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BIODIVERSITY RESEARCH

Genic diversity, genetic structure, and biogeography of *Pinus sabiniana* Dougl.

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Abstract. *Pinus sabiniana* Dougl. (grey pine) forms savanna forests in the foothills surrounding California's Great Central Valley. However, its fossil record, which dates from the late Miocene through the Pliocene and Pleistocene, is found exclusively in southern California, south of the species' present range. A total of twenty-nine isozyme loci, representing eighteen enzyme systems, was assayed to analyse the genetic structure in eight populations of grey pine and attempt to track its migration history from southern to northern California. Expected heterozygosity in the two southernmost samples was 0.128 and 0.150, and heterozygosity tended to decrease with increasing latitude, suggesting the loss of diversity as grey pine dispersed northward. However, genetic distances between populations were very small, even on opposite sides of the treeless Great Central Valley; and estimated time since divergence was 900 to 9000 years at a maximum. Wright's F_{ST}, the proportion of total genetic diversity among populations, was only 0.057, which is similar to values found in many conifers with continuous distributions. *Nm*, the number of migrants among populations per generation, was 4.1 to 6.7, depending on estimator, and indicates that gene flow is extensive, or was so in the recent past. In every population, observed heterozygosity was less than expected heterozygosity, and the fixation index, F_{IS}, for the progeny was 0.128, which indicates a fairly high rate of inbreeding. The genetic similarity of disjunct populations, in combination with paleogeographic and paleoclimatic evidence, suggests that grey pine formed a continuous population throughout the Great Central Valley, perhaps between 12,000 and 8000 yrs BP. Its range became fragmented during the Xerothermic, when it ascended into the foothills. Gaps in its range correlate with late Pleistocene–early Holocene lakes in adjacent basins and with the Sacramento–San Joaquin Delta.

Key words. *Pinus sabiniana*, migration, biogeography, genetic variation, isozymes.

INTRODUCTION

Judging from the fossil record, grey or foothill pine (*Pinus sabiniana* Dougl.), a California endemic adapted to semi-arid conditions, has an interesting history. All of its fossil occurrences are south of its present range. Fossils of *Pinus pieperi* Dorf, which is either a progenitor or identical to grey pine, occur in late Miocene and Pliocene floras and in early and late Pleistocene floras of southern California, south of the Transverse Ranges (Fig. 1; Axelrod, 1938, 1986). The Transverse Ranges separate the Los Angeles Basin from the Great Central Valley, which is formed by the San Joaquin Valley in the south and the Sacramento Valley in the north. grey pine apparently moved northward after or during the late Pleistocene. It was eliminated from southern California, perhaps by increasing winter temperatures or by competition with elements of the chaparral formation, which expanded in the late Quaternary (Axelrod, 1938).

Grey pine is a member of the big-cone pines, subsection *Sabinianae*, of the genus *Pinus*. The subsection includes Coulter pine (*P. coulteri* D. Don), Torrey pine (*P. torreyana* Parry ex Carr.), and grey pine. Grey pine and Torrey pine are endemic to California, and Coulter pine extends into Baja California. Subsection *Sabinianae* is linked to subsection *Ponderosae* via hybrids between Coulter and Jeffrey (*P. jeffreyi* Grev. & Balf.) pines, but otherwise is reproductively isolated from other subsections of the genus (Critchfield, 1966).

Grey pine occupies habitats with as little as 250 mm

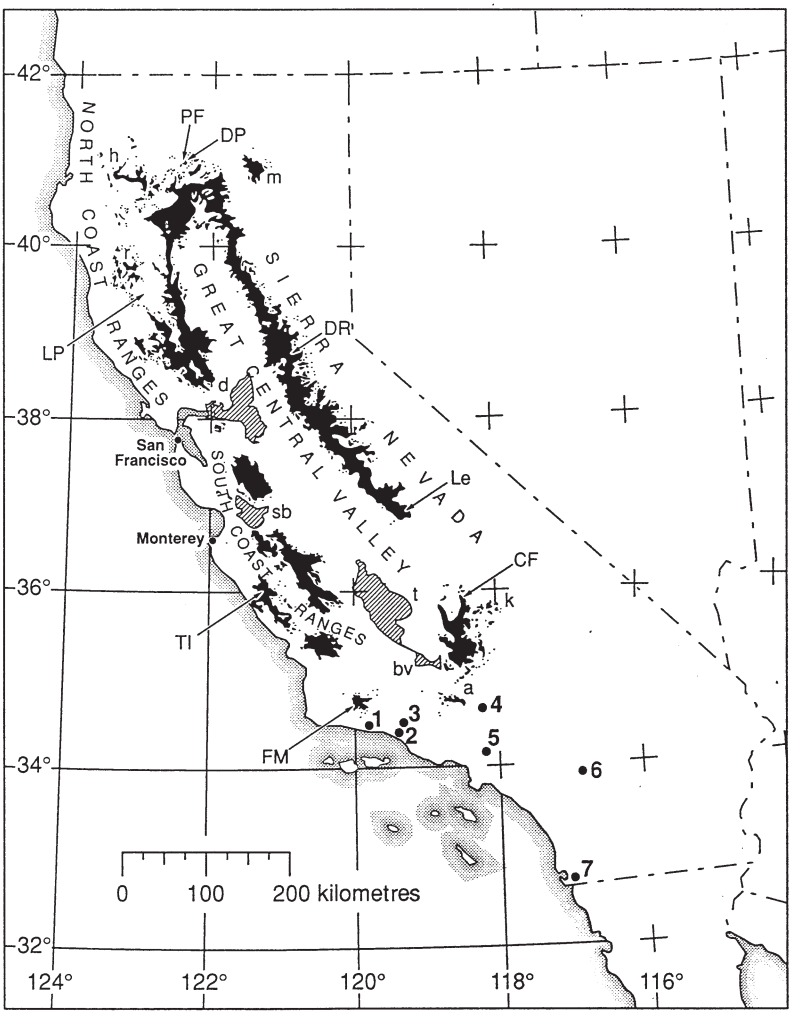

Fig. 1. The natural range of grey pine (after Griffin & Critchfield, 1972), location of fossil sites (Axelrod, 1986), Pleistocene lakes (Goudey & Smith, 1994; Atwater *et al.*, 1986; Jenkins, 1973), and some geographic features mentioned in the text. Sampled populations are indicated by arrows: PF = Pollard Flat; DP = Delta Point; LP = Lake Pillsbury; DR = Dixon Ranch; Le = Lerona; TI = The Indians; CF = Chamise Flat; FM = Figueroa Mountain. Fossil sites are numbered: 1 = Carpinteria; 2 = Pico; 3 = Lake Canyon; 4 = Anaverde; 5 = Rancho La Brea; 6 = Mount Eden; 7 = Chula Vista. Geographic features are lettered: a = Antelope Valley; bv = Buena Vista and Kern Lakes; d = Sacramento-San Joaquin Delta; h = Hoopa Valley Indian Reservation; k = Kern Plateau; m = Modoc Plateau; r = Round Valley Indian Reservation; sb = Lake San Benito; t = Tulare Lake.

of rain a year (Axelrod, 1938). Yearly evaporation exceeds precipitation wherever it grows (Watts, 1959). Thus, its range extends far below the lower elevational limits of any other interior pine. Stands are found as low as 30 m in elevation in the Sacramento Valley. In general, however, its elevational range is between 150 and 1200 m (Sudworth, 1908), where it occurs in woodland or savannah associations, often with blue oak (*Quercus douglasii* Hook. & Arn.). At its upper limits it merges with the ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) belt.

The species may have potential value for forestation in semi-arid environments (e.g., Guseinov, 1972; Maxsimov, 1980; Smola, 1980). Grey pine has little

Table 1. Location of grey pine populations sampled.

Population	County[1]	Latitude N	Longitude W	Elevation[2] (m)
Pollard Flat, Shasta-Trinity National Forest	Shasta	40°59′	122°27′	630
Delta Point, Shasta-Trinity National Forest	Shasta	40°57′	122°26′	420
Lake Pillsbury, Mendocino National Forest	Lake	39°27′	122°57′	560
Dixon Ranch	Eldorado	38°43′	121°02′	310
Lerona, Sierra National Forest	Fresno	37°08′	119°27′	850
The Indians, Los Padres National Forest	Monterey	36°05′	121°24′	460
Chamise Flat, Sequoia National Forest	Tulare	35°55′	118°29′	1070
Figueroa Mountain, Los Padres National Forest	Santa Barbara	34°43′	119°58′	1270

[1] Counties in California.
[2] Rounded to the nearest 10 m.

present commercial value, but was used for mine timbers and general construction 150 years ago during California's gold rush (Watts, 1959). Before that, its large seeds were an important item of food and commerce among Native Americans (Davis, 1974; Farris, 1983, 1993). Grey pine's main disadvantage for forestry is its lack of apical dominance. During the first several years of development, young trees maintain a single, erect stem, but mature trees almost invariably have multiple tops. This might be improved by selection breeding.

The present range of grey pine is essentially a 'bathtub' ring around the Great Central Valley (Fig. 1). Major gaps in this range are considered 'strange', and are not explicable by lack of habitat (Griffin & Critchfield, 1972). Particularly notable is the 100-km gap between the Kings River and the South Fork of the Tule River (mentioned in 1865 by Josiah Dwight Whitney, the first State Geologist and director of the California Geological Survey, as recounted in Griffin & Critchfield, 1972). The elevational gradient is steeper in this part of the Sierra Nevada than it is northward, and Watts (1959) speculated that Indian fires may have sped relatively unchecked up these slopes and eliminated grey pine, which is less fire-tolerant than associated oaks or chaparral. In addition, competition among species is more accentuated on a steep slope than on a gradual one (Beales, 1969), and grey pine may have been squeezed by chaparral from below and by ponderosa pine from above.

The objective of the study reported here was to characterize genic diversity and the genetic structure of grey pine, and relate this to paleoclimatic, paleogeographic, and ethnographic information. It was of particular interest to see whether isozymes could track and time grey pine's access to and migration around the Great Central Valley. Isozymes track the path of migration of Coulter pine northward from southern California into the South Coast Ranges (Ledig, in review). Grey pine, like Coulter pine, is heavy-seeded and, in addition, grey pine seeds are essentially wingless (Krugman & Jenkinson, 1970); therefore, its vagility would seem relatively limited. Range gaps could separate long-isolated populations or recent colonies, and patterns of isozyme diversity might help to distinguish between these possibilities.

MATERIALS AND METHODS

Cones were collected in eight populations of grey pine from 30 September to 19 November, 1981. Populations were systematically chosen to bracket the range of the species (Table 1, Fig. 1). Cones were generally collected by pole pruner.

The goal was to sample thirty-five trees in each population. Within populations, cone-bearing trees were chosen in close proximity until the goal was reached. Samples usually were less than thirty-five because of a paucity of cone-bearing trees or because

Table 2. Allele frequencies for twenty-seven polymorphic loci in eight populations of grey pine.

Locus/allele		Population[1] (sample size)							
		PF (16)	DP (33)	LP (30)	DR (30)	Le (34)	TI (31)	CF (40)	FM (35)
AAP-1	1	1.000	1.000	1.000	1.000	1.000	0.935	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000
AAP-2	1	1.000	1.000	0.983	1.000	1.000	1.000	0.962	1.000
	2	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000
ACO-1	1	0.031	0.182	0.207	0.050	0.059	0.129	0.225	0.157
	2	0.906	0.712	0.741	0.667	0.809	0.726	0.637	0.714
	3	0.031	0.000	0.017	0.067	0.044	0.016	0.075	0.000
	4	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
	5	0.031	0.091	0.034	0.217	0.074	0.113	0.038	0.114
	6	0.000	0.015	0.000	0.000	0.000	0.000	0.025	0.000
	7	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.014
ACO-2	1	1.000	0.985	1.000	1.000	0.985	0.968	0.988	1.000
	2	0.000	0.015	0.000	0.000	0.015	0.032	0.013	0.000
CAT-2	1	1.000	1.000	0.983	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
FEST	1	0.906	0.864	0.933	0.933	0.956	0.935	0.988	0.986
	2	0.094	0.136	0.067	0.067	0.044	0.065	0.013	0.014
GDH	1	1.000	1.000	0.983	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
GOT-1	1	1.000	0.970	1.000	1.000	0.985	1.000	0.975	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	3	0.000	0.030	0.000	0.000	0.015	0.000	0.013	0.000
GOT-3	1	0.969	1.000	1.000	0.983	1.000	0.984	0.938	0.971
	2	0.000	0.000	0.000	0.017	0.000	0.000	0.063	0.014
	3	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.014
G6P-1	1	1.000	1.000	1.000	0.983	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
G6P-2	1	1.000	1.000	1.000	1.000	0.985	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
LAP-2	1	1.000	1.000	1.000	0.983	1.000	0.919	0.962	0.943
	2	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.014
	4	0.000	0.000	0.000	0.000	0.000	0.081	0.013	0.043
LAP-3	1	0.000	0.167	0.217	0.383	0.162	0.516	0.375	0.214
	2	1.000	0.833	0.783	0.617	0.838	0.484	0.625	0.786
MDH-1	1	0.906	0.939	0.950	0.933	0.956	0.984	0.938	0.986
	2	0.094	0.061	0.050	0.067	0.044	0.016	0.025	0.014
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000
MDH-2	1	0.969	0.985	1.000	0.967	1.000	0.935	0.988	0.986
	2	0.031	0.015	0.000	0.033	0.000	0.065	0.013	0.014

continued

Table 2. *continued*

Locus/allele		Population[1] (sample size)							
		PF (16)	DP (33)	LP (30)	DR (30)	Le (34)	TI (31)	CF (40)	FM (35)
MDH-3	1	0.750	0.909	0.783	0.883	0.868	0.661	0.775	0.829
	2	0.031	0.076	0.133	0.117	0.103	0.210	0.213	0.171
	3	0.219	0.015	0.083	0.000	0.015	0.032	0.013	0.000
	4	0.000	0.000	0.000	0.000	0.015	0.097	0.000	0.000
MNR-1	1	0.969	0.970	1.000	0.933	0.882	0.984	0.938	0.957
	2	0.031	0.015	0.000	0.033	0.088	0.016	0.050	0.029
	3	0.000	0.015	0.000	0.033	0.029	0.000	0.013	0.014
MNR-2	1	0.969	0.879	0.917	0.859	0.897	0.919	0.850	0.857
	2	0.000	0.045	0.017	0.078	0.015	0.000	0.063	0.029
	3	0.031	0.076	0.067	0.063	0.088	0.081	0.087	0.114
MPI-1	1	0.938	0.985	1.000	0.867	1.000	1.000	1.000	1.000
	2	0.063	0.000	0.000	0.117	0.000	0.000	0.000	0.000
	3	0.000	0.015	0.000	0.017	0.000	0.000	0.000	0.000
MPI-2	1	0.625	0.379	0.317	0.767	0.515	0.581	0.650	0.443
	2	0.375	0.591	0.667	0.133	0.456	0.306	0.275	0.457
	3	0.000	0.030	0.017	0.100	0.029	0.113	0.075	0.100
PGI-1	1	0.063	0.030	0.100	0.167	0.044	0.339	0.175	0.214
	2	0.938	0.970	0.900	0.833	0.956	0.661	0.825	0.786
PGI-2	1	0.969	0.924	0.800	0.900	0.794	0.903	0.925	0.971
	2	0.000	0.015	0.033	0.033	0.176	0.032	0.000	0.000
	3	0.031	0.045	0.167	0.050	0.029	0.048	0.063	0.014
	4	0.000	0.015	0.000	0.017	0.000	0.016	0.013	0.014
PGM	1	1.000	0.939	0.900	0.983	0.900	0.806	0.775	0.814
	2	0.000	0.015	0.033	0.017	0.033	0.113	0.112	0.086
	3	0.000	0.045	0.050	0.000	0.050	0.016	0.100	0.100
	4	0.000	0.000	0.017	0.000	0.017	0.065	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
6PG-2	1	0.563	0.636	0.633	0.217	0.588	0.403	0.387	0.500
	2	0.063	0.030	0.017	0.100	0.059	0.081	0.050	0.029
	3	0.375	0.303	0.333	0.583	0.324	0.435	0.475	0.371
	4	0.000	0.030	0.017	0.100	0.029	0.081	0.087	0.100
6PG-3	1	0.844	1.000	1.000	1.000	1.000	1.000	0.962	1.000
	2	0.094	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	3	0.063	0.000	0.000	0.000	0.000	0.000	0.025	0.000
SKD-1	1	0.813	0.864	0.900	0.950	0.956	1.000	0.975	0.957
	2	0.156	0.091	0.083	0.017	0.029	0.000	0.013	0.043
	3	0.031	0.045	0.000	0.033	0.015	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	5	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
TO-4	1	1.000	1.000	1.000	0.983	0.971	0.984	0.988	0.986
	2	0.000	0.000	0.000	0.017	0.029	0.016	0.013	0.014

[1] PF = Pollard Flat; DP = Delta Point; LP = Lake Pillsbury; DR = Dixon Ranch; Le = Lerona; TI = The Indians; CF = Chamise Flat; FM = Figueroa Mountain.

cones lacked viable seeds. In one instance, Chamise Flat, seeds were obtained from forty trees. Seeds were extracted after the cones opened and were stored at 1°C until needed. Cones and seeds of each tree were maintained separately.

In July 1982, seeds were germinated for isozyme analysis. When radicles appeared through the seed coat, the megagametophytes and embryos were excised and separated. Knowing the genotype of the megagametophyte, the pollen contribution to the embryo can be deduced by subtraction. Extracts of one megagametophyte-embryo pair from each tree were analysed, resulting in a sample of thirty-two to eighty independent genomes (embryos from sixteen to forty trees) per population, assuming that the pollen parents were drawn at random from a large population. The assumption was tested by comparing gene frequencies in the megagametophytes to frequencies in the pollen contribution. Allele frequencies were generally the same for pollen and megagametophytes.

Using the techniques of starch gel electrophoresis described by Conkle *et al.* (1982), eighteen 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). When several zones of activity were observed for a single enzyme, hyphenated numerals following the enzyme abbreviation were used for identification. Twenty-nine presumptive loci were scored consistently and used in the statistical analysis.

BIOSYS (Swofford & Selander, 1981) was used to estimate genic diversity, genetic relationships among populations, and F-statistics. Inferences about the populations apply to the progeny 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 by two methods. From Wright (1951):

$$Nm = (1 - F_{ST})/4F_{ST},$$

where F_{ST} is the proportion of the total genic diversity among populations. *Nm* also can be calculated from the number and frequency of private alleles (unique alleles found in only one population), using simulations developed by Slatkin (1985):

$$\log_{10}(\bar{p}(1)) = \alpha \log_{10}(Nm) + \beta,$$

where $\bar{p}(1)$ is the mean frequency of private alleles, and α and β are constants determined by fitting simulated data developed for a sample size of twenty-five (Barton & Slatkin, 1986). Estimates of *Nm* were corrected for actual mean sample size, which was thirty.

RESULTS

Only two of the twenty-nine loci, ADH and ALD, were monomorphic in all populations sampled. Five others (CAT-2, GDH, AAP-1, G6P-1, G6P-2) were polymorphic by virtue of rare variants in one population each (Table 2). About one-third, or ten, of the loci were polymorphic in every population sampled. A locus was considered polymorphic when any variants were observed in any population. Fifteen alleles were private, or novel, restricted to a single population each. The distribution of allele frequencies was ∪-shaped (Fig. 2), which is true for most large, natural populations (Chakraborty, Fuerst & Nei, 1980). That is, most alleles are in high frequency (≥ 0.90) or in low frequency (≤ 0.05).

Percent polymorphic loci (P) ranged from 51.7% to 72.4%; alleles per locus (A) ranged from 1.7 to 2.3; and expected heterozygosity (H_e, unbiased estimate) ranged from 0.108 to 0.156 (Table 3). Expected

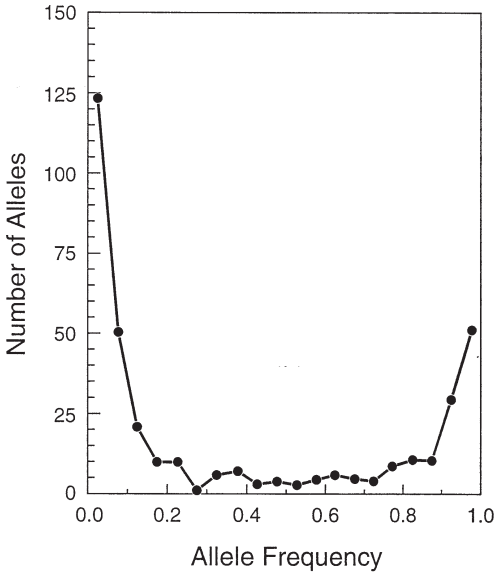

Fig. 2. The ∪-shaped distribution of allele frequencies in eight populations of grey pine.

Table 3. Genic diversity in grey pine: number of seed trees sampled (n), mean expected heterozygosity (H_e, unbiased estimate), observed heterozygosity (H_o), percent polymorphic loci (P), number of alleles per locus (A), and fixation index or mean deviation from Hardy–Weinberg equilibrium (F).

Population	n	H_e	H_o	P	A	F
Pollard Flat	16	0.108 (.029)[1]	0.099 (.028)[1]	51.7	1.7	0.083
Delta Point	33	0.114 (.029)	0.096 (.025)	58.6	2.0	0.158
Lake Pillsbury	30	0.119 (.030)	0.103 (.029)	51.7	1.9	0.134
Dixon Ranch	30	0.134 (.032)	0.099 (.025)	65.5	2.0	0.261
Lerona	34	0.115 (.030)	0.108 (.029)	58.6	2.1	0.061
The Indians	31	0.156 (.039)	0.126 (.034)	62.1	2.0	0.192
Chamise Flat	40	0.150 (.035)	0.131 (.032)	72.4	2.3	0.127
Figueroa Mountain	35	0.128 (.035)	0.117 (.033)	58.6	2.0	0.086
Mean		0.128	0.110	59.9	2.0	0.138

[1] Standard errors in parentheses.

heterozygosity tended to decrease with increasing latitude (Fig. 3). Percent polymorphic loci and alleles per locus also tended to decrease with increasing latitude, although correlations were not statistically significant.

Observed heterozygosity was less than expected heterozygosity in every population, which suggests a degree of inbreeding. Inbreeding coefficients, calculated from heterozygote deficiencies averaged over loci, ranged from 0.061 to 0.261 with a mean of 0.138 (Table 3). Wright's F_{IS} was similar, 0.128 (Table 4). Malecot's coefficient of parental relationship is twice the inbreeding coefficient, or 0.256, suggesting that parents of the embryos were related as half siblings on average.

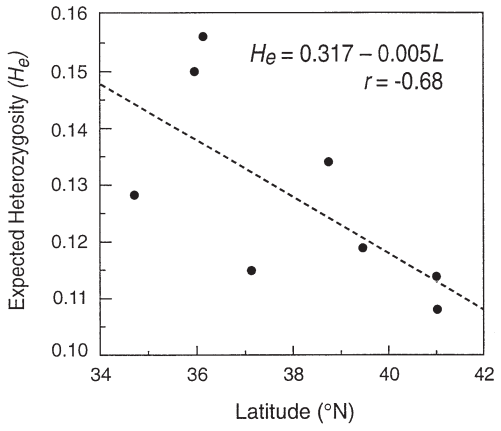

Fig. 3. The correlation of expected heterozygosity with latitude for eight populations of grey pine.

Wright's F-statistics (Table 4) partition genetic diversity into among- and within-population components. Diversity among populations, F_{ST}, was only 0.057, which means that 94.3% of the observed variation was within populations. F_{ST} is considered equivalent to Nei's (1975) G_{ST}. Nei's (1978) unbiased genetic distance (D) further reflects the lack of differentiation among populations (Table 5); $\bar{D}$ was only 0.007. Genetic distance between populations was unrelated to geographic distance. Clustering algorithms using Nei's unbiased genetic distance or other distance measures failed to group populations meaningfully.

Indirect estimates of gene flow between populations were substantial. Nm calculated from Wright's F_{ST} was 4.1 migrants per generation. Using the method of private alleles, estimates were slightly higher, 6.7 migrants per generation. In either case, rates of gene flow are either now or were in the recent past too high to permit extensive differentiation by random genetic drift (Wright, 1969).

Allele frequencies at two of the twenty-seven polymorphic loci (FEST and MDH-3) seemed to be correlated with latitude (P<0.01; Fig. 4). This is more than expected by chance alone, and may suggest the action of selection on FEST and MDH-3 or closely linked segments of the genome.

DISCUSSION

The estimates of genic diversity in grey pine were substantially higher than average for long-lived, woody endemics but close to average for outcrossing endemic

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

Locus	F_{IS}	F_{IT}	F_{ST}
AAP-1	1.000	1.000	0.057
AAP-2	0.444	0.459	0.026
ACO-1	0.034	0.069	0.036
ACO-2	−0.023	−0.009	0.013
CAT-2	−0.017	−0.002	0.015
FEST	−0.094	−0.067	0.025
GDH	−0.017	−0.002	0.015
GOT-1	−0.023	−0.008	0.016
GOT-3	−0.040	−0.014	0.025
G6P-1	−0.017	−0.002	0.015
G6P-2	−0.015	−0.002	0.013
LAP-2	0.120	0.152	0.037
LAP-3	0.657	0.698	0.121
MDH-1	0.001	0.015	0.015
MDH-2	−0.042	−0.022	0.019
MDH-3	0.052	0.096	0.046
MNR-1	0.024	0.045	0.022
MNR-2	0.069	0.082	0.014
MPI-1	0.746	0.765	0.073
MPI-2	0.077	0.157	0.087
PGI-1	0.218	0.279	0.078
PGI-2	0.013	0.060	0.048
PGM	0.155	0.196	0.049
6PG-2	0.050	0.096	0.048
6PG-3	0.067	0.146	0.085
SKD-1	0.035	0.079	0.045
TO-4	−0.020	−0.011	0.009
Mean	0.128	0.177	0.057

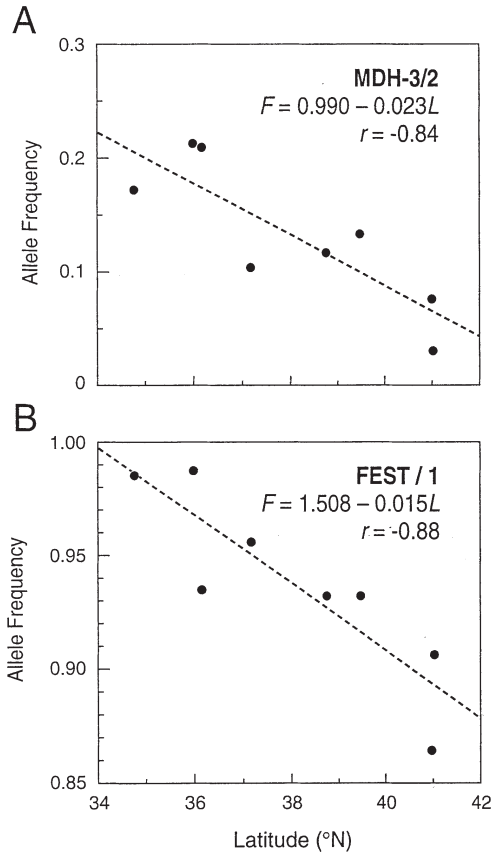

Fig. 4. The correlation of allele frequencies at selected loci with latitude for eight populations of grey pine: (A) FEST/1; (B) MDH-3/2.

plant species in general. Mean expected heterozygosity (H_e) was only 0.056 for twenty long-lived woody endemics (Hamrick, Godt & Sherman-Broyles, 1992) and 0.142 for fifty-seven outcrossing endemic plant

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

Population[1]	PF	DP	LP	DR	Le	TI	CF	FM
PF		0.005	0.007	0.014	0.004	0.018	0.012	0.007
DP	4		0.000	0.015	0.001	0.014	0.009	0.002
LP	176	173		0.017	0.002	0.012	0.008	0.002
DR	279	275	185		0.010	0.006	0.003	0.008
Le	501	496	401	225		0.011	0.006	0.002
TI	551	553	397	293	211		0.002	0.005
CF	660	656	556	384	160	264		0.002
FM	729	724	588	453	271	200	188	

[1] PF = Pollard Flat; DP = Delta Point; LP = Lake Pillsbury; DR = Dixon Ranch; Le = Lerona; TI = The Indians; CF = Chamise Flat; FM = Figueroa Mountain.

species (Hamrick & Godt, 1996). Percent polymorphic loci (P) was generally higher in the grey pine samples than the mean for outcrossing endemics (Hamrick & Godt, 1996; Hamrick *et al*., 1992). H_e and P for grey pine were closer to values for woody plants with 'narrow' ranges; H_e was 0.143 and P was 44.3% for the sixty-one woody species in this category reviewed by Hamrick *et al.* (1992). Although grey pine is a California endemic, it covers a fairly broad latitudinal range; the longest axis of its range extends about 800 km from northwest to southeast.

Genetic structure of grey pine is not typical of endemics either. Differentiation among populations was substantially lower in grey pine than in other endemics; F_{ST} was 0.057 for grey pine versus an average G_{ST} of 0.179 for all outcrossing plant endemics (Hamrick & Godt, 1996) and 0.141 for woody endemics (Hamrick *et al*., 1992). Only 'widespread' woody endemics average as little differentiation among populations as does grey pine.

Compared to California pines of the subgenus *Pinus*, especially those with narrow ranges, grey pine is fairly typical. H_e in species with restricted, fragmented ranges varies from 0.098 to 0.148, excluding the anomalous Torrey pine, which is genetically depauperate (Ledig, 1998). The California *Pinus* with narrow ranges include knobcone (*P. attenuata* Lemm.), Coulter, bishop (*P. muricata* D. Don), Monterey (*P. radiata* D. Don), and Washoe (*P. washoensis* Mason & Stockwell) pines. H_e in common, widely distributed species, such as lodgepole (*P. contorta* Dougl. Ex Loud.), Jeffrey, and ponderosa pines, varies from 0.155 to 0.241. In forty-eight species of pines, which is about half the genus, the mode lies between 0.13 and 0.16 (Ledig, 1998). Hamrick & Godt (1996) report the average for pines as 0.154. Grey pine seems slightly less diverse than average.

Estimates of inbreeding in grey pine ($F_{IS} = 0.128$) were high for pines, including the closely related Coulter pine (Ledig, in review), for which the sampling scheme was similar to that used here. It seems unlikely that the Wahlund Effect is responsible. However, it must be remembered that F_{IS} was estimated for progeny at the late embryo or early germinant stages. As a result of natural selection against homozygotes, mature populations may show substantially less inbreeding (e.g. Plessas & Strauss, 1986). Nevertheless, grey pine may be a species that tolerates inbreeding to some degree. Compared to several other species, isolated single trees of grey pine planted in the Crimea produced full seed of high quality (Podgornyi & Smirnova, 1984), which suggests that the species is fully self-fertile.

No geographic patterns were obvious in the isozymes assayed here, except at the FEST and MDH-3 loci. Clustering algorithms using various genetic distance measures failed to group populations into geographically coherent clusters. Likewise, natural variation studies failed to find any geographic patterns in cone morphology (Griffin, 1964). However, seeds from northern populations tended to germinate more slowly than those from southern populations and to have a stronger requirement for stratification (Griffin, 1971). The same seed sources surveyed here were sown in Spain, and height after 2 years of growth in a nursery was related to latitude and hydrologic balance at the collection sites (Toval and Puerto, 1985). Thus, differentiation is not apparent with respect to predominantly neutral characteristics, like isozymes or cone morphology, but adaptive differentiation with respect to physiological and growth traits is likely.

The tendency for measures of genic diversity to decrease from south to north suggests that some diversity was lost during founder events as grey pine dispersed northward through the Great Central Valley. Decreases in heterozygosity with latitude are common in West Coast conifers (Ledig, in review; Cwynar & MacDonald, 1987; Furnier & Adams, 1986; Steinhoff, Joyce & Fins, 1983; Fins & Libby, 1982; Hamrick, Mitton & Linhart, 1981), and have been noted in other organisms and regions as well (Godt, Hamrick & Bratton, 1995; Moran, Bell & Turnbull, 1989; Gooch & Glazier, 1986). Coulter pine's northern migration was apparently characterized by several long-distance colonization events, and loss of alleles during its northern migration can be readily tracked (Ledig, in review). However, it is not possible to accurately track migration paths in the grey pine data set, which indicates a recent distribution more continuous than that of Coulter pine.

The ∪-shaped distribution of allele frequencies indicates that random genetic drift has been relatively unimportant in differentiating populations of grey pine. Under drift, allele frequencies tend first toward a uniform distribution (excluding frequencies of 0.0 or 1.0) and then, in the extreme, to fixation and a complete lack of polymorphism. The low estimates of genetic distance between populations and the estimates of substantial gene flow (Nm) among them also indicate little role for random genetic drift in differentiating populations of grey pine. Furthermore, the genetic similarity of disjunct fragments, such as the populations

on the Kern Plateau south of the South Fork of the Tule River (e.g. Chamise Flat), to others suggests that they have not been separated for very long.

A rough estimate of time (T) since populations began to diverge can be calculated from Nei's (1975) genetic distance (D) and, therefore, date the time since fragmentation:

$$T = \mathrm{D}/2\mu,$$

where μ is the mutation rate. For assumed μ of 10^{-5} and 10^{-6} and a genetic distance of 0.006 between Chamise Flat and Lerona, the estimate of T is only 300 to 3000 years.

Genetic distances between the Coast Ranges and the Sierra Nevada were also small. For opposite sides of the Great Central Valley, estimates of time since divergence range from 100 to 1000 years in a comparison of Lerona and Lake Pillsbury, to 850 to 8500 years between Dixon Ranch and Lake Pillsbury. Axelrod (1986) speculated that the gaps in the grey pine range dated to the Xerothermic period, which he (Axelrod, 1981) placed at 8000 to 3500 yrs BP. The genetic evidence presented here is consistent with this estimate.

Gaps of 100 km should reduce or eliminate gene flow. However, *Nm* between Lerona, north of Kings Canyon, and Chamise Flat, in the disjunct south of the South Fork of the Tule River, is estimated as 8.7. It is unlikely that this is the current number of immigrants exchanged, but reflects recent contact, perhaps through a chain of populations. *Nm* between Dixon Ranch and The Indians, 293 km across the treeless Great Central Valley, was 9.4, a value that also seems highly unlikely to measure present rates of exchange. *Nm* between Dixon Ranch and Lake Pillsbury was smaller, 3.7, but still substantial for such widely separated populations. Perhaps 100 to 1000 generations are required for *Nm* to reach equilibrium after gene flow ceases among populations (Slatkin & Barton, 1989), which is a long time in a tree species that can live over a century before regenerating. Thus, estimates of *Nm* reflect past contact as well as present gene flow, and probably overestimate present levels of exchange.

Genetic distances between populations on opposite sides of the Great Central Valley and the estimates of substantial recent gene flow between them make it unlikely that grey pine first diverged into separate populations that then followed separate migration routes north through the Coast Ranges and the foothills of the Sierra Nevada, respectively. It is more likely that grey pine spread up the valley floor at low elevations before diverging.

Axelrod (1938) hypothesized that the northward movement of grey pine forest was related to the rising winter temperatures since the last glaciation, and that it finally disappeared from southern California during the postglacial Xerothermic (Axelrod, 1986). The area in which grey pine fossils are found has substantially higher winter temperatures (by about 5°C) than occur within its present range (Axelrod, 1938).

During the cool climate characterizing Pleistocene glaciation, grey pine could probably not invade the Great Central Valley over the Transverse Ranges because temperatures restricted the species to low elevations. During the last glaciation, ranges of several tree species may have been shifted downward in elevation by as much as 1500 m relative to the present (Adam & West, 1983; Cole, 1983; Anderson, 1990). If grey pine was present north of the Transverse Ranges, in all probability it was confined to the floor of the San Joaquin Valley.

How a wingless, heavy-seeded species migrated up the Great Central Valley is a matter of conjecture. One possibility is that American Indians helped transport the species. Grey pine seeds, 'pine nuts', were a traditional trail food and were offered to visitors as a display of hospitality (Farris, 1993). Native Californians broadcast seeds of stickleaf (*Mentzelia* sp.), goosefoot (*Chenopodium* sp.), and other 'wild' plants (Anderson, 1993), but there is no ethnographic evidence that they cultivated pines, in the sense of sowing seeds. However, that is a possibility. In 1980, an 83-year-old man of the Apwaruge attributed the presence of grey pine on the Modoc Plateau to Paiutes, who: 'were eating seeds they brought with them, dropped some' (interview notes obtained from Ramsay Blake, cited in Farris, 1983). While the story is not entirely plausible, it illustrates that Indians themselves can conceive of the spread of grey pine as a result of their actions.

It is noteworthy that the three major extensions of grey pines beyond the Great Central Valley (Fig. 1) all occur in areas strongly associated with Native Americans: (1) the important Indian trade route along the Pit River on the Modoc Plateau (Watts, 1959), where one oral history suggests it was introduced (Farris, 1983); (2) along the Trinity River in and to the southeast of what is now the Hoopa Valley Indian Reservation; (3) along the Eel River, in and south of the Round Valley Indian Reservation. Both Hoopa

Valley and Round Valley were already sites of permanent Indian settlements when California was Anglicized (Anderson, 1956; Hammond, 1972). Another important Indian trail lay along the upper reaches of the Kern River above Isabella, near Chamise Flat. Watts (1959) felt it reasonable to speculate that Indians may have introduced grey pine to that region.

Grey pine was undoubtedly forced higher in elevation and deeper into the foothills during the Xerothermic period. From British Columbia through the Sierra Nevada, climates were warmer and dryer in the early- to mid-Holocene as evidenced from dendrochronological studies at timberline and palynological studies of lake sediments (Clague & Mathewes, 1989; Anderson, 1990; Scuderi, 1987; Davis *et al.*, 1985; Davis & Moratto, 1988; Adam & West, 1983). The exact timing varied from location to location, but generally began about 9000 to 7000 yrs BP and lasted until 6000 to 2000 yrs BP.

Warmer conditions during the Xerothermic may have eliminated grey pine from the floor of the Great Central Valley. However, it can survive present Valley conditions, which are much like those in the late Pleistocene and early Holocene; old trees occur along Oat Creek Wash near Arbuckle at 15 m elevation and the species is found at about 30 m near Folsom and at Oroville, although these two latter locations are probably recent invasions in the wake of placer mining (Watts, 1959). Watts (1959) attributed the absence of grey pine from the Great Central Valley to Indian fires.

If grey pine occupied a near-continuous range that spanned the Great Central Valley from north to south, what accounts for the present gaps in the foothills? Especially in the southern extreme of its range, gaps might result from deteriorating conditions for its growth and survival during the Xerothermic (Axelrod, 1986). This is a logical inference, given its fossil occurrence in southern California up until the late Pleistocene and its current absence there.

However, deteriorating climate does not explain all the gaps in the range of grey pine. I propose a pluvial barrier hypothesis. The gap between the Kings River and the South Fork of the Tule River may have resulted if grey pine had entered the west side of the San Joaquin Valley and been blocked from migrating across the valley into the Sierra Nevada foothills. Tulare Lake, Goose Lake, Buena Vista Slough, Buena Vista Lake, Kern Lake, and interconnecting wetlands were such a barrier (Fig. 1). From the Kings River south, the San Joaquin Valley is internally drained. The northernmost lake, prehistoric Tulare Lake, overflowed and discharged through Fresno Slough only during severe floods (Janda & Croft, 1967). Tulare Lake was fed directly by the Kings, Kaweah, and Tule Rivers. Buena Vista and Kern Lakes, to the south, were fed by the Kern River. During flood years, Buena Vista Lake emptied north into Tulare Lake through the Buena Vista Slough and Goose Lake. Tulare Lake is now a dry lake bed, but it remained full from about 26,780 to 14,000 yrs BP and reappeared again between 12,000 and 10,000 yrs BP. This stage of Tulare Lake lasted until 8000 yrs BP, with an overflow at about the 65 m contour (Croft, 1968). Subsequent to 8000 yrs BP and into historic times, the lakes filled during flood years, and even when they did not overflow, the San Joaquin Valley was marshland covered by a luxuriant growth of reeds, or 'tules' (Gudde, 1969). In 1772 the Spanish explorer, Pedro Fages, described it as 'a labyrinth of lakes and tulares' (Gudde, 1969). Thus, even during dry cycles, grey pine on the west side of the valley could have been blocked from reaching the east side.

Two other gaps in the 'bathtub ring' also may be explained by the presence of Pleistocene lakes and wetlands (i.e. the pluvial barrier hypothesis). These are the gap east of Monterey Bay and the gap east of San Francisco. The Sacramento-San Joaquin Delta (Fig. 1) probably forced the northward migration of grey pine to the east side of the valley. Tidal influence extends throughout the Delta, and the entire area was subject to periodic flooding, even in historic time. The gap east of Monterey Bay may reflect lack of suitable habitat, but it seems more than coincidence that Lake San Benito occupied a 50-km long basin in this area in the late Pleistocene (Fig. 1; Jenkins, 1973).

To summarize: the following scenario takes into account (1) the lack of genetic differentiation and apparently high rates of gene flow between disjunct populations as well as (2) the paleoclimatic evidence. Grey pine occupied southern California during glaciation. However, it could not invade the Great Central Valley until temperatures were warm enough to allow it access to the passes through the Transverse Ranges. As climate warmed in the late Pleistocene, becoming much like the present, grey pine entered the Great Central Valley, perhaps via Tejon Pass, which at an elevation of 1250 m seems relatively accessible. To explain gaps such as that between the Kings River and the South Fork of the Tule River, its migration into the Great Central Valley had to occur before the desiccation of Tulare Lake and the other pluvial lakes that blocked it from the foothills. These considerations seem to restrict the window of opportunity to a period between 12,000 and 8000 yrs BP.

Because grey pine bears seed at an early age, it migrated north rapidly, to the west of Tulare Lake and the wetlands of the southern San Joaquin Valley and to the east of the Delta. By 8000 yrs BP, grey pine occupied a tortuous but near-continuous, north-south range in the valley. A near-continuous range with, at most, small gaps is consistent with the low genetic distances and high values of *Nm* between samples. If gaps were created by long-distance colonization in the migration northward, as suggested by the tendency for heterozygosity to decrease with latitude, evidence of founder effects was largely eradicated by gene flow through subsequent pollen exchange. The distance from Tejon Pass to the north end of the Great Central Valley is about 700 km. Based on known rates of migration of pines in the interglacial period (81 to 400 m yr^{-1} in eastern North America, 240 m yr^{-1} for pinyon pine (*Pinus monophylla* Torr. & Frém.) in the Great Basin, 700 m yr^{-1} in Britain, and 1500 m yr^{-1} in continental Europe; MacDonald, 1993; Lanner, 1983), grey pine may have covered this distance in anywhere from 470 to 8600 yrs. Most estimates would require a span of less than 3000 years, which could be accommodated by the window of opportunity between 12,000 and 8000 yrs BP.

During the Xerothermic period, grey pine began to ascend the foothills, being excluded from lower elevations by increasing aridity sometime after 8000 to 6000 yrs BP (dated from the drying of Tulare Lake and palynological evidence from the Sierra Nevada). Probably simultaneously, it was excluded from its ancient range in southern California. Estimated time since the separation of Coast Range populations from those in the Sierra Nevada, based on genetic distance, are consistent with a breakup between 8500 and 1000 yrs BP. During the present, cooler, mesic period, grey pine has reinvaded lower elevations, particularly where disturbance favoured colonization (e.g. at Folsom and Oroville). More extensive sampling of the grey pine range might provide additional evidence to support or refute this scenario. Samples from Lake Elizabeth southwest of Antelope Valley, the Tehachapi Mountains to its northwest, the Modoc Plateau, and the Diablo Range south of the Delta and north of ancient Lake San Benito would be particularly critical.

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