Founder Effects and the Genetic Structure of Coulter Pine

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Mean expected heterozygosity at 33 isozyme loci decreased with latitude from 0.193 near the southern extreme of Coulter pine's range to 0.107 at its northern extreme. This decrease was paralleled by a loss of alleles north of the Peninsular Ranges of southern California. Fifteen alleles dropped out along the roughly linear range, at points coincident with large gaps in the species' distribution. The pattern may reflect a cascading series of founder events as Coulter pine invaded the Transverse Ranges and the South Coast Ranges from Pleistocene refugia. Alleles were not replaced following colonization, probably because migration, *Nm,* among populations is only 0.74–1.27, depending on estimator, the lowest values reported in any pines. Wright's (F_{ST}) indicated that 16.5% of the total genic diversity is among populations. The fixation index, (F_{IS}) of 0.072 indicated only a moderate excess of homozygotes. However, the northernmost outlier had significant excess homozygosity ($F = 0.253$). Hybridization may also play a role in the genetic structure of Coulter pine: 16 alleles were novel, or private, occurring only where Coulter pine was sympatric with Jeffrey pine, particularly at San Benito Mountain. Some of these novel alleles could be the result of introgression from Jeffrey pine, or possibly represent hybrizymes, products of intragenic recombination between genomes.

The ranges of boreal and temperate zone flora moved back and forth during the transitions between glacial and interglacial climates. The nature of this migration, especially at the end of the last glacial period, is pertinent to understanding the consequences of anthropogenic warming projected for the next century as the result of burning fossil fuels. Migration could occur as a regularly advancing front or erratically through long-distance colonization. Much of what is known about past migration rates comes from analysis of conifer pollen buried in the sediments of ancient lakes and ponds, and long-distance dispersal has been used to explain rapid, postglacial rates of migration (Davis 1981). The spread of exotic pines 16–18 km from their point of introduction on islands and continents in the Southern Hemisphere is a measure of the potential for long-distance colonization, and because self-incompatibility is unknown in pines, even a single colonist can reproduce by facultative selfing (Bannister 1965).

A species' genetic structure should depend, in part, on whether migration occurred by creep or by surge; that is, as a steadily advancing front or a series of long-distance colonization events that created permanently or temporarily isolated populations. Long-distance colonization should result in genetic drift because of the founder effect, and if the advance colonies were the sources for further leaps northward, the result would be a cascading series of founder events. Diversity, especially measured by allelic richness, would be lost at each step in long-distance colonization, and the track of migration should be traceable by an allelic phylogeny.

For many species, however, subsequent spread would reunite colonies, and pollen exchange among them would replace lost alleles. The number of migrants per generation, (Nm) in most conifers is very high; for example, 4.6–17.2 for several species of pines sampled across an extensive geographic distribution (reviewed in Ledig 1998). Thus, in the light-seeded Rocky Mountain lodgepole pine (*Pinus contorta* ssp. *latifolia* Engelm.) allelic loss is only obvious near the northern periphery of its range, where advance colonies have been established as recently as 100 years ago (Cwynar and MacDonald 1987; Wheeler and Guries 1982a,b).

In contrast to many conifers, Coulter

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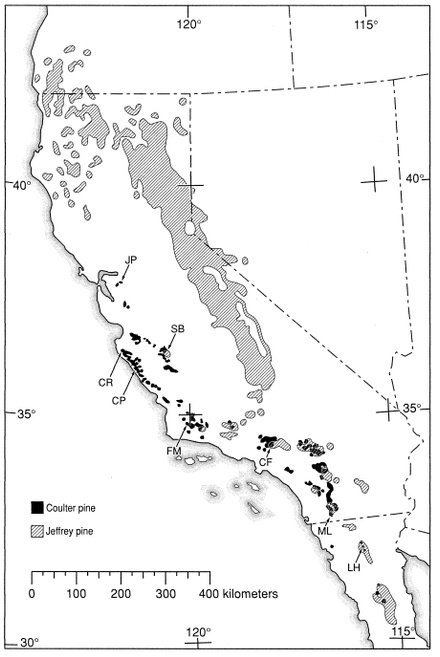

Figure 1. The natural ranges of Coulter and Jeffrey pines [after Little (1971); for more detail, see Griffin and Critchfield (1972) and Minnich (1987)]. Sampled populations are indicated by arrows: JP = Jim's Place; CR = Chews Ridge; CP = Chalk Peak; SB = San Benito Mountain; FM = Figueroa Mountain; CF = Charlton Flat; ML = Mount Laguna; LH = Laguna Hanson.

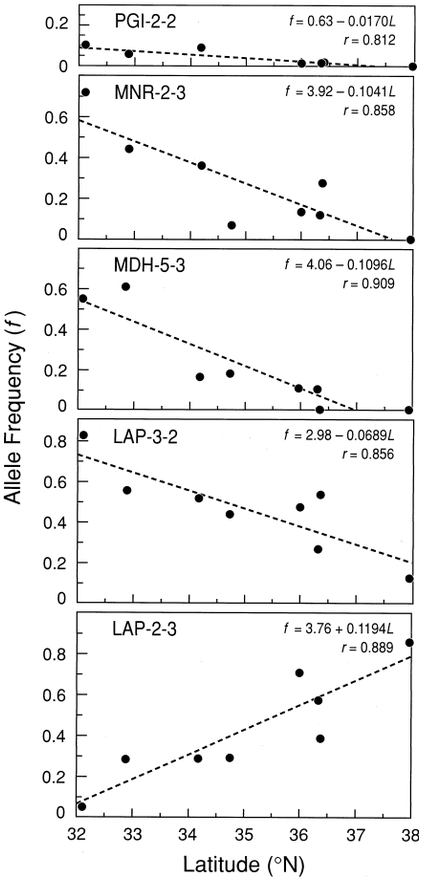

Figure 2. Allele frequencies with respect to latitude at selected loci in eight Coulter pine populations.

pine (*Pinus coulteri* D.Don) provides a good model to track the loss of alleles resulting from founder events. The seeds of Coulter pine are among the heaviest in the genus *Pinus* (averaging 0.32 g; Krugman and Jenkinson 1970). Seed dispersal is by wind, so it is limited under most circumstances. Furthermore, Coulter pine generally occurs at elevations of 900–2100 m (Sudworth 1908) on mountain ranges often separated by semiarid habitats to which it is apparently not adapted. The large gaps in its range must at least restrict gene flow by pollen dispersal, if not eliminate it entirely.

Coulter pine occurs as small, widely scattered stands in the Sierra San Pedro Martir and Sierra Juárez of Baja California (Minnich 1987) and becomes more common further north in the Peninsular Ranges and the Transverse Ranges of Alta California (Griffin and Critchfield 1972). In the Transverse Ranges, a gap of about 50 km separates Coulter pines in the San Bernardino Mountains from those in the San Gabriel Mountains (Figures 1 and 6). An even larger gap of 120 km separates Coulter pines in the San Rafael Mountains of the extreme South Coast Ranges from those in the Transverse Ranges. In the South Coast Ranges, Coulter pine is common along the coast in the Santa Lucia Range, but rarer and more scattered along the inner ranges. In the Diablo Range of the inner South Coast Ranges, a gap of about 50 km separates Coulter pines on the foothills of Mount Diablo, their northernmost occurrence, from the next nearest Coulter pines to the south. A minimum gap of 30 km separates Coulter pines in the Santa Lucia Range from those in the Diablo Range. The inclusive range of Coulter pine is roughly linear along a northwest-southeast corridor.

I hypothesized that genetic drift, operating through the founder effect, was responsible for a decrease in expected heterozygosity from south to north in Coulter pine (Ledig 1987). Sequential loss of alleles, south to north, might map Coulter pine's colonization of California's Transverse and South Coast Ranges. However, the genetic structure of Coulter pine may be complicated by hybridization with Jeffrey pine (*Pinus jeffreyi* Grev & Balf.). Although Coulter and Jeffrey pines are not in the same subsection of the genus, both natural and controlled hybrids are known (Critchfield 1966). Where the species occur together, small colonies, which leads to pollen limitations, and partial self-sterility may be conducive to hybridization.

The objective of the research reported here was to track the migration route of Coulter pine along the Peninsular, Transverse, and South Coast Ranges of California by reference to its genetic structure, including the presence or absence of alleles. In fact, several allozymes were apparent markers of long-distance colonization. However, many other alleles were novel and, perhaps, are associated with hybridization. The results reported below are not meant to be the final answers regarding the effects of migration and hybridization on the genetic structure of Coulter pine, but are meant to stimulate others to test the hypotheses suggested here.

Materials and Methods

Cones were collected from eight populations of Coulter pine chosen to bracket as much of the north-south range of the species as possible and systematically sample across the major gaps in its distribution (see Table 1 and Figure 1 for locations). Because Coulter pine cones do not open immediately upon seed maturity in the autumn and seeds are often retained in cones for months (Borchert 1985), it was possible to extend the collection over several months; five populations were sampled between September 1981 and January 1982 and the other three in May and June 1984. Cones were collected by climbing, by shooting down cone-bearing branches, or by pole pruner, depending on the size of the trees and the location.

The goal was to sample 35–36 trees in each population. Within populations, cone-bearing trees were chosen in as close proximity as possible until the goal was reached, although in two instances the sample was distributed over 1 km or more. In a few cases, samples were actually smaller than 35 trees (as few as 26 trees) because some trees with cones failed to yield viable seeds. Seeds were extracted after the cones opened and were stored at 1°C until needed. Cones and seeds were maintained separate by tree.

In late 1984, seeds were germinated for isozyme analysis, and when the radicles appeared through the seed coat, the megagametophytes and embryos were excised and separated. Extracts of one megagametophyte-embryo pair from each tree were analyzed, resulting in samples of 52–

Table 1. Location of Coulter pine populations sampled

Population	County[a]	Mountain range	Latitude N	Longitude W	Elevation (m)
Jim's Place, Black Diamond Regional Preserve	Contra Costa	Diablo Range	37°57′	121°53′	390
San Benito Mountain Natural Area	San Benito	Diablo Range	36°24′	120°41′	1580
Chews Ridge, Los Padres National Forest	Monterey	Santa Lucia Range	36°19′	121°34′	1380
Chalk Peak, Los Padres National Forest	Monterey	Santa Lucia Range	36°00′	121°26′	1120
Figueroa Mountain, Los Padres National Forest	Santa Barbara	San Rafael Mountains	34°44′	119°59′	1230
Charlton Flat, Angeles National Forest	Los Angeles	San Gabriel Mountains	34°18′	118°01′	1700
Mount Laguna, Cleveland National Forest	San Diego	Laguna Mountains	32°52′	116°25′	1680
Laguna Hanson, Parque Nacional Constitucion de 1857	Ensenada	Sierra Juárez	32°03′	115°56′	1800

[a] Counties in California, except for Laguna Hanson which is in the municipio Ensenada in the state of Baja California, Mexico.

72 independent genomes (embryos from 26–36 trees), assuming that the pollen parents were drawn at random from a large population. Megagametophyte and pollen allele frequencies generally were in good agreement.

Using the techniques of starch-gel electrophoresis described by Conkle et al. (1982), 25 enzyme systems were assayed. The number of loci and alleles were interpreted by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species (Conkle 1981) and by coelectrophoresis of Coulter pine with Jeffrey pine. Where several zones of activity were observed for a single enzyme, hyphenated numerals following the enzyme abbreviation were used for identification. Thirty-three presumptive loci were consistently scored and used in the statistical analysis.

BIOSYS (Swofford and Selander 1981) was used to calculate deviation of genotype frequencies from Hardy–Weinberg expectations, genic diversity, Wright's (1965) *F* statistics, Nei's (1978) unbiased genetic distance, Rogers' (1972) distance, Rogers' modified genetic distance (Wright 1978), and Edwards "E" (Edwards 1974). BIOSYS grouped populations into phylogenetic trees with distance Wagner procedures (Farris 1972). Because Nei's (1978) genetic distance is nonmetric and inappropriate for distance Wagner procedures (Swofford and Selander 1981), Rogers' (1972) distance measure was used. All inferences about the populations apply to the progeny generation of mature, cone-bearing trees.

The degree of genetic isolation among populations was estimated by *Nm,* the number of migrants per generation. *Nm* was calculated from F_{ST}, the proportion of the total genic diversity among populations (Wright 1951), and from the number and frequency of private alleles, the unique alleles found in only one population (Barton and Slatkin 1986; Slatkin 1985). The association between geographic and genetic distances was checked using Mantel's (1967) generalized regression procedure.

Results

Eight loci (*FDP-1, GDH, G6P-2, MPI, PGI-1, 6PG-3, SRDH, UGP-1*) were monomorphic in all populations sampled, and 25 loci (Table 2) had variants in at least one population. Of the 25 polymorphic loci, 11 were variable in only a single or, at most, two populations and 9 were polymorphic in all eight populations. The remaining six were polymorphic in four to seven populations. Populations were distinguished by major differences in allele frequencies. For example, at Laguna Hanson in Baja California, allele 3 at *MNR-2* was most common, at a frequency of 0.724, but at Jim's Place on Mount Diablo its frequency decreased to zero, and allele 1, with a frequency of 0.828, was the most common allele. Other striking changes in frequency occurred at *LAP-2, LAP-3,* and *MDH-5.*

Allele frequencies at the latter four loci (i.e., *MNR-2, LAP-2, LAP-3,* and *MDH-5*) were significantly correlated with latitude at $P \leq .01$, and allele frequency at another locus (*PGI-2*) was correlated with latitude at $.05 \geq P \geq .01$ (Figure 2). Therefore, frequencies at 5 of 25 polymorphic loci, or 20%, were related to latitude. However, if only the 13 loci polymorphic in five or more populations were considered, 39% have allele frequencies correlated with latitude. In either case, it seems that the number of significant correlations exceeded expectations based on chance alone.

Also notable was the number of private alleles. A total of 16 alleles were found in only a single population. Five of these were unique to San Benito Mountain. Each of the three southernmost populations had three unique alleles each. The three southernmost populations and San Benito Mountain are all sympatric with Jeffrey pine. The remaining two private alleles were observed in the sample from Chews Ridge. Although Chews Ridge is not within the native range of Jeffrey pine, Jeffrey pine has been planted there (Griffin 1978), and hybrids between the two species have been known from Chews Ridge for 50 years (Zobel 1951, 1953). In addition to the 16 private alleles, another four alleles appeared in either two or three populations which were in sympatry with Jeffrey pine. The occurrence of unique alleles is not related to sample size; the three populations without rare alleles (Jim's Place, Chalk Peak, and Figueroa Mountain) include those with the largest samples.

Levels of genic diversity were moderate to high in all populations of Coulter pine sampled (Table 3). The percentage of polymorphic loci varied from 33.3 to 48.5%. The number of alleles per locus ranged from 1.4 to 1.9. Mean expected heterozygosity (H_e, unbiased estimate) was highest (0.193) in the southernmost sample, Laguna Hanson from Baja California, and lowest (0.107) in the northernmost, Jim's Place, on a ridge north-northeast of Mount Diablo.

Expected heterozygosity decreased in a clinal fashion from south to north. The correlation between H_e and latitude was −0.912 (Figure 3). The cline was due in large part to a loss of certain alleles and progressive fixation of loci between Laguna Hanson and Jim's Place. Of the 25 loci that were polymorphic elsewhere, 16 were fixed at Jim's Place. Therefore, the percentage of polymorphic loci also was related to latitude ($r = -0.709$; $P \approx .05$); the number of alleles per locus was related to latitude at a lower level of confidence ($r = -0.647$; $P \approx .09$).

Allelic richness was not greatly biased by sample size. The smallest sample ($n = 52$) from Mount Laguna had nearly the greatest number of alleles, 55, compared

to a high of 56 in the sample from Charlton Flat (n = 66) and only 43 at Cone Peak, which had the largest sample (n = 72). When allelic richness was adjusted for sample size using Hurlbert's (1971) "rarefaction" method, the change was about one allele or less (Table 3).

Fifteen alleles present in the sample from Mount Laguna seem to disappear northward (Figure 5). Allele 2 in *GOT-1* drops out between Mount Laguna and Charlton Flat in the San Gabriel Mountains, and does not reappear northward. The greatest number of alleles, five (*ACON* 3, *LAP-3* 4, *PGI-2* 3, *PGM* 3, and *UGP-4* 4), drop out between Charlton Flat and Figueroa Mountain in the San Rafael Mountains and do not reappear northward in the Santa Lucia Range; the largest gap in the range of Coulter pine, about 120 km, is between the San Gabriel Mountains and the San Rafael Mountains. One allele (*PGM* 4) drops out between Figueroa Mountain and Chalk Peak in the Santa Lucia Range. Although Coulter pine is encountered throughout the Santa Lucia Range, gaps are numerous, and four alleles (*FEST* 3, *LAP-2* 2, *LAP-2* 4, and *SKDH* 3) drop out between Chalk Peak and Chews Ridge. Four more alleles (*LAP-3* 3, *MDH-5* 3, *MNR-2* 3, and *PGI-2* 2) drop out between Chews Ridge and Jim's Place in the Diablo Range. The gap between the small populations of Coulter pine on Mount Diablo, such as Jim's Place, and the nearest populations to the south is about 50 km. Of these 15 alleles, *PGI-2* 2 and *LAP-2* 4 are apparently missing at Figueroa Mountain but occur north and south. They may have gone undetected at Figueroa Mountain because of sampling error or may be missing because of genetic drift subsequent to colonization of the San Rafael Mountains.

Observed heterozygosity (H_o) was lower than expected heterozygosity, ranging from 0.083 to 0.179 (Table 3). Excess homozygosity measured by the fixation index across loci and sampled populations (F_{IS}) was 0.072 (Table 4), which suggests some inbreeding. However, the level of inbreeding varied among populations (Table 3). The sample from Jim's Place had the highest inbreeding coefficient, 0.253. Four loci at Jim's Place and two at Laguna Hanson, a southern relict, deviated significantly from Hardy–Weinberg equilibrium (chi square; $P \leq .05$). In the six other populations sampled, of 87 tests, only one locus in each (or none at Mount Laguna) deviated significantly from Hardy–Weinberg equilibrium, which is about equal to expectations based on chance alone.

Table 2. Allele frequencies for 25 polymorphic loci in eight populations of Coulter pine

Locus/allele		Population[a] (sample size)							
		JP (64)	SB (60)	CR (66)	CP (72)	FM (72)	CF (66)	ML (52)	LH (58)
AAP-2/	1	0.828	0.817	0.561	0.944	0.903	0.955	0.923	0.776
	2	0.172	0.150	0.439	0.056	0.097	0.045	0.077	0.224
	3	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
ACON/	1	0.906	0.683	0.939	0.819	0.861	0.742	0.846	0.603
	2	0.094	0.217	0.061	0.181	0.139	0.197	0.115	0.310
	3	0.000	0.067	0.000	0.000	0.000	0.045	0.019	0.086
	4	0.000	0.033	0.000	0.000	0.000	0.015	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
ACP-1/	1	1.000	0.967	1.000	1.000	1.000	1.000	1.000	0.690
	2	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.310
ADH-2/	1	0.734	0.000	0.015	0.000	0.042	0.061	0.038	0.397
	2	0.266	1.000	0.985	1.000	0.958	0.939	0.962	0.603
ALD-2/	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.810
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.190
CAT-2/	1	1.000	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
FEST/	1	1.000	1.000	1.000	0.986	0.944	0.924	0.769	1.000
	2	0.000	0.000	0.000	0.000	0.028	0.000	0.173	0.000
	3	0.000	0.000	0.000	0.014	0.028	0.045	0.058	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000
GOT-1/	1	1.000	1.000	1.000	1.000	1.000	1.000	0.942	0.983
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.017
GOT-3/	1	0.687	0.300	0.273	0.264	0.500	0.409	0.769	0.397
	2	0.250	0.183	0.424	0.528	0.181	0.227	0.058	0.103
	3	0.063	0.500	0.167	0.208	0.319	0.242	0.173	0.500
	4	0.000	0.017	0.136	0.000	0.000	0.121	0.000	0.000
G6P-1/	1	1.000	1.000	0.985	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
IDH-1/	1	1.000	0.983	1.000	1.000	1.000	0.864	1.000	1.000
	2	0.000	0.017	0.000	0.000	0.000	0.136	0.000	0.000
LAP-2/	1	0.859	0.383	0.576	0.708	0.292	0.288	0.288	0.052
	2	0.000	0.400	0.000	0.042	0.250	0.045	0.058	0.155
	3	0.141	0.217	0.424	0.000	0.458	0.606	0.538	0.690
	4	0.000	0.000	0.000	0.250	0.000	0.061	0.115	0.103
LAP-3/	1	0.703	0.250	0.303	0.250	0.319	0.152	0.250	0.172
	2	0.297	0.617	0.409	0.569	0.542	0.606	0.635	0.828
	3	0.000	0.133	0.288	0.181	0.139	0.152	0.058	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.091	0.058	0.000
MDH-1/	1	1.000	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3/	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.776
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.224
MDH-5/	1	1.000	0.950	0.894	0.889	0.819	0.833	0.346	0.448
	2	0.000	0.050	0.000	0.000	0.000	0.000	0.038	0.000
	3	0.000	0.000	0.106	0.111	0.181	0.167	0.615	0.552
ME/	1	0.750	0.533	0.303	0.306	0.472	0.303	0.308	0.328
	2	0.250	0.467	0.697	0.694	0.528	0.697	0.673	0.672
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
MNR-1/	1	0.969	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.031	0.017	0.000	0.000	0.000	0.000	0.000	0.000
MNR-2/	1	0.828	0.683	0.818	0.750	0.861	0.500	0.558	0.103
	2	0.172	0.033	0.061	0.111	0.069	0.136	0.000	0.172
	3	0.000	0.283	0.121	0.139	0.069	0.364	0.442	0.724
PGI-2/	1	1.000	0.983	0.985	0.986	1.000	0.864	0.462	0.862
	2	0.000	0.017	0.015	0.014	0.000	0.091	0.058	0.103
	3	0.000	0.000	0.000	0.000	0.000	0.045	0.481	0.034
PGM/	1	0.906	1.000	0.924	0.972	0.917	0.530	0.596	0.931
	2	0.094	0.000	0.076	0.028	0.028	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.015	0.038	0.069
	4	0.000	0.000	0.000	0.000	0.056	0.455	0.365	0.000
6PG-2/	1	1.000	1.000	0.985	1.000	1.000	1.000	0.788	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.212	0.000
	3	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
SKDH/	1	1.000	0.917	1.000	0.889	0.972	0.955	0.865	1.000
	2	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.017	0.000	0.111	0.028	0.015	0.135	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000
SOD-4/	1	1.000	1.000	1.000	1.000	1.000	0.909	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000

Table 2. Continued.

Locus/allele		Population[a] (sample size) JP (64)	SB (60)	CR (66)	CP (72)	FM (72)	CF (66)	ML (52)	LH (58)
UGP-4/	1	0.422	0.517	0.712	0.597	0.597	0.288	0.096	0.448
	2	0.141	0.233	0.015	0.083	0.014	0.242	0.173	0.121
	3	0.437	0.250	0.273	0.319	0.389	0.439	0.692	0.293
	4	0.000	0.000	0.000	0.000	0.000	0.030	0.038	0.138

[a] JP = Jim's Place; CR = Chews Ridge; CP = Chalk Peak; SB = San Benito Mountain; FM = Figueroa Mountain; CF = Charlton Flat; ML = Mount Laguna; LH = Laguna Hanson.

The proportion of the total genic diversity among populations (F_{ST}) (Table 4) was 0.165, which is high for pines. The number of migrants per generation (*Nm*), calculated from F_{ST}, was only 1.27. *Nm* calculated from the frequency of private alleles was even lower, 0.74. The low level of gene flow indicated by the estimates of *Nm* is reflected in the very high values of Nei's (1978) unbiased genetic distance. The mean genetic distance ($\bar{D}$) was 0.185, and genetic distance between populations was highly correlated with geographic distance (r = 0.82; Table 5 and Figure 5); using Mantel's (1967) generalized regression procedure, r = 0.83.

Discussion

The distribution of alleles suggests that the genetic structure of Coulter pine has been largely determined by a cascading sequence of founder events. Based on the number of alleles and their distribution, the center of diversity for Coulter pine is the Laguna Mountains, represented by the sample from Mount Laguna.

Fifteen alleles were lost northward, with the greatest losses occurring across major gaps in Coulter pine's fragmented range (Figure 4). This pattern seems highly improbable if chance alone was responsible. Considering only those 15 alleles present in three or more populations, 13 show a pattern of presence in the south and absence northward; one is present in the north and absent south of Figueroa Mountain, and one is irregular in distribution, found in three widely separated populations where Jeffrey pines are present. If each of three patterns were equally likely (i.e., present in south, absent in north; present in north, absent in south; or irregular), then by chance only 5 of these 15 alleles would be expected to show a pattern of disappearance northward. However, a chi-square test of the observed versus expected is highly significant ($P <$ .005). The result is still highly significant if *PGI-2* 2 and *LAP-2* 4 (Figure 4) are considered "irregular" patterns.

The pattern of successive loss of alleles is consistent with a scenario of long-distance colonization beginning from southern refugia following late-Pleistocene warming. During the cool climate characterizing Pleistocene glaciation, ranges of several tree species may have been shifted downward in elevation by more than 1000 m. Based on estimates from Clear Lake, about 150 km north of Mount Diablo, maximum Pleistocene cooling was 7°C–8°C (Adam and West 1983). Adam and West (1983) used a lapse rate of −0.6°C per 100 m in elevation to suggest a downward shift of at least 1167 m in vegetational zones. Hopkins' (1938) Law (1°C per 220 m in elevation) suggests a downward shift of 1540 m. Some species of the mixed conifer forest of the Sierra Nevada in California, such as giant Sequoia (*Sequoiadendron giganteum* [Lindl.] Buchholz), occurred at least 500–1000 m lower in elevation during the glacial maximum (Cole 1983). Coulter pine presently occurs at a maximum elevation of only 610 m on Mount Diablo (Vale 1979) and the peak is only 1173 m above sea level, which suggests that Coulter pine might have been excluded from this latitude during the glacial maximum and had to colonize from the south. By contrast, Coulter pine at Laguna Hanson in the Sierra Juárez occurs at an elevation of 1800 m and in the Sierra San Pedro Martir, its southernmost extension, it is found at 2150 m (Minnich 1987).

The little fossil evidence available suggests that the big-cone pines (*Pinus* subsection *Sabinianae*), which consists of Coulter, gray (*Pinus sabiniana* Dougl.), and Torrey pines (*Pinus torreyana* Parry ex Carr.), may have inhabited southern California for millions of years, but there is no

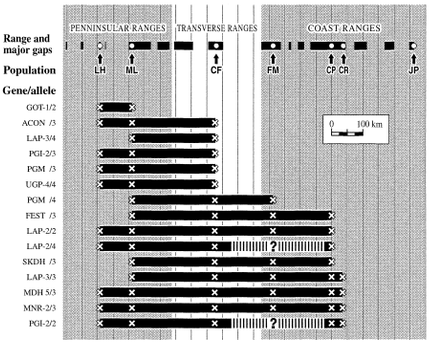

Figure 4. Presence (X) of alleles along the south to north distribution of Coulter pine in the Peninsular, Transverse, and Coast Ranges of Baja and Alta California. "?" indicates that an allele found in populations to the north and south was not detected in a sample of 72 at Figueroa Mountain. A linearized distribution of Coulter pine is shown by the bar at top and major disjunctions are indicated by gaps. Locations of sampled populations are indicated by arrows: JP = Jim's Place; CR = Chews Ridge; CP = Chalk Peak; FM = Figueroa Mountain; CF = Charlton Flat; ML = Mount Laguna; LH = Laguna Hanson.

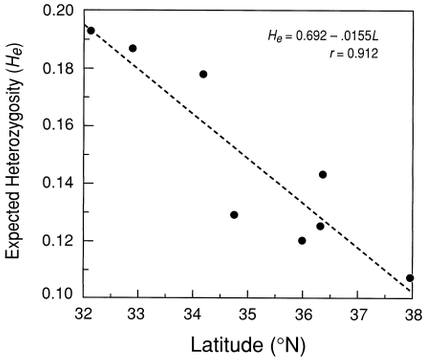

Figure 3. The relationship between expected heterozygosity and latitude in eight Coulter pine populations.

Table 3. Genic diversity in Coulter pine

Population	n	H_e	H_o	P	A	N_O	N_A	F
Jim's Place	32	0.107 (.031)[a]	0.083 (.027)[a]	33.3	1.4	38	38.0	0.253
San Benito Mountain	30	0.143 (.040)	0.130 (.037)	48.5	1.8	51	50.0	0.063
Chews Ridge	33	0.125 (.037)	0.116 (.036)	42.4	1.6	44	42.9	0.058
Chalk Peak	36	0.120 (.035)	0.107 (.032)	39.4	1.5	43	42.4	0.081
Figueroa Mountain	36	0.129 (.037)	0.127 (.037)	39.4	1.6	45	44.4	−0.001
Charlton Flat	33	0.178 (.042)	0.169 (.041)	48.5	1.9	56	55.2	0.051
Mount Laguna	26	0.187 (.040)	0.167 (.035)	48.5	1.9	55	55.0	0.063
Laguna Hanson	29	0.193 (.040)	0.179 (.039)	48.5	1.7	49	48.9	0.057
Mean		0.148	0.134	43.6	1.67			

Number of trees sampled (n), mean expected heterozygosity (H_e, unbiased estimate), observed heterozygosity (H_o), percent polymorphic loci (P), number of alleles per locus (A), total number of alleles observed at 25 polymorphic loci (N_o), number of alleles adjusted to a sample size of 52 (N_A), and fixation index or mean deviation from Hardy–Weinberg equilibrium (F).

[a] Standard errors in parentheses.

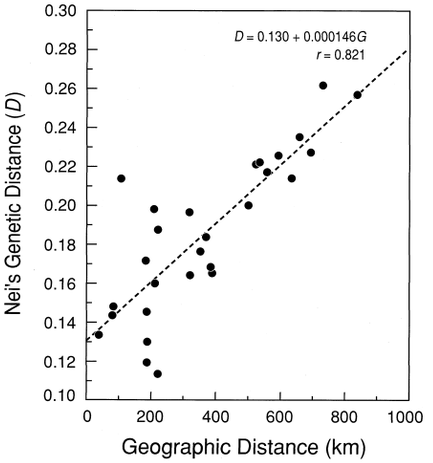

Figure 5. The correlation of Nei's unbiased genetic distance and geographic distance between pairs of Coulter pine populations.

Table 4. Estimates of Wright's (1965) *F*-statistics for 25 polymorphic loci in Coulter pine

Locus	F_{IS}	F_{IT}	F_{ST}
AAP-2	0.182	0.271	0.108
ACON	0.065	0.117	0.055
ACP-1	0.305	0.481	0.254
ADH-2	−0.054	0.431	0.460
ALD-2	−0.010	0.162	0.170
CAT-2	−0.017	−0.002	0.015
FEST	0.082	0.177	0.103
GOT-1	−0.051	−0.009	0.039
GOT-3	0.020	0.136	0.119
G6P-1	−0.015	−0.002	0.013
IDH-1	0.085	0.182	0.106
LAP-2	0.069	0.265	0.211
LAP-3	−0.011	0.089	0.099
MDH-1	−0.017	−0.002	0.015
MDH-3	0.108	0.288	0.202
MDH-5	0.186	0.414	0.281
ME	0.145	0.226	0.094
MNR-1	−0.027	−0.006	0.020
MNR-2	0.024	0.231	0.212
PGI-2	0.134	0.378	0.281
PGM	0.193	0.375	0.225
6PG-2	−0.036	0.147	0.177
SKDH	0.076	0.129	0.058
SOD-4	−0.100	−0.011	0.080
UGP-4	0.059	0.150	0.097
Mean	0.072	0.225	0.165

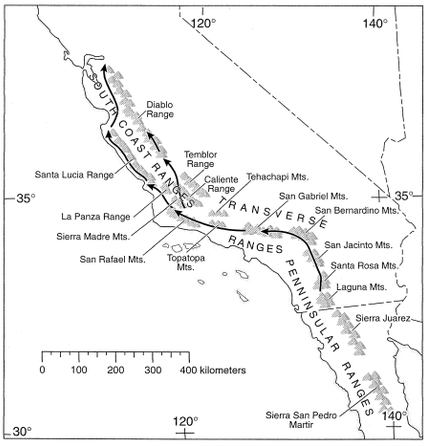

Figure 6. Mountain ranges mentioned in the text, and a possible postglacial migration route of Coulter pine, as inferred by presence or absence of alleles. South Coast Ranges include Diablo Range, Santa Lucia Range, Temblor Range, Caliente Range, La Panza Range, Sierra Madre Mountains, and San Rafael Mountains. For purposes of discussion, Transverse Ranges include Tehachapi Mountains, Topatopa Mountains, San Gabriel Mountains, and San Bernardino Mountains. Peninsular Ranges include, but are not limited to San Jacinto Mountains, Santa Rosa Mountains, Laguna Mountains, Sierra Juárez, Sierra San Pedro Martir.

fossil evidence north of Carpinteria, which is south of Figueroa Mountain. Fossils of *Pinus pieperi* Dorf, which may be synonymous with gray pine, are common, and are found exclusively south of the present range of gray pine (Axelrod 1986). The most recent fossil occurrence of *Pinus pieperi* is from the late Pleistocene (Wisconsin) at Carpinteria and Rancho La Brea. The fossil *Pinus hazenii* Axelrod from the Mount Eden formation of 5–6 million years before present (BP) has been referred to as Coulter pine (Axelrod 1938). The Mount Eden fossil beds lie south of the San Gabriel Mountains in the Transverse Ranges. Coulter pine-like fossils have also been found from the Pliocene-Pleistocene time boundary in the Pico formation, north of Ventura, in southern California (Axelrod DI, personal communication). No recent fossil records of Coulter pine or its relatives are known (Schorn HE, personal communication). Possibly the big-cone pines did not migrate north of the Transverse Ranges until the Holocene.

Once an advance colony, or "beachhead," was established, it was more likely to be the progenitor of (i.e., to colonize) patches of suitable habitat further northward than would its own antecedents to the south. Each colonizing event would provide an opportunity for loss of additional alleles in a cascade effect. The heavy seeds of Coulter pine, and therefore, low vagility, would assure a degree of isolation for the advance colonies, and considerable time might elapse before alleles were reintroduced by pollen dispersal from the south. The low estimates of *Nm* are consistent with poorly dispersed seed and a fragmented range that is a barrier to gene flow through pollen dispersal. For eleven species of pine (reviewed in Ledig 1998), *Nm* was never less than 3.4, and averaged 11.4. The gap separating Coulter pine in the San Gabriel Mountains from the San Rafael Mountains is about 120 km, and the populations on Mount Diablo are separated from the next nearest stands of Coulter pine to the south by about 50 km. These major gaps coincide with major losses in allelic diversity; of 15 alleles diagramed in Figure 4, five apparently disappear between the San Gabriel and San Rafael Mountains and four more disappear between the Santa Lucia Range and Jim's Place near Mount Diablo.

Coulter pine has extremely large seeds, and it is not difficult to believe that very few would be dispersed to advanced outposts. The mechanism of long-distance dispersal is unclear. No birds are known to cache Coulter pine seeds. Native Americans may have had a role; pine nuts other than pinyons are mentioned in many accounts of trade among early tribes in California, but the species identified are either sugar pine (*Pinus lambertiana* Dougl.) or gray pine (Davis 1974). Although Coulter pine seeds are not specifically mentioned by ethnographers, Farris (1983) argues that they were certainly used as food in the Santa Lucia Range, based on accounts of the early Spanish explorers.

In addition to a progressive loss of specific alleles, heterozygosity decreases from south to north in Coulter pine. Loss of alleles is to be expected after a founder event, but loss of heterozygosity occurs only with extreme reduction in the number of founders (e.g., McCommas and Bryant 1990). This suggests that colonies were established from a limited number of seeds. Nevertheless, even the population

Table 5. Half-matrix of Nei's (1978) unbiased genetic distances (*D*, above diagonal) and geographic distances (in km, below diagonal) between pairs of Coulter pine populations

Population[a]	JP	SB	CR	CP	FM	CF	ML	LH
JP		0.198	0.171	0.186	0.165	0.221	0.261	0.256
SB	208		0.147	0.143	0.119	0.164	0.222	0.214
CR	184	84		0.133	0.113	0.168	0.225	0.256
CP	221	82	38		0.130	0.176	0.217	0.234
FM	396	191	229	192		0.145	0.183	0.200
CF	535	332	393	364	186		0.159	0.196
ML	751	548	607	576	389	216		0.214
LH	851	646	703	670	480	316	102	

[a] JP = Jim's Place, SB = San Benito Mountain, CR = Chews Ridge, CP = Chalk Peak, FM = Figueroa Mountain, CF = Charlton Flat, ML = Mount Laguna, LH = Laguna Hanson.

Table 6. Expected heterozygosity (H_e) in northern, central, and southern populations of some Pacific Coast conifers whose ranges were displaced by glaciation

Species	Populations[a]	H_e	Reference
Jeffrey pine	Klamath Mountains (7)	0.184	Furnier and Adams (1986)
	Central Sierra Nevada (5)	0.253	
	Transverse and Peninsular Ranges (2)	0.259	
Western white pine	ID, MT, BC, and northern-interior WA and OR (13)	0.131	Steinhoff et al. (1983)
	Cascades of OR and southern WA (9)	0.190	
	CA (6)	0.266	
Giant Sequoia	Northern Sierra Nevada (8)	0.109	Fins and Libby (1982)
	Central Sierra Nevada (17)	0.131	
	Southern Sierra Nevada (6)	0.156	
Douglas-fir	Glaciated British Columbia	0.15–0.18	Hamrick et al. (1981); Yeh and O'Malley (1980)
	South of the Pleistocene ice sheet	0.26–0.33	

[a] Arranged north to south in each species. Figures in parentheses indicate the number of populations sampled.

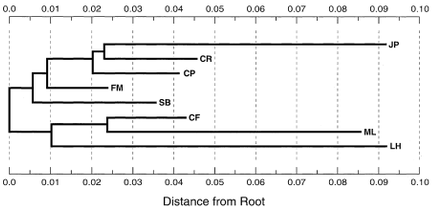

Figure 7. Wagner tree for eight populations of Coulter pine, calculated from Roger's genetic distance: JP = Jim's Place; CR = Chews Ridge; CP = Chalk Peak; SB = San Benito Mountain; FM = Figueroa Mountain; CF = Charlton Flat; ML = Mount Laguna; LH = Laguna Hanson.

at the northern limit, Jim's Place, must have been established by more than one seed, because three alleles were detected at both *GOT-3* and at *UGP-4.* A similar argument was used by Betancourt et al. (1991) in interpreting the origin of a pinyon pine (*Pinus edulis* Engelm.) isolate. An alternative explanation, that alleles may have been replaced by subsequent gene flow through pollen immigration, is unlikely because the nearest population to the south is about 50 km distant. Given the cline in heterozygosity, strong correlations between latitude and alleles per locus or percent polymorphic loci also might be anticipated, but potential relationships were weakened by the presence of several novel, or private, alleles.

The pattern of decreasing heterozygosity and loss of alleles with increasing latitude is not unique to Coulter pine, although Coulter pine provides one of the clearest examples. Others are the herbaceous plant, swamp pink (*Helonias bullata* L.) in the eastern United States (Hamrick and Godt 1996); an amphipod (*Gammarus minus*) inhabiting springs and spring brooks in Pennsylvania (Gooch and Glazier 1986); and river she-oak (*Casuarina cunninghamiana* Miq.) in Australia (Moran et al. 1989). Several other north-south trends in genic diversity have been reported in conifers; for example, Jeffrey pine, western white pine (*Pinus monticola* Dougl. Ex D.Don), giant Sequoia, and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Table 6). It seems likely that all these trends reflect founder effects.

However, only Cwynar and MacDonald (1987) specifically attributed a south-north decline of genic diversity to the founder effect. The arrival of Rocky Mountain lodgepole pine at its fragmented northern limits was dated by the presence of fossils. Migration proceeded in a series of jumps, and the peripheral populations were the youngest, smallest, and had least diversity. The long-distance dispersal events that established the northern colonies involved distances of at least 70 km. Allelic diversity was correlated with time since founding ($r = 0.68$), although heterozygosity was not.

Cwynar and MacDonald (1987) did not, however, attempt to map migration paths from presence or absence of particular alleles, as is possible in Coulter pine. Migration routes from glacial refugia also have been mapped from the geographic pattern of genetic diversity in the widespread Norway spruce (*Picea abies* [L.] Karst.), but founder effects were not as obvious in that light-seeded species as they are in Coulter pine (Lagercrantz and Ryman 1990). The origin of advance colonies of silver beech (*Nothofagus menziesii* [Hook f.] Oerst.) in New Zealand also have been inferred from isozyme frequencies (Haase 1993).

The pattern of allele depletion among populations suggests that Coulter pine dispersed from the Peninsular Ranges, perhaps from a center of diversity in the Laguna Mountains, and migrated northward. Its present distribution (Figure 1) and geographic considerations (Figure 2) indicate that it migrated to the San Gabriel Mountains in the Transverse Ranges through the Santa Rosa Mountains, the San Jacinto Mountains, and the San Bernardino Mountains. From the San Gabriel Mountains it migrated to the San Rafael Mountains, perhaps through the Tehachapi or Topatopa Mountains, although it does not now occur in either range. It then proceeded north by two routes: from the San Rafael Mountains to the Sierra Madre Mountains, the La Panza Range, and the Santa Lucia Range; and from the San Rafael Mountains to the interior mountains of the South Coast Ranges—first to the Sierra Madre Mountains, the Caliente Range or the Temblor Range, and then to the southern end of the Diablo Range. Coulter pine is now absent from the Caliente and Temblor Ranges, low (1556 m maximum elevation), dry mountains that may have become unsuitable for it after the late Pleistocene. The seemingly most obvious route to Jim's Place would be along the Diablo Range. However, five alleles found at Jim's Place on the lower slopes of Mount Diablo are also present in the Santa Lucia Range and southward but are absent on San Benito Mountain in the south of the Diablo Range. This suggests that the northern reaches of the Diablo Range were colonized by long-distance dispersal from the Santa Lucia Range.

A phylogenetic tree (Figure 7), calculated from Roger's genetic distance, parallels the proposed migration route. In the north, the branch furthest from the root of the Wagner tree is between Jim's Place and Chews Ridge, the second furthest is between this pair and Chalk Peak, and the next furthest is between these three and Figueroa Mountain. Each population is closer to the root than the one immediately to the north. In the south, the branch furthest from the root separates Charlton Flat from Mount Laguna, and the next branch joins Laguna Hanson to these two. San Benito branches off between the northern group and the southern. Other distance measures resulted in only slightly different trees.

Migration history does not explain the private, or novel, alleles that occur in only one or a few populations. Sixteen alleles were found in only one population and were missing from the other seven. In every one of these cases, Jeffrey pine was present with Coulter pine, and in all of the five populations in which these alleles were observed, hybridization has been reported or suspected (Libby 1958; Moran 1972; Zobel 1951; notes on file at the Institute of Forest Genetics). If the distribution of novel alleles was the result of chance alone, the probability that every one

of the 16 occurred in one of the five populations where Jeffrey pine was present is $(5/8)^{16} = .0005$, a possibility so small that it must be rejected.

In addition to the 16 alleles unique to single populations of Coulter pine, another five alleles were present in only two populations. In three of these cases (*ACON* 4, *IDH-1* 2, and *MDH-5* 2), the allele was present at San Benito Mountain and one other location where Coulter and Jeffrey pines are sympatric. Hybridization between these taxa was first detected at San Benito Mountain and is, perhaps, more extensive there than elsewhere (Zobel 1951). In one case, an allele (*GOT-3* 4) was found in three widely separated populations where Coulter and Jeffrey pines co-occur: San Benito Mountain, Chews Ridge, and Charlton Flat.

No unique alleles were found in the samples from Jim's Place, Chalk Peak, or Figueroa Mountain, sites from which Jeffrey pine is absent. However, Jim's Place and San Benito Mountain did share one allele (*MNR-1* 2) found nowhere else in the sample. And Figueroa Mountain and Mount Laguna shared *FEST* 2, found nowhere else. Jeffrey pine does occur about 16 km east of Figueroa Mountain on San Rafael Mountain, but the prevailing westerly winds during anthesis would mitigate against pollination of Coulter pine by Jeffrey pine. Furthermore, Jeffrey pine in the San Rafael Mountains occurs at higher elevations than Coulter pine, so phenologic differences do not favor crossing between the two species in this area.

Introgression from Jeffrey pine could account possibly for a few of the novel alleles in Coulter pine. For example, allele 2 at *ALD-2* from Laguna Hanson was present in Jeffrey pine at high frequency (unpublished data). However, several of the novel alleles have not been detected in Jeffrey pine, and may represent hybrizymes.

Hybrizyme is a term coined by Woodruff (1989) to refer to allozymes found in hybrid zones but not present in either parental taxon away from the zone of hybridization. One mechanism proposed for the origin of hybrizymes is intragenic recombination, although a higher mutation rate in hybrids has also been suggested. Hybrizymes have been detected in mammals, birds, reptiles, amphibians, insects, and mollusks (Woodruff 1989). However, in a review of introgression, Rieseberg and Wendel (1993) were unable to find any report of novel alleles generated as a consequence of hybridization or introgression in plants. Nevertheless, in several plant taxa, novel flavonoids have been detected and attributed to the disruption of regulatory mechanisms by hybridization (Levy and Levin 1971).

Additional samples of Coulter pine from the Santa Lucia Range and the Diablo Range could provide further evidence for the hybrizyme hypothesis. Jeffrey pine is not native to the Santa Lucia Mountains or the northern Diablo Range and therefore the hypothesis predicts that Coulter pine should have no novel alleles there.

Genetic drift in the generations following colonization may explain patterns in some alleles. Three alleles (*ADH-2* 1, *LAP-2* 3, and *MNR-2* 2) were found at both extremes of the range but appear to be missing in one or two intermediate populations. No pattern is obvious, and presence or absence of these alleles may reflect the action of random drift and fixation after establishment of the initial colony or, perhaps, failure to detect them with sample sizes of 72 and less.

Five alleles (*FEST* 3, *LAP-3* 3, *LAP-3* 4, *PGM* 4, and *SKDH* 3) were found on Mount Laguna but not in the sample to the south at Laguna Hanson in Baja California. This may reflect long-distance colonization from north to south. However, genetic drift in situ may be a better explanation. The population sampled at Laguna Hanson and other populations in Baja California are southern relicts, highly fragmented, small (Minnich 1987), and may have been reduced to even smaller numbers during the warmer, dryer Xerothermic in the mid-Holocene. Genetic drift may be likely in these relicts.

Genetic drift and fixation in situ also might be an alternative to the founder effect in explaining some of the paucity of alleles at Jim's Place. Coal miners at the site employed great quantities of timber for shoring the mines. Oral history suggests that Coulter pine near Jim's Place and other nearby sites was heavily exploited for the mines, and that stands were much more extensive in the last century than they are now (Waters JT, personal communication). It may be noteworthy that Jim's Place was the only population with a significant inbreeding coefficient ($F = 0.253$). However, the regular pattern in which at least 15 of the alleles were lost over the north-south range of Coulter pine, in addition to the decrease in heterozygosity, suggests that the founder effect is a more likely explanation than genetic drift in situ to explain most of the data.

In summary, 41 alleles were apparently absent from one or more of the eight populations of Coulter pine sampled for this study. Of those 41 alleles, the distribution pattern of 38 may be parsimoniously explained as a result of one of three mechanisms: the founder effect operating through long-distance colonization during postglacial migration northward from southern refugia in the Peninsular Ranges (15 alleles); introgression or the generation of novel alleles in the wake of hybridization (20 alleles); or loss through genetic drift in (1) the small, relict populations of Baja California (5 alleles, a subset of the 15 that drop out to the north) or 2) elsewhere within the range (3 alleles not detected in one or two of the eight populations).

The founder effect following long-distance colonization seems the most plausible explanation for allelic patterns at most loci. Allele depletion predominantly coincides with the two largest gaps in the Coulter pine range: between the San Gabriel Mountains and the South Coast Ranges, and between the Santa Lucia Range and the Diablo Range. Genetic drift after interruption of gene flow in a formerly more continuous range seems unlikely to have generated such regular patterns (Figure 4). Although pollen exchange would tend to eliminate the differences in gene frequency established by founder events (e.g., Haase 1993), the range of Coulter pine remains fragmented, populations are relatively small, and gene flow is minimal. As evidence for restricted gene flow, *Nm* is less than 1.27 and several alleles present at Chalk Peak in the Santa Lucia Range are missing at Chews Ridge in the same range. The explanations offered here to account for the genetic structure of Coulter pine deserve further testing.

Coulter pine provides an interesting system to examine various hypotheses about migration pathways, the role of heterozygosity in fitness, and the relationship between self-fertility, genic diversity, and colonizing ability. For example, if the observed pattern of allelic diversity in Coulter pine is the result of long-distance colonization, then self-fertility may increase northward, inversely to the decrease in heterozygosity, following Baker's (1955, 1967) Law. The pattern of genic diversity in Coulter pine also suggests conservation strategies that may be applicable to other species displaced by glaciation; that is, when resources and knowledge are limited, conservation efforts should be concentrated in southern portions of the range, closest to Pleistocene refugia.

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