

CROSSABILITY AND RELATIONSHIPS OF *PINUS MURICATA* (PINACEAE)

CONSTANCE I. MILLAR¹

Wildland Resources Center, University of California,
Berkeley 94720

WILLIAM B. CRITCHFIELD

Pacific Southwest Forest and Range Experiment Station,
USDA Forest Service, Berkeley, CA 94701

ABSTRACT

Crossing relationships were studied within and among the variable populations of *Pinus muricata* to test hypotheses about crossing barriers among certain populations. Crossability was assessed at the level of viable seed production following planned crosses. Populations north of Sea Ranch, Sonoma Co., California, crossed freely with parapatric but genetically distinct populations in central Sonoma Co., although some reduction in seed-set occurred in the F_2 and backcrosses to F_1 . The distinctness of these adjacent populations is most likely not maintained by post-pollination crossing barriers. Crossability of disjunct *P. muricata* populations generally decreased with distance between populations. Populations north of Sea Ranch crossed freely with the Pt. Reyes population in Marin Co., less readily with the Monterey population, and not at all with the Purisima (southern California) or Baja California populations. Mainland and island *P. muricata* populations south of Monterey were highly interfertile. Test crosses were also attempted between *P. muricata* and the island populations of *P. radiata*, which have been considered closely related to southern *P. muricata* populations. *Pinus muricata* from Baja California did not cross, however, with either Guadalupe Island pine (*P. radiata* var. *binata*) or Cedros Island pine (*P. radiata* var. *cedrosensis*). Together with results from other crossing studies in the Californian closed-cone pines, the patterns of crossability indicate three crossing units in *P. muricata*: 1) northern *P. muricata* populations from Marin Co. northward, which are reproductively isolated from, 2) southern *P. muricata* populations including mainland and Channel Islands populations from Purisima southward, and 3) Monterey *P. muricata*, which is intermediate between the first two units.

Crossing patterns within the three Californian species of closed-cone pines (subsect. *Oocarpae*, Critchfield and Little 1966) are unusual for *Pinus*. In experimental pollinations, *Pinus radiata* D. Don and *P. attenuata* Lemmon hybridize more readily than most other combinations in the genus, whereas crosses between certain northern and southern populations of *P. muricata* D. Don do not produce viable seeds (Critchfield 1967). This is the only known instance in *Pinus* of complete infertility between populations within a species.

Pinus muricata is unique among the California closed-cone pines because of the distribution of genetic variation within and among

¹ Present address: Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, U.S.D.A. Forest Service, Box 245, Berkeley, CA 94701.

populations. The frequencies of several morphological and biochemical traits in northern populations of *P. muricata* change abruptly within continuous stands. Cone morphology in southern populations also differs markedly within and among nearby disjunct stands. Variation is especially complex in the southern and island populations where several traits intergrade between *P. muricata* and *P. radiata*.

This variation has led taxonomists since the early 1800's to apply many species and varietal names to populations and morphological types in these taxa (Millar 1986). It also has led them to explain the origins and relationships of populations with often contradictory hypotheses. Early botanists focused on, and paleontologists still rely on, seed-cone observations in making evolutionary inferences about the closed-cone pines. Observations of other traits often have led to conflicting interpretations. In this paper, we focus on crossability, a measure of genetic relatedness that estimates the potential for gene exchange among taxa. We use this measure to test several hypotheses about relationships among the populations of *P. muricata*. In particular, we consider the hypotheses that genetic distinctness among parapatric races of *P. muricata* in northern California is maintained by crossing barriers, that Monterey *P. muricata* crosses more readily with northern than with southern populations, that *P. muricata* populations at Purisima cross more readily with southern than with northern populations, and that southern *P. muricata* populations are isolated reproductively from the island populations of *P. radiata*.

VARIATION AND HYBRIDIZATION

Variation in Pinus muricata. The three Californian species of subsect. *Oocarpae* are separated from the four taxa restricted to Mexico and Central America by a 640 km gap (Critchfield and Little 1966). The northern group includes *P. attenuata*, a montane, interior element, and *P. muricata* and *P. radiata*, which are maritime/insular elements (Fig. 1; Griffin and Critchfield 1976). Whereas *P. attenuata* ranges widely in southern Oregon, California, and Baja California, *P. radiata* is limited to three mainland populations in California (Año Nuevo, Monterey, and Cambria) and two distinct island populations in Mexico (Cedros and Guadalupe Islands).

Pinus muricata comprises nine disjunct populations that extend from Trinidad in northern California to San Vicente in northern Baja California and to two of the Channel Islands, Santa Cruz and Santa Rosa (Fig. 1). Of the three closed-cone pine species, *P. muricata* has the most interpopulation variability (Fielding 1961, Doran 1974, Millar et al. 1987). Both discontinuous and clinal patterns of variation occur (Millar 1986, Millar et al. 1987). Populations north of Monterey (*P. muricata* var. *borealis*, Axelrod 1983) are distinct in growth and form from the highly variable southern populations

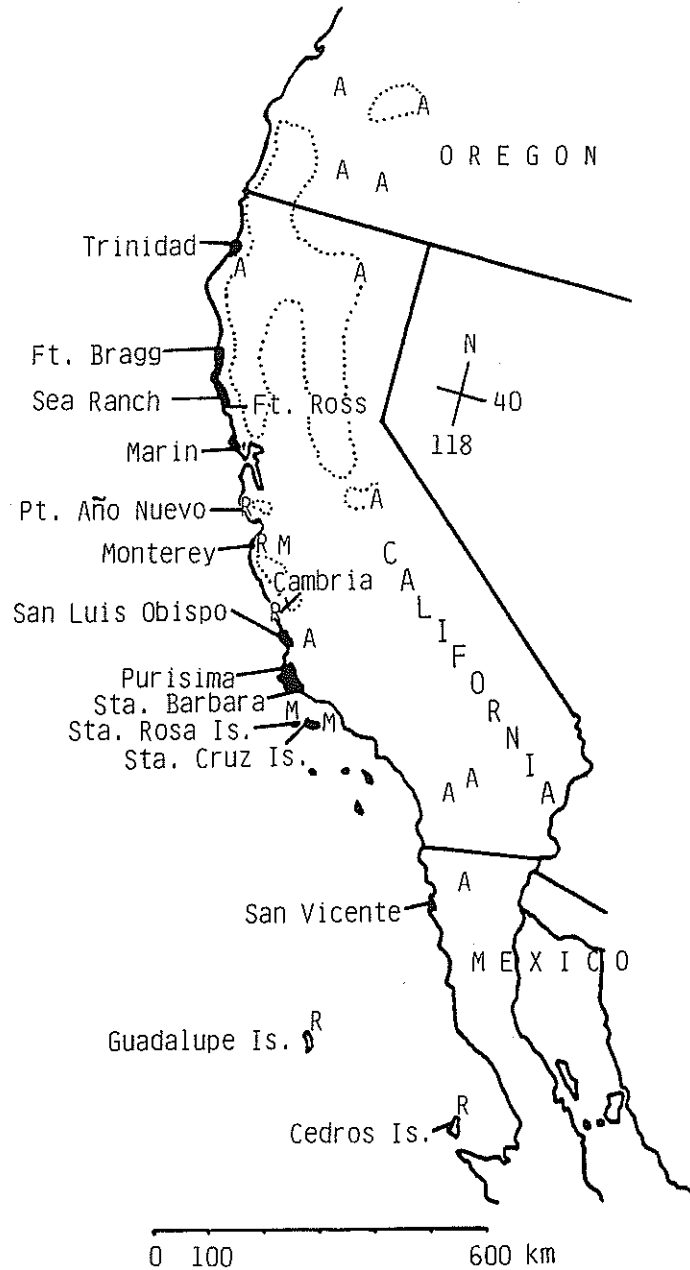


FIG. 1. Distribution of *P. muricata* (shaded areas and areas marked M), *P. radiata* (areas marked with R), and *P. attenuata* (enclosed with dotted lines and areas marked with A) in California and Baja California.

TABLE 1. VARIATION IN *Pinus muricata* POPULATIONS: STOMATAL ANATOMY, MONOTERPENE COMPOSITIONS, GROWTH AND FORM, CONE SHAPE, AND RESIN CANAL NUMBER. 'Blue = open, wax-filled epistomatal chambers, green = partly closed, waxless chambers, Duffield 1951. 'Mirov et al. 1966. 'Composite rank from common-garden studies, including height growth, stem volume, stem straightness, and bark roughness. Rank 1 = greatest height, volume, straightest stem, and roughest bark, Duffield 1951, Fielding 1961, Shelbourne 1974, Shelbourne et al. 1982. '1 = populations with mostly armed, asymmetric cones, 2 = populations with mostly smooth, symmetric cones, 3 = populations with many cone types, Mason 1930, Duffield 1951, Axelrod 1980, 1983, pers. observ. by authors. 'Unpublished data by authors except references noted. 'Averages of trees within populations with armed and unarmed cones. 'Duffield 1951. 'Linhardt et al. 1967.

Population	Stomatal type ¹	Monoterpene composition ²	Growth & form ³	Cone shape ⁴	Mean no. resin canals ⁵		
					All types	Armed cones ⁶	Unarmed cones ⁶
Trinidad, Humboldt Co.	blue	>98% α -pinene	1-2	1	2.0		
Mendocino Co.	blue	>98% α -pinene	1	1	2.3 ⁷		
Sonoma Co.	green	>80% Δ -3-carene	1	1	2.6		
Marin Co.					1.8		
Pt. Reyes Inland	green	>80% Δ -3-carene	2	3	2.1	2.2	2.0
Monterey Co.	green	>80% Δ -3-carene	3	3	2.1		
San Luis Obispo Co.	green	>75% sabinene & terpinolene	4	3	3.2	3.2	3.1
Purisima, Santa Barbara Co.	green	>75% sabinene & terpinolene	4	3, 2	4.1 ⁸		
					4.1 ⁷		
					3.9 ⁸		
Santa Cruz Island	green	>85% α -, β -pinene, sabinene, & terpinolene	5	3, 2	6.8 ⁸	7.5 ⁸	6.1 ⁸
Santa Rosa Island	green	>90% α -, β -pinene, sabinene, & terpinolene	6	2	4.4 ⁸		4.4
San Vicente, Baja CA	green	>75% sabinene & terpinolene	5	3, 2	7.0 ⁷		
					8.6 ⁸		
					7.4	8.0	6.8

(Table 1; Duffield 1951). *Pinus muricata* var. *borealis* is further divided into two discrete genetic races that are separated by a narrow contact zone at Sea Ranch, Sonoma Co., California. The races are distinguished by needle anatomy (Duffield 1951), monoterpene composition (Mirov et al. 1966), phenology, and isozymes (Table 1; Millar 1983). The northern race has foliage that appears blue due to open, wax-filled epistomatal chambers. In this paper we follow the convention of calling all populations with this stomatal type, "blue *P. muricata*". The blue populations also have a distinct terpene composition (Mirov et al. 1966). The partly closed, wax-free epistomatal chambers of the southern race of var. *borealis* and all other *P. muricata* populations southward give the foliage a yellow-green color, here called "green *P. muricata*". Although the green needle anatomy is typical of all populations south of Sea Ranch, the terpene composition found in the green populations immediately south of Sea Ranch occurs elsewhere only in the Monterey population of *P. muricata* (Mirov et al. 1966). The highly variable southern mainland populations (*P. muricata* var. *muricata*) form a third distinct terpene type, whereas the island populations form a fourth terpene type (Table 1). The island pines, variously designated as *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 also occurs in the central and southern mainland populations (Table 1).

Previous reports of hybridization. Natural hybrids between *P. muricata* and *P. radiata* have been reported at Monterey, which is the only place they are sympatric (Mason 1949, Stebbins 1950, Duffield 1951). Lack of intermediates, however, in quantitative traits (Forde 1964) and terpene compositions (Bannister et al. 1962, Forde and Blight 1964), differences in phenology (Duffield 1953, Critchfield 1967), and low crossability of *P. muricata* and *P. radiata* (Critchfield 1967) make this hybridization seem unlikely.

Brown (1966) and Critchfield (1967) studied interfertility among certain populations of closed-cone pines. Using relative numbers of viable seed produced among experimental crosses, they concluded that *P. muricata* consists of three crossing groups: 1) a northern, divergent group, that is isolated from related species and from southern *P. muricata* populations; 2) a southern group that is able to cross with related species; and 3) a central group that is intermediate in crossing relationships. It is noteworthy that all interpopulation crosses in *P. muricata* between northern blue populations and green populations south of Monterey failed. The geographically intermediate population at Monterey crossed poorly with the southern populations, and more readily with the northern blue populations. Crosses

TABLE 2. PARENT TREES OF *Pinus muricata* AND *P. radiata* CROSSES MADE IN NATIVE POPULATIONS AND IN PLANTATIONS AT THE INSTITUTE OF FOREST GENETICS AND RUSSELL RESERVATION.

Race or taxon	Geographic origin	Number of parents	
		Females	Males
1. Native populations			
Blue bishop	Mendocino Co.	—	4
	Sonoma Co.	3	2
Green bishop	Sonoma Co.	4	10
	Marin Co.	—	3
	Monterey	—	2
	Purisima	—	3
2. Plantations			
Russell Reservation (Lafayette, CA)			
Blue bishop	Mendocino Co.	8	15
Green bishop	Sonoma Co.	10	13
Institute of Forest Genetics (Placerville, CA)			
Blue bishop	Mendocino Co.	3	3
Green bishop	Monterey	1	—
	Santa Cruz Is.	1	—
	Santa Rosa Is.	—	1
	San Vicente	3	2
Blue × green bishop (F ₁)	Mendocino & Marin cos., Monterey	4	4
Monterey pine	Guadalupe Is.	—	—
	Cedros Is.	—	1

between trees from contiguous blue and green stands were not included in studies by Critchfield (1967) or Brown (1966).

All crosses attempted by Critchfield and Brown failed between blue *P. muricata* and either *P. attenuata* or *P. radiata*. By contrast, *P. muricata* from Monterey set some sound seeds in combinations with mainland *P. radiata* and *P. attenuata*, whereas Channel Islands *P. muricata* produced many sound seeds in those combinations. *Pinus muricata* from San Vicente produced moderate amounts of viable seed in combination with *P. attenuata*, but none with mainland *P. radiata*, and few with *P. radiata* from Guadalupe Island.

MATERIALS AND METHODS

Parent trees. Crosses on *P. muricata* were made during two periods and in three places (Table 2). In 1965–66 crosses were made on blue and green trees in native stands near Sea Ranch. In the same years, pollen parents from several native stands were crossed with females of different origins planted at the Institute of Forest Genetics (IFG), Placerville, California (elev. 825 m). In 1980–81 crosses were made at IFG on a single blue tree and on four blue × green hybrids. The

hybrids had originated from 1965 crosses and were planted in 1968. Although the trees grew poorly, by 1980 they had been sexually mature for several years. Crosses in 1980–81 also were made on 13- and 14-year-old trees growing in a common-garden plantation at the Russell Reservation, Univ. of California, 16 km east of Berkeley. *Pinus muricata* grows well there, and the trees had been sexually mature for several years prior to pollination.

Pollen for the 1965–66 pollinations was collected from arboretum and native-grown trees, and stored frozen for a year before use. Fresh pollen from plantation trees was used for the 1980–81 crosses.

Breeding techniques and terminology. Trees were pollinated and seeds processed using standard techniques (Cumming and Righter 1948). Seeds from 1965–66 pollinations were sorted using a Clipper mill. If less than 10 viable seeds per cone remained, they were x-rayed to determine viability. Viable seed yields from 1980–81 crosses were determined by germination of all harvested seeds. Crosses that failed to yield cones or had severely insect-damaged cones were excluded from analyses.

An **attempt** in this study refers to the pollination in a single season of a single female parent with pollen from a particular pollen source. All but one set of crosses in this study were single-pair matings with only one male parent contributing pollen. In these matings, the number of attempts for a given cross also specifies the number of male and female parents. In the single cross where a pollen mix was used (San Vicente × San Vicente, pollen mix of two males), each attempt involved three parents: one female and these two males.

Crossability refers to the ease with which two taxa can be successfully crossed, compared to control crosses within the maternal taxon. For accurate estimation of crossability, it is essential to compare between-taxon crosses to within-taxon control crosses, since the amount of viable seed naturally produced within taxa varies. Controls reported here for each cross combine results from control crosses involving the same female used in the interpopulation crosses, and control crosses on other females of the maternal taxon. We quantify crossability as the number of viable seeds per cone produced from between-taxon crosses expressed as a percent of the number of viable seeds from the within-taxon control crosses. Thus, percents less than 100 indicate fewer viable seed produced from between-taxon crosses, percents equal to 100 indicate an equal number from each type of cross, and percents greater than 100 indicate more viable seed produced from between-taxon crosses. Differences in numbers of viable seeds among crosses were tested by analysis of variance with significant differences reported for *p* values less than 0.05. Differences among crosses in germination of seeds were tested by chi-square analyses, with the same significance level.

TABLE 3. SUMMARY OF CROSSES BETWEEN BLUE AND GREEN *Pinus muricata* POPULATIONS (PARENTS FROM MENDOCINO AND SONOMA COUNTIES) MADE ON NATIVE AND PLANTATION TREES. ¹CI = 95% confidence interval, based on average results per attempt, not per cone. ²Expressed as average percent of total number of seeds/cone that germinated. ³The F₁'s in all cases are blue-female/green-male combinations.

Parents		Attempts producing sound seeds	Female strobili pollinated	Percent of strobili producing cones	Total number of seeds/cone		Number of viable seeds/cone		Germi- nable seed (%) ²
Females	Males				Mean	CI ¹	Mean	CI ¹	
I. Native populations									
Interpopulation crosses:									
Green	Blue	3	16	18.8	29.0	—	24.0	—	82.8
Blue	Green	10	101	37.4	75.1	(5.1)	64.8	(5.5)	87.2
Within-population crosses:									
Blue	Blue	6	50	60.0	61.0	(7.7)	48.3	(6.9)	81.2
II. Russell Reservation									
Interpopulation crosses:									
Green	Blue	19	68	79.4	12.9	(3.5)	5.7	(2.1)	44.8
Blue	F ₁	5	17	76.5	14.7	(10.3)	8.4	(9.1)	52.6
Green	F ₁	10	22	86.4	10.1	(3.3)	5.7	(2.5)	51.0
Within-population crosses:									
Green	Green	18	43	86.0	17.3	(5.0)	8.2	(2.5)	46.3
Blue	Blue	6	33	75.8	5.3	(0.9)	1.1	(0.5)	31.9
III. Institute of Forest Genetics									
Interpopulation crosses:									
Blue	Green	8	118	86.4	8.6	(0.9)	4.7	(0.6)	50.1
Blue	F ₁	2	15	100.0	50.8	(1.8)	41.2	(6.0)	86.9
F ₁ ³	Blue	2	4	100.0	8.5	—	2.5	—	14.7
F ₁	Green	2	7	71.4	45.6	—	3.0	—	6.6
F ₁	F ₁	5	19	78.9	18.3	(3.0)	2.2	(0.6)	9.5
Within-population crosses:									
Blue	Blue	10	178	44.9	6.7	(0.6)	4.0	(0.5)	67.2

RESULTS

Crosses of northern Pinus muricata populations. Crossability between blue and green trees from all northern sources averaged 100% (Table 3). In crosses on native trees, the average number of sound seeds from blue $\times$ green crosses was significantly higher than the blue $\times$ blue control crosses. Germination percents of green $\times$ blue and blue $\times$ green crosses did not differ significantly from the blue $\times$ blue control crosses. In crosses at Russell Reservation, the average numbers of sound seeds and germination percents from green $\times$ blue crosses did not differ significantly from the green $\times$ green control crosses. The lower seed set for the blue control crosses at Russell was probably due to premature opening of the unbagged cones, which permitted some seed to shed before harvesting. The percent germination of these crosses was only slightly lower than the other control and blue $\times$ green families. Blue $\times$ green crosses on trees at IFG produced numbers of sound seed similar to those in blue $\times$ blue control crosses.

Crosses on F_1 females produced fewer sound seeds than either the within-population crosses or crosses that used the F_1 as male (Table 3). At IFG, the average numbers of viable seeds and percent germination for crosses involving the F_1 females were significantly lower than results from the blue $\times$ blue cross. At Russell, however, results from backcrosses using the F_1 's as males did not differ significantly from the blue $\times$ blue and green $\times$ green crosses.

Viable seed from all blue $\times$ green crosses and crosses involving the F_1 's were sown in a nursery. Heights of seedlings from all crosses did not differ significantly through their second year. The only differences in survival were between crosses on F_1 females ($\bar{x}$ = 33%) and all other crosses ($\bar{x}$ > 80%).

Crosses of widespread Pinus muricata populations. Crossability generally decreased with increased distance between populations (Table 4). The blue populations north of Sea Ranch crossed easily with the Marin population (crossability > 100%); easily with the Monterey population in one direction (using a blue female, >100%), but less easily in the reciprocal cross (52%); and did not cross at all with green trees from Purisima or San Vicente (0%).

The green population in southern Sonoma Co. crossed less readily than the blue population with Monterey trees (crossability = 27%) and was nearly unsuccessful (<5%) in crosses with San Vicente trees. Marin trees followed a similar pattern: low success with Monterey (26%) and no seed from a single attempt with Santa Cruz Island (0%). Crossability between Monterey and Purisima was very low (6%).

Populations south of Monterey were highly interfertile. Crossability of the San Vicente $\times$ Purisima combination was 82%, and

TABLE 4. SUMMARY OF CROSSES AT THE INSTITUTE OF FOREST GENETICS AMONG WIDESPREAD CLOSED CONE PINE POPULATIONS. ¹CI = 95% confidence interval, based on average results per attempt, not per cone. ²Expressed as average percent of total number of seeds/cone that germinated. ³Each female pollinated with a mix of the same 2 males.

Parents		At-tempts	At-tempts pro- ducing sound seeds	Female polli- nated	Percent of strobili pro- ducing cones	Total number of seeds/cone		Number of viable seeds/cone		Germi- nable seed (%) ²
Females	Males					Mean	CI ¹	Mean	CI ¹	
I. Interpopulation crosses										
Mendocino Co.	Marin Co.	2	2	39	56.4	12.2	(1.5)	4.9	(1.1)	42.2
	Monterey	2	2	29	86.2	21.0	(1.4)	8.5	(0.1)	40.4
	Purisima	2	0	30	83.3	17.4	(0.8)	0.0	—	—
Monterey	Mendo./Son. Cos.-Blue	4	4	97	33.0	7.4	(3.3)	4.7	(2.3)	68.6
	Son. Co.-Green	4	3	74	28.4	5.2	(2.7)	2.4	(0.7)	72.6
	Marin Co.	2	2	58	74.1	6.0	(0.7)	2.3	(0.4)	38.2
	Purisima	2	2	41	60.9	5.8	(1.3)	0.5	(<0.1)	8.1
	Marin	1	1	7	42.8	9.3	—	0.0	—	—
San Vicente	Santa Rosa Island	1	1	6	16.7	15.0	—	14.0	—	93.3
	Mendocino	2	0	24	75.0	3.3	(1.4)	0.0	—	—
	Son. Co.-Green	2	1	35	45.7	0.8	(0.2)	0.1	—	10.0
	Purisima	2	2	25	72.0	11.5	(7.2)	1.8	(1.1)	15.5
	Guadalupe Island	1	0	10	10.0	4.0	—	0.0	—	—
Cedros Island	1	0	19	36.8	5.0	—	0.0	—	—	—
II. Within-population crosses										
Mendocino Co.	Mendo./Son. Cos.-Blue	10	10	178	44.9	6.7	(0.6)	4.0	(0.5)	67.2
Monterey	Monterey	1	1	32	46.8	10.0	—	9.0	—	90.0
San Vicente	San Vicente	3 ³	3 ³	27	81.5	11.0	(5.2)	2.2	(1.1)	22.0

although the control cross was lacking, the Santa Cruz $\times$ Santa Rosa combination set more seed on average than any of the controls. In two attempts to cross San Vicente *P. muricata* with Guadalupe and Cedros Island pines, none of the 39 seeds produced was viable (0%).

First-year nursery heights and survival did not differ significantly between seedlings from interpopulational crosses and control crosses.

DISCUSSION

Crossability of Pinus muricata. Crossing data support conclusions from previous studies of growth, form, and anatomical traits that *P. muricata* is a highly polymorphic species, with great intra- and interpopulation variability, especially in the southern mainland and island populations. Relationships among the populations of Californian closed-cone pines are illustrated in a crossing polygon (Fig. 2). We redefined three distinct breeding units (Critchfield 1967) within *P. muricata*: 1) northern *P. muricata* populations from Marin Co. northward; 2) southern *P. muricata* populations including mainland and Channel Islands populations from Purisima southward; and 3) Monterey *P. muricata*, which remains intermediate between the first two units. *Pinus radiata*, including Guadalupe and Cedros Island pines, and *P. attenuata* remain distinct from northern and central bishop pine, but appear weakly related to certain southern *P. muricata* populations.

The occurrence of barriers to hybridization among widespread *P. muricata* populations led Critchfield (1967) to hypothesize that the genetic differences between blue and green races in northern California also are maintained by crossing barriers. Contrary to this speculation, we found no post-pollination barriers in crosses between contiguous blue (Mendocino and northern Sonoma cos.) and northern green (central Sonoma Co.) populations. These results corroborate prior indications of natural hybridization in Sonoma Co. from terpene (Mirov et al. 1966) and isozyme evidence (Millar 1983). Natural introgression may be inhibited, however, by differences in flowering times and by lower fertility in the hybrid female strobili.

In the limited number of combinations we made, the Marin and Monterey *P. muricata* populations responded more like northern *P. muricata* than the southern populations of the species. Monterey (green) trees set only a few viable seeds in combination with Santa Cruz Island pines, but had moderate crossability in combinations with Marin and Sonoma-green populations and moderate to high crossability to Sonoma/Mendocino blue populations. The southern breeding unit defined by Critchfield now can be expanded to include Purisima.

Phylogenetic inferences. Patterns of crossability supplement previous studies of phylogenetic relationships in *P. muricata*. The ex-

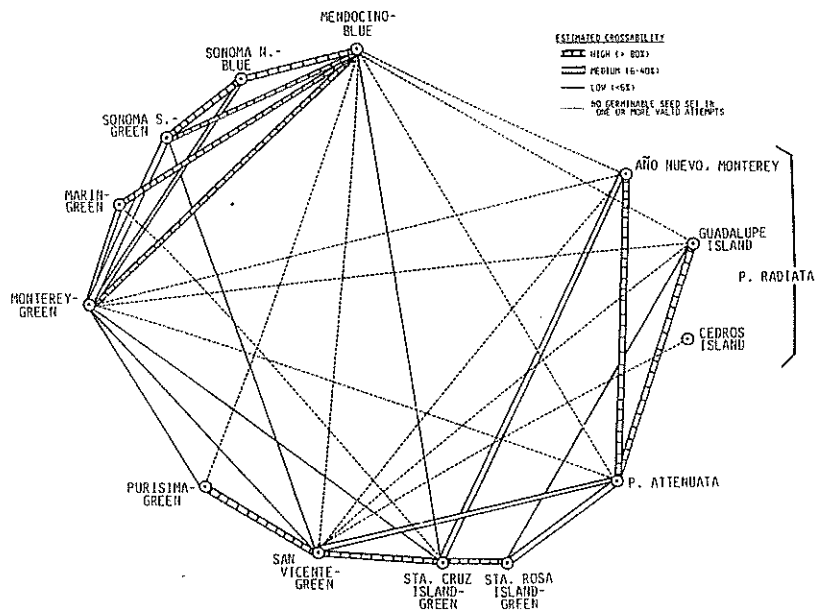


FIG. 2. Crossing relationships in the California closed-cone pines, including all available information to date. All populations not labeled with a species name are *P. muricata*.

istence and maintenance of abrupt genetic discontinuities in morphological and biochemical traits between blue and green races of *P. muricata* at Sea Ranch in northern California are difficult to interpret. Forests of *P. muricata* are continuous through a narrow (2 km) transition, and no environmental or ecological changes coincide with the discontinuity. Our crossing studies showed that the races have remained interfertile. This suggests that, despite genetic differences in several traits, the Sonoma green population and northern Mendocino populations are closely related. The races appear to have evolved recently in a mosaic pattern that did not affect interpopulation fertility except possibly at the F_2 level. Although blue and green populations can hybridize, other barriers, such as differences in phenology (Millar 1983) and different responses to soils (Millar unpubl. data), may contribute to keeping the contiguous races distinct.

Duffield's (1951) proposal that a distinct northern race of *P. muricata* exists is re-enforced by reproductive barriers between northern and southern groups. From an analysis of many traits, Duffield concluded that populations in Humboldt, Mendocino, and Sonoma cos. are a distinct variety and that Marin and Monterey are intermediate between this northern variety and the rest of the species. Axelrod

(1983) formally published the northern variety as *P. muricata* var. *borealis*. He based the description, however, only on cone shape and extended var. *borealis* south to include some "relict trees" at Monterey. Axelrod's designation leaves *P. muricata* var. *borealis* unsatisfactorily heterogeneous in other traits (Table 1), and suggests that two sympatric varieties somehow maintain their genetic integrity at Monterey. Crossing relationships show Marin and Monterey populations to be distinct and intermediate, although they have greater affinities to northern than to southern populations. We suggest that the varietal designation conservatively be limited to populations north of Marin Co.

The infertility between the northern populations of *P. muricata* (e.g., Mendocino) and *P. radiata* and *P. attenuata* suggests that var. *borealis* has diverged considerably from common ancestors of the Californian closed-cone pines, and from closely related species. In contrast, the southern populations of *P. muricata*, especially the San Vicente and Channel Islands populations, are sufficiently similar to allow successful interspecific hybridization. Apparently evolution in these taxa has not affected hybridization potential.

Evolutionary interpretations of central and southern *P. muricata* populations (summarized in Millar 1986) have also differed depending on the emphasis given cone morphology. Mason (1930, 1949) and Axelrod (1967, 1980, 1983) argue that pines having symmetric cones with smooth apophyses represent an independent evolutionary lineage (*P. remorata* Mason). Mason restricts this designation to pines with symmetric cones on the Channel Islands, whereas Axelrod uses the name for all trees with this cone type wherever they occur on the islands and mainland. Both authors suggest that the present variation in cone traits, typical of many stands south of Sonoma Co., resulted from hybridization of *P. remorata* with *P. muricata*. Other authors have concluded that cone shape is just one of many variable traits in *P. muricata* (Duffield 1951, Fielding 1961, Linhart et al. 1967, Doran 1974). In quantitative analyses, Linhart et al. (1967) found that distinct variation in resin canals, terpenes, and several needle anatomy traits did not correlate with cone variation, and concluded that *P. remorata* was "primarily a name given a particular cone type in a variable species".

We found no support from crossing studies for the hypothesis that *P. remorata* is a distinct taxon from *P. muricata*. Although most of our breeding trees were not identified individually by cone type, all our pollen and seed lots from Marin south contained trees with the smooth, symmetric cone type. Pines sampled from the Channel Islands, especially those from Santa Rosa, had high frequencies of smooth cones. We found no pattern of crossability to suggest that these trees were distinct taxonomically. The Channel Islands pines resembled southern populations of *P. muricata* in crossing behavior

among all populations tested. Furthermore, if *P. remorata* extends north to Monterey and Marin (Axelrod 1980), we would expect to find greater crossability between those populations and Channel Islands populations than was found. Observations on resin canals indicated that number of canals varied greatly among trees, and that variation was related to geographical location and not to cone type. Thus, we found no evidence that smooth, symmetric cones found on trees throughout the species are indicators of an independent lineage.

CONCLUSIONS

Crossing results reported here supplemented and corroborated other studies on *P. muricata* which indicate that complex patterns of variation exist in the species. Unique in *Pinus* is the presence of intraspecific post-pollination barriers among *P. muricata* populations. These barriers, together with distinguishing patterns of variation in other traits, suggest that the northern and southern populations have long been isolated and perhaps should be considered distinct species. By contrast, evolution of genetic differences between blue and green races within the northern populations has not been accompanied by evolution of post-reproductive barriers. Genetic differences between these races must be maintained by other factors.

Since southern *P. muricata* populations retain crossability to *P. radiata* and *P. attenuata*, the great variation in these populations may have been imported through prior interspecific hybridization. Patterns of crossability, coupled with evidence from variation in other traits, gave no evidence to suggest that the smooth, symmetric cone type alone is an indicator of a distinct evolutionary lineage within the species complex. This cone type is found in nearly all *P. muricata* populations and appears to be one of many polymorphic traits in the species.

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