0.457	–	Ducci et al. (1999)
A. nordmanniana	11	90.9	0.197	0.066	–	Ducci et al. (1999)
A. nordmanniana	8	62.5	0.188	–	–	Scaltsoyiannes et al. (1999)
A. numidica	8	50.0	0.137	–	–	Scaltsoyiannes et al. (1999)
A. pinsapo	32	≤ 28.0	–	–	–	Pascual et al. (1993)
A. pinsapo	25	16.0	< 0.021	–	–	D. Delany (pers. comm.1993)
A. pinsapo	8	25.0	0.056	–	–	Scaltsoyiannes et al. (1999)
A. religiosa	16	31.8	0.108	0.216	0.250	Aguirre et al. (2000)

[a]Or G_{ST}.
[b]Includes one population of *A. nebrodensis*.
[c]Calculated from the outcrossing rate as $F=(1-t)/(1+t)$.

Furnier 2002). This level of inbreeding probably reflects geographic isolation coupled with limited pollen dispersal; limited pollen dispersal appears responsible for low seed set in open stands of Spanish fir (Arista and Talavera 1996).

Inbreeding levels in Santa Lucia fir embryos contrasted sharply with that in the mature trees. The embryo F_{IS} of 0.388, accumulated in one generation, would be higher than that expected for complete full-sib mating (0.250) and approaches expectations for complete selfing (0.500).

Assuming that the embryo population that gave rise to the present mature trees was initially as inbred as the embryo generation that we sampled, a substantial number of genetic deaths must have intervened during stand development. Selection must weed out the vast majority of inbred embryos during seedling and sapling stages, as inferred for Monterey pine (*Pinus radiata* D. Don; Plessas and Strauss 1986). Therefore, the inbreeding coefficients estimated here are not equilibrium levels but represent recurrent selfing if the inbred progeny are lost each generation. For Ventana Double Cone, F calculated from heterozygote deficiency in the embryos was 0.455, which could be achieved in one generation only with nearly 100% selfing.

In addition to minimal structure within mature populations, there was little detectable structure among populations either; F_{ST} was only 0.080. Nevertheless, this is relatively high for a narrow endemic. F_{ST} in some wide-ranging conifers is even less than 0.080; e.g., most estimates for European silver fir in Table 8, except in the case of populations that originated from different refugia (Mejnartowicz 2004) or for insular-mainland comparisons (Vicario et al. 1995). Our embryo populations showed even less differentiation than mature trees; F_{ST} was only 0.025 for embryos.

Despite the lack of differentiation, the estimate of gene exchange among mature Santa Lucia fir populations was low relative to many conifers; the estimate based on Wright's F_{ST} was 2.88. Because estimates of *Nm* do not reach equilibrium for perhaps 100–1000 generations after gene flow ceases (Slatkin and Barton 1989), 2.88 may reflect past contact, not necessarily the present rate of migration. *Nm* of 2.88 is similar to estimates in Mexican firs with highly fragmented ranges (Aguirre et al. 2000). By comparison, estimates of *Nm* in pines are usually much higher, and the lowest value reviewed in Ledig (1998) was 3.4 for Colorado pinyon (*Pinus edulis* Engelm.), about the same as that estimated for Santa Lucia fir. For embryos, the estimate of *Nm* was 9.75, which suggests gene flow by pollen exchange in the seed year sampled.

The lack of relationship between genetic distance and geographic distance might suggest that genetic drift was important in the structure of Santa Lucia fir; a correlation would be expected with extensive gene flow because near neighbors are more likely to exchange genes than distant ones. Of course, geographic distance may not adequately reflect the complexities of slope, aspect, wind direction, distance from the coast, and a myriad of other factors. Nevertheless, relationships between geographic distance and genetic distance are found in other species that occupy a similar diversity of habitat (e.g., Ledig 2000). Although some near neighbors clustered on the dendrogram calculated from Nei's genetic distance (not shown), there was little phylogeographic pattern that would suggest isolation by distance through a stepping stone model. The lack of any parsimonious relationship among the populations that we sampled may reflect the paucity of polymorphic loci available and the uniformly low estimates of genetic distance. The test for recent bottlenecks found no greater heterozygosity than that expected for populations at mutation-drift equilibrium and, therefore, the low level of diversity in Santa Lucia fir must represent a more ancient restriction in the species numbers.

Lack of diversity within and lack of differentiation among populations, plus little indication of recent bottlenecks in the three populations which we could test, suggest to us that Santa Lucia fir was reduced to a single interbreeding population, at least once, in which drift occurred. Like Axelrod (1976), we feel that this could have happened during the warmer, dryer, mid-Holocene and, subsequently, the species expanded throughout its present range. The estimate of *Nm*, although low compared to values reported for pines, is high enough to suggest recent contact within the last few hundreds to thousands of generations, perhaps, during the last glacial period.

Seed yields

Until now, almost no information on seed yields per cone or seed weights have been published to guide

restoration should it become necessary (Schopmeyer 1974). Our observations of a potential 188 to 262 seeds per cone, realized viable seed counts of about 19–89, and 100-seed weight of about 3.5 g may help in planning restoration projects.

Viable seeds per cone reflect the rate of selfing in the Pinaceae; in experimentally pollinated conifers, seed yields are considerably reduced by self-pollination relative to cross-pollination (Kraus and Squillace 1964; Franklin 1970; Coles and Fowler 1976; Ledig 1986). Among the six Santa Lucia fir populations in the present study, the range in full seeds per cone was large, from 18.6 at Villa Creek and 22.7 at San Simeon, the two southernmost, highly isolated, populations, to 93.7 at Cone Peak, the largest population sampled. The range in total number of seeds per cone was much less, from 236.4 at Villa Creek to 262.2 at Big Sur, so the variation in viable seeds per cone reflects differences in the percentage of the total seeds that are empty, 91.3% at Villa Creek to 53.0% at Cone Peak.

Our estimates of seed yields are similar to Talley's (1974). For 12 trees representing 7 populations, he found 195–370 seeds per cone compared to our population means of 188–262 seeds, based on 20–50 trees per population. Among 11 trees in his sample, there were 20–57 full seeds per cone (and an outlier, 274 seeds, in his 12th tree) compared to our population means of 18.6–93.7. The percent full seeds in the two studies were, therefore, 7–24% versus 8.7–47%, respectively. Our values for percent full seeds may be slightly higher than Talley's (1974) because our Clipper Mill excluded small, undeveloped seeds, whereas he included them in his base count of total seeds per cone.

Like Santa Lucia fir, grand fir (*A. grandis* (Dougl. ex D. Don) Lindl.), Pacific silver fir (*A. amabilis* Dougl. ex Forbes), and subalpine fir all have a low number of filled seeds (Singh and Owens 1982). The percentage of viable seeds in Pacific silver fir may be only 22%; in noble fir (*A. procera*), 24%; in grand fir, 28%; and up to 38% in subalpine fir (Franklin 1968). Much of the loss of reproductive potential in the firs of western North America is due to a paucity of pollen and fir's unspecialized and inefficient pollination mechanism (Owens and Molder 1977; Singh and Owens 1981). Ovules in Pacific silver fir and other fir species fully enlarge and develop seed coats in the absence of pollination, resulting in normal appearing but empty seeds (Owens and Molder 1977). Seed chalcids also reduce seed yields in these and other western firs, and were the most important factor in seed loss of grand fir (Singh and Owens 1982). We did not separate seed losses into chalcid-related versus unpredated empty seeds, but the combined loss was over 50% of the crop in the years that we sampled and may be 100% in many years (Talley 1974).

The differences in full seed yields among Santa Lucia fir populations suggests that either pollen limitation or embryo death following inbreeding were responsible. The three populations with the lowest numbers of full seeds in our study (Big Sur, Villa Creek, and San Simeon) were the smallest populations, suggesting inbreeding. Empty seeds may reflect failure of the embryo to develop after fertilization, which is an early expression of inbreeding depression and an indication of self-pollination (Singh and Owens 1981). In addition, Villa Creek and San Simeon are isolated from other Santa Lucia fir by great distances and unlikely to receive pollen input from other populations. Pollen dispersal in fir is limited relative to that in more extensively studied pines; fir pollen is always under-represented compared to its occurrence in the vegetation (Jackson 1991; Arista and Talavera 1994, 1996; Jackson and Smith 1994). By contrast to Villa Creek and San Simeon, the population that had the highest percentage of full seeds was the most extensive stand of Santa Lucia fir in our sample, Cone Peak, where inbreeding and pollen limitation might be less of a problem.

Among populations, seed weight was negatively associated with the number of full seeds per cone (Figure 2a). One explanation is that developing seeds compete for maternal resources so that the more seeds per cone, the smaller the individual seeds. However, the estimated relationships between seed weight and number of full seeds among trees within populations provides no support for this argument because it was positive in most cases (Figure 2b), although not significantly different from zero except for Ventana Double Cone. Large seeds are associated with long growing seasons and seem an adaptive response to aridity (Baker 1972). Cone Peak with many small seeds experiences a short growing season and high precipitation (Talley 1974) compared to San Simeon and Villa Creek, which have large seeds and occupy a drier environment. Ventana Double Cone and Cone

Peak have similar seed weights, but Ventana Double Cone has many fewer full seeds per cone, perhaps, because of its high rate of inbreeding, which approaches complete selfing. The seed size–seed weight relationship may be the result of all three factors, adaptations to length of growing season, drought, and inbreeding.

Conservation of Santa Lucia fir

Legg (1953) and others believed that Santa Lucia fir was making its "last stand", but Talley (1974) thought this exaggerated. Santa Lucia fir may give the appearance of a vulnerable species because it is rarely seen. Its range is largely within the Ventana Wilderness of the Los Padres National Forest, and most stands are accessible only to backpackers. The exceptions are a seasonal, dirt road along Arroyo Seco from which a few isolated trees of Santa Lucia fir can be glimpsed, and Cone Peak. Cone Peak is relatively accessible and involves only a short walk. Elsewhere, access is difficult. In addition to lack of access, Santa Lucia fir is not commercially valuable and, therefore, has not been exploited for timber. Its

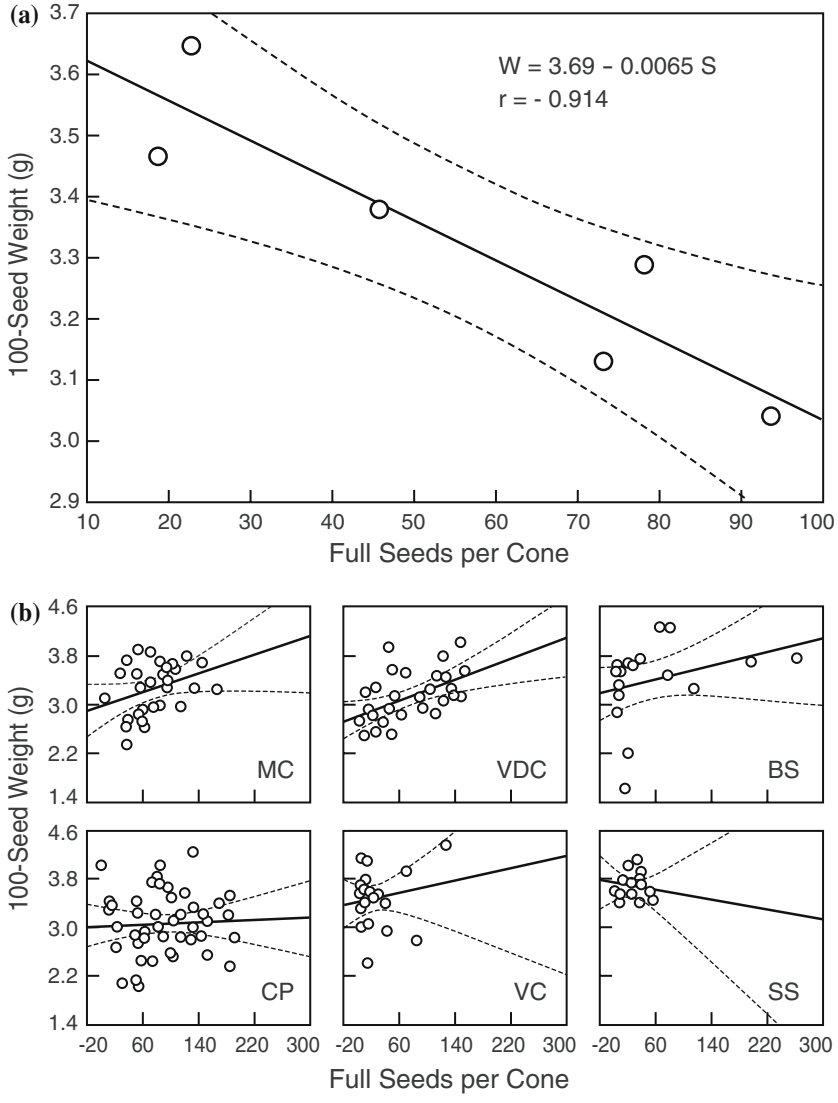

Figure 2. (a) Correlation with 0.95 confidence bands between population means for 100-seed weight and number of full seeds per cone. (b) Estimated relationships with 0.95 confidence bands between 100-seed weight and number of full seeds per cone for trees within each of six populations of Santa Lucia fir: MC = Miller Canyon; VDC = Ventana Double Cone; BS = Big Sur; CP = Cone Peak; VC = Villa Creek; SS = San Simeon.

decline since the Miocene, its limited range and fragmented distribution are the result of long-term geoclimatic changes, not human exploitation or human-mediated habitat loss. Nevertheless, it could be endangered by further anthropogenic warming.

We believe that the real danger to Santa Lucia fir is lack of genetic diversity, either within populations or among populations. Species with little diversity are in danger of any new threat because they lack the ability to field an evolutionary response. Santa Lucia fir became extinct in its former range as the western United States became progressively more arid after the Miocene (Axelrod 1976), and it is now restricted to the coastal fog belt in relatively mesic ravines and on steep, rocky slopes where it is shielded from hot fires (Talley 1974). In the process of range reduction it lost genetic diversity.

A slow attrition in the number of populations is to be expected from century to century, and protection from exploitation alone is unlikely to prevent eventual extinction. At exposed, high elevation sites, effective regeneration may occur only once or twice a century, as inferred from age distributions of trees in natural populations (Talley 1974). The infrequent periods of regeneration may be driven, in part, by climate and fire, but reductions in seed yield due to inbreeding and predation probably play a role. Demographic stochasticity might result in population extinction, given the right sequence of high seed chalcid numbers and climatic conditions unsuitable for pollination, seed maturation, and/or seedling survival. Dispersal of Santa Lucia fir seeds from canyon to canyon is likely weak because of the limited seed crop and the inhospitable terrain between ravines.

As indicated by low full seed yields, never higher in our collections than 47% of the total seed count, Santa Lucia fir's reproductive potential already seems seriously impaired by pollen limitation and by selfing, as well as by seed chalcids which in some years may destroy 100% of the seeds (Talley 1974). However, even our estimates of full seed per cone exaggerate true regeneration potential. In addition to the losses resulting from lack of pollination and seed predation are the losses from genetic deaths as the inbred zygotes are removed by selection during stand maturation, an event that must occur as populations move from a mean fixation coefficient of 0.388 in the embryos to 0.049 in the mature stands. These genetic deaths occur as seeds fail to germinate and as a high proportion of the seedlings that do germinate succumb to competition or developmental failures before maturation.

Santa Lucia fir's remaining genetic resources should be protected *ex situ* in seedbanks and field gene banks in case restoration is needed or to establish new populations in suitable habitat if climate change scenarios unfold as projected. Because differences among populations were small, the whole range could possibly be treated as a single seed zone for restoration. Common garden tests and investigations using other markers should be used to confirm or reject this supposition; lack of isozyme diversity does not rule out adaptive variation at other loci. However, Talley (1974) found no adaptive variation in photosynthetic rate, temperature response, or seedling growth in phytotron experiments, and perhaps little adaptive variation is to be expected because environmental variation is limited within the species' narrow range.

Theoretically, both commonly distributed rare alleles and common alleles could be captured for *ex situ* conservation with any sampling scheme; that is, by sampling a large number of trees from either a single variable population like Cone Peak or distributed among several populations (Marshall and Brown 1975). It is important to sample large numbers of trees if the objective is to capture as many alleles as possible. Rare isozyme variants probably represent neutral mutants, but some may become advantageous under changed conditions, and they mark segments of chromosomes that may include potentially adaptive variants. The results of this survey suggest that among the populations sampled, Cone Peak is one of the best candidates for *ex situ* conservation and use as a seed source for restoration. The maximum number of alleles and polymorphic loci were found in Cone Peak and, to a lesser degree, Big Sur. No novel alleles were found in other populations. Furthermore, the greatest number of sound seeds per cone was found at Cone Peak and it is the most accessible population of Santa Lucia fir. Nevertheless, a cautious approach would ensure that multiple populations were conserved *ex situ*.

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