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Allozyme Differentiation and Biosystematics of the Californian Closed-cone Pines (*Pinus* subsect. *Oocarpae*)

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ABSTRACT. Allozyme differentiation at 32 loci was studied in the three Californian species of *Pinus* subsect. *Oocarpae: P. attenuata, P. muricata,* and *P. radiata,* and in a small sample of a Latin American species of the subsection, *P. oocarpa.* The Californian species were previously known to comprise highly differentiated, disjunct populations, but with uncertain phylogenetic relationships among several populations and species. All populations had clear affinities for single species. The controversial Channel Islands (Santa Cruz Island) population of *P. muricata* and the Mexican Island (Guadalupe and Cedros islands) populations of *P. radiata* were distinct within their respective species, but clearly fell within each species complex. Contrary to evidence from other traits, the Californian species were equally differentiated from one another allozymically, with no evidence of close relationships among pairwise comparisons of the three species. *Pinus oocarpa,* the putative ancestral species, was about two times more variable, and at substantial and approximately equal genetic distance from each of the three Californian species. Divergence of populations within species was generally clinal. The initial radiation of *P. attenuata* was in the Sierra Nevada, and subsequent divergence was toward the coast in the Siskiyou Mountains, and then south through the coast range to southern California. Divergence in both *P. muricata* and *P. radiata* occurred northward along the coast, with the southern island populations retaining ancestral alleles, and differentiation from *P. oocarpa* increasing northward within species. Genetic differentiation among species was twice that among populations within species.

Three Californian pines within the subsect. *Oocarpae* Little and Critchfield, *Pinus attenuata* Lemmon, *P. muricata* D. Don, and *P. radiata* D. Don, compose a natural and distinct group in an otherwise heterogeneous subsection (Duffield 1952). They resemble one another biochemically (Mirov 1961), physiologically, and morphologically (Critchfield 1967; Doran 1974; Fielding 1961; Shaw 1914), hybridize in certain combinations (Brown 1966; Critchfield 1967; Millar and Critchfield 1988), and are isolated by complete crossing barriers from all other species, including the four Latin American species that have been placed in the same subsection (Critchfield 1967). The Latin American species are heterogeneous in morphology and crossing relationships (Duffield 1952). Some cross more readily with species in other subsections than with other Latin American members of subsect. *Oocarpae* (Critchfield 1967).

Although the Californian species are a distinct group, the many disjunct and genetically differentiated populations have variability that has thwarted satisfactory phylogenetic analysis, especially for the island populations. In the northern parts of the ranges, the species are generally distinct from one another. The southern and insular populations, however, are highly variable. Their characteristics sometimes have intermediate values, and species relationships suggested by different traits have often contradicted one another. The divergent insular populations have variously been included with *P. radiata, P. muricata,* and an independent lineage,

P. remorata Mason (reviewed in Millar 1986). The two maritime species, *P. radiata* and *P. muricata*, share many morphological similarities, especially in the southern and island populations. Relative crossability, however, is much higher between *P. radiata* and the interior species, *P. attenuata*, than between the two maritime species (Critchfield 1967).

One reason why the phylogenetic relationships among and within the Californian species in subsect. *Oocarpae* have been difficult to assess is the nature of traits that have been studied. Cone morphology, tree form, bark texture, and needle characteristics have been the basis for most genetic analyses in this group. These traits, however, seem particularly subject to evolutionary modification by natural selection in the closed cone pines (Plessas and Strauss 1986). They are, therefore, poor traits for phylogenetic analyses because homoplasies induced by convergent adaptations can obscure historic relationships.

Biochemical characteristics such as allozyme frequencies are better than morphological traits for phylogenetic studies. They are relatively insensitive to natural selection (Endler 1986) and environmental modification, and are therefore suitable for studying genetic variation among and within natural populations. Allozymes have been used to interpret evolutionary history in subsect. *Oocarpae* in *P. attenuata* and *P. muricata* (Millar 1983; Strauss 1986).

In the present study, allozyme variation was assessed within and among the Californian species of *Pinus* subsect. *Oocarpae*, and evolutionary relationships were analyzed among the species. The Californian species were emphasized but the study also included samples of the Latin American species, *P. oocarpa* Schiede, to serve as an outgroup for the Californian species and to clarify relationships among the Latin American and Californian groups.

Subsect. *Oocarpae*

Three primarily Californian species (*P. attenuata*, *P. muricata*, and *P. radiata*) and four Latin American species (*P. oocarpa*, *P. patula* Schiede & Deppe, *P. greggii* Engelm., and *P. pringlei* Shaw) compose subsect. *Oocarpae*. Within several species, formal varieties and unnamed but genetically distinct populations have been recognized.

Current Studies. *Pinus attenuata* is widely dispersed on dry, interior sites of southern Oregon, California, and at one location in northern Baja California. *Pinus muricata* and *P. radiata* occur in disjunct mainland populations along the California and Baja California coasts and on four offshore islands (fig. 1). Pairs of species are sympatric in only two places, one on a part of the Monterey Peninsula, where *P. radiata* and *P. muricata* co-occur and another in a few stands near Pt. Año Nuevo, where *P. attenuata* and *P. radiata* co-occur.

Geographic variation in *P. attenuata* has been studied less than in the two maritime species. It appears, however, that variation within populations is smaller, and variation among populations is less distinct, than in *P. muricata* and the island populations of *P. radiata*. In northern and central populations of *P. attenuata* (Klamath, Cascade, Sierra Nevada, and Coast ranges), morphological and growth traits vary clinally with latitude (Brown and Doran 1985; Newcomb 1962). Discontinuous variation occurs in cone morphology among the disjunct southern and Baja Californian populations, where cones resemble cone variants in the *P. muricata* complex (Newcomb 1962). No systematic studies of crossability within *P. attenuata* have been made, but related work indicates that populations are interfertile (Critchfield 1967 and pers. comm.).

Pinus muricata contains high amounts of genetic variation within and among populations (fig. 1) and both discontinuous and clinal patterns of variation among populations (Millar 1986). Populations north of Monterey (*P. muricata* var. *borealis* Axelrod, Duffield 1951; Axelrod 1983) are distinct in growth and form from the highly variable central and southern populations, and are characterized as large trees with excurrent stems, thick bark, and cones with low serotiny (Duffield 1951). *Pinus muricata* var. *borealis* is further divided into two discrete genetic groups that are distinguished by needle anatomy (Duffield 1951), monoterpene composition (Mirov et al. 1966), phenology, and allozyme frequencies (Millar 1983). The needle anatomy of the southern populations of var. *borealis* is typical of all *P. muricata* populations to the south, but their monoterpene composition is found elsewhere only in the Monterey population of *P. muricata*. The highly variable southern mainland populations (*P. muricata* var. *muricata*) form a third distinct monoterpene type,

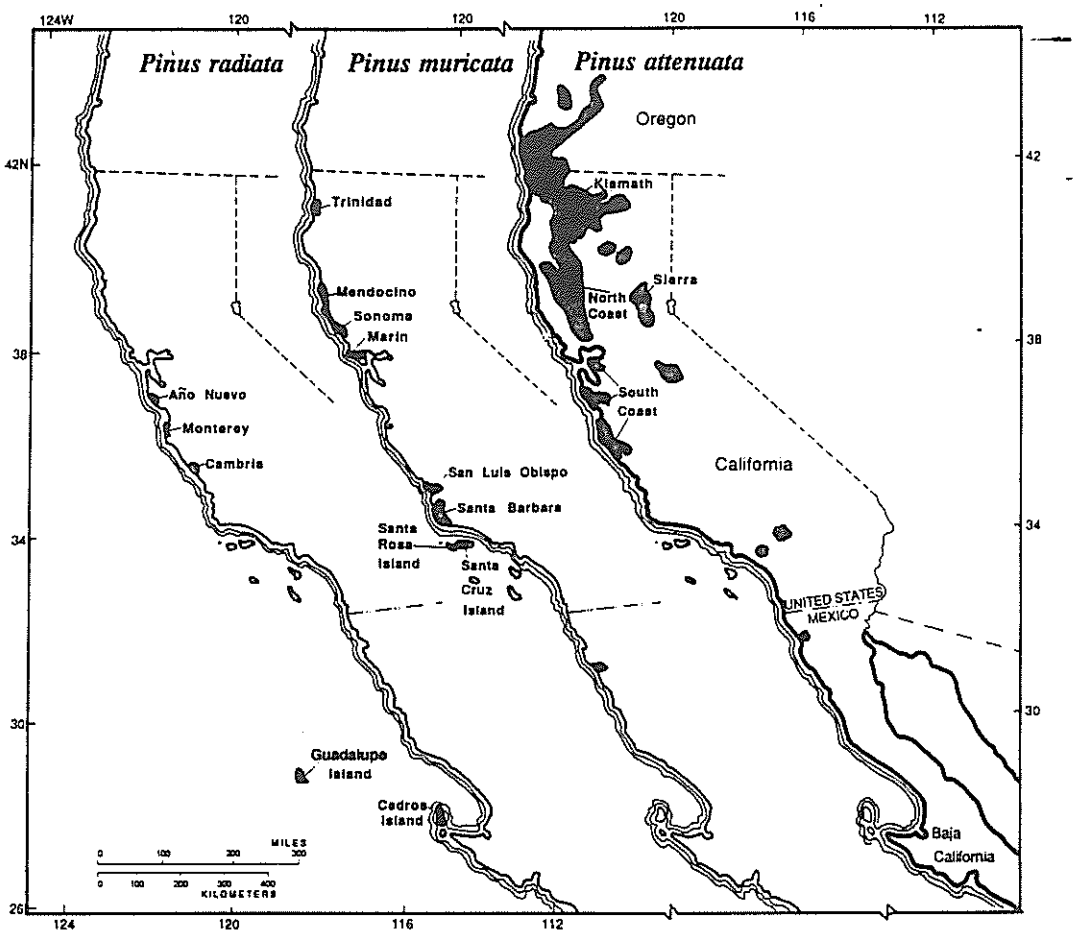

FIG. 1. Map of Californian species of subsect. *Oocarpae*, showing distributions of *Pinus radiata*, *P. muricata*, and *P. attenuata*.

whereas the island populations form a fourth monoterpene type. The island pines (called *P. remorata* Mason, 1930, *P. muricata* var. *remorata* Duffield, 1951, or *P. muricata* f. *remorata* Hoover, 1966) have high frequencies of a deviant cone type (symmetric cones with smooth apophyses) that occurs also in the central and southern mainland populations.

Complete crossing barriers within *P. muricata* exist between populations north of Monterey and both southern mainland and insular populations (Critchfield 1967; Millar and Critchfield 1988). The northern populations are reproductively isolated from *P. attenuata* and *P. radiata*, whereas the southern mainland and insular populations cross with these species.

Pinus radiata occurs in mainland California at Año Nuevo, Monterey, and Cambria, and on the Mexican islands of Guadalupe and Cedros (fig. 1). Despite high diversity within the three mainland populations, interpopulation divergence is low, and these populations compose a closely related unit (Bannister et al. 1962; Fielding 1961; Forde 1964; Murphy 1981; Plessas and Strauss 1986). The Guadalupe (var. *binata* Lemmon) and Cedros (var. *cedrosensis* (Howell) Axelrod) Island populations differ from one another and from the three mainland populations in many morphological traits (Millar 1986). *Pinus radiata* crosses freely in all combinations attempted with *P. attenuata* (Critchfield 1967), and all five *P. radiata* populations appear to be interfertile (W. J. Libby, unpubl. data).

Pinus oocarpa is the most widespread of the Latin American species of subsect. *Oocarpae*. It extends at subtropical latitudes from north-

western Mexico to northern Nicaragua, extending up to elevations of 2000 m. *Pinus oocarpa* is highly variable, with at least four varieties recognized (Martinez 1948). The other three Latin American species in subsect. *Oocarpae*, *P. greggii*, *P. patula*, and *P. pringlei*, are restricted to a few small populations in interior Mexico (Martinez 1948; Shaw 1909).

Evolutionary Hypotheses. Evolutionary origins of the Californian species of subsect. *Oocarpae* have been investigated from several perspectives. Axelrod (1958, 1967, 1980) presented floristic and tectonic evidence that subsect. *Oocarpae* originated in Mexico in the early-mid-Tertiary. He speculated that the Californian pines were part of a larger floristic association that migrated northward into what is now southwestern United States and that is currently separated from Mexican relatives by the Sonoran desert. Northward movement of the oceanic plate along the San Andreas fault (with a cumulative movement of 450–500 km since the early Miocene) accounts for the displacement of the Mexican and Californian elements; isolation of these elements was related to a general warming and drying climate 6000–8000 years ago (Axelrod 1980). Critchfield's (1967) crossing results, which show interfertility among some members of subsect. *Oocarpae* and some Mexican species, corroborate the hypothesis of a Mexican origin of subsect. *Oocarpae*.

Axelrod (1967, 1980) further speculated that *P. oocarpa* is closest to the ancestral form of the subsection because it contains characteristics considered prototypic of two lines that diverged early in the radiation of the subsection. One line remained in continental habitats and evolved into *P. greggii*, *P. patula*, and *P. pringlei* in the mountains of Mexico. He hypothesized that an offshoot from this line migrated north into Baja and southern California and evolved into *P. attenuata*, while a second line exploited coastal habitats. Two maritime groups evolved as the pines migrated northward. One group resulted in *P. radiata*, while the other evolved through *P. masonii* Dorf (a Miocene species with cones indistinguishable from northern *P. muricata*) and *P. remorata* to *P. muricata*.

Earlier, Axelrod (1967) had proposed but later apparently abandoned a monophyletic origin for the Californian species. An ancestral Latin American pine, diverging from a *P. oocarpa*-like pool reached California in the Oligocene. The subsequent radiation of variants into different ecological niches resulted in an interior taxon that led to *P. attenuata*, and a divergent coastal element (unique in subsect. *Oocarpae*) that split into the *P. radiata* and *P. muricata* lines.

Phylogenetic relationships among the populations within the Californian species are debated (Millar 1986). The genetic history of the *P. muricata* populations on the Californian Channel Islands is unclear and three different hypotheses have been proposed to explain them. First, Mason (1930, 1949) postulated that trees having cones with smooth scales and symmetric axes, which are found in high frequencies on Santa Cruz and Santa Rosa islands, represented an ancient and distinct lineage, *P. remorata*. Mason argued that this species is being diluted and lost due to late Pleistocene hybridization and introgression with mainland *P. muricata*.

Axelrod (1980, 1983) interpreted the extent of *P. remorata* differently. He hypothesized that *P. remorata* was a distinct and widespread species until the late Wisconsin, when hybridization with another distinct but now relictual lineage, *P. "borealis,"* gave rise to the Quaternary species, *P. muricata*. He considered *P. "borealis"* to be a taxon directly descended from the fossil species, *P. masonii*. Other authors have proposed a third origin for trees with unusual cones. They interpret cone variation as polymorphism within the highly variable *P. muricata* complex (Duffield 1951; Linhart et al. 1967; Millar and Critchfield 1988).

Pines on Cedros and Guadalupe Islands also have uncertain phylogenetic affinities. In many traits, the populations are distinct from each other and from the other closed-cone pine populations (Millar 1986). Guadalupe Island pine has long been considered a variety of *P. radiata* (var. *binata* Engelmann, 1880). Until recently, Cedros Island pine was included with *P. muricata* (Critchfield and Little 1966; Griffin and Critchfield 1976); it is now considered a variety of *P. radiata* (var. *cedrosensis*, Axelrod, 1983). After comparing fossil and extant cones, Axelrod (1980) inferred that the Cedros and Guadalupe Island pines had retained ancestral *P. oocarpa*-like characteristics, and that they are the oldest taxa in *P. radiata*.

Materials and Methods

Bulked seed collections were obtained from 31 rangewide stands of *P. attenuata*. These stands

TABLE 1. Origin of seeds, numbers of stands, and sample sizes used in allozyme analyses of closed cone pines.

Taxon	No. of stands	Total no. of trees	Origin
Pinus muricata	1	10	Trinidad, CA
	5	15	Mendocino Co., CA
	5	15	Sonoma Co., CA
	7	36	Marin Co., CA
	3	15	San Luis Obispo, CA
	5	15	Santa Barbara, CA
	6	29	Santa Cruz Is., CA
Pinus radiata	5	15	Año Nuevo, CA
	5	15	Monterey, CA
	5	15	Cambria, CA
	5	14	Cedros Is., Baja, Mexico
	4	15	Guadalupe Is., Baja, Mexico
Pinus attenuata	10	50	Sierra (central CA, Sierra Nevada)
	11	30	Klamath (coast ranges of SW OR and NW CA)
	7	53	North Coast (north central coast ranges of CA north of S.F. Bay)
	3	15	South Coast (CA coast ranges, south of SF Bay to San Luis Obispo)
Pinus oocarpa	2	unknown	Morelia, Michoacan, Mexico La Palma, El Salvador

were subsequently pooled for analysis into four populations to approximate the sampling structure used in the other species. Each population sample comprised 15–53 trees (table 1). Bulked seed collections were obtained from 10 to 36 trees in each of seven populations of *P. muricata* and five populations of *P. radiata* (table 1). Seed from an unknown number of trees from each of two widely separated stands of *P. oocarpa* were pooled into one group for analysis.

Seeds of all taxa were either cold-wet stratified for 30 days or treated with 1% hydrogen peroxide for 48 hours to induce rapid germination. Seeds were germinated until the radicles emerged from the seed. Female gametophytes were electrophoresed on starch gels using techniques of Conkle et al. (1982), Millar (1985), and gel systems of Strauss and Conkle (1986). The number of gametophytes analyzed for each population was twice the number of trees composing the seedlot.

Enzymes assayed were aconitase (ACO), acid phosphatase (ACP), alcohol dehydrogenase (ADH), alanine aminopeptidase (ALA), beta-esterase (EST), fructose diphosphatase (FDP), fumarase (FUM), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (GPD), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), mannose phosphate isomerase (MPI), peptidase (PEP), 6-phosphogluconic dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and UDP-glucose pyrophosphatase (UGP). Enzyme commission numbers can be found in Strauss and Conkle (1986). Loci within these enzyme systems were designated by numerals that follow the italicized enzyme acronyms (loci with the lower numbers are closest to the anode on gels). Thirty-two loci from these 19 enzyme systems resolved consistently in all taxa.

Electrophoresis of conifer gametophytes yields haploid genetic information about the maternal seed parent. Allele frequencies, expected heterozygosities, hierarchical diversity statistics (Chakraborty 1980; Nei 1973), Nei's (1978) unbiased genetic distance measures, UPGMA cluster analyses (Sneath and Sokal 1973), and Wagner trees (Farris 1972) based on modified Roger's distances (Wright 1978) were calculated using BIOSYS-1 (Swofford and Selander 1981). The UPGMA, which assumes equal evolutionary rates in all taxa, was used to enable comparison to other studies (it has frequently been applied to allozyme data, e.g., Wheeler et al. 1983); the Wagner method, which is free of this assumption, was also calculated. Nei's distance measures could not be used for construc-

TABLE 2. Allozyme frequencies for *Pinus attenuata, P. muricata, P. radiata,* and *P. oocarpa* populations. [1] Sie = Sierra Nevada, Kla = Klamath-Siskiyou Mountains, NC = North Coastal Ranges, SC = South Coastal Ranges, Tri = Trinidad, Men = Mendocino, Son = Sonoma, SLO = San Luis Obispo, SBa = Santa Barbara, SCI = Santa Cruz Island, AñN = Año Nuevo, Mon = Monterey, Cam = Cambria, Ced = Cedros Island, and Gua = Guadalupe Island. [2] Alleles are identified by their mobilities relative to the most common allele (1.00); the anodal migration distance (mm from the origin) of the 1.00 allele and the letter designation of the gel buffer system used for electrophoresis are in parentheses following each locus's abbreviation. [3] Bands of this locus were not resolved.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
	Sample size—mean number of megagametophytes analyzed per locus																
	135	168	97	59	101	59	47	219	19	27	58	83	93	84	65	46	22
Aco1 (21.5, D)																	
1.21			0.01	0.05	0.02	0.28	0.26	0.73		0.14	0.08	0.13					0.26
1.14		0.01	0.01	0.10													0.04
1.09	0.33	0.25	0.17	0.22								0.13	0.26	0.03		0.05	
1.05	0.04	0.03			0.04							0.02	0.03	0.03	0.15	0.03	0.37
1.00	0.58	0.71	0.81	0.63	0.94	0.72	0.74	0.27	1.0	0.82	0.92	0.70	0.68	0.87	0.82	0.92	0.29
0.93												0.02					
0.84	0.05									0.04			0.03	0.07	0.03		0.04
Acp1 (19.5, D)																	
1.00	0.96	0.93	0.84	0.05	1.0	0.92	1.0	1.0	1.0	1.0	1.0	0.99	0.92	0.90	0.60	0.76	0.55
0.87	0.01		0.16	0.95		0.08						0.01	0.05	0.01	0.17		0.25
0.79													0.01		0.21	0.24	
Null	0.03	0.07											0.02	0.09	0.02		0.20
Acp2 (14.5, D)																	
1.00	0.33	0.30	0.15	0.02	1.0	1.0	1.0	1.0	1.0	0.60	1.0	0.59	0.62	0.67	0.67	0.50	0.47
0.93		0.05															0.12
0.79	0.67	0.65	0.85	0.98						0.40		0.39	0.37	0.33	0.31	0.50	0.29
0.62												0.02	0.01		0.02		0.12
Adh2 (21.0, E)																	
1.19						0.10									0.01		1.0
1.00	0.63	0.86	0.94	0.93	1.0	0.90	0.95	0.93	0.81	0.67	0.26	0.33	0.17	0.21	0.31	0.59	
0.79							0.05	0.06	0.19	0.33	0.48						
0.64	0.03											0.52	0.50	0.32	0.13	0.13	
0.38	0.34	0.14	0.06	0.07				0.01			0.26	0.15	0.33	0.47	0.55	0.28	

TABLE 2. Continued.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
Ala1 (42.0, A)																	
1.10								0.03				0.02	0.05	0.02		0.08	0.10
1.05			0.01		0.12			0.23		0.03	0.03	0.02	0.05	0.20	0.16	0.11	0.38
1.00	1.0	1.0	0.99	1.0	0.88	1.0	1.0	0.70	1.0	0.97	0.97	0.96	0.90	0.78	0.84	0.81	0.52
0.93								0.04									
Ala2 (41.0, A)																	
1.00	0.90	0.81	0.48	0.53	1.0	0.96	1.0	1.0	0.85	0.87	0.74	1.0	0.97	0.75	0.65	0.64	0.38
0.97	0.10	0.16	0.52	0.47		0.04			0.10	0.13	0.26		0.03	0.24	0.32	0.27	0.57
0.92		0.03							0.05					0.01	0.03	0.09	0.05
Est2 (29.5, A)																	
1.15		0.05		0.06											0.01	0.40	0.29
1.10													0.04		0.03		
1.03	0.04	0.16	0.07	0.12													
1.00	0.96	0.79	0.93	0.82	1.0	0.92	1.0	1.0	1.0	0.96	1.0	1.0	0.96	1.0	0.96	0.60	0.71
0.92										0.04							
0.76						0.08											
Fdp2 (15.0, E)																	
1.10	0.20	0.15	0.28	0.20			0.05										
1.00	0.79	0.85	0.70	0.80	1.0	1.0	0.95	1.0	1.0	0.97	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.93	0.01		0.02														
0.73										0.03							
Fum (27.0, D)																	
1.26	0.01		0.02	0.13								0.02	0.01	0.01			0.04
1.00	0.97	1.0	0.98	0.87	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.98	0.99	0.99	1.0	1.0	0.96
0.89	0.01																
0.81	0.01																
Gdh (18.5, B)																	
1.16											0.02					0.02	0.44
1.08																	0.13
1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.91	0.98	1.0	0.99	1.0	1.0	0.92	0.13

TABLE 2. Continued.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
0.95										0.09							0.04
0.86													0.01				0.26
0.76																0.06	
Got1 (25.5, B)																	
1.57													0.02			0.04	
1.43															0.01		
1.18																	0.15
1.16	0.86	0.96	0.83	0.83	0.97	0.39	0.58	0.95	0.48	0.54	0.49		0.03		0.01	0.04	0.33
1.14							0.10										0.11
1.00	0.14	0.04	0.17	0.17	0.03	0.61	0.32	0.05	0.52	0.46	0.51	1.0	0.95	1.0	0.98	0.92	0.41
Got3 (16.5, B)																	
1.58																	0.15
1.30			0.01	0.03	0.01				0.10				0.01				0.41
1.21																	0.19
1.09												0.35					0.18
1.00	1.0	1.0	0.99	0.97	0.99	0.97	1.0	1.0	0.90	1.0	0.94	0.65	0.99	1.0	1.0	1.0	
0.76						0.03					0.06						0.07
G6pd1 (29.5, B)																	
1.07						0.02							0.01				
1.00	0.99	1.0	1.0	1.0	1.0	0.98	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	0.84
0.92																	
Null	0.01																0.16
G6pd2 (17.5, B)																	
1.11										0.05							
1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0	1.0	1.0	0.89	1.0	0.50
0.83															0.05		0.50
0.54															0.06		
Idh (17.5, D)																	
1.89																	
1.20															0.01		

TABLE 2. Continued.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
1.09														0.04			
1.00	0.99	0.98	0.84	1.0	1.0	0.99	0.95	0.99	1.0	1.0	1.0	1.0	0.93	0.92	0.99	1.0	0.96
0.86		0.02	0.16					0.01						0.01			0.04
0.63	0.01												0.07	0.03			
0.77						0.01	0.05										
Lap1 (47.0, A)																	
1.06										0.03		0.02	0.03	0.03	0.16	0.17	
1.03	0.57	0.82	0.87	0.83					0.24	0.19	0.05	0.11	0.10	0.10	0.18	0.09	0.57
1.00	0.43	0.18	0.13	0.17	1.0	1.0	0.89	0.99	0.57	0.78	0.95	0.85	0.82	0.87	0.54	0.74	
0.96									0.19			0.02	0.05		0.12		
0.87																	0.38
Null							0.11	0.01									0.05
Lap2 (38.0, A)																	
1.00	0.95	0.68	1.0	0.91	0.89	0.38	0.24	0.55	1.0	0.84	1.0		0.01	0.22	0.69	0.15	N.R.[3]
0.95	0.05	0.32		0.09	0.11	0.62	0.76	0.45		0.16		1.0	0.99	0.78	0.31	0.85	
Mdh1 (30.0, D)																	
1.05							0.02						0.01				
1.00	1.0	1.0	1.0	1.0	1.0	1.0	0.98	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0
Mdh2 (24.5, D)																	
1.18																	0.08
1.00	0.96	1.0	1.0	1.0	1.0	1.0	0.88	0.94	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	0.92
0.96								0.03									
0.92							0.12										
0.63	0.04							0.03					0.01				
Mdh4 (8.0, D)																	
1.62					0.02			0.09									0.07
1.00	1.0	0.97	1.0	1.0	0.98	1.0	1.0	0.91	1.0	1.0	1.0	0.99	0.98	1.0	1.0	1.0	0.93
0.75		0.03										0.01	0.02				

TABLE 2. Continued.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
Mdh5 (3.0, D)																	
2.00														0.14			
1.00	1.0	1.0	1.0	1.0	1.0	0.99	1.0	0.99	1.0	1.0	1.0	1.0	0.99	0.86	1.0	1.0	1.0
0.33						0.01		0.01					0.01				
MdhM																	
1.10									0.05			0.05	0.04		0.01		
1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0	0.95	0.96	1.0	0.99	1.0	1.0
Mpi1 (56.0, B)																	
1.03																	0.07
1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	0.86
0.96													0.01				0.07
Pep2 (42.0, A)																	
1.02													0.01				
1.00	1.0	1.0	1.0	1.0	1.0	0.97	1.0	1.0	1.0	0.97	0.98	1.0	0.98	1.0	1.0	1.0	0.82
0.98						0.03				0.03	0.02		0.01				0.18
Pep4 (20.0, A)																	
1.10		0.01					0.17								0.03	0.02	
1.00	1.0	0.99	1.0	1.0	1.0	0.88	0.54	0.85	1.0	0.93	0.86	0.99	0.89	0.89	0.92	0.96	1.0
0.92						0.12	0.29	0.15		0.07	0.14	0.01	0.11	0.11	0.05	0.02	
6Pdg1 (32.5, D)																	
1.21	0.06												0.02				
1.11	0.18	0.44	0.48	0.42									0.03	0.01	0.04		
1.03													0.01		0.01		
1.01													0.01				0.15
1.00	0.76	0.56	0.52	0.58	1.0	0.94	0.93	1.0	0.95	0.78	1.0	1.0	0.93	0.99	0.95	1.0	0.67
0.98										0.06							
0.92						0.06	0.07		0.05	0.16							0.07
0.88																	0.11
6Pgd2 (26.0, D)																	
1.15																	0.04

TABLE 2. Continued.

Locus/ allele[2]	Species and populations[1]																
	Pinus attenuata				*P. muricata*							*P. radiata*					*P. oocarpa*
	Sie	Kla	NC	SC	Tri	Men	Son	Mar	SLO	SBa	SCI	AñN	Mon	Cam	Ced	Gua	
1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.97	1.0	0.89	0.87	0.95	1.0	0.98	0.85
0.96												0.11	0.13	0.05		0.02	
0.92																	
0.83																	0.04
0.69										0.03							
Null																	0.07
Pgi2 (34.0, E)																	
1.00	0.84	0.88	0.75	0.82			0.44		0.14	0.25	0.48	0.76	0.57	0.65	0.33	0.89	0.54
0.91	0.16	0.11	0.22	0.18	1.0	0.91	0.53	1.0	0.86	0.69	0.23	0.10	0.23	0.18			0.04
0.76		0.01	0.03			0.09	0.03			0.06	0.29	0.14	0.20	0.17	0.67	0.11	0.42
Pgm1 (42.0, D)																	
1.00	0.89	0.91	1.0	0.97	0.48	0.73	0.60	0.20	0.10	0.28	0.32	1.0	0.86	1.0	0.87	1.0	0.64
0.95		0.01		0.03	0.41	0.06	0.21	0.06		0.03	0.05		0.10				0.36
0.90	0.07				0.11	0.21	0.19	0.74	0.90	0.69	0.63		0.04		0.13		
0.74	0.04	0.08															
Skdh1 (25.0, D)																	
1.08													0.01		0.01		0.20
1.00	0.99	0.97	1.0	1.0	1.0	0.91	0.95	0.98	0.81	0.97	1.0	1.0	0.97	1.0	0.94	0.97	0.75
0.96						0.09	0.05	0.02	0.19	0.03							0.05
0.84	0.01												0.02		0.05	0.03	
Null		0.03															
Ugp1 (47.0, E)																	
1.00	0.17	0.13			0.36					0.20	0.29	0.79	0.77	0.79	1.0	1.0	1.0
0.97	0.83	0.85	1.0	1.0	0.64	1.0	1.0	0.16	1.0	0.80	0.71	0.21	0.23	0.21			
0.94		0.02						0.84									
Ugp2 (24.0, E)																	
1.25		0.04			0.01			0.03									0.30
1.12	0.03	0.09	0.06	0.03	0.48			0.30									0.44
1.00	0.94	0.87	0.94	0.97	0.51	0.63	0.66	0.62	1.0	0.69	0.50	0.53	1.0	1.0	1.0	1.0	0.22
0.88	0.03					0.37	0.34	0.05		0.31	0.50	0.47					0.04

tion of Wagner trees because they violate the triangle inequality; Roger's distances were therefore used instead.

Results

Allozyme frequencies varied greatly among the three Californian species (table 2). Differences were especially marked at *Aco1, Acp2, Adh2, Got1, Lap1, Lap2, Pgd1, Pgi2, Pgm1, Skdh1,* and *Ugp1*. The differences were manifest as a combination of dissimilar frequencies of shared alleles (e.g., *Lap2*-1.00 high frequencies in *P. attenuata*, moderate in *P. muricata*, and mostly low in *P. radiata*; *Pgi2*-1.00 high frequencies in *P. attenuata*, low in *P. muricata*, and moderate in *P. radiata*), and the presence and absence of unique alleles (e.g., *Got1*-1.14 present only in *P. muricata*, and -1.43 and -1.57 only in *P. radiata*; *Pgd1*-0.98 and -0.92 present only in *P. muricata*, -1.03 and -1.01 only in *P. radiata*). Allele frequencies at other loci revealed similarities among pairs of the Californian species. *Pinus attenuata* and *P. radiata* had similar allele frequencies at *Aco1, Acp1, Est2, Mdh4, Pgm1,* and *Ugp2*; *P. attenuata* and *P. muricata* had similar frequencies at *Adh2, Lap2, Pgd2,* and *Ugp1*; and *P. muricata* and *P. radiata* had similar frequencies at *Fdp2, Lap1, Pep4,* and *Pgd1*.

Genetic distance (table 3; fig. 2A), and genetic diversity (table 4), clearly demonstrated the distinctness of each of the three Californian species. Each group diverged at about the same genetic distance from the others. Average genetic distances were the same (0.11) between *P. attenuata* and *P. muricata* and between *P. muricata* and *P. radiata* (table 3). *Pinus attenuata* and *P. radiata* had the largest interspecific genetic distance values of the three combinations (0.13). The affinities of the three Californian species differed between the two cluster methods (fig. 2A, B), with *P. attenuata* and *P. muricata* most closely related in the UPGMA, and *P. muricata* and *P. attenuata* most closely related in the Wagner method. These differences occurred due to the methods of clustering and not due to differences in distance measures, further emphasizing the lack of a definitive relationship among the three species. Diversity values indicated differentiation at many loci, with 24% of the pooled total variation for the three Californian species distributed among species, 12% among populations, and 64% within populations (table 4). Assuming a common progenitor for the Californian taxa, all three species were on average about equidistant from the presumed ancestor, *P. oocarpa* (fig. 2B). Each species had a wide and similar range of affinities among its populations.

Analysis of the *P. oocarpa* sample revealed substantial divergence between this single representative of the Latin American taxa and the Californian species. Allele frequencies in *P. oocarpa* differed greatly from the three Californian species at many loci (table 2), and only the loci with low polymorphism had similar frequencies. *Pinus oocarpa* had several loci with alleles not present in the Californian species, including *Adh2*-1.19, *Gdh1*-1.16, -1.08, -0.86, *Got1*-1.18, *Got3*-1.58, -1.21, -1.09, and *Lap1*-0.87. Although *P. oocarpa* had many of the same alleles as the Californian species, there were some common alleles that occurred in one or more of the Californian species but not in *P. oocarpa*. These included *Aco1*-1.09, *Acp1*-0.79, *Adh2*-0.79, -0.64, -0.38, *Est2*-1.03, *Fdp2*-1.10, *Got3*-1.00, *Lap1*-1.06, -1.00, -0.96, *Pep4*-0.92, *Pgd1*-1.11, *Pgm1*-0.90, and *Ugp1*-0.94 (table 2). Average genetic distances (table 3) of *P. oocarpa* from the Californian species were twice the values of the distances among the Californian taxa, and ranged from 0.21 for *P. radiata* to 0.26 for *P. muricata*.

The three Californian species had similar average amounts of allelic variation but different amounts from *P. oocarpa* (table 5). *Pinus muricata* had the lowest average number of alleles per locus (1.6), expected heterozygosity (0.118), and percent polymorphic loci (47%). *Pinus attenuata* and *P. radiata* had values about equal to each other, slightly higher than *P. muricata*, but lower than *P. oocarpa*. *Pinus oocarpa* had higher diversity values than the Californian species for all the statistics, especially expected heterozygosity (0.270), which was twice the values of any of the other species.

There was substantial allelic divergence among populations within the Californian species, as indicated by allele frequency differences (table 2), genetic distances (table 3), expected heterozygosities, and the partition of genetic diversity (table 4). When expected heterozygosities were calculated for the whole species disregarding subdivision (table 4), the values for *P. attenuata* and *P. radiata* were slightly higher than when expected heterozygosities were averaged over populations within species.

TABLE 3. Matrix of Nei's unbiased genetic distance coefficients among populations and species for four pines of the subsection *Oocarpae* based on 32 loci. Interpopulation distances are above the diagonal, average species distances with ranges of values are below.

Species/population	*Pinus attenuata*				*P. muricata*							*P. radiata*					*P. oocarpa*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Pinus attenuata																	
1 Sierra	—	0.01	0.02	0.05	0.08	0.09	0.08	0.11	0.08	0.05	0.07	0.11	0.10	0.09	0.10	0.10	0.21
2 Klamath		—	0.01	0.04	0.10	0.10	0.08	0.13	0.10	0.07	0.10	0.12	0.12	0.11	0.12	0.11	0.23
3 N. Coast		0.03	—	0.03	0.12	0.13	0.12	0.16	0.11	0.08	0.12	0.17	0.16	0.14	0.13	0.14	0.23
4 S. Coast		(0.01–0.05)		—	0.16	0.16	0.15	0.19	0.15	0.12	0.16	0.20	0.18	0.17	0.15	0.16	0.25
P. muricata																	
5 Trinidad					—	0.04		0.03	0.05	0.04	0.06	0.14	0.13	0.12	0.13	0.15	0.25
6 Mendocino						—	0.05	0.04	0.04	0.03	0.05	0.08	0.08	0.08	0.11	0.11	0.29
7 Sonoma							0.01	0.04	0.06	0.04	0.06	0.08	0.08	0.08	0.12	0.11	0.30
8 Marin							—	—	0.05	0.04	0.07	0.15	0.14	0.14	0.16	0.18	0.30
9 San Luis Obispo									—	0.02	0.04	0.15	0.13	0.12	0.12	0.15	0.27
10 Santa Barbara		0.11						0.04		—	0.02	0.10	0.09	0.09	0.09	0.11	0.23
11 Santa Cruz Isl.		(0.05–0.19)						(0.01–0.07)			—	0.10	0.10	0.08	0.08	0.11	0.22
P. radiata																	
12 Año Nuevo												—	0.02	0.02	0.06	0.03	0.22
13 Monterey													—	0.01	0.04	0.03	0.24
14 Cambria														—	0.03	0.02	0.22
15 Cedros Isl.		0.13						0.11						0.03	—	0.04	0.17
16 Guadalupe Isl.		(0.09–0.20)						(0.08–0.18)						(0.01–0.06)		—	0.21
P. oocarpa																	
Latin America		0.23						0.27						0.21			—
		(0.21–0.25)						(0.22–0.30)						(0.17–0.24)			

TABLE 4. Mean (32 loci) expected heterozygosities (He) and apportionments of gene diversity (Chakraborty 1980) between species, among populations within species, and within populations for the Californian closed-cone pines. [1] The mean expected heterozygosities assume taxon panmixis and their standard errors are between 0.032 and 0.035.

Taxonomic level	He[1]	Pct. diversity		
		Between spp.	Among populations within spp.	Within populations
Pooled total for the three Californian closed-cone pines:	0.200	24	12	64
Individual species:				
Pinus attenuata	0.138		12	88
P. muricata	0.149		22	78
P. radiata	0.160		13	87

This occurs due to variance among populations, as indicated by percent variation among populations (12% within *P. attenuata*, 13% within *P. radiata*). *Pinus muricata* had nearly twice the amount of diversity attributed to differences among populations (22%) than the other species, and also had a considerably inflated average heterozygosity value (table 4).

All three species had geographic trends in allelic variation. A cline of allele frequencies among contiguous populations of *P. attenuata* from the Sierra Nevada through the Klamath region and into the North and South Coast ranges was apparent at many loci (tables 2, 3; fig. 2). Alleles that showed roughly clinal patterns were *Aco1*-1.09, *Acp1*-1.00, *Acp2*-1.00, *Adh2*-1.00, *Ala2*-1.00, *Fum*-1.00, and *Ugp1*-0.97 (table 2). Genetic distances increased with geographic distance between the populations of *P. attenuata*. The geographically adjacent Sierra and Klamath populations and adjacent Klamath and North Coast populations were most similar (distance = 0.01, table 3). The South Coast was the most distinct population. It was most closely related to the North Coast (distance = 0.03) and most distantly related to the Sierran population (distance = 0.05). By Wagner procedures, there was a geographic cline in distance from the presumed ancestor, *P. oocarpa* (fig. 2B), with the Sierran population most ancestral and the South Coast population most derived.

The large genetic divergence among *P. muricata* populations (tables 3, 4; fig. 2) stemmed primarily from major geographic differences between northern (Trinidad, Mendocino, Sonoma, and Marin) and southern (San Luis Obispo, Santa Barbara, and Santa Cruz Island) populations, notably at *Aco1*, *Adh2*, *Ala2*, *Got1*, *Lap1*, *Lap2*, *Pgi2*, *Pgm1*, and *Ugp4*.

Substantial divergences occurred among populations within the northern and southern clusters of *P. muricata*. The Trinidad and Marin populations were distinct within the northern group of *P. muricata* populations, differing most from the Sonoma populations (table 3; fig. 2). The Trinidad population was notable for having the least allelic variation of any population in the four species studied, with expected heterozygosity of 0.07 (table 5). This may result, in part, from only a single stand being included in the Trinidad sample.

In the southern group, the Santa Cruz Island population was distinct, set apart by having alleles occurring in high frequency that were uncommon elsewhere (e.g., *Adh2*-0.79, -0.38, *Ala2*-0.97, *Got3*-0.76, *Pgi2*-1.00, -0.76, *Ugp2*-0.88). Relative to the other southern populations, the Santa Cruz Island population was most similar to the Santa Barbara population, as indicated by the genetic distance to Santa Barbara (0.02) being half the value to the San Luis Obispo population (0.04). The Santa Cruz Island population differed substantially from the two southern mainland populations at *Adh2*, *Ala2*, *Gdh*, *Lap1*, and *Pgi2*. At all these loci except *Adh2*, the Santa Cruz Island population had similar allele frequencies to *P. radiata*, especially to the Cedros Island population, where the genetic distance (0.08) value was the lowest of all *P. muricata–P. radiata* combinations. At several of these loci, the unusual allele frequencies also resembled *P. oocarpa* and *P. attenuata*. The distances between the Santa Cruz Island and the northern populations of *P. muricata* were the largest interpopulation distances in *P. muricata* (0.05–0.07, table 3). Relative to all other *P. muricata* populations, the Santa Cruz Island population had fairly high expected heterozygosity, although the average number of alleles and percent polymorphic loci were slightly below the *P. muricata* population averages (table 5).

A clinal trend from north to south in *P. muricata* occurred in its divergence from a hypothetical progenitor of the Californian closed-cone pines as shown by the Wagner tree (fig. 2B). The northern populations were the most

FIG. 2. Phenograms for the Californian closed-cone pines. A) Hierarchical clusters produced using the unweighted pair-group algorithm (UPGMA) with Nei's unbiased genetic distances (Nei 1978), and B) Phylogenetic tree produced using the Wagner procedure with modified Roger's distances (Wright 1978).

TABLE 5. Genetic variability at 32 loci for 4 species of the genus *Pinus* subsect. *Oocarpae* (standard errors in parentheses). [1] A locus was considered polymorphic if more than one allele was detected. [2] Average heterozygosity within populations. [3] Values averaged over two population samples.

Species/population	Pct. loci polymorphic[1]	Mean no. alleles per locus	Mean Hardy-Weinberg expected heterozygosity[2]
Pinus attenuata			
Sierra Nevada	59	1.9 (0.2)	0.14 (0.03)
Klamath	59	1.9 (0.2)	0.14 (0.03)
North Coast	50	1.6 (0.1)	0.12 (0.03)
South Coast	50	1.6 (0.1)	0.12 (0.03)
Mean	55	1.7	0.131
P. muricata			
Trinidad	28	1.4 (0.1)	0.07 (0.03)
Mendocino	56	1.6 (0.1)	0.12 (0.03)
Sonoma	47	1.6 (0.1)	0.14 (0.04)
Marin	56	1.9 (0.2)	0.11 (0.03)
San Luis Obispo	31	1.4 (0.1)	0.09 (0.03)
Santa Barbara	66	1.8 (0.1)	0.17 (0.03)
Santa Cruz Isl.	44	1.5 (0.1)	0.14 (0.04)
Mean	47	1.6	0.118
P. radiata			
Año Nuevo	44	1.8 (0.2)	0.13 (0.04)
Monterey	91	2.4 (0.2)	0.15 (0.03)
Cambria	50	1.8 (0.2)	0.14 (0.03)
Cedros Isl.	56	2.0 (0.2)	0.16 (0.04)
Guadalupe Isl.	47	1.7 (0.2)	0.13 (0.03)
Mean	58	1.9	0.141
P. oocarpa[3]	65	1.9 (0.2)	0.27 (0.04)

distant from the root, with San Luis Obispo and Santa Barbara populations intermediate. The Santa Cruz Island population was most like the hypothetical progenitor.

The five populations of *P. radiata*, including the three mainland populations and Cedros and Guadalupe Islands populations, were distinct from all other populations and species. Allele frequency differences (table 2), genetic distances (table 3), and cluster analyses (fig. 2) suggested a north-to-south cline from Año Nuevo to Cedros Island at several loci. Alleles in high frequency in Año Nuevo and Monterey that decreased southward were *Acp*-1.00, *Adh2*-1.00 (mainland), *Adh2*-0.64, *Ala1*-1.00, and *Ala2*-1.00. The three mainland populations were a closely related group, and had some of the lowest interpopulation genetic distances of all the Californian species (table 3). Genetic distances among the mainland populations generally increased with geographic distance, and allele frequencies at *Aco1*, *Ala1*, *Ala2*, *Lap1*, *Mdh-m*, and *Pgd2* sugggested that Año Nuevo and Monterey were the most closely related pair of mainland populations. Another subset of loci, however, *Adh2*, *Idh*, *Pep4*, and *Ugp2*, suggested that Monterey and Cambria were the most closely related pair of mainland populations. The affinities of the mainland *P. radiata* populations differed in the two cluster analyses (fig. 2A, B). This was due to differences in the method of clustering and not to differences in distances, thus further emphasizing the lack of definitive relationships among these populations in the allozyme loci.

The island populations of *P. radiata*, like the Santa Cruz Island population of *P. muricata*, were distinct from the mainland populations. They differed in having high frequencies of several alleles, including *Aco1*-1.05, -1.00, *Acp1*-0.79, *Acp2*-0.79, *Ala2*-0.97, -0.92, *Est2*-1.15, *Gdh1*-0.76, *Lap1*-1.06, -0.96, *Pgi2*-0.76, and *Pgm1*-0.90, and in lacking alleles present in mainland populations (*Fum*-1.26, *Idh*-1.09, -0.63, *Pgi2*-0.91, and *Ugp1*-0.97). The genetic distances among the island populations (0.04) were high, comparable to genetic distances between the islands and the more distantly related mainland populations (table 2: *Adh2*, *Est2*, *Gpd2*, *Lap2*, *Pgi2*, and *Pgm1*). The Cedros Island population had the greatest distances to other *P. radiata* populations and most resembled the hypothetical progenitor (fig. 2A, B). Genetic distances from this common root increased clinally from south to north (Cedros, Guadalupe, Cambria, Monterey, and then Año Nuevo). Compared to populations of all the species, the Cedros and Guadalupe islands populations retained normal levels of genetic variability (heterozygosities of 0.16 and 0.13, respectively, table 5).

The ancestral nature of certain populations within each of the three Californian species indicated by the Wagner tree (fig. 2B) was corroborated by Nei's genetic distances (table 3), with the putative ancestral populations having mostly the lowest genetic distances to other species. This was especially true for *P. attenuata*, where genetic distances were lowest for all combinations between the ancestral-like Sierran population and all *P. muricata* and *P. radiata* populations, and highest for all combinations involving the South Coast *P. attenuata* popu-

lation (table 3). This pattern also occurred for genetic distance combinations between the Cedros Island population of *P. radiata* and *P. attenuata* populations and, with less consistency, *P. muricata* populations. The situation in *P. muricata* was not as clear: the ancestral-like Santa Cruz Island population had low but not always the lowest genetic distances to *P. radiata* and *P. attenuata* populations, but the presumed divergent populations (Trinidad and Sonoma) in some cases also had low genetic distances to *P. radiata* and *P. attenuata* populations.

Discussion

Previous work on phylogenetic relationships of the Californian closed-cone pines of subsect. *Oocarpae* focused on morphological characteristics, primarily of seed cones (Axelrod 1980, 1983; Mason 1932, 1934, 1949). The current studies of allozyme variation shed new light on both intra- and interspecific evolution of the Californian taxa, and their relationship to the Latin American species, *P. oocarpa*.

A major result of our analyses was the unambiguous distinction of the three species, *P. muricata*, *P. radiata*, and *P. attenuata*. Despite variability within the species, all allozyme measures differentiated the species as independent taxa. Each species had unique alleles at many loci, and for many loci where alleles were common among the three species, each species had distinguishing average frequencies. Twenty-five percent of total allozyme diversity was distributed among the three species. Their average genetic distances were comparable to values reported among species of subsect. *Contortae* (Wheeler et al. 1983). Genetic distance measures indicated that each species was about equidistant from the other two, with no obvious relationships between pairs of species, in contrast to morphometric evidence (Doran 1974; Fielding 1961) and crossability (Critchfield 1967).

Allozyme evidence was equally clear in showing that, although the Californian species were distinct from each other, they formed a closely related species-cluster remote from *P. oocarpa*. Genetic distances between the Californian species and *P. oocarpa* were twice the distances among the Californian species (Nei's average distance for *P. oocarpa* and the Californian species was 0.24, compared to 0.12 among the Californian species). This evidence corroborates results of other systematists who have reported, from morphological and crossing evidence, that the Californian species are the only natural and related group of species in an otherwise heterogeneous and unnatural subsection (Critchfield 1967; Duffield 1952).

Several possible phylogenetic paths have been suggested for the Californian species, including mono- and polyphyletic origins (Axelrod 1967, 1980). Of these paths, a monophyletic origin for derived species evolving at similar rates, and having separated at similar times, is most likely to show the derived species equidistant genetically from each other, and the derived species at a greater but approximately equal genetic distance from the progenitor. There was no evidence suggesting either a polyphyletic origin for the group, or that one of the extant Californian species more closely resembles the progenitor lineage. Although our analyses included only one Latin American species, *P. oocarpa* had attributes that might be found in a progenitor lineage of the Californian species. It was highly variable; two- to threefold more variable in several measures than the Californian species. Allelic variation in the Californian species was mostly a subset of variation in *P. oocarpa*, both for alleles common to all Californian species and for alleles unique to one of the three species. Allozymic data thus corroborate a hypothesis that *P. attenuata*, *P. muricata*, and *P. radiata* evolved monophyletically from a *P. oocarpa*-like complex and diverged from both *P. oocarpa* and from each other at similar times and rates. An alternative hypothesis, that the three species diverged at different times from *P. oocarpa* but had compensating differences in their rates of evolution is possible, but less parsimonious.

There were some inconsistencies with postulating that our sample of *P. oocarpa* was progenitor-like. Several alleles in relatively high frequency in the Californian species were not found in *P. oocarpa*. *Pinus oocarpa* was fixed for *Adh2*-1.19 yet it was nearly absent in the Californian species; two *P. oocarpa* loci (*Got3*, *Lap1*), were highly variable, but lacked the *Got1*-1.00 and *Lap1*-1.00 alleles that were fixed or in high frequencies in the Californian populations. These apparent inconsistencies might stem from our limited sampling of the highly variable *P. oocarpa* and/or to an ancient time of divergence of the Californian species.

Our conclusions about the origins of the Cal-

ifornian species are in agreement with previous studies on phylogeny in this group. Axelrod's (1967, 1980) paleontological evidence and Critchfield's (1967) crossing studies elucidated the unity of the Californian species and pointed to the possibility of a Mexican origin for the group. Axelrod presented a detailed argument for *P. oocarpa* as the progenitor of this group, although he argued at different times for polyphyletic and monophyletic origins of the group (Axelrod 1967, 1980). Our evidence corroborated, with reservations noted, his conclusion that the Californian closed-cone pines derived monophyletically from a Mexican taxon, specifically *P. oocarpa*-like.

Major geographic clines of allozymic variation were noteworthy within the three Californian species. Allozyme relationships of *P. attenuata* populations were similar to latitudinal patterns observed in growth and morphological traits (Newcomb 1962). Clinal variation followed a gene flow path, from Sierra Nevada to the Klamath mountains, then southward through the North and South Coast populations. Genetic distances increased with geographic distance along this path. The populations at the ends of the species distribution were the ones most divergent (South Coast) and most ancestral (Sierran).

Pinus muricata was divided into northern and southern allozyme groups, and these two groups contained divergent populations. The distinctness of the northern cluster, including Trinidad, Mendocino, Sonoma, and Marin populations, corroborates studies on morphological and resin traits (Duffield 1951; Forde and Blight 1964; Mirov et al. 1966). The genetic barriers to hybridization of the northern and southern groups (Critchfield 1967; Millar and Critchfield 1988) suggested that these groups may be two species. Allozymically, however, both groups clustered tightly within *P. muricata*, and genetic distances among the groups were not substantially higher than among distant populations in *P. attenuata* and *P. radiata*. The northern allozymic group included the Marin population, which by some studies was put in an undetermined position intermediate between northern and southern groups (Duffield 1951). There was no evidence for prominent differentiation of the Sonoma and Mendocino populations from other *P. muricata* populations; in other traits, the Sonoma and Mendocino populations show marked racial-level variation (Duffield 1951; Millar 1983; Mirov et al. 1966). The northern group was lower in intrapopulation allozyme variability than other populations of *P. muricata* and of *P. radiata* and *P. attenuata*. This group of populations also has less variation in quantitative traits (Doran 1974; Fielding 1961; Shelbourne et al. 1982). The northern *P. muricata* populations were allozymically more divergent than the southern populations from the other two California species, a finding consistent with the occurrence of reproductive barriers between this group and southern *P. muricata* populations, and between this group and *P. radiata* and *P. attenuata* (Critchfield 1967).

The distinct southern group of *P. muricata*, including San Luis Obispo, Santa Barbara, and Santa Cruz Island populations, contained high allozyme variation within populations. These populations are highly variable in other biochemical (Mirov et al. 1966) and morphological (Doran 1974; Fielding 1961; Shelbourne et al. 1982) traits. The southern populations had alleles found in the other Californian species, and also in *P. oocarpa*, and are the only elements in *P. muricata* that cross with *P. radiata* or *P. attenuata* (Critchfield 1967).

The Santa Cruz Island population stood apart from other *P. muricata* populations allozymically, as it does in other traits, notably cone morphology (Mason 1930) and monoterpenes (Mirov et al. 1966). Several lines of evidence, including shared allozymes with *P. radiata* and *P. attenuata*, a monoterpene composition that has *P. radiata* and *P. attenuata* elements, and relative ease of crossing with *P. radiata* and *P. attenuata*, point to the Santa Cruz Island population as being a relict population, rich in variation, of an ancestral lineage common to the Californian taxa. It is unlikely that the island population was founded by a few genetically unusual individuals, but rather is a representative of an older variable taxon. The debate over the status of the Santa Cruz Island pines as either, 1) relictual remnants of an independent lineage, *P. remorata* (Mason 1930), that is hybridizing with *P. muricata* both on the islands and extensively on the mainland (Axelrod 1980, 1983; Mason 1949), or 2) a population with extreme values in the highly polymorphic *P. muricata* lineage (Duffield 1951; Linhart et al. 1967; Millar and Critchfield 1988), cannot be resolved by present evidence. Both scenarios

could lead to the clinal patterns of allele frequencies we found.

In *P. radiata*, the mainland populations were closely related to one another and the two island populations were distinct from each other and from the mainland populations. Genetic distance increased with geographic distance clinally. Isozyme data did not clearly indicate whether the Año Nuevo-Monterey or the Monterey-Cambria pair was most closely related, although by Nei's genetic distance, the Monterey-Cambria pair was closest. This conclusion independently corroborates the relationship suggested by Plessas and Strauss (1986).

Notable from our data was the close clustering of the Guadalupe and Cedros islands populations with the other *P. radiata* populations in comparisons with *P. attenuata* and *P. muricata*. Cedros Island pines were long thought to be a population of *P. muricata*, and were named *P. muricata* var. *cedrosensis* (Howell 1941). Analyses of morphology (Newcomb 1959; Axelrod 1980), monoterpenes (Bannister et al. 1962; Bannister and McDonald 1983), seed proteins (Murphy 1981), and breeding behavior (W. J. Libby, unpubl. data), however, indicate a closer relationship of Cedros Island pines to *P. radiata*; recently the island pines were renamed *P. radiata* var. *cedrosensis* (Axelrod 1983). Our analyses indicated that these pines of Cedros Island, and to a lesser extent, Guadalupe Island, are highly variable, distinct populations of *P. radiata*, which contain relictual variation in the same way the Santa Cruz Island population of *P. muricata* does. Axelrod (1980) came to the same conclusion about the relictual nature of the insular populations of *P. radiata* when he concluded, using evidence from cone morphology, that Cedros and Guadalupe islands populations most resembled a *P. oocarpa*-like progenitor.

In sum, phylogenetic evidence from allozyme data on species and population divergence indicated a monophyletic origin for the Californian closed-cone pines. The highly variable Latin American species had several attributes that suggested it is a representative of the lineage from which the Californian species diverged. Genetic evidence suggested that the Californian species radiated approximately equally from each other. The most ancestral populations of *P. attenuata* occurred in the interior mountains of the Sierra Nevada, with divergence increasing clinally northward in the interior and then southward along the coast. By contrast, in both *P. muricata* and *P. radiata*, radiation initially occurred along the coast from south to north, with the southern island populations retaining ancestral alleles, and the northern mainland populations being most divergent.

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