Genetic Studies of Dissimilar Parapatric Populations in Northern Bishop Pine (*Pinus muricata*)

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Constance Irene Millar

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Approved: Philip T. Spieth 3/18/85
Chairman Date
William J. Libby Jr.
William B. Critchfield
Kevin Padian

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ABSTRACT

Reported here are the results of studies on the unusually abrupt genetic transition that occurs in several traits in continuous bishop pine stands of northern Sonoma County, California. The genetic nature of this anomalous cline was investigated using allozyme, common garden, crossability, and controlled-soil analyses. Data from these analyses allowed a preliminary interpretation on the evolutionary relationships of the pines of this region.

Initially, seeds were collected from 78 green foliaged trees in 8 stands south of the transition at Sea Ranch, from 27 blue, green or intermediate foliaged trees in 3 transition stands, and from 96 blue foliaged trees in 8 stands north of Sea Ranch. Allozyme frequencies in maternal trees of alleles at one locus (Got2) differed significantly between the blue population (average frequency of allele 1, .96) and green population (average frequency of allele 1, .24). The frequencies changed over 2 km in a steep cline at Sea Ranch that coincided with the transition in foliage color.

The Got2 allele frequencies in the pollen grains that effected fertilization of the analysed seeds differed significantly from maternal allele frequencies in green stands south of Sea Ranch. The frequencies differed in a pattern that suggested unidirectional gene flow via pollen from north (blue) to south (green): south of Sea Ranch, the frequency of $Got1^1$ was lower in pollen than in maternal trees. The gene flow explanation was corroborated by the north-northwestern direction of prevailing winds along the coast during the pollination season.

Furthermore, differences in phenology were found in blue and green plantation bishop pines; the pattern of differences (blue flowering first) suggested that if interpopulation pollen flow occurs, then it is more likely to occur from the blue (north) population to the green (south) population. Gene flow was detected as far as 27 km south of the cline at Sea Ranch.

A sample of seeds from this preliminary allozyme study was grown in in the nursery to provide seedlings and rooted cuttings for common garden experiments. Seedlings and rooted cuttings of the green population planted in six plantations at three native locations (north of, south of, and in the cline) grew faster in all plantations than did plants from the blue or transition populations. The interactions of population samples and plantation environments did not significantly depart from linear expectations.

On remote ridgetops south of the cline, local patches of highly podsolized soils support stunted pygmy forests that resemble those farther north near Ft. Bragg, California. Although this is far into the region of the green population, pines with blue foliage grow on some of these patches, surrounded by forests dominated by Douglas-fir (Pseudotsuga menziesii) and coast redwood (Sequoia sempervirens). Pines with green foliage grow in meadow-edge stands as close as 1 km.

For a second, intensive allozyme study, seeds were collected from 40 trees at each of 6 blue coastal stands north of Sea Ranch, from 40 trees at each of 6 green coastal stands south of Sea Ranch, and from 20 trees at each of 6 blue or green ridgetop stands south of Sea Ranch. Average overall gene diversity for these populations based on 46 loci was .0504, and the average genetic distance between blue coastal and green coastal populations was .014. Allozyme frequencies in 13 of 20 polymorphic loci differed significantly between the blue and green coastal stands. Allele frequencies in the ridgetop stands differed from the coastal stands at several loci.

Seedlings of blue and of green origin were grown in controlled soil experiments, using two native soils and one nursery soil. Blue and green seedlings differed significantly from each other in top height, root length, number of side roots, and dry root weight. A strong interaction between population and soils suggested that, especially in some root traits, blue seedlings grew better in pygmy soils than green seedlings, whereas in other soils, green seedlings outperformed blue seedlings.

To estimate crossability, crosses were made on blue, green, and blue X green plantation trees. There was no evidence for a barrier (in amount of sound-seed production) to crossing between blue and green trees from these northern populations: All blue x green crosses among trees from the two parapatric northern populations produced amounts of sound seed similar to those of within-population control crosses. Early nursery growth and survival also did not differ significantly between the within-population control families and between-population hybrid families. Crossability may, however, be lower when the F_1 (blue x green) is the female parent.

Evidence from allozyme, common garden, controlled-soil, and crossability analyses suggests that blue and green bishop pine populations evolved genetic differences while the populations were isolated from one another. Blue bishop pine may have coevolved with other plants of the pygmy-soils ecosystem, and become adapted to the extremely stressful conditions of those soils. Green bishop seems best

adapted to growth on less stressful soils, and is capable of rapid growth on fertile soils under conditions where grass competition is high. Blue and green populations most likely expanded and met at Sea Ranch recently, and the steep cline that occurs there now may lessen in time as interbreeding proceeds. On patches of pygmy soils, however, blue bishop pine seems to have an adaptive advantage over green bishop, and the genetic differences between pines on and off these soils may be maintained by selection.

In addition to studies involving the genetic and evolutionary nature of clinal variation, this dissertation also reports the results of successful rooting studies, and the inheritance of 30 polymorphic loci from 19 isozyme loci in bishop pine.

to my father

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PREFACE

This dissertation focuses on the genetic events that occur during the process of population divergence. Despite an abundance of mathematical models and theories, this is a challenging process to study empirically, due to its historic nature. Once populations are distinct genetically, it is very difficult to determine what events led to their divergence. If, on the other hand, populations differ from one another only slightly, their evolutionary fate--continued divergence or loss of differentiation--is not easily predicted.

There are several patterns of genetic variation that arise as populations evolve from one another. I have been interested in studying two of these patterns, clinal and ecotypic variation. Clinal variation occurs in a gradual manner geographically, with genetic distance proportional to geographical distance, whereas ecotypic variation occurs discontinuously in a mosaic pattern.

Often the pattern of genetic variation corresponds to changes in the environment of the populations. For example, in clinal variation, climate may change gradually or rapidly with distance, and the genetic cline responding to this is correspondingly gradual or steep. In ecotypic variation, there may be patchiness in the environment, such as soil types, that correlates to the genetic mosaic. These associations between environment and genetic variation suggest, but do not prove, that natural selection is involved in the process of population divergence. There are, however, mechanisms other than selection that give rise to similar patterns of variation. For example, hybridization between two genetically dissimilar groups can lead to clinal variation across the regions of contrast between the groups.

The main object of the present research has been to gather genetic and ecological evidence from an unusual ensemble of populations in a local pine species, with the goal of clarifying the process of evolutionary divergence and hybridization among these populations. At the same time, other questions have arisen, pertaining to phylogenetic relations among other populations of the species, and among related species. Similarly, other genetic and forestry-related aspects were addressed during the investigation; several of these are described in the dissertation.

The species under investigation is bishop pine, *Pinus muricata* D. Don, a maritime member of the subsection *Oocarpae,* the western closed cone pines. It is closely related to Monterey pine (*P. radiata*), another maritime pine growing in California and on two Mexican islands, and to knobcone pine (*P. attenuata*), a scrubby pine of Baja California, interior California, and Oregon.

Bishop pine grows in several habitats, close to the ocean and in areas where coastal fogs moderate extreme temperatures. It is a colonizing species, invading grassy openings and disturbed sites. In some parts of its range, it grows into a substantial timber tree with closed stand structure, whereas elsewhere, bishop pine occurs as a member of the coastal sage and chaparral communities, where individual pines are scrubby and solitary.

Bishop pine ranges in 10 discontinuous populations from Trinidad in northern California to the vicinity of San Vicente in Baja California. It is very diverse genetically, and several subspecies and varieties have been proposed. The southern populations converge in several traits with insular members of related species, a situation that has resisted satisfactory taxonomic classification.

The bishop pine populations of primary concern in this dissertation are in northern California, consisting of stands that occur from Ft. Ross in Sonoma County to Ft. Bragg, 200 km north, in Mendocino County, and extending less than ten kilometers from the ocean. The pine stands in this region compose the largest continuous group in the species.

About 30 km north of Ft. Ross, near the south end of Sea Ranch, an unusual transition occurs in several traits between pines north and south of this region. One of the changes is a difference in foliage color. In all populations north of Sea Ranch, bishop pines typically have "blue" foliage; at Sea Ranch, there is a rapid transition to the "green" foliage typical of all populations to the south. The color difference is related to qualitative differences in the anatomy and waxiness of the epidermal cells surrounding the epistomatal chambers. The transition between foliage types occurs in a single area of only two kilometers length.

Geographically coincidental to the change in foliage color is a large change in the composition of monoterpenes in pines of this area. North of Sea Ranch, alpha-pinene is the dominant terpene; at Sea Ranch alpha-pinene decreases abruptly in frequency, and frequencies of delta-3-carene increase to over 90%. This terpene remains in high frequencies in bishop pine populations south of Sea Ranch and through central California. Intermediates in both foliage color and terpene composition occur in the short transition area at Sea Ranch, forming patterns of clinal variation in both traits.

There were no obvious explanations for the stepped clines that occur at Sea Ranch. The environment from Ft. Ross to Ft. Bragg is very uniform, and no major differences north and south of Sea Ranch were known that might have contributed to the evolution or maintenance of this abrupt transition. The only clue came from crossing work done by Dr. W.B. Critchfield of the Institute of Forest Genetics in the mid 1960's. Critchfield was unable to successfully cross, in controlled pollinations, blue bishop pines from Mendocino with green bishop pines from central and southern California and from Mexico. The presence of an intra-specific reproductive barrier suggested that the clines at Sea Ranch might be maintained by crossing barriers.

The five chapters composing this dissertation discuss various aspects of this problem on the origin and evolution of abrupt geographic differences between blue and green bishop pine populations. The chapters are written as independent units; thus, there is overlap in several cases in material presented. The dissertation begins with two technical chapters, the first on rooting of bishop pine. This chapter addresses the nursery potential for vegetatively propagating bishop pine, and discusses differences in rooting between the populations, time of year for setting, maturation effects and clonal repeatability. The second chapter describes inheritance of 46 isozyme loci that were used in studies of population variation.

The following three chapters focus on phylogenetic and population genetic issues in the species, especially concerning the blue/green transition at Sea Ranch. Chapter three describes results from crossing studies performed at two different times, one in the late 1960's, by Dr. W.B. Critchfield, and the latter by the author in the early 1980's. All data from the former study were supplied by Dr. Critchfield, and are presented here formally for the first time. The crosses involved combinations between trees from adjacent blue and green populations in northern California, and also wide crosses between northern blue trees and central and southern green trees.

Chapters four and five describe intensive studies of variation in blue and green populations from Ft. Ross to Ft. Bragg. Chapter four presents initial data from an allozyme study of this region; chapter five presents results from an intensive study of allozyme variation. Common garden studies, and soil experiments are also summarized in chapter five. This final chapter also discusses the discovery of ecotypic differences between blue and green bishop pines growing on and off the highly podsolized soils of the pygmy forests. A tentative explanation for the origin and evolution of the steep clines and ecotypic variation in northern populations of bishop pine, drawing on all available lines of evidence, is outlined in the discussion of the fifth chapter.

CHAPTER 1

Rooting Bishop Pine (*Pinus muricata* D. Don) Cuttings

ABSTRACT. Presented here are results of four years of rooting studies using hedges established from juvenile seedlings of blue- and green-foliaged bishop pine populations from Mendocino and Sonoma Counties, California. Rootability, averaged over all clones and all setting dates, was 88%. The average time for 50% of the cuttings to root was 6 months. Regardless of the time of year when cuttings were set, all cuttings began to root rapidly in late winter/early spring. The time of year when cuttings were set determined how soon they began a phase of rapid rooting, with cuttings set in late winter and early spring beginning faster than other setting dates. The period of rapid rooting lasted 2-3 months until mid/late summer, beyond which time rooting was very slow. The major maturation effect was between cuttings taken from juvenile seedlings and cuttings taken from hedges; cuttings from seedlings rooted faster than cuttings taken from hedges. Within the group of cuttings taken from hedges, there was a weak and inconsistent trend of reduction in rooting as hedges matured. Population differences in rooting of blue-, green-, and transition-origin hedges were not significant; family differences in rooting were similarly inconsequential. Differences in 8-month rooting among 14 clones, however, were large and significant, and analyses of these clones in two experiments indicated that rooting was highly heritable. Overall, bishop pine promises to be a species that is possible to propagate clonally.

INTRODUCTION

Although Monterey pine (*Pinus radiata*) is now the primary plantation conifer in New Zealand and Australia, foresters in these countries have sought alternative species for sites where Monterey pine grows poorly. The three northern populations of bishop pine (*Pinus muricata* D. Don) from Sonoma, Mendocino, and Humboldt Counties, California, have proven in several different trials to be promising replacements on high elevation and infertile sites that are too cold or sterile for Monterey pine (Doran 1974; Shelbourne 1974; Shelbourne, Bannister and Wilcox 1983). This result was somewhat surprising, since earlier trials with bishop pine, a highly variable California maritime species, had shown the species to be inferior to Monterey pine. Those earlier trials, however, used provenances from the central and southern parts of the species range, and only in the last decade have trees from the northern populations been studied systematically.

The bishop pines of Sonoma and Mendocino Counties grow in nearly continuous stands for about 180 km between Ft. Ross and Ft. Bragg. An unusual division occurs in the northern populations. Several morphological, phenological, and biochemical characteristics change abruptly at Sea Ranch, Sonoma Co., 27 km north of the southernmost extent of this population (Fig. 1) (Duffield 1951; Mirov et al. 1966; Millar 1983). Most notable is the change in foliage color, which is related to differences in stomatal morphology. Over a distance of 2-3 km, trees with "green" foliage, typical of all the central and southern populations, replace trees with "blue" foliage, typical of the northern populations.

The plantation trials in New Zealand cited above tested population samples from the green stands of the Sonoma population, and blue stands of Mendocino and Humboldt County populations as well as samples from other central and southern populations of green bishop pine. They demonstrated that, although growth among the northern population samples differed, these three northern populations were the most promising sources of bishop pine for New Zealand and Australia.

If the northern populations of bishop pine are to be used more commonly in the future, information will be needed on nursery practices for the species. Nursery characteristics relating to seedling establishment resemble those for Monterey pine, except that bishop pine has 3-1/2 times as many seeds per kilogram (Schopmeyer 1974) and its seedlings usually grow much slower than those of Monterey pine in the first two years (Shelbourne 1974). The techniques of vegetative propagation for bishop pine, however, are not well known. Many of the same reasons that make clonal propagation so promising for Monterey pine forestry (Fielding 1964, 1970; Thulin and Faulds 1968; Libby, Brown and Fielding 1972) apply to bishop pine, and create a demand for practical knowledge on rooting techniques in this species.

Over the past six years, I have been studying the blue and northern green populations of bishop pine, using rooted cuttings from hedges established in 1980. I report here the effects on rooting bishop pine cuttings of varying the time of year of collection; of maturation; of population, family and clone; and heritability of rooting.

MATERIALS AND METHODS

Open-pollinated seeds were collected in 1978 from three regions of the two native bishop pine populations in Sonoma and Mendocino Counties: 1) the green population in Sonoma Co. (8 stands), 2) the blue populationa north of Sea Ranch in Sonoma and Mendocino Counties (8 stands), and 3) the transition stands at Sea Ranch (3 stands) (Fig. 1). Seeds were germinated in early summer 1979 and grown in leach tubes containing a light potting soil in a Berkeley greenhouse. In February 1980, 20 seedlings from each population were transplanted into the same soil mixture in 15 cm clay pots, fertilized biweekly (10N-8P-7K plus micronutrients) and grown in a lathhouse. These seedlings were to be hedged at 0.2-0.3 m and kept as cutting donors for rooting studies.

In April 1980, the first cuttings were taken from the terminal and first-order branches of these seedlings and hedging was thus begun. These and all subsequent cuttings were treated according to the following standard procedure: immediately after collection, the 8-10 cm cuttings were soaked in a benomyl solution (150 ppm in water) for 30 minutes, then were basally trimmed to 6-8 cm, the basal 2 cm were dipped in an indole-butyric-acid solution (4000 ppm in 95% ethanol) for five seconds, and the cuttings were stuck in a rooting medium in opaque plastic leach-tubes (25 cm^3). The bottom two-thirds of the tubes were filled with the same standard light potting-soil (1 peat: 1 sand: 1 redwood soil conditioner, plus micro-nutrients) and topped with our standard rooting medium (1 peat: 1 N-charged redwood sawdust: 1 oak-mold). The tubes were put on greenhouse benches under intermittent mist and 16-hour daylight. Bench temperature was not controlled, although summer temperatures in the greenhouse were modified by whitewashing the windows. Cuttings were fertilized weekly with a 3N-10P-3K solution.

The cuttings were considered to have rooted when roots emerged out the bottom of the leach tubes. The experiments were continued until all the cuttings were either rooted or dead.

After the April 1980 set, cuttings were taken from the hedges periodically, on 27 August 1980, 23 February 1981, 1 September 1981, 25 June 1982, 8 September 1982 (green cuttings only), and 7 November 1983. To increase the numbers of cuttings per clone, several hedges were replicated, using cuttings from the original hedges as the new donors.

The number of cuttings per hedge varied. Only 2-3 cuttings per hedge could be taken initially; several years later, 20 or more cuttings could be taken at a time, although the number varied by hedge.

RESULTS

Overall Rootability. Shoot cuttings of juvenile and hedged-juvenile bishop pine rooted rapidly. The average time until 50% of the cuttings was rooted was six months, and the average final rooting percent for cuttings from all clones and all setting dates was 88%. The root systems formed were multiply branched and generally were symmetric, and unrooted cuttings remained healthy and free of damaging infection for 12-16 months on the mist benches.

Time of Year and Maturation. The rooting curves for the different setting dates differed significantly (by chi-square analyses) in both rooting percents at 6 and 12 months and rates of rooting, as measured by the time to 50% rooting (Fig. 2). Time of year and maturation state of the cutting donors affected rooting and confounded the interpretation of the differences.

The overall shape of the rooting curves was similar for all setting dates (Fig.2). There was an initial period of varying duration when rooting was slow, then a 2-3 month period when rooting increased rapidly, followed by a final phase of slow rooting. The inflection points, where rooting began to increase rapidly, occurred at about the same time of year for all seven experiments. Those points were in late winter and early spring, generally regardless of the time of year that the cuttings were collected.

The duration of the initial period of slow rooting was related to the length of time between the setting date and early spring. Generally, cuttings set in summer (25 June 1982) and early fall (1 September 1981 and 1 September 1982) had a longer initial period of low rooting than cuttings set in late fall (7 November 1983), which in turn had a longer initial period than cuttings set in late winter (23 February 1981) and spring (14 April 1980). One curve, 27 August 1980, was exceptional in having a very short time before rapid rooting began.

The deflection points in the curves, where the rate of rooting once again decreased, also came at the same time of the year for all the experiments, in mid-late summer. Beyond this time, rooting was very slow and most of the mortality happened during this final period.

The shape of the rooting curves of Figure 2 was related to the type of cutting donor. Cuttings taken from juvenile seedlings, set on 14 April 1980, rooted rapidly and had low mortality. By contrast, cuttings taken from hedges, which included all other setting dates, rooted slower and most had higher mortality. Within the group that derived from hedges was a weak and inconsistent trend showing an effect of maturation. With several exceptions, notably the cuttings set on 7 November 1983, the cuttings taken when the hedges were chronologically younger rooted better, at six months after setting, and rooted faster, as measured by the time to 50% rooting, than cuttings taken when the hedges were older. The relative juvenility of the cuttings taken on 27 August 1980 may explain their early rapid rooting despite being taken at a time of year when early rapid rooting was not expected.

Population, Family and Clonal Differences. To investigate population, family and clonal variation, I analyzed percent rooting for those two experiments with over 400 cuttings (1 September 1981 and 25 June 1982), using chi-square analyses. Mean rooting percents at 12 months for the September 1981 experiments were 64.9% (blue population), 78.3% (green) and 79.4% (transition); for the June 1982 experiment, the 12-month rooting percents were 77.2% (green population), 89.1% (transition) and 93.0% (blue). The means within each experiment were not significantly different, nor were the population rankings consistent between the two experiments.

Despite the lack of statistical significance of these means, rooting curves for each population for the two experiments (Figures 3 and 4) suggest possible differences between populations. For the September 1981 experiment, throughout the 12-month rooting period, the blue population rooted consistently slowest and lowest of the three populations, and the transition population rooted fastest and highest. The same trend occurred in the late June 1982 experiment in the first 8-1/2 months. After this, the rooting rates of the blue and transition populations increased while that of the green population remained approximately constant.

The means for 12-month rooting-percent of families within populations did not differ significantly. Since not all families had large numbers of cuttings in both experiments, I could not effectively compare the relative ranks of families in the two experiments.

In contrast to the population and family levels, both the rate of rooting and the final rooting percent varied tremendously among clones. To determine whether rooting differences had a heritable basis, I graphed my data following Duffield and Liddicoet's (1949) approach, since my data were not adequate for a regression analysis. The September 1981 and September 1982 experiments were used, as these had large enough numbers of cuttings of the same 14 clones in both experiments, and as the time of year when the cuttings were collected was nearly identical. Fourteen clones were used with 10-20 cuttings per clone for each experiment. Since the September 1982 experiment included only cuttings from the green population, I could only compare green clones from the September 1981 cuttings. The clones were grouped into five classes based on their rooting percent at eight months (0-0.10, 0.11-0.20, 0.21-0.30, 0.31-0.40, 0.41-0.50) in the 1981 experiment. Means were computed for each class for each experiment, and graphed, using 1982 means at 8 months as the dependent variable (Fig. 5). The straight line indicates that clonal rooting-percent was highly heritable for the two years. In other words, clones with low 8-month rooting in 1981 were low in 1982, and those high in 1981 were high in 1982.

Variation in Rooting Duration Within Clones. For both the September 1981 and June 1982 experiments, I analyzed the time it took for all live cuttings from 15 clones to root (5 clones per green, blue and transition population; each clone had 10-20 cuttings). The clonal mean times varied from 204 to 348 days for the 1 September 1981 experiment, and from 210 to 337 days for the 25 June 1982 experiment, with significant differences between the clones within each experiment. The standard deviations, however, which varied form 20 to 106 days (average = 60.1

days) indicated that the time it took all live cuttings within a clone to root varied considerably. The actual rooting period within clones, from first rooted to last rooted, ranged from four to nine months.

DISCUSSION AND CONCLUSIONS

The apparent superiority of trees from the three northern populations of bishop pine when grown on certain sites in New Zealand, Australia, and elsewhere make them logical alternatives to Monterey pine on such sites. Bishop pine's potential as an important forestry species depends, among other things, on the ease with which it can be grown in the nursery. Prior studies have shown that nursery practices for growing bishop pine seedlings are similar to Monterey pine.

The results presented here indicate that vegetative propagation by rooting shoot-cuttings from hedges is feasible for bishop pine. Overall rooting percents were generally higher for bishop pine than those reported for juvenile Monterey pine (Libby 1964; Puffer 1971; Libby, Brown and Fielding 1972; Barr 1973; Puffer and Maire 1974). The relatively simple nursery procedures used in this study led to rooting percents over 70% even when cuttings were rooted during the worst time of year. After only two years, potted hedges of bishop pine regularly produced over 20 cutting-sized shoots. No harmful fungal infections occurred up to 16 months on the mist bench, probably as a result of the benomyl fungicide. No topophytic problems resulted during subsequent growth of the rooted cuttings, regardless of the position on the hedge from which cuttings were taken. Ten months after the cuttings were set, the rooted cuttings had full, symmetric root systems and were ready for outplanting. Many of the cuttings rooted in this study were outplanted to native bishop pine sites in spring 1982 and as of spring 1984, over 85% had survived.

Many factors can influence the final rooting percent and the rate of rooting of cuttings. The time of year when cuttings begin to root rapidly followed a consistent pattern for all but one experiment. Regardless of the setting date, cuttings began to root rapidly in late winter/early spring, continued at a high rate through spring and early summer, and slowed down in mid/late summer. In general, cuttings set in late winter and early spring began the period of rapid rooting sooner than cuttings set in late summer or fall. The variation among experiments in the exact date when the cuttings began rapid rooting probably resulted from annual seasonal differences in the rooting environment. In a year that had a longer winter than average (1981), cuttings did not begin to root rapidly until mid-spring, which was later than in other experiments.

These results compare with the general pattern in Monterey pine. In early studies, when cuttings were not soaked in a fungicide prior to setting, Monterey pine cuttings rooted best when collected from dormant trees in mid-winter just prior to the spring flush (Libby 1964; Puffer 1971; Barr 1973; Cameron and Rook 1974; Puffer and Maire 1974). Cuttings set in spring died of fungus infections. In more recent rooting tests, when cuttings were soaked in fungicide, Monterey pine cuttings rooted most rapidly when collected during spring (Libby, pers. comm.). At that time, trees usually have high foliar nitrogen concentrations, a condition that Fielding (1954) suggested might improve rootability.

The major effect of maturation on rooting was related to the type of cutting donor. Cuttings from juvenile seedlings rooted better than those from hedges. This pattern was also found in Monterey pine, where hedges matured in their first few years (Libby pers. comm.). In the present study, within the group of cuttings taken from hedges, rooting did not seem to be strongly associated with hedge age. In a similar experiment with Monterey pine, Libby, Brown and Fielding (1972) found the same pattern, namely that rooting fluctuated significantly from year to year, and was only weakly associated with chronological age of the hedges. Other effects, such as annual weather conditions and vigor of the hedges may affect rooting in a particular year.

Population and family differences in rooting were not large and thus will little affect the choice of plantation stock should rooting be used. Cuttings from the blue population did root somewhat slower than the green and transition populations, which may reflect genetic differences in rootability between the populations, as in Monterey pine (Libby, Brown and Fielding 1972). Operationally, however, the three populations can be treated as one.

By contrast, clonal differences in rooting were significant and appeared to be heritable. High heritability in rooting has been found for other species (Duffield and Liddicoet 1949; Fielding 1954; Nienstaedt et al. 1958) and in bishop pine, at 12-months clones varied from 43-100% cumulative rooting (for clones with more than 5 cuttings per experiment). Other factors for the variation in rooting among clones should be considered, however, such as health differences among the individual hedges.

To take advantage of the many benefits of clonal forestry, it is desirable to maintain rooting consistency of cuttings within clones, so that within-clone variation of rooted-cuttings for outplanting will be as low as possible. The average duration of rooting within a particular clone of bishop pine was long. Although there were peaks for most clones, rooting was often scattered throughout the rooting period. Possible causes for this variation include differences in size or nutrient status of individual cuttings, position of cuttings on the hedges, and variation in conditions on the rooting bench. As we become more experienced in pine rooting, the relative importance of these (and other) factors should be clarified, and techniques can be used that minimize the duration of rooting within clones.

Overall, bishop pine promises to be a species readily propagated by rooted cuttings. This potential should allow efficient testing of this species in plantation trials, and eventually lead to mass propagation for reforestation on sites where bishop pine is superior.

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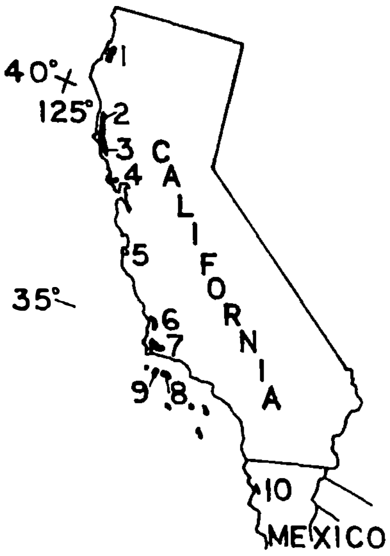
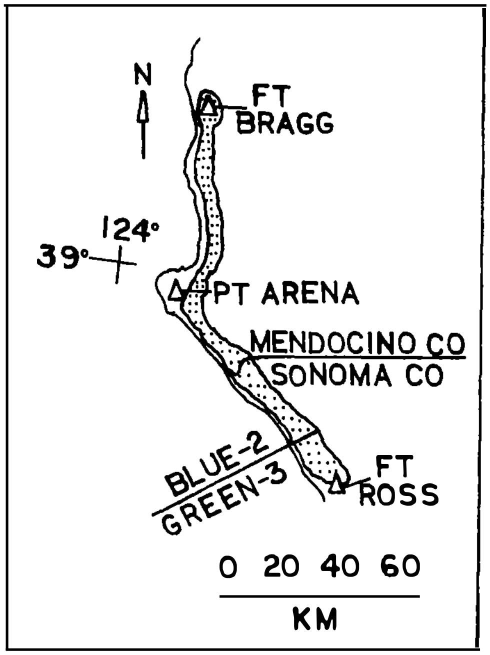
FIGURE 1. Distribution of bishop pine in California and Mexico, and detailed distribution of the northern populations of bishop pine in Sonoma and Mendocino Counties (inset).





LEGEND

1 TRINIDAD (BLUE)
2 MENDOCINO (BLUE)
3 SONOMA (GREEN)
4 MARIN (GREEN)
5 MONTEREY (GREEN)
6 SAN LUIS OBISPO (GREEN)
7 SANTA BARBARA (GREEN)
8 SANTA CRUZ IS (GREEN)
9 SANTA ROSA IS (GREEN)
10 SAN VICENTE (GREEN)

FIGURE 2. 12-month cumulative rooting curves of bishop pine cuttings set on 7 different dates. Sample sizes and mortality given for each experiment.

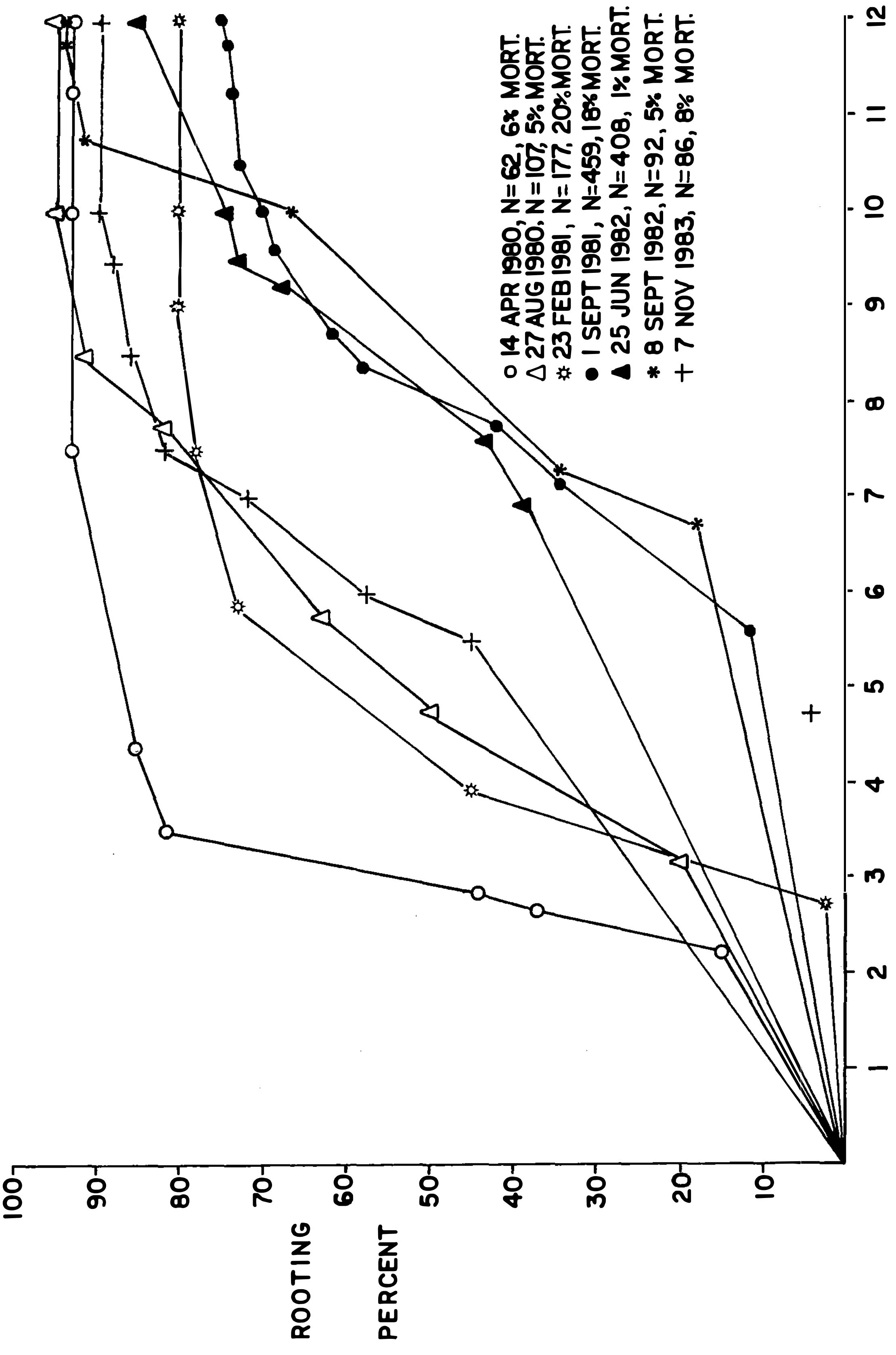

ROOTING
PERCENT
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
MONTHS SINCE CUTTINGS WERE SET
○ 14 APR 1980, N=62, 6% MORT.
△ 27 AUG 1980, N=107, 5% MORT.
✲ 23 FEB 1981, N=177, 20% MORT.
● 1 SEPT 1981, N=459, 18% MORT.
▲ 25 JUN 1982, N=408, 1% MORT.
* 8 SEPT 1982, N=92, 5% MORT.
\+ 7 NOV 1983, N=86, 8% MORT.

FIGURE 3. 12-month cumulative rooting curves of bishop pine cuttings by population for the blue, green, and transition populations. Cuttings set 1 September 1981.

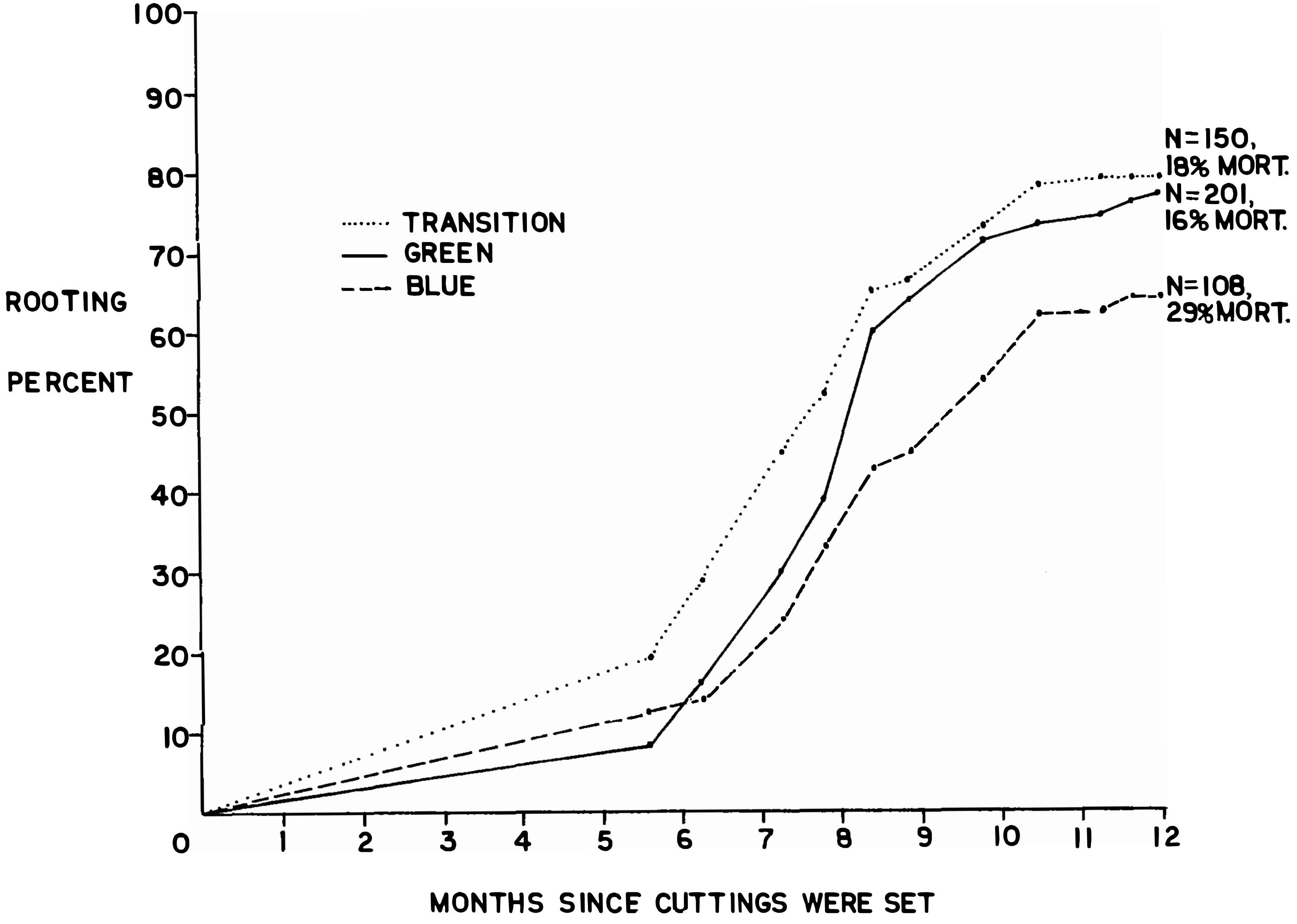

ROOTING
PERCENT
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
MONTHS SINCE CUTTINGS WERE SET
TRANSITION
GREEN
BLUE
N=150,
18% MORT.
N=201,
16% MORT.
N=108,
29% MORT.

FIGURE 4. 12-month cumulative rooting curves of bishop pine cuttings by population for the blue, green, and transition populations. Cuttings set 25 June 1982.

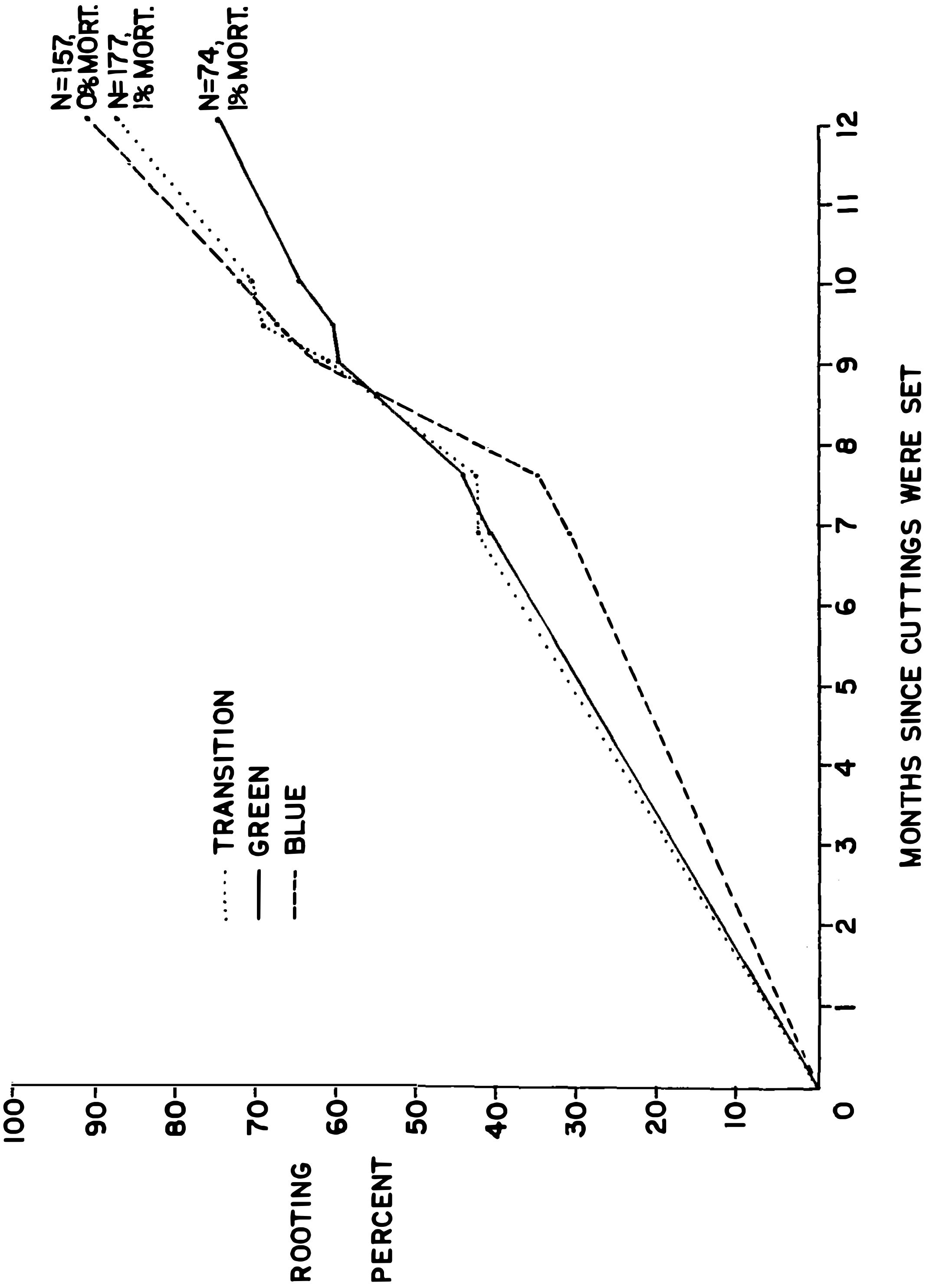
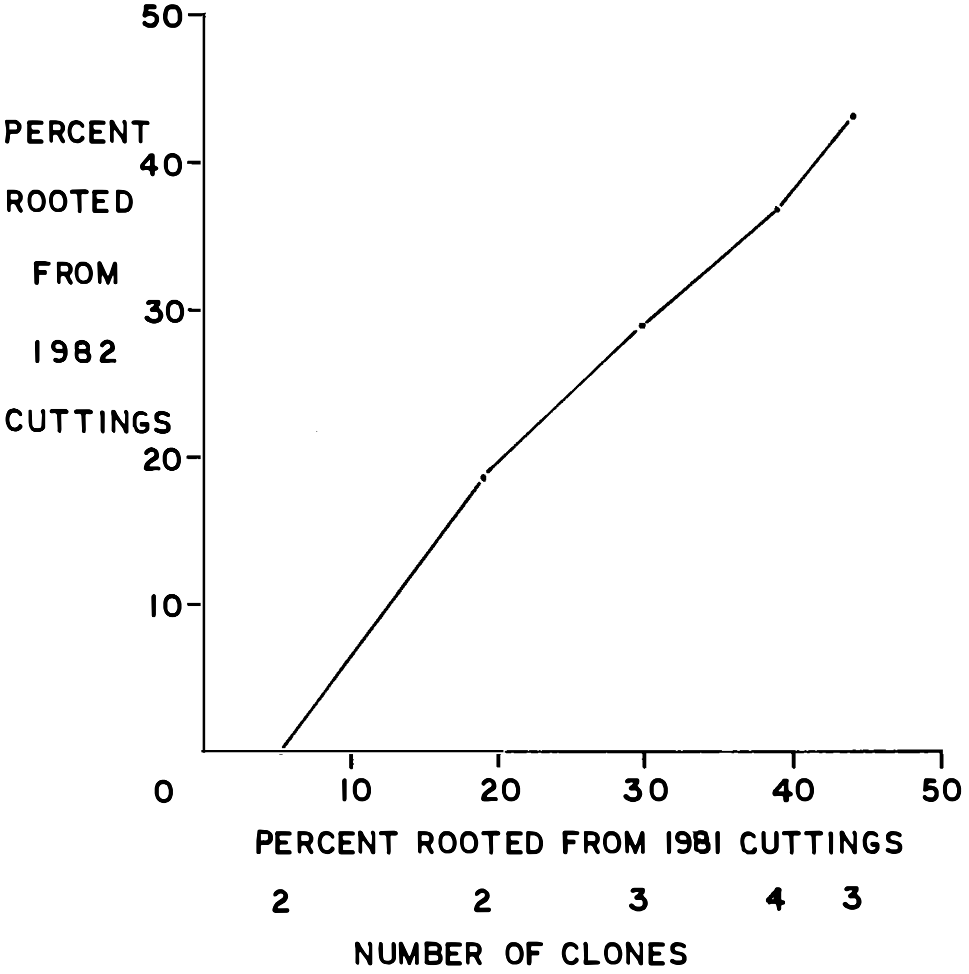

ROOTING
PERCENT
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
MONTHS SINCE CUTTINGS WERE SET
TRANSITION
GREEN
BLUE
N=157, 0% MORT.
N=177, 1% MORT.
N=74, 1% MORT.

FIGURE 5. Relation of September 1982-collected to September 1981-collected rooting of cuttings from 14 clones of bishop pine. Data grouped by 1981-outcome classes.


50
40
30
20
10
PERCENT ROOTED FROM 1982 CUTTINGS
0
10
20
30
40
50
PERCENT ROOTED FROM 1981 CUTTINGS
2
2
3
4
3
NUMBER OF CLONES

CHAPTER 2

Inheritance of Allozyme Variants in Bishop Pine (*Pinus muricata* D. Don)

Abstract. Isozyme phenotypes are described for 45 structural loci in bishop pine (*Pinus muricata* D. Don), and segregation data are presented for 30 polymorphic loci from 19 enzyme systems. All polymorphic loci had alleles that segregated within single-locus Mendelian expectations, although one pair of alleles at each of three loci showed significant segregation distortion. Other alleles at these loci segregated 1:1. In addition to the structural loci, a polymorphic non-structural locus of malate dehydrogenase is described. This locus modified the mobility of one of the isozyme loci and segregated 1:1.

INTRODUCTION

The analysis of allozyme variation allows biologists to rapidly and extensively examine genetic variation in wild and domesticated populations of many species. In the past decade, forest geneticists have routinely applied this technique to clarify problems of basic biological and applied nature, such as estimating genic diversity and genetic architecture within and among species (Brown and Moran 1981; Guries and Ledig 1981; Yeh 1981; Wheeler and Guries 1982), clarifying hybrid relationships (Copes and Beckwith 1977), estimating mating system and gene flow parameters (Brown et al. 1975; Mitton et al. 1977; El-Kassaby et al, 1981; Shaw and Allard 1981; Millar 1983), distinguishing among seed-orchard clones (Copes 1975; Adams and Joly 1980a), and evaluating relative heterozygosity of trees in seed orchards and wild stands (Rudin and Lindgren 1977; Adams and Joly 1980a; Moran, Bell and Matheson 1980).

The successful application of electrophoretic techniques for any of these purposes requires an understanding of the inheritance of each locus used, and benefits from using as many loci as possible. Early reports of allozyme inheritance in conifers described only a few loci (eg, Conkle 1971; Lundkvist 1975; Rudin 1975), whereas several recent studies reported inheritance at many loci (Guries and Ledig 1978; O'Malley, Allendorf and Blake 1979; Adams and Joly 1980b; El-Kassaby, Yeh and Sziklai 1982; King and Dancik 1983; Cheliak and Pitel 1984).

Taken together, these studies reveal many similarities and differences among species. Some of the differences between species include: different numbers of loci (isozymes) between species for the same enzymes (Adam and Joly 1980b; El-Kassaby 1981), different band phenotypes for similar loci in different species (eg, single vs double bands, different stain intensities) (Adams and Joly 1980b; El-Kassaby, Yeh and Sziklai 1982), variable appearance among species of heterodimeric bands caused by interaction of products at two loci (El-Kassaby 1981), and variable expression among species of post-translational modification for some loci (Harry 1983). Because of these and other differences among species, and because incorrect interpretation may lead to erroneous conclusions (El-Kassaby 1981; Harry 1983), it is important to document inheritance for those loci in a species whose allozyme variation is contemplated for analyses such as those described above.

Because the haploid, multicellular megagametophyte of conifers is a large organ that gives rise to an egg of identical genotype, it not only can be easily electrophoresed, but allozyme inheritance can be studied without making control crosses. The data are obtained more efficiently and are more clearly interpretable than similar data from other organisms. First, segregation between different megagametophytes from putative heterozygotes is analyzed, and then observed frequencies of bands are compared with expected Mendelian frequencies (1:1). When embryos and megagametophytes are electrophoresed side by side, the bands from the two tissues can be compared and the structure of the enzyme (monomeric or dimeric) can be deduced. These analyses of specific embryo-megagametophyte combinations lead to unambiguous determination of maternal and paternal contributions to each embryo, which is particularly useful for assessing gene flow, pollen contamination, and mating systems.

This paper reports an analysis of inheritance using megagametophyte segregation in bishop pine (Pinus muricata D. Don) of 30 polymorphic isozyme loci and one non-structural locus from 19 enzyme systems. It further describes 15 monomorphic, putative loci from three additional enzyme systems. Bishop pine (subsection Oocarpae, Critchfield and Little 1966) is an island and maritime species distributed in 10 disjunct populations in California and Baja California. An analysis of allozyme variation at one locus has already clarified problems of population architecture in bishop pine (Millar 1983). Further applied and basic research might benefit by using the many isozyme loci that resolve in this species.

MATERIALS AND METHODS

Wind-pollinated cones were collected from 6 stands (40 trees each) of the northern "green" population of bishop pine (see Millar 1983 for explanation of racial types) between Ft. Ross and Sea Ranch, Sonoma Co., California; 6 stands (40 trees each) of the northern "blue" population between Sea Ranch and Ft. Bragg, Sonoma and Mendocino Cos., California; and 6 mixed stands (20 trees each) growing on podsolized soils of the pygmy forests, Sonoma Co., California, making a total of 600 trees. Extracted seeds, identified by wind-pollinated parents, were cleaned, sorted, and stored in sealed plastic bags at -4° C. Seeds were prepared for electrophoresis by placing them on moistened filter paper in petri dishes and refrigerating at 5° C overnight. The petri dishes were then moved to an incubator (22° C) where the seeds were left to germinate until the radicles emerged about 1 cm from the seed coat.

Dissected megagametophytes and embryos were electrophoresed following the techniques and using the gel systems of Conkle et al. (1982). Briefly, I used horizontal 12.5% starch gels at 3 different pH's (gel system A--tris citrate, pH 8.3; gel system B--tris citrate, pH 8.8; and gel system D--morpholine citrate, pH 6.1). Twenty-two enzyme systems stained consistently enough to be included in this study (Table 1). Some stain recipes and electrophoretic procedures differed from ora were additional to those reported by Conkle et al. (1982). These modifications are given in Table 2.

I initially electrophoresed 5 megagametophytes and 1 embryo from each of the 600 trees, and thus identified trees that were putative heterozygotes for each putative locus. For the rest of this paper, I refer to putative loci and putative heterozygotes simply as loci and heterozygotes, and make note of all cases where analysis either does not confirm this designation or is insufficient to determine inheritance.

Assuming 1:1 segregation at heterozygous loci, by sampling megagametophytes as described, it is expected that 6% of the heterozygotes will be missed in such a survey. If, however, severe segregation distortion exists for certain loci, it is more likely to miss heterozygotes of such loci.

For each polymorphic locus, I pooled segregation data from all heterozygotes identified. When there were at least 10 heterozygous trees for each locus (ie, at least 50 megagametophytes per locus), I calculated chi-square values testing the expected 1:1 ratios on the pooled data, and the heterogeneity chi-square values among trees. For loci with fewer than 10 heterozygous trees (ie, less than 50 megagametophytes), I re-electrophoresed, where possible, 1-5 heterozygous trees, using 10-42 additional megagametophytes per identified heterozygous tree per locus. These data also were pooled, and the above-described chi-square values calculated. Whenever the pooled total was fewer than 200 megagametophytes, a continuity correction was used for calculating chi-square values (Sokal and Rohlf 1969). In several cases, especially those involving rare alleles, I had insufficient seed to electrophorese a statistically sufficient number of megagametophytes. When I had fewer than 25 megagametophytes segregating for a locus, chi-square tests were not made, as the power of such a test to identify significant departures from 1:1 is low. Nonetheless, I report the segregation ratios for those combinations where I was reasonably certain that the bands represented alleles. One locus (of GOT) appeared only in embryos, and although I could not verify segregation ratios, I describe it here because it stained clearly and was highly polymorphic.

The following nomenclature was used: An enzyme and its band phenotypes were referred to by the enzyme's abbreviation in capital letters (Table 1); each locus of an enzyme was referred to by the enzyme's abbreviation with the 2nd and 3rd letters in lower case. When multiple loci occurred for an enzyme, the enzyme phenotypes and loci were numbered, with the most anodally migrating locus designated as 1. Within each locus, the most frequent allele was assigned the value of 1.00. Other alleles of the locus were designated by a mobility value expressed relative to the frequent allele (1.00). Negative mobility values were given to cathodally-migrating alleles. An allele lacking stain activity (null), observed at only one locus, was designated with a lower case "n". Nomenclature for the apparent non-structural locus is described below, and follows Harry's (1983) convention.

RESULTS AND DISCUSSION

General Comments. Fifteen monomorphic loci and 31 polymorphic loci (frequency of common allele $\leq .99$) resolved consistently in the 592 trees that had sufficient sound seeds and interpretable band patterns (Table 3). Additional zones that appeared inconsistently in some systems were excluded. The number of alleles observed and verified at polymorphic loci varied from 2 to 5 (mean of all trees = 2.7), and megagametophyte band phenotypes at a locus varied from null to quadruple-banded. The megagametophyte phenotypes for all analyzed loci alleles are drawn schematically in Figure 1. Seven enzymes had a single zone of activity (ACO, ACP, ADH, GDH, IDH, SOD, SRDH), while the rest had multiple zones (maximum, 4 zones in MDH). Co-electrophoresis of embryos and megagametophytes showed substantial variability between tissues in band expression for many enzymes.

The patterns of bishop pine's isozymes closely resembled those found in other species, both in number of loci for given enzymes, and in band phenotype (number of bands and pattern of stain intensity). Differences that arise may result from variation in electrophoretic techniques used in different laboratories (eg, different gel components and pH's, different staining techniques), as well as species-specific differences.

Compared to many other conifers, bishop pine is low to moderate in amount of allozyme variation. When rare alleles are excluded (frequency less than 0.05), there are 26 polymorphic loci (57% of all investigated loci) and an overall average of 1.56 alleles/locus. These values are generally lower than those reported for other conifers (Hamrick, Mitton and Linhart 1981), with the exceptions of *Pinus resinosa, Pinus torreyana* and *Thuja plicata* (Fowler and Morris 1977; Copes 1981; Ledig and Conkle 1983). These values may be conservative for the entire bishop pine species, however, since they are based on samples that included only northern populations, which are known to be less variable in other traits than the southern populations of the species.

Monomorphic Loci. Three enzyme systems, GDH, SRDH and SOD, were entirely monomorphic; each had a single band in megagametophytes and embryos. The phenotypes of bands in these zones resembled those of corresponding polymorphic loci of other conifers, where single-locus Mendelian segregation has been demonstrated (Table 3). The other 11 monomorphic zones (ALAP2, CAT2, FDP2, FDP3, G6PD2, MDH4, MPI2, 6PGD2, PGI1, SKDH2, and SKDH3) occurred as invariant bands in enzyme systems that also had polymorphic loci banding in different zones. The monomorphic MDH, 6PGD, and PGI zones resembled polymorphic loci in other conifers where single-locus Mendelian segregation was reported, suggesting that these monomorphic zones in bishop pine are also loci (Table 3). Inheritance of the remaining monomorphic zones has not been investigated in other conifers, and they should best be considered as putative loci in bishop pine.

Polymorphic Loci. The 30 polymorphic structural loci comprised 29 loci that were analyzed in megagametophytes and 1 locus that was analyzed in embryos. Table 3 lists characteristics of these loci, as well as the monomorphic loci, and Figure 1 depicts the band phenotypes. Megagametophyte band phenotypes at a single locus were either all single (most common), all double (either with 2 equally staining bands, FDP1, or with one strongly-staining band plus a weaker staining "trailer" band, ACO, IDH, and PGI2), single and double (ACP and PGM), double and triple (GOT3), triple (ALD2), or quadruple (CAT1, with bands of equal staining intensity, and MDH2, with 2 strongly staining bands in the middle, plus fast and slow weakly staining bands). A single locus (Got2) did not stain in megagametophytes but stained clearly and consistently in embryos. Its homozygous phenotypes were single banded and the heterozygous phenotype was triple banded.

Non-structural Loci. In addition to the 30 structural loci, a single non-structural locus seemed to modify the mobility of the MDH2 structural locus. The MDH2 phenotype has 4 bands that move in unison. Most of the observed MDH2 variation was between the 2 slowest-migrating morphs (MDH2-1.00 and MDH2-0.95). Phenotype MDH2-0.95 was present in low-moderate frequencies in many stands and seemed associated with two of the three populations sampled. Initially I scored these two morphs as alleles. It was not until the rare fast morph appeared (MDH2-1.62), and I found all three megagametophyte morphs segregating from a single tree, that a non-structural locus was inferred. The structural locus seems to express a fast and slow morph, resulting in a major mobility difference between phenotypes, while the non-structural locus caused a minor mobility shift in the slow morph, from MDH2-1.00 to MDH2-0.95. Although four phenotypes are expected given this interpretation, I observed only three. The structural alleles can be designated Mdh2-1.00 and Mdh2-1.62, and the modifier alleles, Mmd2-f (fast) and Mmd2-s (slow) (after Harry 1983). Unfortunately I had very few seeds from trees with the MDH2-1.62 phenotype and I only detected one fast-migrating structural phenotype. The genotypes of the three observed megagametophyte phenotypes are: MDH2-1.00 = Mdh2-1.00/Mmd2-f; MDH2-0.95 = Mdh2-1.00/Mmd2-s; MDH2-1.62 = Mdh2-1.62/Mmd2-f. The last genotype was inferred from several heterozygous combinations where I found only MDH2-1.00 and MDH2-1.62 segregating. Since these trees appeared to be homozygous for Mmd2-f (ie, MDH2-0.95 was absent), the genotype of MDH2-1.62 must be Mdh2-1.62/Mmd2-f. If enough trees with the Mdh2-1.62 allele could be electrophoresed, I would expect to find the fourth phenotype migrating slightly slower than MDH2-1.62.

Analogous patterns of inheritance involving a non-structural, modifier locus have been described for MDH in other conifers and angiosperms (Harry 1983 and references therein). Harry (1983) thoroughly investigated the complex inheritance of this MDH zone in megagametophytes and embryos of incense-cedar (Calocedrus decurrens), concluding that the observed band patterns and frequencies were consistent with those theoretically expected if an unlinked, polymorphic, non-structural locus modifies a polymorphic, structural locus. In incense-cedar, although the alleles of the structural locus are co-dominant, the alleles of the modifier locus show dominance. The megagametophyte phenotypes and mobility shifts of MDH2 in bishop pine are similar to those described for incense-cedar.

Embryo Phenotypes. When megagametophytes and embryos were co-electrophoresed on the same gel, several patterns resulted. I interpreted the different embryo band patterns as falling into 4 classes (Table 3): In most cases, the embryo bands stained at the same mobility as one and/or the other megagametophyte bands, or at both megagametophyte mobilities plus a mobility halfway between the two. In these cases, I assumed that the same genes were expressed in both tissues. A second pattern showed positive staining in the embryos, but at a different mobility than the megagametophytes and not clearly related to the megagametophyte bands. In these cases, I assumed that genes were differentially expressed or modified in the two tissues. Sometimes the embryo bands were very similar in mobility to the megagametophyte bands. It became clear that such embryo and megagametophyte bands were not identical only when rare variant megagametophyte bands stained at a different mobility from the embryos. A third pattern resulted when no bands stained in the embryo at the megagametophyte zone. I interpreted this to mean that the megagametophyte loci were not active in embryo tissue (although possibly the locus is expressed in embryos and my technique could not detect it). Finally, in some cases, staining in the embryo was inconsistent and I did not attempt to interpret embryo activity in these zones.

In cases where the megagametophyte locus was clearly expressed in the embryo and where heterozygous embryos were found, the quaternary structures (subunit associations) of the enzyme could be inferred from embryo phenotypes (Table 3). When the phenotype of the megagametophyte was single-banded and the corresponding embryo 2-banded, the enzyme structure was inferred to be monomeric (good examples, LAP2 and SKDH1); if the embryo phenotype was 3-banded, the structure was inferred to be dimeric (good examples, ADH and 6PGD1). In ACO, where the megagametophyte phenotype was double-banded, I interpreted the 4-banded embryo phenotype to represent a monomeric enzyme, as no intermediate bands appeared in embryos. In IDH and PGI2, where the megagametophytes were also double-banded, the embryo phenotype was 3-banded, lacking the trailer bands of the megagametophyte, but expressing an intermediate band between the two darkly-staining megagametophyte bands. These enzymes I inferred to have dimeric structure. Enzyme structure of monomorphic loci, or of polymorphic loci where no heterozygote embryos were found, could not be inferred without additional information.

Additional Observations on Specific Loci. The following list contains additional observations that are not described above, or in the tables or figure.

GOT3: Although heterozygous embryos were rare and difficult to interpret, the clearest gels seemed to have six bands. Four of these were identical in mobility to the fastest bands and the slowest bands of the two megagametophyte alleles, with the remaining two bands staining halfway between the two fastest and the two slowest bands (ie, these last two locations did not stain in megagametophytes). A rare third allele of Got3 (1.92a) had 2 bands identical in mobility to Got3-1.92, but did not have the third band. This pattern has been reported for GOT3 in incense-cedar (Harry 1984). LAP1: When the genotype of a female parent tree was inferred from megagametophytes as Lap1-n/Lap1-n (homozygous null), a paternal contribution of Lap1-1.00 or Lap1-1.06 could be detected and resulted in an embryo phenotype of a single band.

If, however, the maternal genotype was heterozygous with one of the alleles a null allele, the paternal contribution could not always be clearly determined in the embryo (since a 1.00/1.00 phenotype was not distinguishable from a 1.00/n phenotype. MNR1: The megagametophyte phenotype, although drawn schematically as a single band in Figure 1, is actually a darkly-staining diffuse band. PER: Major differences in stain intensity among megagametophytes on the same gel occurred roughly related to different stages of seed germination. My designation of Per3 as a single locus with a double-banded phenotype must be considered tentative since no variation was seen in these bands. The two bands at this zone could just as well be the expression of two independent loci. The former interpretation agrees with results from other conifers. Segregation Analysis. Tests of deviation from single-locus 1:1 segregation for 22 putative polymorphic loci involving 31 combinations of alleles gave small and statistically non-significant chi-square values for all but three loci ($p < 0.05$) (Table 4). For many of these 22 loci, segregation of several common alleles could be tested, but rare alleles often were insufficiently represented to test. However, in most of these insufficiently represented cases, and also in the nine remaining polymorphic loci where sample sizes were too small to use chi-square tests, actual segregation frequencies conformed reasonablly well to 1:1 expectations. Heterogeneity among parents was never significant; in all cases, p-values were large (Table 4).

Included in the group of 22 loci with adequately analyzed heterozygotes is the Mmd2 non-structural modifier locus, as well as its affected Mdh2 structural locus. To test Mmd2, I scored 307 MDH2-1.00 (= Mmd2-f) and MDH2-0.95 (= Mmd2-s) phenotypes segregating from a single mother. Similarly, I tested Mdh2 by counting 54 MDH2-1.00 and MDH2-1.62 phenotypes segregating in the only available heterozygous mother, which apparently was homozygous for Mmd2-f.

Alleles of ACP (heterozygote combination 1.00/1.02) and ALD2 (heterozygote combination 1.00/1.52) showed significant deviation from 1:1 segregation favoring the common allele (1.00). In other heterozygote combinations at these two loci, the common allele did not segregate significantly different from the Mendelian expectation. Possibly certain enzyme forms of these less common alleles function poorly, and early selection reduces the number of seeds containing these enzymes in megagametophytes. Segregation ratios in Aco 1.21/0.89 heterozygotes also differed significantly from 1:1, favoring the 1.21 allele. Both alleles, however, when in combination with allele 1.00, conformed to Mendelian expectations. The observed deviations from 1:1 segregation in these 3 allelic pairs may not indicate actual segregation distortion, since in 31 trials, 2 (1%) are expected to differ at the 5% level. Segregation distortion has been observed in other conifers sporadically when recorded either for single trees or pooled data (ACP, *Pinus sylvestris,* Rudin and Ekberg 1978, *P. strobus,* Eckert, Joly, and Neale 1981; GOT, *P. sylvestris,* Rudin 1975, Rudin and Ekberg 1978; *P. virginiana,* Witter and Feret 1978; LAP, *P. taeda,* Adams and Joly 1980b, *P. sylvestris,* Rudin and Ekberg 1978, *P. strobus,* Eckert, Joly, and Neale 1981, *Picea abies,* Lundkvist 1974; MDH, *P. sitchensis,* Simonsen and Wellendorf 1975, *Pinus sylvestris,* Rudin and Ekberg 1978, *Pseudotsuga menziesii,* El-Kassaby, Yeh and Sziklai 1982; PER, *Pinus taeda* and *P. palustris,* Snyder and Hamaker 1978; G6PD, *P. taeda,* Adams and Joly 1980b; PGI, *P. taeda,* Adams and Joly 1980b, *Picea glauca,* Cheliak and Pitel 1984).

CONCLUSIONS

The 46 isozyme loci described here for bishop pine comprise 30 polymorphic structural loci, one polymorphic non-structural locus, and 15 monomorphic loci. Although the variability in northern bishop pine populations may be lower than that of many other conifers, sufficient allozymic variability exists to encourage the use of isozymes in evolutionary and forest genetic studies of this species. Furthermore, since prior studies suggest that allelic variation for many traits is associated with population of origin, isozyme analyses may be useful in analyzing patterns of population variation. Side-by-side comparisons of megagametophytes with embryos from the same seed showed that many megagametophyte loci are expressed in the embryo. Thus, embryos can be used in studies to further investigate population variability, gene flow and pollen contamination.

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TABLE 1: List of enzymes, enzyme commission reference numbers and gel buffer systems used for bishop pine electrophoresis.

Gel Buffer System[1]	Enzyme Name	Abbreviation	E.C. Number
A	Alcohol dehydrogenase	ADH	1.1.1.1
	Alanine aminopeptidase	ALAP	3.4.11.1
	Leucine aminopeptidase	LAP	3.4.11.1
	Menadione reductase	MNR	1.6.99.2
	Peroxidase	PER	1.11.1.7
	Phosphoglucose isomerase	PGI	5.3.1.9
B	Acid phosphatase	ACP	3.1.3.2
	Catalase	CAT	1.11.1.6
	Glucose-6-phosphate dehydrogenase	G-6PD	1.1.1.49
	Glutamate dehydrogenase	GDH	1.4.1.3
	Glutamate oxaloacetate transaminase	GOT	2.6.1.1
	Mannose phosphate isomerase	MPI	5.3.1.8
	Sorbitol dehydrogenase	SRDH	1.1.1.14
	Superoxide dismutase	SOD	1.15.1.1
~~C~~ D	Aconitase	ACO	4.2.1.3
	Aldolase	ALD	4.1.2.13
	Fructose diphosphatase	FDP	3.1.3.11
	Isocitric dehydrogenase	IDH	1.1.1.42
	Malate dehydrogenase	MDH	1.1.1.37
	6-Phosphogluconic dehydrogenase	6PGD	1.1.1.44
	Phosphoglucomutase	PGM	2.7.5.1
	Shikimate dehydrogenase	SKDH	1.1.1.25

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[1] Gel systems of Conkle et al. 1982.a

TABLE 2: Modifications to electrophoretic procedures of Conkle et al. (1982) used for bishop pine studies. A. Miscellaneous modifications. B. Modifications to stain recipes. C. Additional stain recipes.

A. Wicks: Paper wicks were cut narrower than recommended (14mm x 2mm).
Extraction buffer: Add to buffer,

D-glucose-6-phosphate	20 mg/100ml buffer
bovine albumin	40 mg/100ml buffer

B. Modifications to stain recipes:

Enzyme	Stain Components that Differ	
ACO	cis-aconitic acid isocitrate dehydrogenase	200 mg 60 units
ADH	95% ethyl alcohol	2 ml
ALAP	L-alanine-napthylamidea fast black K salt	50 mg 40 mg
ALD	glyceraldehyde-3-phosphate dehydrogenase	780 units
GOT	fast blue BB salt	50 mg
G6PD	D-glucose-6-phosphate	250 mg
IDH	DL isocitric acid 1% $MgCl_2$	200 mg 1 ml
LAP	black K salt	40 mg
MNR	menadione	25 mg
PGM	a-D-glucose-1-phosphate glucose-6-phosphate-dehydrogenase	250 mg 80 units
SKDH	shikimic acid	250 mg

C. Additional recipes:

Enzyme	Stain Buffer	Stain Components	
FDP	25 ml 0.2M tris-HCL pH 8.0	fructose 1,6 diphosphate	150 mg
		NADP	1 ml
		NBT	1 ml
		PMS	0.5 ml

Enzyme	Stain Buffer	Stain Components	
		glucose-6-phosphate dehydrogenase	100 units
		phosphoglucose isomerase	100 units
		1% $MgCl_2$	1 ml
	Procedure:	Add stain components to warm stain buffer and incubate gels in dark.	
MPI	25 ml 0.2M tris-HCL pH 8.0	mannose-6-phosphate	20 mg
		NADP	1 ml
		NBT	1 ml
		PMS	0.5 ml
		glucose-6-phosphate dehydrogenase	100 units
		phosphoglucose isomerase	100 units
	Procedure:	Add stain components to warm stain buffer and incubate gels in dark.	
SRDH	60 ml 0.2M tris-HCL pH 8.0	sorbitol	5 gm
		NAD	1 ml
		NBT	1 ml
		PMS	0.5 ml
	Procedure:	Add stain components to warm stain buffer and incubate gels in dark.	

TABLE 3. Descriptions of megagametophyte and embryo phenotypes, number of alleles in 46 bishop pine loci, quaternary enzyme structure of 22 bishop pine enzymes, and references to inheritance studies in other conifers.

Locus	Gametophyte Phenotype	Number of Alleles	Expression of Gametophyte Bands in Embryo[1]	Gametophyte Enzyme Structure[2]	References Reporting Inheritance in Other Conifer Species
Aco	double	3	same[3]	monomer	Guries and Ledig 1978; Yeh and Layton 1979; Adams and Joly 1980; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983
Acp	single and double	5	different	---	Tigerstedt 1973; Copes 1975; Lundkvist 1975; Lundkvist and Rudin 1977; Rudin and Ekberg 1978; Yeh and Layton 1979; Adams and Joly 1980; Eckert, Joly, and Neale 1981; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; Fins and Libby 1982; King and Dancik 1983
Adh	single	4	same	dimer	Conkle 1971; Rudin and Ekberg 1978; O'Malley Allendorf and Blake 1979; Yeh and Layton 1979; Eckert, Joly, and Neale 1981, Fins and Libby 1982; Wheeler and Guries 1982; King and Dancik 1983
Alapl	single	2	same		
Alap2	single	1	same		
Aldl	single	2	different	---	Wheeler and Guries 1982; Cheliak and Pitel 1984
Ald2	triple	4	different		
Catl	quadruple	2	no bands	---	Copes 1975
Cat2	single	1	same		
Fdpl	double	3	same		
Fdp2	single	1	same		
Fdp3	single	1	same		
Gdh	single	1	same	---	Copes 1975; Yeh and Layton 1979; Mitton et al. 1979; Adams and Joly 1980; Eckert, Joly, and Neale 1981; El-Kassaby, Yeh and Layton 1982; Wheeler and Guries 1982; King and Dancik 1983; Loukas, Vergini, and Krimbas 1983; Cheliak and Pitel 1984
G6pdl	single	2	indeterminable	---	Yeh and Layton 1979; O'Malley, Blake and Allendorf 1979; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983; Loukas, Vergini, and Krimbas 1983; Cheliak and Pitel 1984
G6pd2	single	1	same		

Locus	Gametophytee Phenotype	Number ofe Alleles	Expression of Gametophyte Bands in Embryo[1]	Gametophyte Enzyme Structure[2]	References Reporting Inheritance in Other Conifer Species
Got1	single	4	same	dimer	Rudin 1975; Conkle and Adams 1977; Yang, Ching and Ching 1977; Guries and Ledig 1978 Rudin and Ekberg 1978; Witter and Feret 1978; O'Malley, Allendorf, and Blake 1979; Yeh and Layton 1979; Adams and Joly 1980b; Eckert, Joly, and Neale 1981; El-Kassaby, Yeh and Sziklai 1982; Fins and Libby 1982; Wheeler and Guries 1982; King and Dancik 1983; Cheliak and Pitel 1984
Got2	no bands	2	embryo locus	dimer	
Got3	triple and double	3	same	---	
Idh	double	4	same[3]	dimer	Guries and Ledig 1978; Yeh and Layton 1979; O'Malley, Allendorf and Blake 1979; Adams and Joly 1980b; Eckert, Joly, and Neale 1981; El-Kasssaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983; Loukas, Vergini, and Krimbas 1983; Cheliak and Pitel 1984
Lap1	single ande null	3[4]	same	monomer	Bergmann 1973; Tigerstedt 1973; Lundkvist 1974; Conkle 1971; Somonsen and Wellendorf 1975; Lundkvist and Rudin 1977; Rudin 1977; Yang, Ching and Ching 1977; Guries and Ledig 1978; Rudin and Ekberg 1978; O'Malley, Allendorf and Blake 1979; Adams and Joly 1980b; Eckert, Joly, and Neale 1981; Fins and Libby 1982; King and Dancik 1983; Loukas, Vergini, and Krimbas 1983; Cheliak and Pitel 1984
Lap2	single	2	same		
Mdh1	single	2	same	dimer	Simonsen and Wellendorf 1975; Guries and Ledig 1978; Rudin and Ekberg 1978; O'Malley, Allendorf and Blake 1979; Yeh and Layton 1979; Adams and Joly 1980b; El-Kassaby 1981; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983
Mdh2	quadruple	2	same		
Mdh3	single	3	no bands		
Mdh4	single	1	no bands		
Mmd2	modifier	2	indeterminable		
Mnr1	diffuse band	2	different		
Mnr2	single	2	same		
Mnr3	single	4	same		
Mpi1	single	2	same	---	El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982
Mpi2	single	1	different		
Per1	single	2	same	---	Mitton et al. 1977; Synder and Hamaker 1978; Copes 1979
Per2	single	2	same		

Locus	Gametophytee Phenotype	Number of Alleles	Expression of Gametophyte Bands in Embryo[1]	Gametophytee Enzyme Structure[2]	References Reporting Inheritance in Other Conifer Species
Per3	double	1	same		
6Pgd1	single	2	same	dimer	Guries and Ledig 1978; O'Malley, Allendorfe and Blake 1979; Yeh and Layton 1979; Adams and Joly 1980b; Eckert, Joly, and Neale 1981; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983; Loukas, Vergini, and Krimbas 1983; Cheliak and Pitel 1984
6Pgd2	single	1	same	---	
Pgi1	single	1	different	---	Simonsen and Wellendorf 1975; O'Malley, Allendorf and Blake 1979; Yeh and Layton 1979; Adams and Joly 1980b; El-Kassaby, Yeh and Sziklai 1982; King and Dancik 1983
Pgi2	double	3	same	dimer	
Pgm1	single and double	2	same	monomer	Simonsen and Wellendorf 1975; Guries and Ledig 1978; Mitton et al. 1979; O'Malley, Allendorf and Blake 1979; Yeh and Layton 1979; Adams and Joly 1980b; Eckert, Joly, and Neale 1981; El-Kassaby, Yeh and Sziklai 1982; Wheeler and Guries 1982; King and Dancik 1983; Loukas, Vergini,and Krimbas 1983
Pgm2	single	3	same	monomer	
Pgm3	single	2	same	monomer	
Skdh1	single	4	same	monomer	
Skdh2	single	1	same	---	
Skdh3	single	1	same	---	
Sod	single	1	same	---	El-Kassaby, Yeh and Sziklai 1982; Fins and Libby 1982; King and Dancik 1983
Srdh	single	1	same	---	O'Malley, Allendorf and Blake 1979; Wheeler and Guries 1982

[1] Comparison within present study of bands from megagametophytes to bands from their derived embryos.

[2] Structure inferred from band phenotypes of heterozygous embryos--see text. Dash(---) indicates structure was indeterminable because 1) a different locuse was expressed in the embryo, 2) a heterozygote embryo was not seen, 3) no bands appeared for the embryo, or 4) the embryo bands were indeterminable.

[3] Embryo had the same main band as the gametophyte, but sometimes lacked the weaker trailer band(s).

[4] Including a null phenotype.

TABLE 4: Segregation data for 29 bishop pine isozyme loci and 1 non-structural locus.

Locus	Segregating Phenotypes	No. Phenotypes Observed	Chi-Square Tests[1] Pooled (df = 1)		Among Parents		
			X^2	p	X^2	df	p
Aco	1.00/1.21	191/209	0.81	0.437	57.19	99	0.995
	1.00/0.89	26/24	0.02	0.896	4.16	7	0.749
	1.21/0.89	33/15	6.02	0.016*	3.19	2	0.276
Acp	1.00/1.09	183/217	2.89	0.092	60.11	99	0.995
	1.00/1.02	65/43	4.08	0.045*	1.78	8	0.985
	1.00/1.13	1/3					
	1.00/0.91	6/2					
	1.09/1.13	1/3					
Adh1	1.00/0.81	57/79	3.24	0.076	13.76	33	0.995
	1.00/1.08	74/62	0.89	0.422	19.11	33	0.974
	1.00/0.65	24/26	0.02	0.896	4.13	6	0.655
Alap1	1.00/0.98	13/7					
Ald1	1.00/0.88	3/1					
Ald2	1.00/1.28	157/163	0.11	0.814	44.99	79	0.995
	1.00/1.52	41/23	4.52	0.036*	6.48	10	0.756
	1.00/0.80	7/9					
	1.28/1.52	9/11					
Cat1	1.00/0.94	40/40	0.00	1.000	4.00	20	0.995
Fdp1	1.00/0.79	15/17	0.03	0.887	2.97	7	0.884
	1.00/1.26	11/13					
Got1	1.00/0.76	205/195	0.25	0.687	60.75	99	0.995
	0.76/1.07	24/15	1.64	0.289	3.16	1	0.080
	1.00/0.98	5/7					
Got3	1.00/1.92	37/35	0.01	0.928	6.99	17	0.981
	1.00/1.92a	2/2					
G6pd1	1.00/1.09	30/32	0.02	0.900	2.10	5	0.828
Idh	1.00/0.86	123/121	0.02	0.900	34.98	60	0.995
	1.00/1.36	1/3					
	0.86/0.96	1/3					
Lap1	1.00/n	209/191	0.81	0.437	61.19	99	0.995
	1.00/1.06	3/1					
Lap2	1.00/1.06	197/203	0.09	0.833	44.91	99	0.995

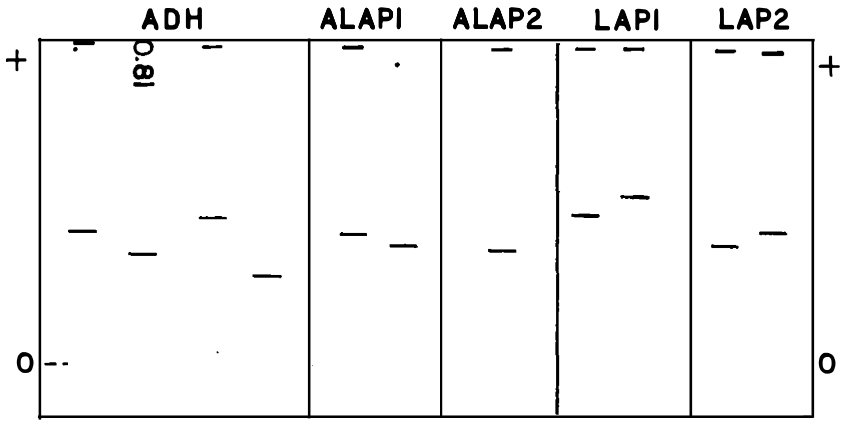
Locus	Segregating Phenotypes	No. Phenotypes Observed	Chi-Square Tests[1]				
			Pooled (df = 1)		Among Parents		
			X^2	p	X^2	df	p
Mdh1	1.00/1.09	30/26	0.16	0.769	1.74	4	0.782
Mdh2	1.00/1.62	31/23	0.91	0.419	2.54	4	0.643
Mmd2	f/s[2]	169/138	2.93	0.090	33.80	64	0.995
Mdh3	1.00/.091 1.00/1.29	58/62 2/2	0.75	0.448	11.93	29	0.995
Mnr1	1.00/0.71	23/17	0.63	0.469	8.38	9	0.498
Mnr2	1.00/1.13	2/2					
Mnr3	1.00/0.71	31/21	1.56	0.304	6.44	9	0.682
	1.00/1.42	41/43	0.01	0.928	11.00	20	0.938
	1.00/0.17	29/31	0.02	0.900	2.18	10	0.995
Mpi1	1.00/0.97	3/1					
Per1	-1.00/-0.88	3/5					
Per2	-1.00/-0.55	3/1					
6Pgd1	1.00/0.91	49/39	0.92	0.417	14.08	21	0.853
Pgi2	1.00/0.81 1.00/1.38	181/179 3/1	0.01	0.928	52.99	89	0.995
Pgm1	1.00/0.95	199/201	0.01	0.928	52.99	99	0.995
Pgm2	1.00/0.94	2/2					
Pgm3	1.00/1.11 1.00/0.86	4/4 2/2					
Skdh1	1.00/1.05 1.00/0.85	72/68 1/3	0.06	0.860	19.94	35	0.979

[1] Chi-square values calculated only when more than 25 gametophytes observed.

[2] Segregation of MDH2-1.00 and -0.95 used to assess Mmd2.

* .05 > p > .01.

FIGURE 1. Haploid isozyme phenotypes for bishop pine. A. Isozymes from A system. B. Isozymes from B system. C. Isozymes from D system.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ALAP2
LAP1
LAP2
+
+
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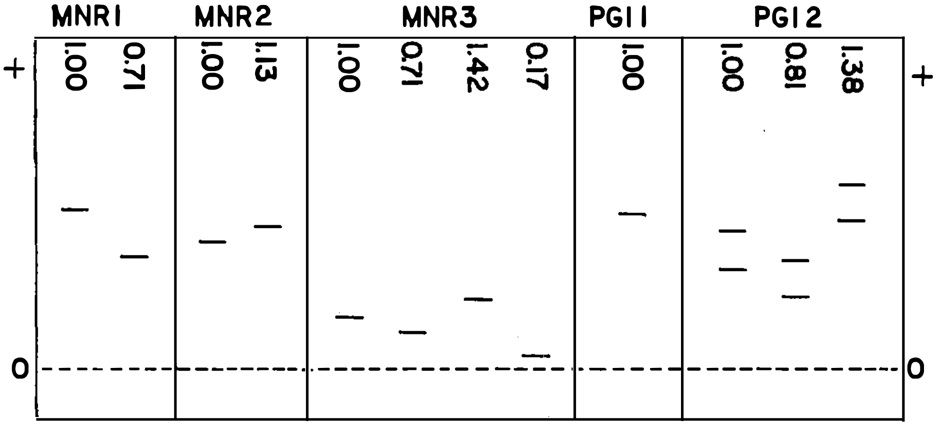
0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MNR2
MNR3
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PGI2
+
+
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1.00
1.00
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1.38
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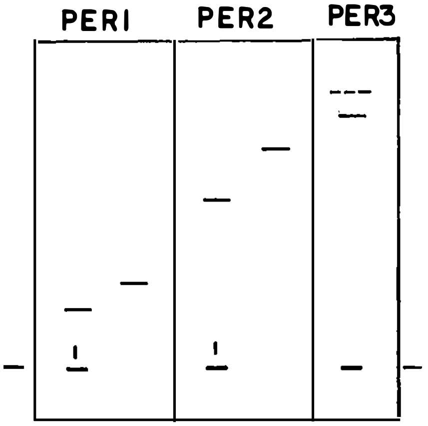
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PER3
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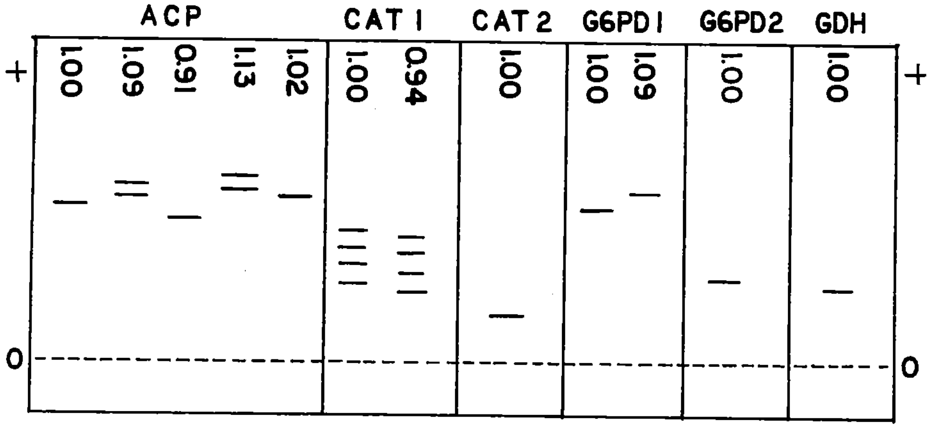
ACP
CAT 1
CAT 2
G6PD 1
G6PD 2
GDH
+
1.00
1.09
0.91
1.13
1.02
1.00
0.94
1.00
1.00
1.09
1.00
1.00
+
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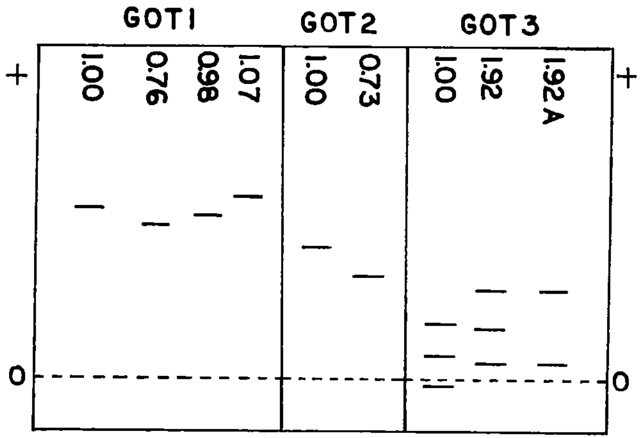
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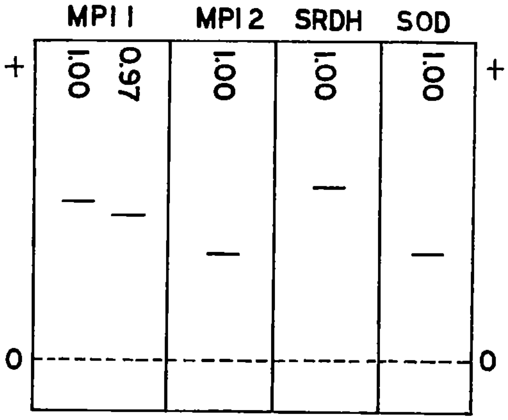
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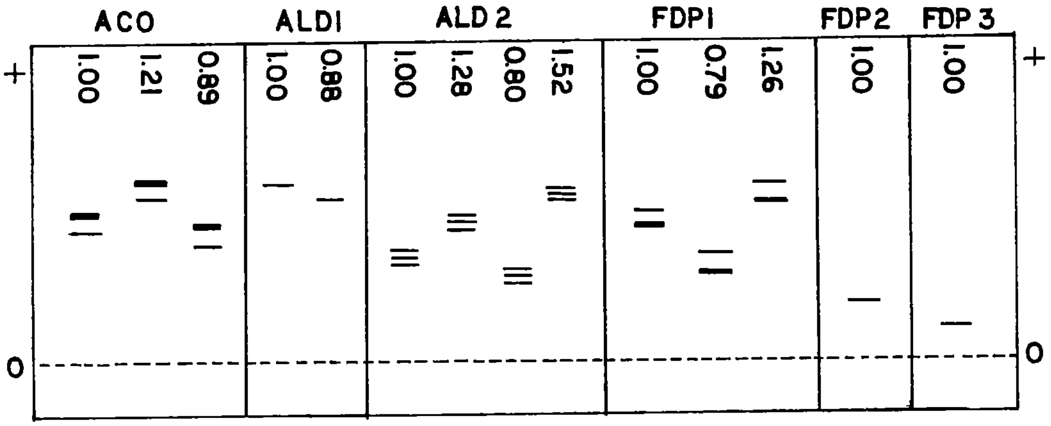
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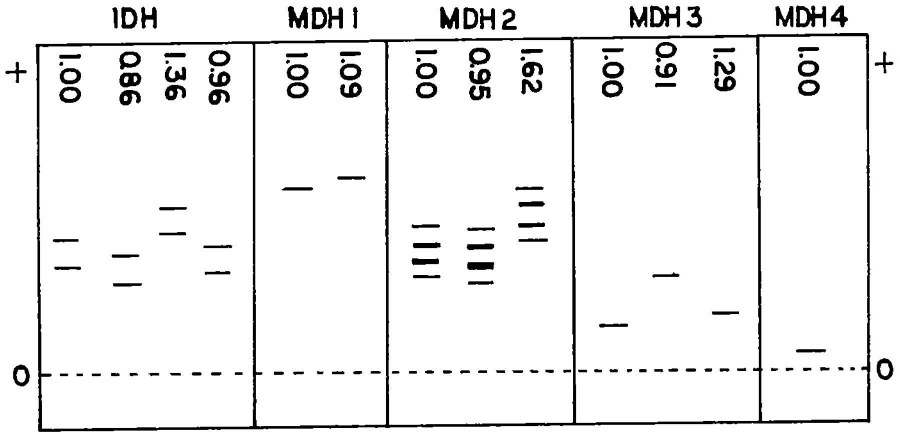
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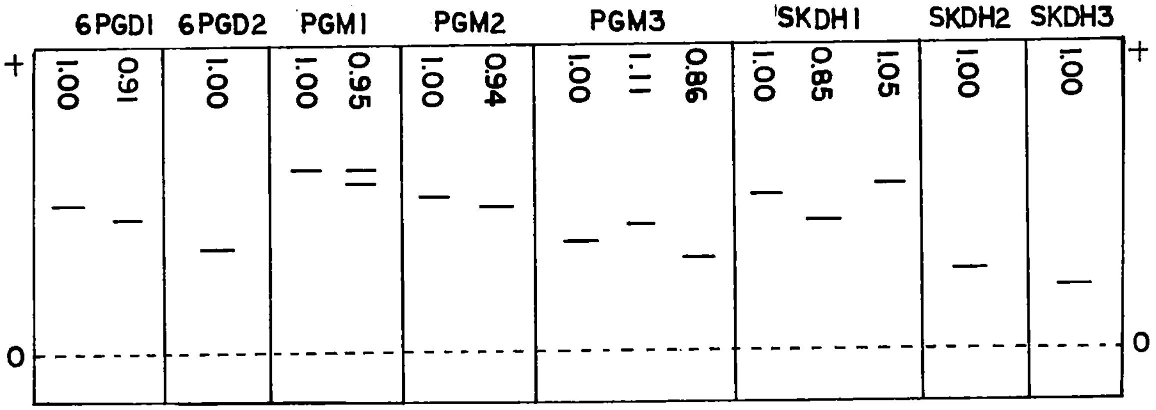
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CHAPTER 3

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A STEEP CLINE IN *PINUS MURICATA*

CONSTANCE I. MILLAR
Department of Genetics, University of California, Berkeley, California 94720

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Clines, including hybrid zones, have long been studied empirically and theoretically, especially for the opportunity they present to study evolutionary forces (Sumner, 1929; Haldane, 1948; Barber and Jackson, 1957). Recent theoretical studies have emphasized that clines may be important in speciation (summarized in Endler, 1977). This emphasis has motivated mathematical modelling of gene flow and selection (Endler, 1973; Slatkin, 1973; Nagylaki, 1975, 1976), and has renewed interest in the origin and evolution of steep clines (Endler, 1977; Moore, 1977).

Steep clines may evolve due to differential selection along a geographic gradient, hybridization of formerly separate populations, or a combination of these factors. The cause for the clinal origin may be apparent, as in differential selection of metal tolerance in grasses (Antonovics et al., 1971), or in hybridization of hooded and carrion crows in Europe (Mayr, 1963). Occasionally the cause is less apparent, as in *Cepaea* area-effects, where evidence for selection gradients or hybridization is lacking (Jones et al., 1977).

A steep cline occurs in bishop pine (*Pinus muricata* D. Don, subsection *Oocarpae,* Critchfield and Little, 1966), a species with a discontinuous coastal and island distribution in California and Baja California. In northern California, a continuous population of bishop pine extends 180 km from Ft. Ross to Ft. Bragg (Fig. 1). Several morphological and biochemical characteristics change abruptly at Sea Ranch, 27 km north of Ft. Ross. Green foliage, typical of all southern populations, replaces, over approximately 2 km, the blue foliage typical of all northern populations (Duffield, 1951). Distinct differences in the shape and waxiness of the chambers above the stomata determine foliage color. Geographically coincidental with this change is an abrupt transition in the composition of the monoterpene fraction of xylem resin (Mirov et al., 1966). South of the transition, delta-3-carene predominates, whereas to the north, alpha-pinene is the major terpene. Genetic analyses in related species have shown that single or a few loci control stomatal traits and monoterpene composition (Stockwell and Righter, 1946; Forde, 1964). Trees with intermediate terpene composition and stomatal type occur over approximately 2 km. In a major investigation of morphological variation in the species, Duffield (1951) found no other differences between blue and green populations of this region.

Neither ecological nor genetic factors readily explain the origin or maintenance of this steep cline. The coastal environment between Ft. Ross and Ft. Bragg is remarkably uniform in climate, topography, vegetation, and soil pattern. No environmental source for selection is apparent. Critchfield (pers. comm.) found no reproductive barriers in controlled crosses between trees in contiguous blue and green stands, although other populations of this species are isolated by strong reproductive barriers (Critchfield, 1967).

In this study I used variability at allozyme loci in conjunction with ecological information to investigate gene flow and origin of the cline in bishop pine. Using a marker locus I mapped the genotype distribution in standing trees and embryos, and postulate unidirectional pollen flow across the cline. From this and other evidence, interpretations regarding clinal origin and maintenance are proposed.

MATERIALS AND METHODS

Electrophoretic assays were made on haploid female gametophytes and diploid embryos extracted from freshly germinated seeds. These tissues have been used repeatedly in conifer allozyme research, and the enzyme systems and loci expressed are well known. Bishop pine has predominantly closed (serotinous) cones, so seeds can be collected any time of the year. In all collections, I sampled either current-year or one-year-older cones. To reduce the probability of relatedness between trees, I collected from trees separated by more than 100 m. I scored sample trees and at least ten additional trees in each stand for stomatal type. "Green" (G) stands had all trees with green-type stomata; "blue" (B) stands had all trees with blue-type stomata; and "transition" (T) stands had both types in the same stand.

Immersion in boiling water for 10 sec and drying at approximately 28 C opened the serotinous cones. Seeds were soaked overnight in cold water (5 C) and germinated in petri dishes in an incubator (22 C) until the radicles emerged.e

A preliminary study surveyed allozymee variability in samples of green and blue trees considerably south and north of the transition. I collected cones in April 1978 from: (a) 50 trees from five green stands (G1–G5) more than 15 km south of Sea Ranch, and (b) 45 trees from two blue stands (B3, B4) more than 20 km north of Sea Ranch (Table 1). Extracts from one gametophyte per tree were subjected to starch gel electrophoresis (Conkle, 1972). Fourteen enzyme systems were assayed: (a) buffered with tris citrate at pH 8.3—alcohol dehydrogenase (ADH), esterase (β-EST), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), peroxidase (PER), tetrazolium oxidase (TO); (b) buffered with tris citrate at pH 8.8—glutamate-oxaloacetate transaminase (GOT), glucosee6-phosphate dehydrogenase (G6PD), acid phosphatase (ACPH), glutamate dehydrogenase (GDH), catalase (CAT); and (c) buffered with tris citrate at pH 6.2—malate dehydrogenase (MDH), 6-phosphate-glucose dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH). The scored loci segregate according to Mendelian expectations in related conifers (Conkle, 1981). When enzymes had more than one scored locus, I numbered the loci by their relative mobilities, the lowest number (1) designating the locus that migrated the farthest.

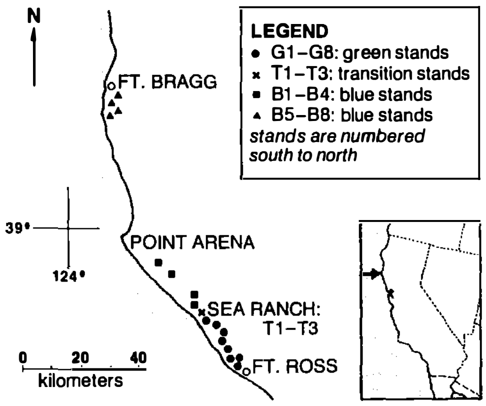

FIG. 1. Location of bishop pine stands sampled in northern California.

A more intensive study sampled trees in the transition area, as well as additional blue trees near Ft. Bragg. Cones were collected in August and September 1978 and July 1979 from 108 trees in green (G6–G8), blue (B1, B2, B5–B8), and transition (T1–T3) stands (Table 1).

I assayed only GOT-1 in the intensive study since preliminary work had shown that it was the most useful marker for blue and green populations. Electrophoretic segregation in gametophytes from 25 heterozygous trees confirmed the Mendelian nature of GOT-1: departure from the expected 1:1 segregation was not significant. GOT-1 was assayed in five gametophytes and two embryos for every tree. Genotype frequencies of standing trees were estimated from five gametophytes per tree. The probability of misclassifying a heterozygote as a homozygote in this case is small (0.06; Morris and Spieth, 1978).

For each tree, I analyzed the genotypes of two embryos with their respective gametophytes. Since the maternal contribution to the embryo is genetically identical to the gametophyte, the paternal contribution can be determined by subtraction.

I recorded onset and duration of flow-

TABLE 1. *Seed collections and enzyme assays performed.*

Stand	Foliage type	Number of trees	Collection date	Tissues[1] assayed	Enzymes[2] assayed
G1	green	5	April 1978	1g 4g, 2e	all GOT-1
G2	green	8	April 1978	1g 4g, 2e	all GOT-1
G3	green	6	April 1978	1g 4g, 2e	all GOT-1
G4	green	15	April 1978	1g 4g, 2e	all GOT-1
G5	green	16	April 1978	1g 4g, 2e	all GOT-1
G6	green	10	September 1978	5g, 2e	GOT-1
G7	green	7	September 1978	5g, 2e	GOT-1
G8	green	11	September 1978	5g, 2e	GOT-1
T1	mixed	10	July 1979	5g, 2e	GOT-1
T2	mixed	10	September 1978	5g, 2e	GOT-1
T3	mixed	7	September 1978	5g, 2e	GOT-1
B1	blue	9	September 1978	5g, 2e	GOT-1
B2	blue	10	September 1978	5g, 2e	GOT-1
B3	blue	29	April 1978	1g 4g, 2e	all GOT-1
B4	blue	16	April 1978	1g 4g, 2e	all GOT-1
B5	blue	4	August 1978	5g, 2e	GOT-1
B6	blue	14	August 1978	5g, 2e	GOT-1
B7	blue	4	August 1978	5g, 2e	GOT-1
B8	blue	10	August 1978	5g, 2e	GOT-1

[1] Per tree; g = gametophyte, e = embryo.
[2] See list of enzymes, Table 2.

ering in male and female strobili during 1980 in 22 blue and 24 green trees at the University of California's Russell Reservation, 16 km east of Berkeley. These trees originated as open-pollinated seed collected in 1966 from populations between Ft. Ross and Ft. Bragg. The trees were planted in 1968 as part of a common garden experiment, and many have been sexually mature for several years. Phenological observations in the native stands were made periodically.

The National Climatic Center provided wind-direction data collected at the Pt. Arena Weather Station, 30 km north of the transition.

RESULTS

Of the 14 enzyme systems assayed in gametophytes, 26 loci were scored (Table 2). Eight loci were polymorphic: GOT-1 had one allele common in the blue population, and another allele common in the green population; LAP-1, GOT-2, MDH-2, ADH, and PER-1 had one allele with frequencies greater than 0.75 in both populations, and; LAP-2 and β-EST had two alleles in approximately equal frequencies in both populations. Since allele frequencies in GOT-1 were significantly different between blue and green populations, it was used as a marker.

Multi-locus enzymatic differentiation within and between green and blue populations was measured by Nei's (1972, 1973) diversity and identity statistics. With the exception of GOT-1, green and blue populations are very similar enzymatically (Table 3). When GOT-1 is excluded from calculations, $\bar{G}_{st}$ (0.029) compares with the

TABLE 2. *Allele frequencies of 50 trees south of the cline (green) and 45 trees north of the cline (blue).*

Locus	Allele[1]	Frequencies Green	Frequencies Blue	Locus	Allele[1]	Frequencies Green	Frequencies Blue
Lap-1	*1*	0.16	0.12	*Mdh-3*	*1*	1.00	1.00
	2	0.00	0.12	*Adh*	*1*	0.02	0.00
	3	0.84	0.76		*2*	0.00	0.06
Lap-2	*1*	0.54	0.48		*3*	0.96	0.94
	2	0.46	0.52		*4*	0.02	0.00
Got-1	*1*	0.96	0.24	*Pgm*	*1*	1.00	1.00
	2	0.04	0.76	*Acph-1*	*1*	1.00	1.00
Got-2	*1*	0.96	0.90	*Acph-2*	*1*	1.00	1.00
	2	0.04	0.10	*Per-1*	*1*	0.90	0.98
Got-3	*1*	1.00	1.00		*2*	0.10	0.02
Got-4	*1*	1.00	1.00	*Per-2*	*1*	1.00	1.00
G-6pd-1	*1*	1.00	1.00	*Per-3*	*1*	1.00	1.00
G-6pd-2	*1*	1.00	1.00	*Cat*	*1*	1.00	1.00
Idh	*1*	1.00	1.00	*To-1*	*1*	1.00	1.00
Gdh	*1*	1.00	1.00	*To-2*	*1*	1.00	1.00
6-Pgd-1	*1*	1.00	1.00	*β-Est*	*1*	0.33	0.42
6-Pgd-2	*1*	1.00	1.00		*2*	0.67	0.58
Mdh-1	*1*	1.00	1.00				
Mdh-2	*1*	0.92	1.00				
	2	0.08	0.00				

[1] Alleles at locus are numbered according to migration rates: 1 = fastest, 4 = slowest.

lowest values in comparable conifer studies (Brown and Moran, 1981; Guries and Ledig, 1981; Yeh, 1981).

A steep cline in GOT-1^1 frequencies in standing trees occurs at Sea Ranch. Figure 2 summarizes the allele frequencies of GOT-1^1 in both standing trees and in the pollen contribution to embryos. In standing trees, allele frequencies averaging 0.97 in the green stands decreased to 0.23 in the blue stands over only 2.3 km. The gradient in the pollen allele frequencies was not as steep (Fig. 2): allele frequencies averaged 0.89 in green stands and 0.25 in blue stands.

When analyzed by Chi-square tests, allele frequencies in green stands as a group were homogeneous both among standing trees and among pollen. Blue stands were similarly homogeneous, with the exception of stand B1. In this stand, the pollen frequency of GOT-1^1 was significantly higher than pollen frequency of other blue stands. Transition stands T2 and T3 were homogeneous, whereas T1 had significantly higher GOT-1^1 frequencies in both standing trees and pollen than T2 and T3. Based on these homogeneities, I used average frequencies in subsequent analyses.

Differences in GOT-1^1 frequencies between standing trees and pollen were tested using an arcsine transformation (Sokal and Rohlf, 1969). Frequencies of GOT-1^1 in

TABLE 3. *Measures of gene diversity, genetic identity and genetic distance in bishop pine.*

Statistics[1]	26 loci Green stands	26 loci Blue stands	25 loci (excl. *Got-1*) Green stands	25 loci (excl. *Got-1*) Blue stands
$\bar{H}_c$	0.064	0.075	0.062	0.067
$\bar{D}_{cs}$	0.005	0.006	0.006	0.004
$\bar{H}_t$	0.085		0.069	
$\bar{G}_{st}$	0.141		0.029	
I	0.974		0.998	
D	0.026		0.002	

[1] $\bar{H}_c$ = average gene diversity within stands within blue or green populations.
$\bar{D}_{cs}$ = average interstand gene diversity within blue or green populations.
$\bar{H}_t$ = average total gene diversity in both populations.
$\bar{G}_{st}$ = relative interpopulational gene diversity between composite blue and composite green populations.
I = Nei's genetic identity between composite blue and composite green populations.
D = Nei's genetic distance between composite blue and composite green populations.

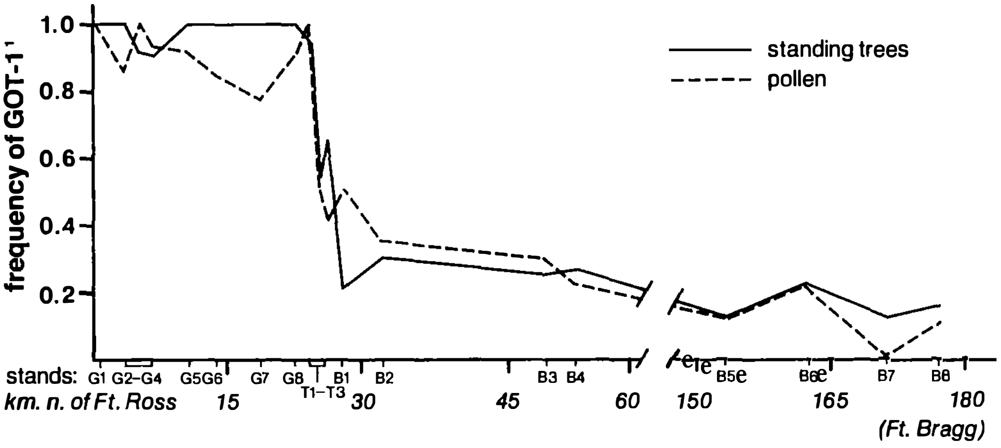

FIG. 2. Cline in frequency of *Got-1*^{1} in bishop pine.

green stands (G1–G8) differed significantly in standing trees (0.97) and pollen (0.89). Differences in allele frequencies between standing trees and pollen in transition and blue stands were not significant.

Genotypes of GOT-1 and stomatal type were associated in the transition area. Of 19 green trees, 15 were homozygous for GOT-1^1, four were heterozygous, and none was homozygous for GOT-1^2. By contrast, of seven blue trees, none was homozygous for GOT-1^1, four were heterozygous, and three were homozygous for GOT-1^1.

Blue trees flowered earlier than green trees in the Russell Reservation plantation (Fig. 3). Modal dates for pollen shedding were 15 days apart (23 April and 8 May). Nevertheless, pollen shedding in the populations overlaps greatly. The periods of female-strobilus receptivity also differed, with modal dates 20 days apart (13 April and 3 May). No overlap between populations occurred. Seven days separated the end of female receptivity in blue trees and its onset in green trees. No green pollen was shed during the period of blue female-strobilus receptivity, but blue pollen was shed almost throughout the entire period of green female-strobilus receptivity. Phenology did not differ significantly in stands within blue or green populations. Observations in natural stands during 1978–1981 were consistent with results in the plantation.

DISCUSSION

The cline in bishop pine is atypical for conifers both because of its steepness and because of the apparent absence of an associated environmental gradient. The steepest intra-specific clines previously reported in conifers extended for tens to hundreds of kilometers (Ledig and Fryer, 1972; Adams, 1975). Inter-specific hybrid zones in conifers may be narrow but the pattern of variation often is not clinal (Wright, 1976). In general, clines in conifers cover large distances, and follow broad environmental and climatic gradients (e.g., Langlet, 1936; Hamrick and Libby, 1972; Conkle, 1973; Griffin and Ching, 1977).

Despite the large differences in stomatal form, terpene composition, and flowering, the blue and green bishop pine populations are little differentiated enzymatically. Only GOT-1 allele frequencies differ significantly in blue and green populations; the remaining loci appear unaffected by the cline.

Gene Flow.—The disparity in GOT-1^1 allele frequencies between standing trees and pollen may be attributed to pollen flow

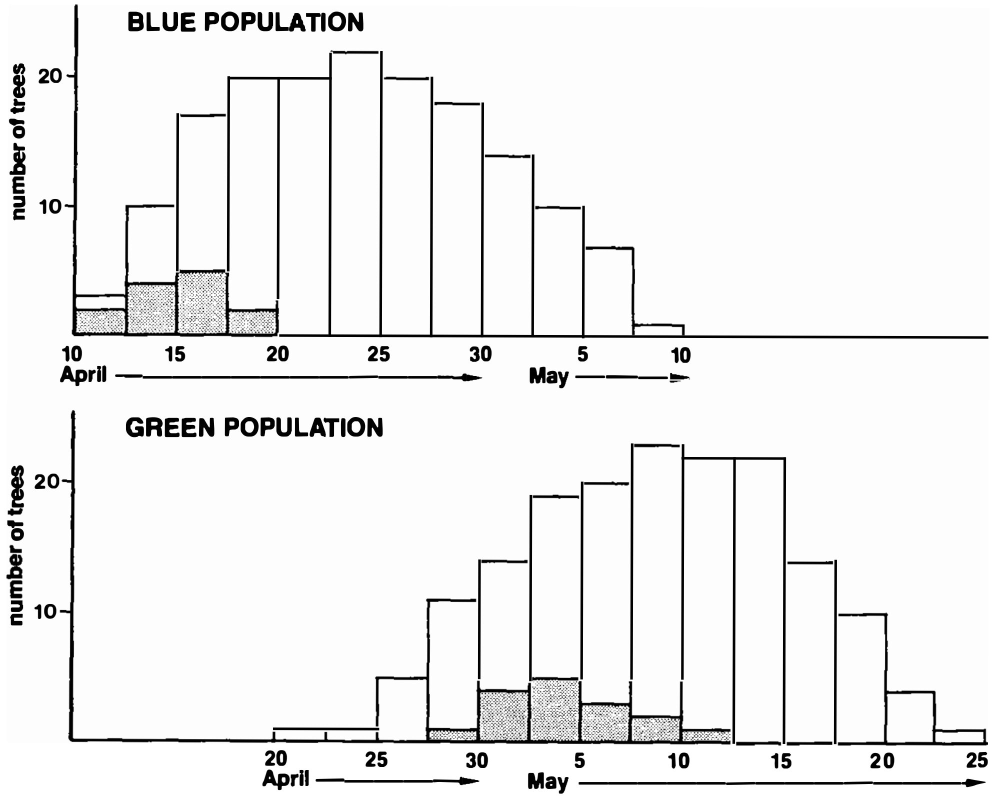

FIG. 3. Distribution of pollen shedding (open bars) and female strobile receptivity (shaded bars) in 22 blue and 24 green trees at Russell Reservation, April and May, 1980.

across the cline. In five of the eight green stands, the allele frequency of pollen and embryos was significantly less than that of standing trees. Pollen flow of GOT-1^2 alleles from northern blue stands to southern green stands would create this disparity. Similarly, Camin and Ehrlich (1958) credited the recurring disparity between juvenile and adult banding frequencies in *Natrix sipedon* to gene flow.

Wind direction and phenology support the north to south gene flow interpretation of the allozyme data. Sixty-five percent of the winds during the pollination season on the coast (April and May) are from the NNW and have velocities averaging 13 mph (National Climatic Center, 1980). These winds occur during fair weather and are probably responsible for most of the pollen dispersal. The differences observed in phenology of pollen shedding and female-strobilus receptivity between blue and green populations similarly would favor north to south pollen dispersal and inhibit pollen movement in the opposite direction (Fig. 3).

GOT-1^1 frequencies in pollen increase with distance from the transition in a pattern consistent with pollen flow (Fig. 2). The higher frequencies in pollen from stands T1 and G8 may result from the location of these stands on points in the shoreline near the transition: northwesterly winds would flow over the ocean before reaching these stands, decreasing the potential for interpopulational pollen flow. Since allele frequencies have been estimated from embryo and adult populations, the following single locus estimator approximates the amount of pollen flow between the blue population and increasingly distant green stands. Only GOT-1 is useful here since no other loci varied significantly between populations. This model has been used in admixture studies in humans (Workman, 1968; Adams and Ward, 1973).

$$m = (p_e - p_g)/(p_b - p_g)$$

where m is the proportion of blue immigrants, p_e is the GOT-1^1 frequency in embryos, p_g is the GOT-1^1 frequency in green standing trees, and p_b is the GOT-1^1 frequency in blue standing trees (average frequency = 0.23). For stands G8 to G1 respectively, m is 0.06, 0.09, 0.09, 0.06, 0.01, 0, 0.09, 0. These values decrease roughly with distance as expected with long distance pollen flow.

Other explanations currently under study for the disparity in allele frequencies between standing trees and pollen include (1) greater production of pollen by trees with GOT-1^2 alleles than by trees with GOT-1^1 alleles, or (2) competitive advantage of pollen with GOT-1^2 alleles in the nucellus, or of embryos with GOT-1^2 alleles over those with GOT-1^1.

If the disparity in allele frequencies between embryos and adult bishop pine trees is due to pollen flow, then a large amount of long distance gene flow occurs in this species. In other conifers, estimates of gene flow are highly variable. Pollen dispersal studies have estimated gene flow as short (less than one km: Colwell, 1951; Wright, 1953; Strand, 1957; Wang et al., 1969; Sarvas, 1967) or long (tens of kilometers: summarized in Lanner, 1966; Koski, 1970). These studies measured pollen transport, whereas actual gene flow may be considerably different. Values calculated in this study estimate effective pollen flow, and are consistent with reports of long distance pollen dispersal in conifers.

Implications for the Origin and Maintenance of the Cline.—Endler (1977) stressed the difficulty in discerning the mode of origin of steep clines once they are established. Several lines of evidence suggest that the cline in bishop pine did not develop by differential selection in continuous populations but evolved following secondary contact of formerly allopatric populations. Theoretically, the lack of evidence for an environmental gradient coupled with the possibility of widespread gene flow argue against non-allopatric development of the cline. Furthermore, paleobotanic, geologic, and climatic evidence indicates a history of repeated fragmentation of bishop pine populations. Abundant fossil cones of bishop pine from central and southern California document frequent shifts in distribution during the late Tertiary and Pleistocene (Axelrod, 1980). Orogenic activity caused a constant change in land-sea relations during the late Tertiary and Pleistocene (Bailey and Jahns, 1954; Bailey, 1966), and Mason (1934) proposed that pine populations were isolated at one or more times on islands created by such tectonic events. Expansions and contractions of pine populations appear correlated with cyclic climatic changes during the Pleistocene (Mason, 1934; Johnson, 1977), and Axelrod (1967*a*, 1967*b*, 1980) attributed a recent fragmentation of pine populations to the drought and heat of the Xerotherm (8000–4000 years before present). Since the Xerotherm, the California climate has become cooler and wetter, and populations of several species are expanding in areas where they are not inhibited by other reasons (Axelrod, 1981). Thus the Tertiary and Pleistocene history of bishop pine is replete with population fragmentation and expansion events, the most recent probable contraction occurring during the Xerotherm.

Thus we may be witnessing in bishop pine a recent meeting of blue and green populations. The fate of the cline is unpredictable from current knowledge, although selection for clinal maintenance is implicated from several clues. First, if pollen flow is long distance (e.g., more than 3 km), then differences in neutral traits between the populations would have become more gradual as the populations met and interbred, and a steep cline (less than 3 km) would never have formed. Second, the disparity in allele frequency between embryos and mature trees suggests post-embryonic selection, and the association of allozyme genotypes and foliage type in mature trees in the transition suggests selection against hybrid recombinants. The disparity in flowering time of blue and green populations may indicate an early stage in the reduction of gene exchange between the populations. The flowering time of the green population in this study is completely out of an ordered latitudinal sequence that has been observed for the remaining California and Baja California populations (Wilcox et al., 1979; pers. obser. Russell Reservation 1978–1981). The green population of this study flowers last of all the bishop pine populations.

SUMMARY

Two northern California bishop pine populations differing in stomatal form, monoterpene composition, flowering time, and allozyme frequency are separated by a cline of less than 3 km width. Allelic frequencies at a marker allozyme locus in standing trees changed from an average of 0.97 north of the cline to 0.23 south of the cline. Differences in allelic frequencies between mature trees and embryos are attributed to long distance pollen flow across the cline. Data on wind direction and flowering phenology support this hypothesis. Genetic, ecological, and paleontological evidence suggests that the cline resulted from recent contact of formerly isolated populations.

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CHAPTER 4

Crossability and Relationships of Bishop Pine

Abstract. Crossability data help to clarify the relationships among the taxonomically confusing populations of the maritime California closed-cone pines. Inconsistent patterns of variation at different levels (morphological, physiological and biochemical) have led to the large number of contradictory names and evolutionary scenarios proposed for these pine populations. Previous studies have included crosses among the different populations of bishop pine (Pinus muricata), but not all combinations have been attempted, and some of those that were attempted produced insufficient data for confident interpretation. The present study completed some of the missing or insufficient interpopulation crosses, with particular emphasis on crosses among subpopulations of the southernmost "blue" and northernmost "green" populations. All blue x green crosses among trees from these northern parapatric populations of bishop pine produced amounts of sound seed similar to those of within-population control crosses; early nursery growth and survival also did not differ significantly between the within-population control families and between-population hybrid families. Crossability may, however, be lower when the F_1 (blue x green) is the female parent. In crosses amonga widespread populations, the trees from Marin grouped with the northern populations in their limited crossability to bishop pines from Monterey and their inability to cross with bishop pine populations further south. In crosses with bishop pines from Monterey, the four more northern blue and green populations were similar to each other. Southern populations (San Vicente, Purisima) and the two Channel Island populations (Santa

Rosa and Santa Cruz) were highly interfertile. Average nursery survival and height growth did not differ significantly among those widespread crosses that yielded sound seeds. Patterns of crossability, resin-canal numbers and other traits presented here support the argument that the smooth-coned variant of bishop pine (*Pinus remorata*) is not phylogenetically distinct, but represents one polymorphic trait within a highly variable pine complex.

INTRODUCTION

The three California species of closed cone pines (subsection *Oocarpae*, Critchfield and Little 1966) exemplify unusual crossing behavior for *Pinus*. In controlled pollinations, knobcone pine (*Pinus attenuata*) and Monterey pine (*P. radiata*) intercross more readily than most other species combinations in the genus, whereas repeated crosses among certain different populations within bishop pine (*P. muricata*) have not to date produced viable seeds (Critchfield 1967, 1984). These unsuccessful crosses are, with similar results in *P. ponderosa*, the only two known instances in *Pinus* of breeding barriers between populations of a species (Critchfield 1967, 1984).

In addition to their unusual crossing relationships, the closed cone pines vary tremendously within and between populations in morphological, physiological and biochemical traits, and some populations of these species are much more variable than others. Variation among the mainland populations of bishop and Monterey pines is augmented by even greater variation among populations occurring on four islands, where patterns of variation form gradients, not always consistent among traits, between these species. A rich fossil record documents variation since the late Miocene in these lineages.

Several contradictory hypotheses on the origins and genetic relationships of bishop and Monterey pines have been proposed. Many of these hypotheses are based on morphological features, especially of the seed cones, and incorporate relatively less evidence based on other kinds of data. Since any unbalanced emphasis may be misleading, it is preferable to compare many traits and to study properties that clarify origins of species and population relationships. Crossability is one measure of genetic relatedness within species and between closely related taxa. Unlike other types of comparative evidence, crossability also gives a measure of the ability of these taxa to exchange genes. I report here new studies on crossing relationships in bishop pine and summarize the knowledge to date on crossability within the species. Using these data, I re-evaluate evolutionary hypotheses proposed for bishop pine and its related taxa.

TAXONOMY, DISTRIBUTION, AND VARIATION

A 640 km gap in Mexico separates the three primarily Californian species of <u>Oocarpae</u> from the four exclusively Central American taxa. The northern group includes a montane, mostly interior element (knobcone pine) and a maritime/insular element (bishop pine and Monterey pine) (see Fig. 1 for locations mentioned in this article, and maps in Griffin and Critchfield 1976). Knobcone pine extends from southern Oregon to northern Baja California, growing on dry, fire-prone sites mostly inland from the coast. The highly discontinuous mainland populations of bishop and Monterey pines, by contrast, never extend far from coastal fogs, and range from Trinidad in northern California to San Vicente in Baja California. Closed cone pines also grow on four islands: Santa Cruz and Santa Rosa in California (part of the Channel Islands), and Guadalupe and Cedros Islands in Mexico.

Ever since Lois de Longchamp's vague description in 1812 of a closed cone pine from Monterey that he named Pinus californiana (Lemmon 1893), the taxonomy and phylogenetic interpretation of the maritime group have been contradictory and inconsistent. Even today, authors disagree over the taxonomic status of all but a few populations. For example, whereas Axelrod (1983) recognized three taxa on Santa Cruz Island, Linhart, Burr and Conkle (1967) suggested that there was only one. Since the same specific epithets are often used by different authors to refer to different pines, and to suggest dissimilar genetic affinities, Latin names have often added to the confusion in this group. To clarify my later use of names in this paper, and to outline the present problems, I review both the historic use of names (before 1900) and present taxonomy (since 1900) of the California closed cone pines, with emphasis on the bishop pine complex. In the following sections, the phrase "formally described" or "formally named" implies that an author has described a taxon according to officially acceptable rules.

Taxonomy Before 1900. On the basis of cones collected by T. Coulter at San Luis Obispo, David Don described a new pine species and presented a paper to the Linnaean Society of London formally naming Pinus muricata in 1835 (Don 1837). Eleven years later, Hartweg (1848) described a similar pine growing in a small area on the Monterey peninsula, and also south of San Luis Obispo at Purisima, as Pinus Edgariana. By the 1880's, this name was subsumed under P. muricata D. Don, which by that time included pine populations at Mendocino, Pt. Reyes, Monterey, San Luis

Obispo and Purisima/Lompoc (Gordon 1858; Engelmann 1880; Lemmon 1888). Although pines had been described from Santa Cruz, Santa Rosa, Guadalupe and Cedros Islands prior to 1900 (Engelmann 1880; Greene 1887; Brandegee 1888, 1890; Lemmon 1895), only the Cedros Island population seems to have been described as *P. muricata* (Greene 1888), and not all authors agreed with this. A small mainland population of small-coned pines growing near San Vicente in Baja California was described as *P. muricata* var. *Anthonyi* by Lemmon (1895).

In the same year that he described *P. muricata,* Don also formally described *P. radiata* from cones collected on the Monterey peninsula by Coulter (Don 1837). Don formally described yet another species from Monterey, *P. tuberculata,* related to *P. radiata,* but distinguished by the form of the cone (Don 1837). Earlier names with incomplete descriptions for a similar pine were *P. californiana* Lois (in Loudon 1838); *P. californica* Hooker and Arnott (Hooker and Arnott 1841); *P. montereyensis* Godefroy; *P. montheragensis* Ranuch; *P. Llaveana* Otto; *P. adunca* Bosc. and *P. Sinclairii* Hooker and Arnott (Hooker and Arnott 1841; Lemmon 1893). All of these names were used to describe pines (probably not *P. muricata)* from Monterey, and some of these were championed for priority later in the nineteenth century.

From cones of a pine collected in Monterey by David Douglas shortly before his death, Loudon formally published a description of a species he called *P. insignis* (Loudon 1838). Owing to the completeness of the description and the wide circulation of Loudon's book, this name and many varietal names became commonly used, although from all appearances *P. insignis* was the same pine as Don's *P. radiata.* Watson (1876) described but did not name a two-leaved variety of *P. insignis* collected by E. Palmer in 1875 on Guadalupe Island. Engelmann mentioned, in his description of *P. insignis* in 1880, a variety "*binata,* collected by Dr. Palmer on Guadalupe Island, with the normal cones of *P. insignis* but leaves in pairs" (Engelmann 1880). Eight years later, Lemmon also mentioned "an outlying variety (?) [sic] *binata* on the Island of Guadalupe" (Lemmon 1888). Some authors also described the Santa Cruz and Santa Rosa Island pines as *P. insignis* var. *binata* (Greene 1887; Brandegee 1888). Concurrent with his description of *P. radiata* var. *binata,* Lemmon named two more varieties, *P. insignis* var. *radiata* (large cones) and *P. insignis* var. *laevigata* (smooth cones), both from Monterey (Lemmon 1888). By the late 1880's, pines at Año Nuevo were ascribed to *P. insignis* (Engelmann 1880, Lemmon 1888) while pines at Cambria were named *P. insignis* var. *macrocarpa* (Hartweg 1846). In several places, Lemmon argued for Don's priority with *P. radiata,* and in 1895 he transferred *P. insignis* to *P. radiata,* a transfer that also applied to the varieties (Lemmon 1888, 1893 and 1895). At that time Lemmon listed only two varieties, *P. radiata* var. *binata,* which included "a few trees on the islands of Santa Cruz and Guadalupe", and var. *tuberculata,* the small-coned pine from Monterey that Don had originally called *P. tuberculata.* In subsequent English literature, the transfer from *P. insignis* to *P. radiata* seems to have been accepted, and *P. radiata* became widely used.

The interior pine, knobcone pine, was named *P. californica* (but not the *P. californica* of Hooker and Arnott) by Hartweg (1847) based on trees growing in the hills near Monterey. Two years later, this pine was described by Gordon using the name Don had given a pine similar to *P. radiata,* namely *P. tuberculata* (Gordon 1849). Gordon's name was then commonly used, and Mayr appended to it the varietal name *acuta* to describe pines from a southern California population that had deviant cone shapes (Mayr 1890). Arguing against a name that previously had been used for a different species, Lemmon formally renamed this pine P. attenuata in 1892 (Lemmon 1892a, 1892b), a name that has been undebated ever since.

Taxonomy Since 1900. 1. Bishop Pine. There is increasing recognition that the northern populations of bishop pine, from Ft. Ross north to Trinidad and sometimes including the Marin and Monterey populations, differ greatly from the rest of the species in many physiological and morphological characteristics, and in their within-population coefficients of variation. (Metcalf 1921; Duffield 1951; Fielding 1961a; Doran 1974; Everard and Fourt 1974; Shelbourne, Bannister and Wilcox 1982). Although these observations apply to all stands north of Ft. Ross, an unusual transition occurs in other traits in the continuous pine forests of this region. Several morphological and biochemical characteristics change abruptly near the south end of Sea Ranch, 27 km north of Ft. Ross: There is a transition over approximately 2 km, from "green" foliage typical of all populations south of Sea Ranch to "blue" foliage typical of populations north of Sea Ranch (Duffield 1951). Distinctive differences in the shape and waxiness of the chambers above the stomata determine foliage color. Geographically coincidental with this color change is an abrupt transition in the composition of the monoterpene fraction of xylem resin (Forde and Blight 1964; Mirov, et al. 1966). South of the transition, from Ft. Ross to Monterey, delta-3-carene predominates, whereas to the north, alpha-pinene is the major terpene. Flowering phenology and allozyme frequencies of an enzyme locus also differ greatly north and south of this narrow transition (Millar 1983). The causes for this unusually sharp transition are enigmatic, and Critchfield (1967) speculated that crossing barriers between blue (= blue-foliaged) and green (= green-foliaged) trees may maintain the abrupt transition.

The Pt. Reyes/Inverness Ridge stands are the best studied bishop pine stands in Marin County. The pines there resemble northern trees in some aspects of growth, and in bark and needle characteristics, but have higher coefficients of variation in all traits studied (Duffield 1951; Fielding 1961a; Doran 1974; Shelbourne, Bannister and Wilcox 1982). Furthermore, cone sizes and shapes vary and resemble those in southern populations more than they resemble those of the Sonoma green population. The high percent of delta-3-carene in the terpenes of the Pt. Reyes/Inverness Ridge stands is similar to the terpene composition of the population from Ft. Ross to Sea Ranch and of the bishop pine population near Monterey.

In addition to the bishop pines on Pt. Reyes, there are several scattered stands on dry exposed ridges up to 13 km inland. These stands have not been carefully studied, although I visited them and noted that the trees have green foliage and resemble the Pt. Reyes stands in growth form and cone variation.

The small Monterey population of bishop pine is unique in its proximity to native stands of Monterey pine, which completely surround these bishop pines. These trees are distinct from those of all northern populations in growth, bark, and cone traits. In other morphological traits, and coefficients of variation, this population is intermediate between northern and southern populations (Duffield 1951; Fielding 1961a; Doran 1974; Shelbourne, Bannister and Wilcox 1982). Like the southern Sonoma and Marin populations, delta-3-carene is the major terpene (Forde and Blight 1964; Mirov et al. 1966).

Although recent authors have agreed that all the northern populations described above belong in *P. muricata,* the taxonomic importance of the differences between the groups and locations of boundaries between groups have been treated differently. Duffield (1951) proposed but never published the name "*P. muricata* var. *borealis*" for a grouping of several northern populations. It is unclear, however, just which populations this name applied to: In several places he stated that this variety comprised all populations from Ft. Ross northward (including the green population from Ft. Ross to Sea Ranch), whereas in his abstract he described var. *borealis* as having "white-waxy stomatal courts", thus implying only blue populations from Sea Ranch northward. Duffield left the Marin and Monterey populations in an undetermined status, suggesting that the Marin population is intermediate between his "var. *borealis*" and typical *P. muricata.*

Duffield's "var. *borealis*" has been commonly used in the literature. Usually the name refers to populations north of Sea Ranch characterized by blue foliage, although the relationship of the green population from Ft. Ross to Sea Ranch is often unclear or unspecified (Fielding 1961b; Forde and Blight 1964; Mirov et al. 1966; Doran 1974; Shelbourne 1974). Other authors, without specifying a name, have referred to blue and green races, or alpha-pinene and delta-3-carene races as distinguishable groups (Everard and Fourt 1974; Cobb and Libby 1968; Critchfield 1967; Doran 1974). Based on cone observations, Axelrod (1980) invalidly described a "*Pinus 'borealis'* Duffield" as a relictual species extending northward from Ft. Ross. In 1983, he formally published Duffield's name, *P. muricata* var. *borealis,* which, contrary to his earlier descriptions, included the Marin population and all populations northward, plus a few "relict trees...on Huckleberry Hill, Monterey" (Axelrod 1983, p. 76).

On the mainland south of Monterey, bishop pines may be distinguished from all northern populations by many traits, and by a north to south cline of increasing within-population morphological and biochemical variation. In all traits studied, the San Vicente stands are the most variable, both within and between stands (Duffield 1951; Crowley 1974). The terpenes of the mainland populations south of Monterey differ from both of the two northern terpene groups, with sabinene and terpinolene predominant and alpha-pinene and delta-3-carene present in only trace amounts (Forde and Blight 1964; Mirov et al. 1966).

In particular, variation in cone-scale armament and cone symmetry increases southward from Marin. Whereas trees from the populations north of Ft. Ross have nearly uniform, asymmetric, heavily armed cones, pines from Marin and Monterey populations bear cones with more variable shapes. Those from the southern mainland populations show a great range of cone variation from one extreme of unarmed, symmetric cones through intermediates to another extreme of heavily armed, asymmetric cones (see Axelrod 1980 for photos). Although the cone variation occurs mostly within stands, two stands near San Luis Obispo, one stand near Lompoc, and two stands near San Vicente have cones that are primarily smooth-scaled (Axelrod 1983, p. 56-57).

Bishop pines growing on Santa Cruz Island (3 stands) and Santa Rosa Island (1 stand) differ little from those on the southern mainland in growth form and coefficients of morphological variation, except in two ways: The Santa Rosa stand seems to be the slowest growing of these populations (Duffield 1951; Crowley 1974; Shelbourne, Bannister and

Wilcox 1982), and Libby (pers. comm.) observed large differences in spring flushing patterns between the island and southern mainland bishop pine populations. A substantial difference between mainland and island populations is the relative abundance of trees with smooth-scaled, symmetric cones on the islands (Mason 1930; Howell 1941; Linhart, Burr and Conkle 1967; Linhart 1978; Axelrod 1983). Although all stands on both islands have intermediate cone types, the Santa Rosa stand and the western stand on Santa Cruz Island show a full range of cone variation. Terpene compositions of these island pines resemble the southern mainland populations in their relatively high percentage of terpinolene and sabinene and trace amounts of delta-3-carene, but differ in having high amounts of alpha-pinene (similar to the northern mainland populations) and beta-pinene (unique in bishop) (Forde and Blight 1964; Mirov et al. 1966; Linhart, Burr and Conkle 1967).

The extreme variation in cone-scale shape and cone symmetry has been the basis for the taxonomic controversy over the southern mainland and island populations. Some taxonomists continued the earlier claim that _P. radiata_ var. _binata_ grew on the California islands (on Santa Cruz, Sargent 1947; on Santa Rosa, Abrams 1923). Mason (1930), however, elevated the smooth-scaled, symmetric-coned pines of Santa Cruz Islands to species status, formally naming them _Pinus remorata._ Although the primary basis for this designation was the cone shape, Mason further distinguished _P. remorata_ as having more resin canals (6-12) and stouter, darker-green foliage than _P. muricata._ At the time, resin-canal counts were not available from all mainland populations, nor did Mason seem aware of the occurrence of trees with remorata-like cones scattered throughout the mainland populations from Marin south. Mason suggested that *P. muricata* grew with *P. remorata* on the islands.

Subsequent investigators have disagreed on the existence of *P. remorata* as a distinct species. Some paleobotanists, who have found cones of the remorata type from Pleistocene deposits, have regarded the species as a lineage distinct from *P. muricata* (Chaney and Mason 1930; Mason 1930, 1949; Johnson 1977; Axelrod 1967, 1980, 1983). In stressing this distinctiveness, Axelrod (1983 p. 56) emphasized that *P. remorata* is not restricted to the island populations, but is growing rather extensively on the central and southern mainland with *P. muricata* wherever the remorata-type cone is found.

Several neobotanists, however, concluded that, with the exception of cone-scale armament and cone symmetry, *P. remorata* does not differ distinctly from the southern mainland bishop pines, and deserves at most varietal rank. Duffield (1951) described but never published *P. muricata* var. *remorata,* which nevertheless has been widely used in the literature. In an intensive comparative study of the island pines, Linhart, Burr and Conkle (1967) found no within-tree correlations among smooth, symmetric cones, stout needles and high resin-canal numbers, and concluded that "*P. remorata* is primarily a name given to a particular cone type in a variable species". Terpene compositions varied among their samples of island stands, with differences related to population of origin and not to cone type (Linhart, Burr and Conkle 1967).

Another taxon growing in two of the Santa Cruz Island stands has recently been formally described. *P. muricata* var. *stantonii,* sympatric with P. *remorata* and *P. muricata,* is distinguished by "broadly triangular apophyses that often are directed outward, and...cones [that] have fewer scales than the associated cones of *P. muricata* var. *muricata*" (Axelrod 1983). Four fossil cones found in a conifer deposit dated 1.0 million years before present at Seacliff (southern California) are similar to *P. muricata* var. *stantonii,* and were used as evidence that this variety was formerly more widespread (Axelrod 1983).

Aside from the taxonomic problems created by the presence of smooth-coned pines in the southern mainland populations, the bishop pine stands near San Luis Obispo and Santa Barbara are commonly agreed to be typical *P. muricata* as described by Don. Lemmon's (1895) varietal designation for the San Vicente population, *P. muricata* var. *Anthonyi,* was retained by a few modern authors (Abrams 1923; Mason 1932), but mostly has been rejected. More commonly, pines at San Vicente are referred to simply as typical southern *P. muricata,* with affinities to the Santa Barbara and San Luis Obispo populations.

2. Guadalupe and Cedros Island Pines. The pines of Guadalupe and Cedros Islands, which gave 19th-century naturalists such problems, have continued to perplex modern taxonomists. Characteristics that have led to confusion are the predominantly two-leaved fascicles; the small, often smooth-scaled cones; resin-canal numbers; phenology (closer to mainland *P. radiata* than to *P. muricata,* Libby, pers. comm.), terpenes (similar to mainland *P. radiata,* Linhart, Bannister and Libby 1968; Bannister and McDonald 1983), and crossing relations (Libby, pers. comm.). Most modern authors accept that the Guadalupe pines are a population of *Pinus radiata;* many use the infraspecific name, var. *binata.* The validity of this name is unclear, since Engelmann (1880) first described it as *P. insignis* var. *binata,* Lemmon (1895) transferred the variety name *binata* to *P. radiata,* and Howell later formally published it as *P. radiata* forma *binata* (Howell 1941). Some authors have suggested that both *P. radiata* and *P. radiata* var. *binata* grow on

the island (Mason 1932). The pines of Guadalupe Island also have been put into other taxa: *P. radiata* forma *guadalupensis* (Howell 1941, formally described); *P. muricata* (Mason 1932); and *P. remorata* (McMinn and Maino 1935; Mason 1949).

Cedros Island pines also have had many names. The first formal description was by Howell (1941), as *P. muricata* var. *cedrosensis.* Other authors have classified these pines as *P. muricata* var. *Anthonyi* (Abrams 1923), *P. remorata* (Mason 1930, 1949; Axelrod 1967), *P. muricata* (Greene 1888; Mason 1932), and *P. radiata* var. *binata* (Axelrod 1967). Forest geneticists in California, New Zealand and Australia have confirmed the suggestions that the Cedros Island pines are related to the Guadalupe Island pines, and that both are more closely related to *P. radiata* than to *P. muricata* or *P. remorata* (Newcomb 1959; Fielding 1961b; Brown 1966; Libby pers. comm.). Axelrod (1980) formally transferred the varietal name to *P. radiata,* creating the new combination, *P. radiata* var. *cedrosensis* (Axelrod 1980). Subsequent genetic studies continue to confirm this relationship to *P. radiata* (Bannister and McDonald 1983; Libby pers. comm.).

3. Monterey and Knobcone Pines. The taxonomy of the Mexican island pines and their relation to *P. radiata* are discussed above. The mainland Monterey pine populations at Año Nuevo and Monterey are widely accepted as composing typical *P. radiata,* with Monterey pine at Cambria distinguished from these two populations by its large cones. An old idea that two separate species, *P. radiata* and *P. insignis* grow together at Monterey was again suggested by Todd (1934), although common garden experiments do not support this contention. Current interest has focused on the tremendous infraspecific variation within and among the Monterey pine populations (Fielding 1953, 1961a; Bannister et al. 1962;

Forde 1964; Cobb and Libby 1968; Hood and Libby 1980; Guinon, Hood and Libby 1982; Bannister and McDonald 1983). Similarly, knobcone pine is widely recognized as a single highly variable species (Newcomb 1962), notwithstanding Axelrod's (1983) suggestion that some pines near the coast may actually be P. linguiformis, a fossil species that resembles P. attenuata.

4. Related Members of Subsection Oocarpae. The four Latin American closed cone pines, P. patula in eastern and southern Mexico, P. greggii, in eastern and northeastern Mexico, P. pringlei, in southern Mexico, and P. oocarpa, extending from northwestern Mexico to northern Nicaragua, form a less coherent assemblage than their Californian relatives (Critchfield 1967). They differ from the California group in cone shape, cone serotiny and resin-canal position (Martinez 1948; Critchfield 1967). Plate tectonic and floristic evidence suggest that the Oocarpae originated in western Mexico before the Miocene with a P. oocarpa-like ancestor (Critchfield 1967; Axelrod 1980).

PREVIOUS STUDIES ON NATURAL AND ARTIFICIAL HYBRIDIZATION IN BISHOP PINE

Natural Hybridization. Many of the puzzling patterns of variation in bishop pine have been attributed to natural hybridization and introgression. The intermediacy of the Monterey and Marin populations was interpreted as hybridization between southern and northern races of bishop pine (Duffield 1951): variation in the Monterey bishop pine population was ascribed to hybridization with Monterey pine (Mason 1949; Stebbins 1950; Duffield 1951; questioned by Forde 1964 and Critchfield 1967); and the sharp transition between blue and green races at Sea

Ranch was explained as very recent hybridization between formerly isolated groups (Mirov et al. 1966; Millar 1983).

The origin of trees with symmetric smooth-scaled cones (ie, remorata-type cones) has been explained as introgression between Monterey and bishop pines. Newcomb (1959) explained this cone variant as the result of introgression of mainland Monterey pine genes into bishop pine growing on the Channel Islands; Mirov et al. (1966) presumed that Monterey pine was on the islands first, and infiltration of mainland bishop pine genes created the cone variant. Mason (1949) used *P. remorata* to exemplify a species that is vanishing due to hybridization with introgression from aggressive, invading *P. muricata.*

In two recent accounts based on cone variation, Axelrod (1980, 1983) reinterpreted much of the bishop pine history. Although details differ between his reports, a general proposal of pervasive hybridization and introgression remains central: present-day bishop pine is a recent hybrid between *P. remorata* and *P. muricata* (or "*P. 'borealis'* Duffield" in the 1980 article). *P. remorata sensu* Axelrod refers to a distinct lineage of pines with smooth-scaled, symmetric cones, which presently grows on the islands and mainland, but formerly ranged widely on sterile, well-drained sites; *P. muricata* (or "*P. 'borealis'* Duffield") *sensu* Axelrod designates a pine that coexisted with *P. remorata* during the Pleistocene and that bore exclusively heavily armed cones.

Axelrod's evidence for hybridization came from comparisons of cone morphology in fossil suites and extant populations. Whereas modern populations of bishop pine (especially mainland populations south of Marin) have a wide range of cone-scale variation, Axelrod stressed that fossil cones fall into only two classes--either smooth-scaled or heavily scaled (eg, remorata-like or muricata-like, sensu Axelrod). Arguing that increasing drought in the late Pleistocene and Xerotherm (the hot, dry period that occurred about 2000-4000 years before present) favored P. remorata traits, Axelrod suggested that P. remorata hybridized in varying degrees with P. muricata throughout its range to produce the cone variation present in modern populations. Axelrod (1983, p. 58) also observed that P. remorata "now crosses with...vars. borealis and stantonii", although to a limited extent with the former, as "there are few parental remorata trees...in the northern populations, just their back-, back-, back-cross introgressive progeny" (p. 55).

The dependence on cone morphology in most of these hybridization scenarios leaves them open to challenge as additional and different kinds of evidence accumulate. For instance, terpene data are inconsistent with the hypotheses that southern and northern bishop pine populations hybridized to form the Monterey and Marin populations (Mirov et al. 1966), and terpene and crossing data do not support the claim that bishop and Monterey pines hybridize at Monterey (Forde and Blight 1964; Critchfield 1967).

Artificial Hybridization. Artificial crossing studies often give the best clues to the likelihood of possible natural hybridizations, and add powerful evidence to phylogenetic relationships among taxa. An extensive study of crossability in the closed cone pines, conducted between 1927 and 1963 at the Institute of Forest Genetics, Placerville, California, was summarized by Critchfield (1967). Brown (1966) independently studied crossing relations, flowering phenology and other reproductive traits in the closed cone pines. Taken together, these studies give the following picture of crossing relationships within the bishop pine complex. In these studies, crossability was based on the relative numbers of sound seeds produced from the different crosses.

In artificial crossing studies, bishop pine did not act as a single unit in breeding behavior. Trees from several northern sources easily interbred, as did trees from southern populations. However, trees of the blue race were almost completely isolated by reproductive barriers from the southern (south of Monterey) elements of the species. The Monterey population, used in a small number of crosses, was intermediate to these two units, and crossed poorly with the southern populations, and somewhat more easily with the northern populations. San Vicente and Channel Island trees crossed more readily among populations than within populations.

Crosses of bishop pines to pines of other taxa also suggested that bishop pine is not a single breeding unit. All crosses failed between northern bishop pines (blue population) and Monterey pines, Guadalupe and Cedros Island pines, and with all attempted knobcone pine, whereas Monterey bishop pine as a parent set some seeds in these combinations, and Channel Island bishop pines as parents produced many sound seeds in the same crosses. Bishop pines from San Vicente crossed fairly easily with knobcone pines, but not at all with mainland Monterey pines, and poorly with Guadalupe Island pines.

Nursery studies suggested that when sound seeds result from inter- and intra-specific crosses, there is no hybrid breakdown in growth and vigor of the resulting F_1 seedlings. Hybrid seedlings from all crosses that yielded sound seeds grew as well or better than within-population control-cross seedlings in their first year.

Crosses of _P. patula_ with northern bishop pines (blue population) and the Channel Island pines failed to set sound seed.

These results suggest that bishop pine is divided into three crossing groups--a northern, divergent group, isolated from related species as well as from its southern congeners; a southern group, able to cross with related species; and a central group, intermediate in its crossing relations.

The results of these studies by Critchfield and Brown suggested the need for further testing of crossing relations in the bishop pine complex. Certain combinations that had not been included became important in light of these first results; for instance, the combination involving parapatric blue and green bishop pine populations near Sea Ranch, and the combination involving Mendocino and Monterey bishop pine populations. Other combinations had been based on small numbers of crosses, and required verification. The present study reports and then integrates further crossing work in the bishop pine complex.

METHODS

Parent Trees. Crosses on bishop pine were made during two different periods, and in three localities (Table 1). In 1965-66, crosses were made by Dr. W. B. Critchfield of the Institute of Forest Genetics (IFG) on blue and green trees growing in native stands near Sea Ranch, and on bishop pines of different origins planted at IFG in the Sierra Nevada. Although bishop pine grows under stress in the foothill climate at IFG (825 m), trees of all these populations have thrived sufficiently to have been used as parents in crossing studies since the 1920's.

In 1980-81, crosses were made by the author at IFG on a single blue bishop pine and on four blue x green F_1 hybrids (Table 1). These hybrids originated from crosses made by Critchfield in 1965, and had been planted in 1968. Although the trees were spindly and weak, they had been flowering for several years. Crosses in 1980-81 were also made by the author on 13- and 14-year-old bishop pines growing in a common-garden plantation at the University of California's Russell Reservation, 16 km east of Berkeley. Bishop pines grow 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 (Table 1), and stored frozen for a year prior to use. All pollen for the 1980-81 crosses was collected from plantation trees, and for 1980-1981 studies, only crosses using fresh pollen are reported since pollen that had been stored at 4° C died.

Breeding Techniques and Terminology. The crosses that yielded harvestable cones are listed in Table 2-1 (1965-66 crosses) and Table 2-2 (1980-81 crosses). Trees were pollinated and seeds processed using standard techniques (Cummings and Righter 1948). Sound seeds from 1965-66 pollinations were sorted using a clipper mill or, if less than 10 seeds per cone were harvested, by x-ray; from the 1980-81 crosses, sound seeds yields were determined by germination of all harvested seeds.

Excluded from this summary are crosses that failed to yield cones or had severely insect-damaged cones, crosses with incomplete data, and crosses that used one-year-old refrigerated (not frozen) pollen.

An attempt in this study is 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, that is, only one male parent contributed pollen to each of these crosses. In these single-pair matings, the number of attempts for a given cross is also the number of males and the number of females involved in the cross. In the single cross where a pollen mix was used (San Vicente X San Vicente, pollen mix of the same two males), each attempt involved three parents: one female and two males.

Crossability refers to the ease with which two taxa can be successfully crossed, compared to control crosses within the maternal taxon. Controls reported here for each cross combine results from control crosses using the same female as used in the experimental crosses, and also control crosses using different females of the maternal taxon. Crossability is the relative amount of sound seeds per cone produced from between-population crosses expressed as a percent of the number of sound seeds from their within-population control crosses.

This method of calculating crossability was the most appropriate for my data. Other methods incorporate conelet abortion data into crossability indices, and require a knowledge of actual conelet abortion as distinct from conelet loss through experimental accidents (branches breaking, cone insects, inviable pollen). Since I did not distinguish between these events, the crossability estimate involving relative seed production is more biologically meaningful. Because crossability figures have been calculated in different ways, I have deemphasized the use of actual crossability percents in this paper. Instead, I used the figures as indicators of relationships, and, where appropriate, grouped together crosses with similar crossability estimates.

Differences in sound-seed set and percent germination were tested using analyses of variance and chi-square methods, with a rejection significance level of $p = 0.05$.

Nursery Techniques. Sound seeds from 1965 and 1966 pollinations were stratified 60 days and sown in randomized block designs at the IFG research nursery in May 1967 and May 1969, respectively. By contrast, seeds from the 1980 Russell, 1981 IFG, and 1981 Russell pollinations each were soaked overnight, and germinated in petri dishes in an incubator (22°C) in June 1982, May 1983, and November 1983, respectively. Germinants were transferred to leach tubes in a UC Berkeley nursery soon after germination. Seedlings from 1980 pollinations were in a lathhouse continuously since planting, whereas 1981 seedlings grew in a glasshouse with 16-hour photoperiod for five months before transfer to the lathhouse. First-year survival was recorded and heights were measured on all seedlings; second-year survival and height data were taken only for the 1980 pollination seedlings.

2. Resin Canal Numbers. Counts of the numbers of needle resin canals were made for several populations that had not been sampled previously, including Marin, Sonoma (green race) and Monterey bishop pine. Recounts were made on the Sea Ranch, San Vicente, Guadalupe and Cedros Island pine populations to compare with previously reported numbers. In several populations, trees were divided into two cone classes (smooth-scale vs. protruding-scale) for resin canal counts. Two to five needles per tree (one per fascicle) were sampled from 10-25 trees for each population. Foliage was collected from trees growing in native stands of the Sonoma, Marin, Monterey and San Vicente populations, whereas foliage was collected from 20-year-old plantation trees (which originated as wind-pollinated seeds collected in wild stands) from the Cedros and Guadalupe Island populations. Needles were hand-sectioned near the midpoint, and sections were stained with phloroglucinol prior

to observation.

RESULTS

Crosses of Northern Bishop Pine Populations.

1. Seed Data. The results indicate that, under conditions of artificial pollinations, blue and green trees from northern sources easily interbreed (Table 3-1). In 1965-66 crosses, the average yield of sound seeds from blue x green crosses did not differ significantly from blue x blue control crosses (Table 3-1). This occurred both when native-grown and plantation trees were used as parents, despite large differences in magnitude of overall seed-yield from the two parent-tree locations. Average numbers of sound seeds produced by native-grown parents were 24.0 for green x blue crosses, 64.8 for blue x green crosses, and 48.3 for blue x blue; for plantation trees, blue x green crosses yielded 4.7 seeds compared with 4.0 for blue x blue control crossesa

Germination percents, expressed as the average percent of the total number of seeds per cone that germinated, estimate the proportion of sound seeds per cone, independent of cone size (Table 3-1). The 1965-66 figures show the same pattern as above, namely that germination percents of green x blue (82.8% on native-grown trees) and blue x green families (87.2% on native, 50.1% on plantation trees) did not differ significantly from germination percents of blue x blue control cross families from either native-grown (81.2%) or plantation (67.2%) trees.

Results from crosses made in 1980-1981 at Russell corroborated the 1965-66 evidence that the blue and green trees easily interbreed: green x blue and green x green control crosses did not differ significantly in average numbers of sound seeds produced per cone (5.7 and 8.2, respectively), or in percent germination (44.8% and 46.3%, respectively) (Fig. 3-1). The lower seed set for the blue control crosses at Russell (1.1 sound seeds/cone) probably was due to seeds shedding from these cones--which prematurely opened prior to harvest--especially since germination percent of this cross (31.9%) resembled the other control and blue x green families.

Crosses in 1981 that involved the blue x green F_1 hybrids gave a different picture of crossing relations (Table 3-1). At IFG, in crosses using the F_1's as females (namely, F_2 crosses and backcrosses with blue and green pollen parents), the overall average numbers of sound seeds per cone (2.6) and overall percent germination (10.3%) were very low compared to crosses on the unrelated single blue female parent at IFG (overall mean sound seed/conea= 41.2, and overall mean germinationa= 86.9%). Even if data from this single blue female parent are not representative, seed set and percent germination on the F_1 hybrids were significantly lower than seed set and percent germination at IFG for any of the 1965-66 crosses.

In contrast to the results obtained when the four F_1's were used as female parents, when these same four F_1's were used as the pollen parents at Russell, the values for sound-seed set and percent germination resembled the Russell blue x blue and green x green control crosses and crossability estimates were high (Table 3-1).

2. Nursery Data. Overall first-year (all pollinations) and second-year (1980 and 1981-IFG pollinations only) heights of blue x green F_1 hybrids, F_2 hybrids and backcross hybrids were not significantly different from their within-population control families (Table 3-2). The ranking of seedling mean heights (from Table 3-2) of the different

crosses for each pollination group are summarized below. The letters B, G, and F_1 stand for blue, green and F_1 trees, respectively, each pair of letters indicates the cross type, with females given first in the pair.

First-year heights:

1965 pollinations (IFG)	$BG \geq BB$
1966 pollinations (IFG)	$BG \geq GB \geq BB$
1980 pollinations (Russell)	$BB \geq GG \geq GB$
1981 pollinations (Russell)	$GF_1 \geq GB \geq GG \geq BB \geq BG \geq BF_1$
1981 pollinations (IFG)	$BB \geq BF_1 \geq F_1F_1 \overset{=}{a} F_1B \geq F_1G$

Second-year heights:

1980 pollinations (Russell)	$GG \geq GB \geq BB$
1981 pollinations (IFG)	$F_1B \geq BF_1 \geq F_1G = BB \geq F_1F_1$

First- and second-year survival of F_1 hybrids and their controla families was mostly over 80% and, with the exception of the 1966-pollinated seedlings, there were no significant differences in survival. In the 1966-pollinated data, survival of green x blue seedlings (83.3%) was marginally but significantly lower than survival of either blue x green (91.5%) or blue x blue control (96.0%) seedlings.

Overall first- and second-year survival of seedlings from crosses on F_1 female parents (33%) was significantly lower than survival ofa seedlings from blue x blue control crosses (55%) and from blue x F_1 crosses (72.1%). However, the seedlings that survived from F_1 femalesa grew as well as the blue x blue control seedlings and the blue x F_1 seedlings in their first and second years. With few exceptions, seedlings that survived the first year also survived the second year.

Crosses of Widespread Bishop Pine Populations.

1. Seed Data. The 1965-66 crosses at IFG among trees sampling bishop

pine populations from throughout the species range supported the patterns of relationships suggested by pre-1965 crossing studies (Table 4-1). On the basis of sound seeds produced and germination percents, the blue populations north of Sea Ranch crossed easily with the Marin population (crossability over 100%); easily with the Monterey population in one direction (using a northern blue female parent, crossability over 100%) but less easily in the reciprocal cross (52% crossability); and did not successfully cross at all to green trees from Purisima and San Vicente (0% crossability).

The green population in southern Sonoma County crossed with less success than the blue population to Monterey trees (27% crossability), and was nearly unsuccessful (crossability $< 5\%$) in crosses with San Vicente trees. A similar pattern followed in crosses with Marin trees: Green trees from Marin crossed with low success to Monterey (26% crossability), and in the single attempt made, Marin was not successfully crossed with Santa Cruz Island trees (0% crossability). Similarly, populations south of Monterey easily interbred (Santa Cruz x Santa Rosa Islands, and San Vicente x Purisima), whereas these populations did not successfully cross, or set germinable seeds very rarely, with the Monterey, Marin, and northern populations of Sonoma and Mendocino. In most crosses between the Monterey population and populations to the north (blue and green), crossability was at a medium level (26-52%), although the Mendocino x Monterey cross had over 100% crossability. Monterey trees were also crossed with San Vicente and Channel Island pines, and crossability was very low (less than 6%). The results of crosses with Marin population trees again resembled the results of similar crosses on trees from the Sonoma and Mendocino populations.

In two attempts to cross San Vicente bishop pines with Guadalupe and Cedros Island pines, none of the 39 seeds produced was sound (Table 4-1).

2. Nursery Data. Mean first-year heights of seedlings from interpopulational crosses were similar to the means of seedlings from their control crosses (Table 4-2). There were no cases where inter-population hybrid seedlings had means significantly different than their within-population control cross seedlings. Means for the different within-population controls differed significantly: the Monterey x Monterey control crosses (1965) averaged the shortest ($\overline{x}$ = 23.8 cm), the Mendocino x Mendocino control crosses (1965-66) were intermediate ($\overline{x}$ = 26.3 cm), and the San Vicente x San Vicente control crosses (1965) averaged the tallest ($\overline{x}$ = 34.2 cm). Only one interpopulation family, Montery x Purisima, had a very short height (6.5 cm), but this was the height of a sole surviving seedling. Seedlings grown from 1966 inter-population pollinations grew slightly taller than the 1965 inter-population cross seedlings, although there were only two comparisons, and unfortunately, the Monterey control cross was lacking. The interpopulation cross between Santa Cruz and Santa Rosa Islands (no controls available) stood out as having the greatest nursery mean first-year height (47.0 cm) of all hybrid and control crosses.

Survival of hybrid seedlings did not differ significantly from survival of the within-population control-cross seedlings, although survival varied considerably between years and among the female parents. Over 95% of the seedlings from 1966 pollinations survived, whereas less than 80% of those from 1965 pollinations survived. Similarly, 72% of the seedlings from 1965 crosses on Mendocino and San Vicente females survived, whereas only an average of 42% of the seedlings from 1965 crosses on Monterey mothers survived.

Resin Canal Counts. The average numbers of resin canals are near two in northern bishop pine populations, and increase clinally to 6 and more in the southern populations (Table 5). In the four populations where trees were divided into two cone types (armed and unarmed), the mean numbers of resin canals did not differ significantly between the groups. The mean numbers of resin canals in the Guadalupe and Cedros Island pines were low (2.0 and 2.6, respectively), and although they were slightly higher than the means for mainland Pinus radiata, they differed substantially from the nearest bishop pine populations (Table 5). Bannister's (1958) report from one tree of 7.0 resin canals for Guadalupe Island seems exceptionally high.

DISCUSSION

The relationships of the populations of California closed cone pines may be visualized by a web of crossabilities, with links of varying degrees of strength (Fig. 2). Under artificial pollination conditions, some populations appear to be joined by bonds of high crossability and others are not linked to each other, due to apparent reproductive barriers. When crosses from the present study are summarized with older data (Critchfield 1967), three distinct breeding units, which were observed in previous crossing work, stand out (Fig. 2): 1) northern bishop pine populations from Marin northward, 2) southern bishop pine populations including mainland and Channel Islands populations from Purisima southward, and 3) Monterey pine, including Guadalupe and Cedros

Island pines, and knobcone pine. (This latter group is further strengthened by unpublished breeding data from Libby, which indicate that all mainland populations of Monterey pine can be crossed readily with both Cedros and Guadalupe Island pines, and also that the two Mexican island pines can be crossed easily together.)

Critchfield (1967) discussed the significance of these units, especially the unusual presence of crossing barriers within bishop pine between northern and southern populations, the intermediacy of the Monterey bishop pine population, and the link of the southern bishop pine populations to knobcone and Monterey pines. The present work filled in some missing links among the bishop pine populations (shown by Fig. 2), and strengthened the conclusions of former reports about the delineation of breeding units in bishop pine.

The most significant new finding is the lack of crossing barriers between a northern blue population (Mendocino and northern Sonoma Counties) of bishop pine and the northern green populations (southern Sonoma Co., Marin, and, less so, Monterey). When evidence for crossing barriers had been found earlier between northern blue and southern green populations (Critchfield 1967), it seemed logical to speculate that the anomalous, abrupt transition at Sea Ranch between these blue and green morphs also involved crossing barriers. Subsequent observations, however, indicated that these two adjacent populations could interbreed: Native trees with intermediate terpene and foliage types were found in very low frequency at Sea Ranch (Duffield 1951; Mirov et al. 1966), and isozyme analyses of a marker locus pointed to the probability of hybridization (Millar 1983).

The problem of understanding how the abrupt transition is maintained becomes harder to understand once high crossability is demonstrated. Despite the ease of artificial hybridization between these contiguous populations, however, other barriers may reduce natural gene flow across the blue-green transition. Although flowering periods of the two populations overlap, significant differences in peak flowering times (Millar 1983) must reduce natural hybridization. Prevailing northwesterly winds during the pollination season appear to interact with the pattern of flowering phenology to further confine gene flow to a north-to-south (blue to green) direction, as substantiated by isozyme profiles (Millar 1983). Possibly also, alien pollen may not compete well with native pollen, for example, green pollen on blue females.

The possibility of a second barrier at the level of F_1 female infertility was suggested by the poor seed yield of the F_1 female parents in all crossing combinations, and the poor seed germination and seedling survival from these crosses. If this is a barrier, and not just an effect of a weak condition of the F_1 trees at IFG, it would be unusual for pines. Generally in *Pinus,* when interpopulational or interspecific crosses succeed, the progeny of such crosses are vigorous and fertile, although meiotic irregularities may occur more frequently in hybrid than in non-hybrid megagametogenesis (Saylor and Smith 1966). If real, this late-acting barrier might reinforce phenological differences and reduce effective natural hybridization and introgression.

Finally, recent evidence that blue bishop may have evolved on local, highly podsolized, acidic soils (of the so-called pygmy forest) and only recently spread and met green bishop pine on more typical forest soils at Sea Ranch suggests an edaphic explanation for the sharp transition (Millar in prep.).

The present study supported the previous placement of the Monterey bishop pine population as intermediate to the northern and southern bishop pine populations in crossability. Because Monterey bishop pine can be crossed in both directions, it is a link between the reproductively isolated northern and southern populations. The two attempts to cross trees from the Monterey population to trees further south suggest that the Monterey population may be related more closely to northern bishop pine populations than to southern populations. This requires additional work to clarify the relationships of this central population to the northern and southern populations. Furthermore, the next bishop pine population south, in San Luis Obispo County, has never, according to the published records, been included in any interpopulation matings. The crossing relations of this population deserve further study also.

The Marin population seems more closely related than the Monterey population to the northern populations. Marin bishop pine freely interbred with Mendocino blue bishop pines, and resembled the northern populations by failing to cross with Channel Island pine. Unfortunately, no reported attempts have been made to intercross pines from Marin with any of the three southern mainland populations. These crosses also deserve further attention.

Based on our current data, the southern breeding unit may now be expanded to include the Purisima populations, whose trees freely interbred with those of the San Vicente population, and, like San Vicente, were unable to successfully cross with those of Mendocino. The affinities of the Purisima population to other bishop pine populations remains uncertain.

In all cases discussed above, crossing relations among populations were discriminated at the level of relative sound-seed production, and not nursery height of the progeny. In general, first- and second-year nursery mean heights of interpopulational cross seedlings did not differ significantly from the intrapopulational cross seedlings. However, one set of seedlings, which resulted from the cross between Santa Cruz and Santa Rosa Islands, was exceptional in the rapid early height growth of the seedlings compared to seedlings from other crosses. Their mean heights significantly exceeded those from other crosses, including all intrapopulational crosses. A similar pattern of rapid early height growth by the pines of these islands relative to other bishop pine populations was observed by Libby (pers. comm.) and Crowley (1974). These same populations as older trees, however, are among the shortest of the bishop pine populations (Shelbourne, Bannister and Wilcox 1982).

The relationship between crossability and morphological, biochemical, and physiological resemblances among populations is not as clear in the California closed cone pines as in other pines. The numerous, conflicting taxonomies and evolutionary histories proposed for this group reflect the inconsistent patterns of variation at different levels in these pines. As Critchfield (1967) noted, this situation breaks the general rule in *Pinus*, where morphological resemblances usually parallel phylogenetic relationships that are suggested by crossing behavior. Confusion and misunderstandings have resulted when biologists tried to circumscribe certain closed cone pine populations under arbitrary taxa based on single traits such as cone shape. Biological nomenclature is troublesome in that names imply a uniformity among members of the taxon in all traits that may in fact not exist. It is perhaps better to accept the bishop pine ensemble as a polymorphic lineage or group of lineages that is actively evolving, with evolutionary forces affecting it non-uniformly, than to attempt to force certain variants into named taxa. In this ensemble, the most relevant evolutionary unit is probably the individual geographic population, although in some cases (blue and green) there may be reason to further subdivide the units, and in other cases, several populations may be linked by bonds of crossability and other resemblances.

In this regard, I agree with those who have recommended that the number of formal species designations be minimized, and even that the existence of well-defined subspecies or varieties be downplayed. The two northern (blue) populations of bishop pine may be exceptional, given their set of obvious differences from the rest of the complex. However, delineating the southern extent of var. borealis is problematic, should such nomenclature be retained. Crossability and some morphological traits unite the populations as far south as the Marin population, possibly including Monterey. Within this group, however, is the major discontinuity in stomatal anatomy, terpenes, isozymes, and phenology at Sea Ranch. Thus, one could argue that the southern boundary of var. borealis should be Sea Ranch; the variety would then be restricted to the blue, alpha-pinene populations. I take a third option, namely, to discourage the use of the varietal name borealis, and encourage instead the use of geographic names (eg, Mendocino Co. population) or particular variants (eg, blue populations).

Similarly, the cone variant referred to as P. remorata or unofficially as 'P. muricata var. remorata' does not have coherence among the other studied traits. The demonstration on the Channel Islands that cone type is uncorrelated with many other polymorphic traits (Linhart, Burr and Conkle 1967) holds for other populations as well. Not only is cone variation uncorrelated with other traits within populations (eg, resin-canal numbers within Monterey, Purisima and San Vicente populations), but trees with smooth cones do not have a coherence in other traits among different populations. For instance, resin-canal numbers of smooth-coned trees vary by population, as does terpene composition. In the present and former study (Critchfield 1967), parent trees with smooth scales and protruding scales from the Channel Islands behave similarly in crosses to different populations and were fully intercrossable.

The arguments of some paleontologists that the smooth-cone pine (*P. remorata*) is an evolutionary lineage distinct from bishop pine are based on the old age of *remorata-like* fossil cones, and the supposed lack of intermediate cone types in the fossil record. Fossil cones similar to heavily armed bishop pines (*P. masoni*) have been found in small numbers from the late Tertiary in central and southern California. Quaternary deposits from northern Marin to San Diego Counties have been far more prolific, yielding hundreds of fossil cones resembling bishop pine cones. Of these only about 25 have been the remorata-type. Axelrod (1980, 1893) argued that the disproportionately large number of bishop pine cones (ie, heavily armed cones) is a bias of the fossil record, and that *P. remorata* grew in upland dry sites that were not disposed to preservation.

Axelrod further argued that a habitat difference separates extant *P. remorata* and *P. muricata* stands. I disagree that the smooth-cones mark an ecotype within the species, as there are too many mixed-cone stands, with no pattern to the ecological preference of individual trees within these stands. Furthermore, the stands with high frequencies of smooth cones do not appear to differ consistently in their habitat from other stands (Vogl et al. 1977). The present locations of the pure smooth-coned stands in all cases are close to mixed stands, and do not suggest that they would suffer preservational disadvantage relative to other bishop pine populations. In at least one location (San Vicente), the pure smooth-coned stands are in a better position for cone preservation than other stands in the area. Thus, I suggest that the fossil record is not overly biased in representing smooth cones as a minor proportion of cones that existed in southern Californian Quaternary forests. The greater abundance of these smooth cones in central and especially southern extant forests may reflect a distribution already established by the Quaternary, since smooth-coned fossils have been found only in deposits southward from Pt. Sal (near San Luis Obispo).

The distinctness of *P. remorata* has also been claimed on the basis of the absence of intermediate cone types in the fossil record. The existence of extensive cone variation within many extant bishop pine populations was used by Axelrod (1983) to develop his hypothesis that present-day bishop pine resulted from hybridization and introgression between *P. remorata* and Quaternary bishop pine (heavily armed cones). The spread of *P. remorata* genes by introgression supposedly was favored by selection for drought-adaptedness of *P. remorata* during the last interglacial.

From fossil specimens I have seen (University of California at Berkeley, Dept. of Paleontology), I question the interpretation that the fossil cone record lacks intermediates. Of 104 cones labelled *Pinus "borealis"* from Axelrod's (1980) collection, I interpreted 20 as having intermediate cone scales.

In sum, I agree with Linhart, Burr and Conkle (1967) in stating that

"*P. remorata* is primarily a name given a particular cone type in a variable species" (p. 173), and that this cone variant has probably existed within bishop pine populations for a long time over a wide range. Why the frequency of cone variants should vary in space and time in the particular way it has is a question of population genetics. This variation is not unique to bishop pine among the closed cone pines, since a parallel pattern of cone-scale variation occurs in Monterey pine, the pines on Guadalupe and Cedros Islands, and also in knobcone pine (Lemmon 1888; Howell 1941; Newcomb 1962; Strauss and Millar in prep.). Several authors have proposed stochastic and selective explanations for cone variation in Monterey pine populations (Linhart 1978; Axelrod 1980).

Axelrod's proposal of hybridization between *P. remorata* and bishop pine as a means of explaining cone variation may seem conceptually similar to my suggestion that this variation results from the flux of gene flow and other evolutionary forces within an interbreeding ensemble. There is, however, a major difference: When the cone variation is explained as the result of hybridization and introgression between two species, we expect to see correlated patterns of variation in other traits. These patterns are, in fact, not found. Rather, trees with smooth cones resemble the other bishop pines of the population to which they belong more than they resemble each other over the range of the ensemble. This is expected if cone shape is just one polymorphic trait among many within the species. The only other explanation is that *P. remorata* itself is polymorphic with respect to terpenes, resin-canal numbers, etc. This surely must be rejected, since the co-variation with bishop pine in all stands is much too high to be purely coincidental.

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TABLE 1. Parent trees used in crosses of bishop pine in 1965-1966 at the Institute of Forest Genetics (IFG) and in 1980-1981 at the Russell Reservation.

Taxon	Location of Parent	Race or Type	Number of Parents		Pollination Year	Geographic Origin	
			Females	Males			
Bishop Pine	Native Forests	Green	4	2	1966	Sea Ranch S	So Son Co
				6	1965-66	Timber Cove	
				2	1965	Salt Pt	
				3	1965	Inverness	
				2	1965	Monterey	
				3	1965	Purisima	
		Blue	3	2	1966	Sea Ranch N	No Son Co
				4	1966	Gualala	
	IFG	Green	1		1965-66	Monterey	
			1		1965	Sta Cruz Is	
				1	1965	Sta Rosa Is	
			3	2	1965	San Vicente	
		Blue	3	3	1965,1981	Ft Bragg	Men Co
		F_1 Hybrid	1	1	1981	Ft Br x Inv	
			1	1	1981	Ft Br x Mont	
			2	2	1981	Mendo x Inv	
	Russell Plantation	Green	5	5	1980-81	Ft Ross	So Son Co
			2	3	1980-81	Salt Pt	
			3	5	1980-81	Stewart's Pt	
		Blue	2	4	1980-81	Pt Arena	Men Co
			1	3	1980-81	Navarro Riv	
				2	1980-81	Gualala	
			2	2	1980-81	Mendocino[1]	
			3	4	1980-81	Ft Bragg	
Other Taxa	IFG	Guadalupe Is		1	1965	Guadalupe Is	
		Cedros Is		1	1965	Cedros Is	

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[1] Trees from Mendocino pygmy forests.

TABLE 2-1. Crosses made at the Institute of Forest Genetics and in native stands, 1965-66[1].

♂♂ / ♀♀	Green Bishop Pine								Blue Bishop Pine				Other Taxa	
	Sea RanchS[2]	Tmbr Cv	Marin	Mon-terey	Puri-sima	Sta Rosa Is	San Vic	Salt Pt	Ft Bragg	Mendo-cino	Gua-lala	Sea RanchN[3]	Guad Is	Cedros Is
Sea RanchS[2]	(1)	(2)						(3)			(2)	1		
Monterey		2	2	1	2			2			2	2		
Sta Cruz Island			(1)		(1)	1								
San Vicente		2			2		3		2				(1)	(1)
Sea RanchN[3]	3	4						3			3	3		
Mendo-cino		3	1	1	(1)			1	1		3	1		
Ft Bragg	1	3	1	1	1					1	2	2		

[1] Crosses in parentheses produced cones but did not yield sound seeds.
[2] Green trees.
[3] Blue trees.

TABLE 2-2. Crosses of bishop pine made at the Institute of Forest Genetics and Russell Reservation, 1980-1981[1].

	Green Bishop Pine			Blue Bishop Pine					Blue X Green Bishop Pine F_1's			
♀♀ \ ♂♂	Ft Ross	Salt Pt	Stewart's Pt	Gualala	Pt Arena	Navarro	Mendocino	Ft Bragg	Ft Bragg X Invern	Ft Bragg X Mont	Mendo X Tm Cv	Mendo X Invern
Ft Ross	3	3	3	1	2	1	2	2	(1)	1	2	2
Salt Pt	2	2		1				1		1		
Stewart's Pt	2	1	2			4	2	3	(1)		2	
Pt Arena	2		2		2	1	1			1	(1)	1
Navarro	(1)		1		3	3	1		1			1
Mendo	(1)	(1)	(2)	1		4		2	1		1	
Ft Bragg		1	1		1	(1)	(1)				2	
Ft Bragg X Invern	(1)											
Ft Bragg X Mont			1		1				1			1
Mendo X Tm Cv											(1)	
Mendo X Invern	(1)				1					1	1	

---

[1] Crosses in parentheses produced cones but did not yield sound seeds.

TABLE 3-1. Summary of the results of artificial crosses among bishop pines from two contiguous populations north of Ft. Ross.

Parent: Females	Parent: Males	Location of Female	Attempts	Attempts Producing Sound Seed	Female Strobili Pollinated	Percent of Strobili Producing Cones	Total Number of Seeds/Cone: Mean	Total Number of Seeds/Cone: + CI[1]	Number of Sound Seeds/Cone: Mean	Number of Sound Seeds/Cone: + CI[1]	Germinable Seed (%)
I.	1965-1966 Pollinations										
Green	Blue	Native	3	1	16	18.8	29.0	--	24.0	—	82.8
Blue	Green	Native	10	8	101	37.4	75.1	(5.1)	64.8	(5.5)	87.2
Blue	Green	IFG	8	8	118	86.4	8.6	(0.9)	4.7	(0.6)	50.1
	Within-Population Controls										
Blue	Blue	Native	6	6	50	60.0	61.0	(7.7)	48.3	(6.9)	81.2
Blue	Blue	IFG	10	10	178	44.9	6.7	(0.6)	4.0	(0.5)	67.2
II.	1980-1981 Pollinations										
Green	Blue	Russell	19	15	68	79.4	12.9	(3.5)	5.7	(2.1)	44.8
F_1[3]	F_1	IFG	5	3	19	78.9	18.3	(3.0)	2.2	(0.6)	9.5
F_1	Blue	IFG	2	1	4	100.0	8.5	(14.6)	2.5	--	14.7
F_1	Green	IFG	2	1	7	71.4	45.6	--	3.0	--	6.6
Blue	F_1	IFG	2	2	15	100.0	50.8	(1.8)	41.2	(6.0)	86.9
Blue	F_1	Russell	7	5	17	76.5	14.7	(10.3)	8.4	(9.1)	52.6
Green	F_1	Russell	10	6	22	86.4	10.1	(3.3)	5.7	(2.5)	51.0
	Within-Population Controls										
Green	Green	Russell	18	10	43	86.0	17.3	(5.0)	8.2	(2.5)	46.3
Blue	Blue	Russell	6	6	33	75.8	5.3	(0.9)	1.1	(0.5)	31.9
Blue	Blue	IFG	1	1	2	100.0	32.5	--	28.5	--	87.8

[1] CI = 95% Confidence interval, based on average results per attempt,not per cone

[2] Expressed as average percent of total number of seed/cone that germinated.

[3] The F_1's in all cases are blue female/green male combinations.

TABLE 3-2. Summary of first-year height (all pollination years) and second-year height (all 1980 pollinations and only 1981 pollinations at IFG) and survival from crosses among and within tow northern bishop pine populations.

Parents		Number of Seeds or Germinants Sown[1]	Average Heights (cm) ± (CI)[2]							Survival %	
			1st-Year					2nd-Year			
Females	Males		1965 poll IFG	1966 poll IFG	1980 Poll Russell	1981 Poll Russell	1981 Poll IFG	1980 Poll Russell	1981 Poll IFG	1st-year	2nd-year
Green	Blue	36		27.1 (2.1)						83.3	
		37			7.5 (0.7)			22.6 (1.7)		83.8	80.4
		149				11.4 (0.7)				85.9	
Blue	Green	96	27.6 (1.0)							79.2	
		365		27.9 (0.5)						91.5	
		13				10.6 (2.4)				100.0	
F_1	F_1	23					9.9 (3.5)		16.2 (1.8)	26.1	26.1
F_1	Blue	4					9.9 (2.9)		25.3 (6.5)	100.0	100.0
F_1	Green	12					9.0(13.2)		20.7 (1.5)	25.0	25.0
Blue	F_1	49				9.8 (1.0)				91.8	
		104					10.2 (1.0)		23.2 (1.4)	72.1	72.1
Green	F_1	100				11.5 (1.0)				88.0	
Within-Population Controls											
Blue	Blue	48	26.4 (1.6)							75.0	
		327		26.1 (0.5)						96.0	
		43			10.2 (1.2)			20.2 (1.5)		90.7	90.7
		20					10.7 (2.0)		20.7 (3.6)	55.0	55.0
		15				10.8 (2.5)				86.7	
Green	Green	46			9.0 (0.9)			25.5 (2.1)		86.9	84.8
		171				11.2 (0.6)				91.2	

[1] Seeds sown from 1965-66 pollinations; germinants planted from 1980-81 pollinationse

[2] Each group planted at a different time, see text; CIe= 95% confidence level.

TABLE 4-1. Summary of crosses at the Institute of Forest Genetics among widespread closed cone pine populations.

Parents										
Females	Males	Attempts	Attempts Producing Sound Seeds	Female Strobili Pollinated	Percent of Strobili Producing Cones	Total Number of Seeds/Cone		Number of Sound Seeds/Cone		Germinable Seed (%)[2]
						Mean	+ CI[1]	Mean	+ CI[1]	
I. 1965-1966 Pollinations										
Mendocino	Marin	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./N.Son.Co.[3]	4	4	97	33.0	7.4	(3.3)	4.7	(2.3)	68.6
	S. Son. Co.-Green	4	3	74	28.4	5.2	(2.7)	2.4	(0.7)	72.6
	Marin	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	(<.1)	8.1
Sta. Cruz I.	Marin	1	1	7	42.8	9.3	---	0.0	---	---
	Santa Rosa Island	1	1	6	16.7	15.0	---	14.0	---	93.3
San Vicente	Mendocino	2	0	24	75.0	3.3	(1.4)	0.0	---	---
	S. 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 Controls										
Mendocino	Mendo./N.Son.Co.[3]	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[4]	3[4]	27	81.5	11.0	(5.2)	2.2	(1.1)	22.0

[1] CI = 95% confidence interval based on average results per attempt, not per cone.

[2] Expressed as average percent of total number of seeds/cone that germinated.

[3] Blue trees.

[4] Each female pollinated with a mix of the same 2 males.

TABLE 4-2. Summary of first-year nursery heights and survival from crosses among widespread bishop pine populations.

Parents		Number of	Average First-Year Heights(cm) + (CI)[1]		First-Year Survival
Females	Males	Seeds Sown	1965 Poll.	1966 Poll.	(%)
Mendocino	Marin	48	25.6 (1.8)		75.0
	Monterey	48	26.5 (1.8)		62.5
Monterey	Mendo./Sonoma[2]	45		26.9 (1.4)	95.6
	Sonoma Green	24	23.9 (3.0)		54.2
	Sonoma Green	5		31.9 (7.2)	100.0
	Marin	48	24.0 (1.3)		50.0
	Purisima	10	6.5 ---		10.0
Sta. Cruz I.	Sta. Rosa I.	12	47.0 (2.6)		91.7
San Vicente	Purisima	26	38.5 (3.9)		76.9
Within-Population Controls					
Mendocino	Mendocino	48	26.4 (1.5)		75.0
		327		26.1 (0.5)	96.0
Monterey	Monterey	24	23.8 (2.9)		54.2
San Vicente	San Vicente	36	34.2 (3.6)		72.2

---

[1] CI = 95% confidence interval

[2] blue population

TABLE 5. Summary of resin canal counts in the maritime closed cone pines. Counts made on trees in native stands except where noted.

Population, Stand, and Cone Type (where assessed)	Average Number of Resin Canals	Range	Number of Trees	Source
1. Bishop Pine				
Mendocino Blue				
Mendocino	2.3	2-3	30	Duffield 1951
Sea Ranch	2.0	2	30	Duffield 1951
	2.6	2-6	17	present study
Sonoma Green				
Ft. Ross	1.8	1-2	10	present study
Marin				
Pt. Reyes-unarmed	2.2	1-7	15	present study
-armed	2.0	0-2	15	present study
Inland Marin[1]	2.1	1-3	20	present study
Monterey				
Huckleberry Hill-unarmed	3.2	2-7	10	present study
-armed	3.1	2-6	10	present study
Southern California Mainland				
San Luis Obispo	4.1	2-8	--	Linhart et al 1967
Purisima	4.1	2-10	30	Duffield 1951
	3.9	2-7	--	Linhart et al 1967
Channel Islands				
Sta. Rosa Is.	4.4	3-6	--	Linhart et al 1967
Sta. Cruz Is.-unarmed	--	6-12	--	Mason 1930
	7.5	2-11	--	Linhart et al 1967
-armed	6.1	2-10	--	Linhart et al 1967
Baja California				
San Vicente	7.0	3-10	30	Duffield 1951
	8.6	2-14	--	Linhart et al 1967
-unarmed	8.0	3-13	25	present study
-armed	6.8	4-10	25	present study

Population, Stand, and Cone Type (where assessed)	Average Number of Resin Canals	Range	Number of Trees	Source
2. <u>Monterey Pine</u>				
Mainland				
Ano Nuevo	0.98	0-2	100	Forde 1964
Monterey	1.16	0-2	100	Forde 1964
Cambria	0.82	0-2	100	Forde 1964
Islands				
Guadalupe Is.	--	2-several	--	Newcomb 1959
	7.0	3-9	1	Bannister 1958
	2.0[2]	1-3	10	present study
Cedros Is.	--	2-several	--	Newcomb 1959
	2.6[2]	2-5	10	present study

---

[1] Includes 5 trees from each of 4 stands (San Geronimo Ridge, Oat Hill, Bolinas Ridge, and Little Carson Creek).

[2] Counts made on 20-year-old plantation trees.

-- Data not available.

FIGURE 1. Distribution of bishop pine (shaded areas), and locations mentioned in the text.

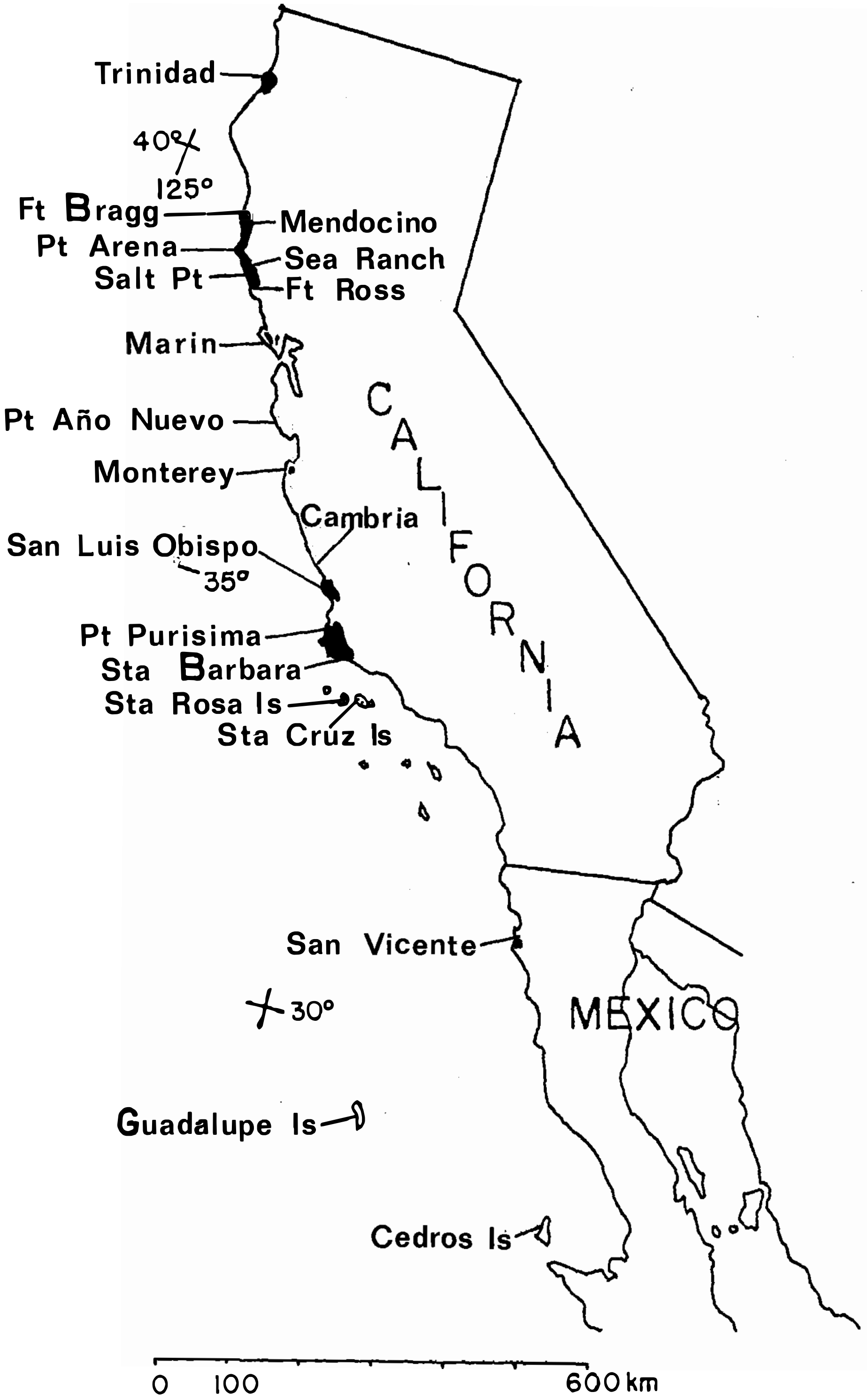
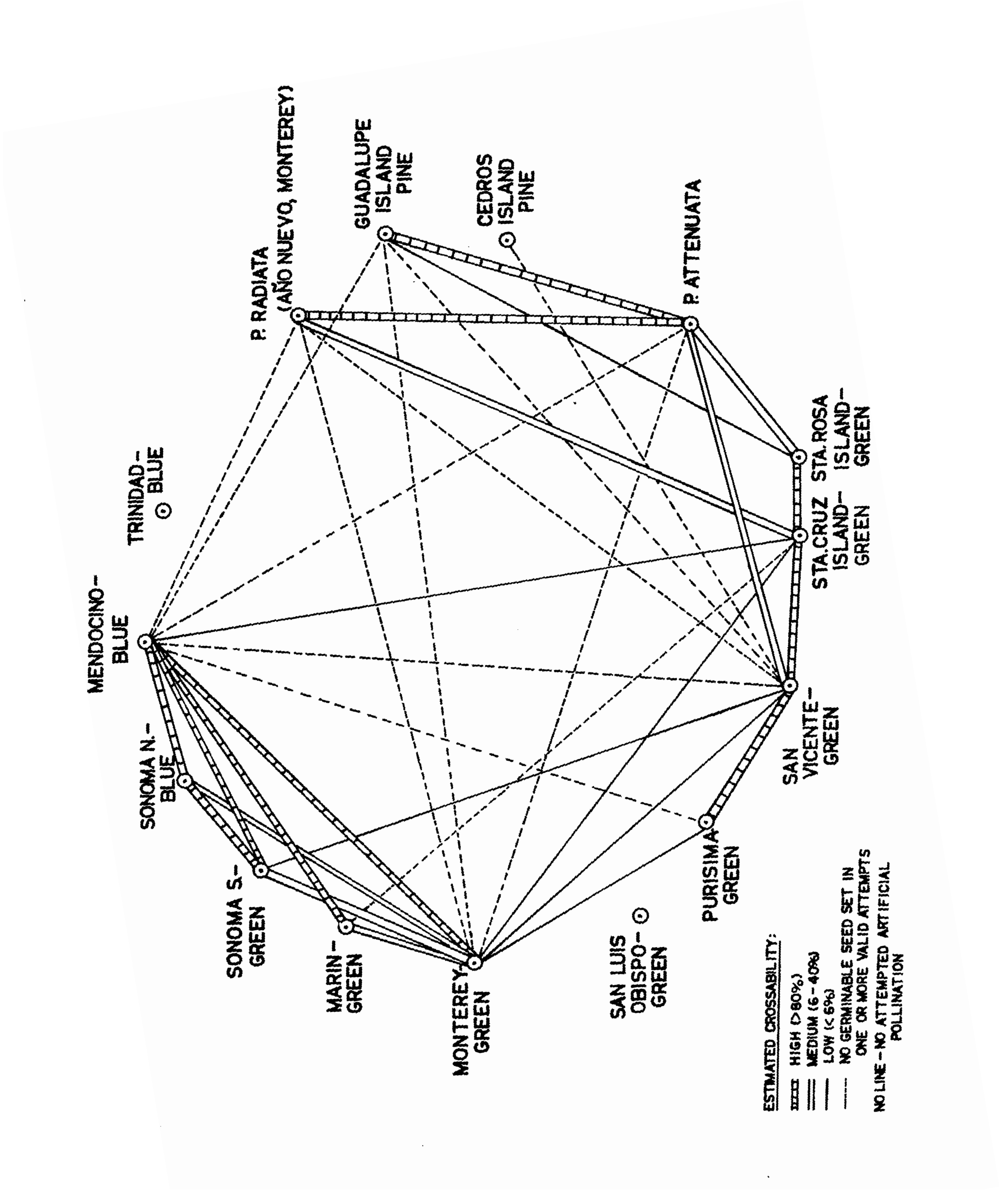

Trinidad
40°
125°
Ft Bragg
Mendocino
Pt Arena
Sea Ranch
Salt Pt
Ft Ross
Marin
Pt Año Nuevo
Monterey
Cambria
San Luis Obispo
35°
Pt Purisima
Sta Barbara
Sta Rosa Is
Sta Cruz Is
CALIFORNIA
San Vicente
30°
MEXICO
Guadalupe Is
Cedros Is
0 100 600 km

FIGURE 2. Crossing relationships in the California closed cone pines, including all available information to date. Populations with similar terpene frequencies are grouped by curved lines.


P. RADIATA (AÑO NUEVO, MONTEREY)
GUADALUPE ISLAND PINE
CEDROS ISLAND PINE
P. ATTENUATA
TRINIDAD-BLUE
MENDOCINO-BLUE
STA. ROSA ISLAND-GREEN
STA. CRUZ ISLAND-GREEN
SAN VICENTE-GREEN
SONOMA N.-BLUE
SONOMA S.-GREEN
MARIN-GREEN
MONTEREY-GREEN
SAN LUIS OBISPO-GREEN
PURISIMA-GREEN
ESTIMATED CROSSABILITY:
HIGH (>60%)
MEDIUM (6-40%)
LOW (<6%)
NO GERMINABLE SEED SET IN ONE OR MORE VALID ATTEMPTS
NO LINE - NO ATTEMPTED ARTIFICIAL POLLINATION

CHAPTER 5

Clinal and Ecotypic Variation in Two Populations of Bishop Pine *(Pinus muricata)*

ABSTRACT. An abrupt cline that separates blue- and green-foliaged bishop pine *(Pinus muricata)* populations over an edaphically and climatically uniform 3 km north-south strip of the northern California coast was investigated with common-garden and isozyme analyses. Bishop pine seedlings and rooted cuttings of the green population planted in six plantations at three locations (north of, south of, and in the cline) grew faster in all plantations than did plants from the blue or transition populations. The interactions of population samples and plantation environments that combined to produce the tree phenotypes did not significantly depart from linear expectations. Allozyme frequencies in 13 of 20 polymorphicaloci differed significantly between the blue and green populations, and the average genetic distance (over all 46 loci) was .014. On remote ridgetops south of the cline, local patches of highly podsolized soils support stunted pygmy forests that resemble those farther north near Ft. Bragg, California. Trees typical of the northern blue populations grow on some of these patches. Trees typical of the green populations grow as close as 1 km away on normal soils, separated from the blue disjunct pygmy forests by stands of other species. The ridgetop stands differed significantly from the coastal populations at 11 isozyme loci, although usually the blue-ridgetop stands resembled the blue-coastal stands and the green-ridgetop stands resembled the green-coastal stands. Average overall gene diversity for coastal and ridgetop populations was .0504. Seedlings of blue and of green origin growing in controlled soil experiments differed significantly from each other in top height, root length, number of side roots, and dry root weights. The interaction of population samples and soils that produced tree phenotypes sharply and significantly departed from linear expectations in the root analyses. Present and prior evidence suggests that the blue and the green bishop pines evolved while in isolation, with the blue bishop pine adapting to the more sterile soils and stressful conditions of the pygmy forests. Where the populations meet on normal soils, the green bishop pines are apparently competitively superior to the blue bishop pines, whereas on the highly podsolized pygmy forest soils, blue bishop pine dominates.

INTRODUCTION

Abrupt geographic variation found within many plant and animal species has long interested biologists who study clinal patterns of selection and historic effects of hybridization. Members of coniferous species on average rank with some of the invertebrates as being among the most genetically variable organisms known. For over two centuries, forest geneticists have studied how that variation is partitioned geographically. Early studies investigated rangewide provenance variation in growth, phenology and morphology by planting trees from widespread natural populations in common gardens. These studies identified several orders of geographic variation, subsequently found to be common in many conifers. First, in many species with large distribution ranges, widely separated groups of populations differ in suites of characteristics typical of geographic subspecies (eg *Pinus contorta,* Critchfield 1957; *Pseudotsuga menziesii,* Munger and Morris

1936; *Pinus ponderosa,* Wells 1964). A second order of variation exists among populations within these major geographic units, and among populations in species that are not divided into obvious subspecies. Commonly, populations differ tremendously in growth traits along environmental gradients, with temperature and moisture figuring prominently (eg *Abies concolor,* Hamrick and Libby 1972, *Pinus ponderosa,* Callaham and Liddicoet 1961, *Pseudotsuga menziesii,* Rehfeldt 1979). Recent interest in a third order of variation, local adaptation among stands within populations and among trees within stands, has demonstrated fine-scale geographic partitioning in some species (*Pseudotsuga menziesii,* Campbell 1979) and little or none in others (*Calocedrus decurrens,* Harry 1984, *Pinus radiata,* Hood and Libby 1980). Overall, scales of clinal variation are typically on an order of tens to hundreds of kilometers, and are only rarely of more abrupt nature.

In some species, morphological traits not obviously related to climatic gradients (eg cone/needle/bark traits) differ significantly over relatively short distances. For example, in jack pine (*Pinus banksiana),* moderately sharp variations in cone curvature, cone angle, percent of trees with prickles, and needle width occur in the lake states (Schoenike 1976). Similarly, bark thickness, number of hypodermal layers in shade leaves and percent of leaves with notched apices in subalpine fir (*Abies lasiocarpa*) vary considerably over moderately short distances in the western United States (Critchfield 1984). Critchfield interpreted these patterns as resulting from Pleistocene impacts on fragmentation and coalescence of populations. Even in the most extreme cases reported to date, however, the smallest transition regions are on an order of tens of kilometers.

Variation in biochemical traits in conifers (eg, terpene composition and allozyme frequency) is often inconsistent with patterns of variation in growth and morphology. Provenance variation is much less prominent in these traits, and by far most genetic variation is within stands (Mirov 1961, Brown and Moran 1979, Hamrick, Mitton, and Linhart 1979). Differences among regions are generally characterized by frequency differences and not by discrete, presence/absence statistics. Genetic distance is often related to geographic distance, but is usually interpreted as a measure of time since the populations separated, or degree of isolation with respect to migration or exchange of alleles. In some cases, terpene frequencies vary more abruptly in a step-wise fashion (Zavarin, Critchfield and Snajberk 1971, Zavarin and Snajberk 1975, Adams 1975), but again, the smallest scales are of tens of kilometers.

Abrupt edaphic ecotypic differentiation occurs in several angiosperm species. For example, steep clinal variation for heavy metal tolerance in several British grass species is associated with discontinuities in soil minerals and pH on and off mine tailings (Antonovics, Bradshaw and Turner, 1971). Similarly, plants of several herbaceous species originating on ultramafic soils in the western United States tolerate mineral deficiencies and toxicities of serpentine soils much better than plants originating from populations on non-serpentine soils, although the existence of steep clines has not been documented (Kruckeberg 1951, 1967).

In conifers, however, the extent of genetic adaptations to substrate is inconclusive, but appears to be much less than in angiosperms. Digger pine (Pinus sabiniana), for example, is one of only a few conifers that can grow vigorously on and off serpentine soils in

California and Oregon. In nursery tests with different native soils, seedlings originating from populations on non-serpentine soils grew better in all soils than those from areas of serpentine soils (Griffin 1965). Although seedlings within each population differed greatly in growth and survival, family rankings were consistent for all soils tested, and the data showed no special adaptation to serpentine soil conditions.

Comparable trends resulted from studies of ponderosa pine seedlings grown on several soil types and originating from populations on fertile and ultramafic soils (Jenkinson 1974, 1977). The seedlings from open-pollinated families varied considerably in their tolerance of extreme soil conditions. Little correlation was found, however, between population of origin and performance on different soils. These results with young seedlings may be misleading, since tests with Pinus contorta latifolia showed clear-cut ecotypic differences related to soils of origin on serpentine and non-serpentine soils, but only after 38 months (Kruckeberg 1967). In Canada, black spruce (Picea mariana) growing in widely separated upland (well-drained) and lowland (boggy) sites differ in allozyme frequencies, with the upland sites supporting more variable stands than the lowland sites (Parker, et al. 1983).

Northern populations of bishop pine (Pinus muricata subsection Oocarpae, Critchfield and Little 1966) are unusual among conifers in their pattern of abrupt genetic change over a distance of 1-3 km. A maritime and insular species, bishop pine ranges in disjunct populations from San Vicente in Baja California to Trinidad in northern California, rarely extending more than a few kilometers inland.

The northern populations of bishop pine, from Marin north to

Trinidad (see map in Critchfield and Little 1966, and Fig. 1) differ enough from the rest of the subspecies in several growth and morphometric traits that several taxonomists have described these populations as a specific variety (var. borealis, Duffield 1951, Axelrod 1983). Within these populations, however, an unusual transition occurs in other traits within the continuous pine forests of this region. Alternative forms of several morphological and biochemical characteristics occur in an abrupt clinal pattern near the south end of Sea Ranch, 27 km north of Ft. Ross (Fig. 1): Over approximately 2 km, there is a rapid change in the frequency of foliage types in the populations, from "green" foliage, typical of all populations south of Sea Ranch, to "blue" foliage, typical of populations north of Sea Ranch (Duffield 1951). Distinctive differences in the shape and waxiness of the chambers above the stomata determine foliage color. Geographically coincidental with this cline in needle color is an abrupt transition in the composition of the monoterpene fraction of xylem resin (Forde and Blight 1964; Mirov, et al. 1966). South of the transition, from Sea Ranch to Monterey, delta-3-carene predominates, whereas to the north, alpha-pinene is the major terpene. Flowering phenology and allozyme frequencies of one enzyme locus also differ north and south of this narrow transition (Millar 1983).

The causes for this unusually abrupt transition are enigmatic. Critchfield (1967) speculated that crossing barriers between blue (=blue-foliaged) and green (=green-foliaged) trees may maintain the discontinuity. In control-cross experiments, however, numbers of sound seeds set and the nursery growth of F_1 individuals (blue X green crosses) did not differ significantly or consistently from the values for intra-population crosses (Millar 1985a). Some reduction in interpopulation crossing may occur due to lower F_2 and backcross fertility (Millar 1985a) or by phenological barriers to effective pollination (Millar 1983); nevertheless, isozyme analyses indicate that inter-population crosses probably occur in at least modest frequency in these native stands (Millar 1983).

Ecological and environmental factors do not readily explain the origin or maintenance of this steep cline either. Although there is topographic, floristic, and edaphic variation in this region, the environmental variation occurs in an east-west pattern that repeats itself regularly along the coast between Ft. Ross and Ft. Bragg: Along the coast for about 3-8 km inland between Ft. Ross and Ft. Bragg, the continent rises from the ocean in a series of more or less distinct terraces, which are the remains of Pleistocene coastlines sequentially exposed by tectonic events and sea-level changes (Jenny, Arkeley and Schultz 1969, Westman 1975). The lowest terrace (at about 30 m) supports lush grasslands bordered by a steep ridge that rises in four more terraces to elevations over 300m. The greatest frequencies of bishop pine occur along the grasslands and on the lower slopes of this ridge on relatively productive soils of the Hugo, Empire, and Caspar series (Westman 1975).

The zone of nearly pure bishop pine abruptly gives way at middle elevations on the ridge to nearly pure or mixed redwood (*Sequoia sempervirens*) and Douglas-fir (*Pseudotsuga menziesii*) forests, where bishop pine is a minor element. The slope of the ridge flattens at successively higher elevations into four more former sea terraces, distinguishable most clearly between Navarro and Ft. Bragg. Due to their relative age and soil-plant relations, each higher terrace presents successively more extreme sites for forest growth. On these sites, redwood and Douglas-fir are excluded due to soil sterility and acid/mineral toxicities of the extremely podsolized Blacklock and Noyo soils (Gardner and Bradshaw 1954, Westman 1975), and are replaced by stunted Bolander pine (Pinus contorta bolanderi), pygmy cypress (Cupressus pygmaea), bishop pine and many acidophilic ericaceous shrubby species. The highest and oldest terraces have extremely stunted (pygmy) vegetation and low site occupancy.

The distinctness of the upper terraces diminishes southward and the abundance of pygmy forests decreases concomitantly, although the coastal ridge still rises above the first grassy terrace to elevations over 300 m. Noyo soils are more abundant than Blacklock soils in these southern pygmy forests. Pygmy cypress grows primarily north of Pt. Arena and bishop pine has replaced Bolander pine south of Pt. Arena. Near the south end of Sea Ranch, where the abrupt blue/green transition in bishop pine occurs, the top of the coastal ridge has a maximum elevation (100 m) that is lower than any ridgetop point between the Gualala and Russian Rivers. Bishop pine grows on productive Hugo, Empire, and Caspar soils throughout this blue/green transition area, and no north-south soil differences have been observed in this region.

Several patches of little-known pygmy forests (on Noyo, Blacklock, and Gualala soils) occur on the remote ridgetops south (1-15 km) of the Sea Ranch transition. Bishop pine is the sole conifer on most of these patches, although on one patch it grows with pygmy cypress. Initial visits to these sites revealed that, although far south of the Sea Ranch transition, most of the trees on these patches had blue foliage. Other ridgetop patches of bishop pine in this region, usually 1-3 km distant from the pygmy forest patches, included pines that had mostly green foliage. These patches of green bishop pines were growing on or around grassland "prairies" that are underlain by more typical forest soils (Hugo, Empire, and Caspar). This was my first clue to a possible edaphic/ ecological correlation with the observed genetic differences in bishop pine.

The present study examines the blue-coastal, green-coastal (both on typical forest soils), and ridgetop bishop pine populations in an attempt to understand genetic differences among these groups and historical sources of differentiation. Presented here are results of growth studies in common-garden plantations, more intensive allozyme analyses than previously reported (Millar 1983), and nursery experiments involving plant growth in different soils.

MATERIALS AND METHODS

Plantation Tests.

These experiments consisted of six common-garden plantations, designed to assess genetic differences in growth of blue-, green-, and transition-origin plants on three coastal sites, north of, south of, and in the Sea Ranch transition zone. Two plantations were established at each of three sites. At each site, one plantation contained plants of clonal (rooted-cutting) origin, and one contained plants of seedling origin. Because the individual seedlings can be clonally replicated, such plantations allow more accurate genetic analyses within and among plantations than would an equal commitment to seedling plantations, with each seedling genetically different from the others. Seedling plantations were planted to compare growth of juvenile- or seedling-origin plants *per seaat* these three sites, and to provide a more conventional test of the population x site interactions.

1. Seed Collection and Nursery Procedures. In 1978, seeds were collected from 19 natural coastal stands of bishop pine for preliminary isozyme analyses (see Millar 1983, Figure 1, for details and map of collection locations). Seeds remaining from some of these collections were used to establish hedges that gave cuttings for clonal plantations and some were used for the seedling plantations.

A. Clonal Tests. In spring 1979, seeds were germinated in petri dishes, then transferred to leach tubes and grown in a Berkeley lathhouse. In spring 1980, randomly selected seedlings were transplanted to 15 cm clay pots, and maintained as hedges (cutting donors) at 0.3 m height in the lathhouse (Millar 1985b). Of these hedges, 7 families from 4 blue stands north of the transition, 10 families from 3 stands in the transition area, and 9 families from 4 green stands south of the transition were included (Table 1).

In August 1981, at least 12 cuttings were taken from each of these hedges, and set in a rooting medium in leach tubes at a UC Berkeley nursery (further details on rooting techniques in Millar 1985b). Rooting occurred over a 10-month period. Total height of all rooted cuttings in the nursery was measured in May 1982.

B. Seedling Tests. Additional seeds from the 1978 collections were germinated in June 1980 so that they would be comparable in size to the cuttings at about the time the cuttings rooted. Twenty-five families from 3 blue stands north of the transition, 20 families from the three transition stands, and 23 families from 3 green stands south of the transition were included (Table 1). At least five germinants from each wind-pollinated family were planted in leach tubes containing a light potting mix, and these were maintained for one year in a UC Berkeley greenhouse (16 hour photoperiod) before being moved to the lathhouse. In November 1981, I repotted the seedlings into steel folding-cans. Total seedling heights were measured in the nursery in April 1981, November 1981, and April 1982. In the Berkeley climate, the seedlings did not uniformly set hard winter buds, whereas under natural conditions along the north coast, winter buds commonly form even on yearling seedlings.

2. Plantation Design and Outplanting. Three plantation sites were chosen along the north coast in typical bishop pine sites, one in the blue forests north of the transition, one in a transition zone forest, and one in the green forests south of the transition (Fig. 1). At the time of these tests, the presence of pygmy forests south of the transition was unknown to me, and edaphic differentiation related to pygmy forests was not yet suspected. Plantation site 1 is 3 km north of the mixed stands of the transition zone. It is on a slightly sloping meadow edge (southwestern aspect), surrounded by vigorous young and old native pines (blue-foliaged), and underlain with Caspar soils. Plantation site 2 is on a forested ridgetop in the transition zone, near the pygmy forest of P1 but on deeper, less acidic soils (Josephine series). The site is flat and surrounded by mature blue and green bishop pines, coast redwood and Douglas-fir. Plantation site 3 is 8 km south of the transition at a meadow edge, surrounded by young and old green bishop pines and underlain by Caspar soils.

Two plantations were established at each of these three sites--one of seedling origin and one of rooted cutting origin. Each plantation was to comprise 99 plants, 33 each of blue-, transition-, and green-stand origin. The plantations were laid out in a random, noncontiguous, interlocking field-design (Libby and Cockerham 1980) with three replicates each planned to contain 11 blue-, 11 transition-, and 11 green-origin plants. This field layout is designed to provide early competitive interactions among neighboring trees due to the spacing around each tree, and by incorporating high levels of genetic dissimilarity among neighbors. The 11 rows of 9 trees each were set 30 cm apart, with trees spaced 30 cm apart within rows. The close spacing was chosen to enhance competitive interactions while the trees were young. The plantations were left unthinned through the course of this experiment.

A. Clonal Plantations. In late May-June 1982, rooted cuttings were planted at the three sites. Every clone was included at each site, with one to three ramets at each site, and the number of ramets per clone at each plantation balanced as much as possible. Not all of the plants of correct origin were available, and for plantation site 1, 23 blue-, 35 green-, and 33 transition-origin trees were outplanted; at plantation site 2, 25 blue-, 32 green-, and 33 transition-origin trees were outplanted; and at plantation site 3, 25 blue-, 33 green-, and 33 transition-origin trees were outplanted. The following spring, 8 more blue trees were planted at each site. The plantations were watered frequently for three months following planting. Survival was monitored and total height measured in February 1983, February 1984, and September 1984.

B. Seedling Plantations. All seedlings were planted within the same week as their companion clonal plantations in late May-June 1982. Each wind-pollinated family was included in each plantation with one to three seedlings per site. Not all plants of correct origin were available, and for plantation site 1, 37 blue-, 38 green-, and 24 transition-origin trees were outplanted; for plantation site 2, 38 blue-, 38 green-origin, and 23 transition-origin trees were outplanted; and for plantation site 3, 36 blue-, 38 green-, and 25 transition-origin trees were outplanted. Plantations were watered, and survival and height were measured at the same times as for clonal plantations.

3. Analyses. Seedling and rooted-cutting nursery heights were analyzed separately by nested analyses of variance, with seedlings nested within families within stands within populations, and rooted cuttings nested within clones within families within stands within populations. Analyses of field heights were similarly structured for seedling and clonal plantations; each plantation, however, was analyzed by a separate analysis of variance for February 1983, February 1984, and September 1984 measurements.

Isozyme Analyses

Preliminary isozyme analyses showed distinct differences between coastal blue and green populations (Millar 1983). After discovery of blue bishop pines in the pygmy ridgetop forests south of the transition, I conducted a more comprehensive isozyme survey, which supplemented previous analyses by 1) increasing the number of loci studied, 2) including samples from pygmy forests south of the transition, 3) increasing sample sizes from all stands, and 4) spacing the coastal collection areas more evenly.

1. Seed Collection. To obtain seeds for electrophoresis, cones were collected from 18 natural bishop pine stands in northern California, including 6 coastal stands north of the Sea Ranch transition (N1-N6), 6 coastal stands south of the transition (S1-S6), and 6 ridgetop stands (P1-P6) south of the transition (Fig. 1). These collections, although

in the same general region as the 1978 collections (see Millar 1983), were in different specific locations. Two- to four-year-old serotinous cones were taken from 40 trees in each coastal stand in summer 1982 and 20 trees in each ridgetop stand in spring 1983. Collection trees were mature (over 25 years old) and spaced at least 15 m apart. In addition to cone collections, foliage from these and 100 additional trees was collected at each stand, and stomatal type was scored with the aid of a dissecting scope.

The coastal stands (N1-N6, S1-S6) were all located on the eastern edge of the first coastal terrace adjacent to the broad grasslands that compose much of that terrace (elevations less than 50 m). Underlying these stands were deep, well-drained, moderately acidic (pH = 5.4-6.8) soils that are typical of luxuriant bishop pine forests at this elevation (Hugo, Caspar, and Empire series).

The ridgetop collection stands (P1-P6) sample the patches of higher sea terraces, where bishop pine grows abundantly if not vigorously on the sterile, podsolized soils of these pygmy forests. Stand P1 (elevation 240 m), at the southern edge of the transition zone, is in a moderately developed pygmy forest where bishop pine with primarily blue foliage is the most abundant conifer. Soils are shallow, undeveloped, sandy, and acidic (Gualala series, pH = 4.2). Stands P2 and P4 (300 and 185 m) are floristically similar but are underlain by more highly developed, podsolized and acidic soils of the Noyo series (clay hardpan at 45-90 cm, bleached surface horizon, pH 3.7-4.0). Stand P4 differs in having trees mostly with green foliage, in occupying the lowest elevation of the sampled ridgetop stands, and in being the only one of the six "P" stands not actually on a flat ridgetop. The aspect of stand

P4 is southwestern and slopes at about 5%. Stand P5 (285 m) is in the most stunted of the pygmy forest patches south of the transition. In many ways it resembles the pygmy forests farther north near Ft. Bragg: This is the only location south of the transition where pygmy cypress (Cupressus pygmaea) grows, and the only site where the most severely podsolized soils (Blacklock series, pH 2.8-3.9) occur. Both the Noyo and Blacklock series have hardpan horizons, but in the Blacklock soils this horizon evolves through leaching of irons and is more impenetrable to roots than the clay hardpan of the Noyo series. All of the stands in the pygmy forests share the sites with acidophilic, shrubby members of the Ericaceae, including several endemic species. In stands P1, P2, and P4, these shrubs are very dense and the pygmy pines are well spaced; in stand P5, even the shrubs are sparse and bare ground abounds. Most of the pines have blue foliage.

Bishop pine also grows in narrow, pure stands at the edges of small (25 ha) grass-covered prairies that occupy several ridgetop locations south of the transition very near the pygmy forests. Pines of these prairies are not at all stunted, and these forests resemble the coastal pine forests in stature and vigor of trees, floristic associates and soils (Caspar series). The two prairie-edge stands that I sampled, P3 (primarily blue-foliaged) and P6 (all green-foliaged) (at 295 and 280 m respectively), were less than 1 km from pygmy forests P2 and P5, respectively, and were separated from them by coast redwood forests.

The closed cones that were collected opened after being immersed in boiling water for 10 seconds and dried at 28°C for several days. Seeds were stored at -4° C.

2. Electrophoresis. To enhance electrophoretic resolution, seeds were germinated prior to analysis. Gametophytes were dissected from the

seeds and five different gametophytes from each of 600 trees were electrophoresed on horizontal starch gels using techniques of Conkle et al. (1982) and Millar (1985c). Twenty-two enzyme systems comprising 46 loci stained consistently enough to be included in this study: a) buffered with tris citrate at pH 8.3--alcohol dehydrogenase (ADH), alanine aminopeptidase (ALAP), leucine aminopeptidase (LAP), menadione reductase (MNR), peroxidase (PER), phosphoglucose isomerase (PGI); b) buffered with tris citrate at pH 8.8--acid phosphatase (ACP), catalase (CAT), glucose-6-phosphate dehydrogenase (G-6-PD), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), mannose phosphate isomerase (MPI), sorbitol dehydrogenase (SRDH), superoxide dismutase (SOD); and c) buffered with morpholine citrate at pH 6.1--aconitase (ACO), aldolase (ALD), fructose diphosphatase (FDP), isocitric dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconic dehydrogenase (6-PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH). Inheritance studies demonstrated that, of the 31 polymorphic loci, all but 3 pairwise band combinations (in ACO, ALD, and ACP) behaved as Mendelian units (Millar 1985c). All 46 loci were used in diversity analyses.

Nomenclature follows Millar (1985c): upper-case abbreviations designate the enzyme and its band phenotypes, lower-case abbreviations designate the corresponding loci. The most anodally migrating zone of multi-locus enzymes was numbered 1. Within each locus, the most frequent allele was assigned the value of 1.00, and other alleles were given a mobility value expressed relative to that of the most frequent allele. A locus that modifies the phenotype of a malate dehydrogenase is symbolized Mmd2 (Millar 1985c).

3. Analyses. Genotypes of the 600 female parent trees were estimated from five gametophytes per tree. The probability of misclassifying a heterozygote as a homozygote with this sample size is 0.06 (Morris and Spieth 1978). Allele frequencies for each stand for each locus were based on estimated female-parent genotypes, and differences in allele frequencies among populations were tested with chi-square. Nei's genetic distance and diversity statistics (Nei 1972, 1973) were calculated among populations, and an unweighted-pair-group-method (UPGMA) dendrogram was constructed (Sneath and Sokal 1973).

Nursery Experiments with Three Different Soils

Discovery of the blue ridgetop pygmy and prairie sites south of the transition made apparent the need to investigate the role of edaphic factors in the growth of blue and green trees. Two experiments were set up, using two native soils and one nursery control soil: 1) Blacklock (top 15 cm) from site P5 2) Caspar (top 15 cm) from stand S4, and 3) Davis mix, a standard conifer nursery potting soil containing equal parts peat, sand, and redwood soil conditioner, plus nutrients.

1. Soil Experiment I. A. Seed Collection and Planting Design. In summer 1982, thirty sound seeds from each of three wind-pollinated families (one family each from three green stands G4, G6, and G7) and thirty sound seeds from each of three wind-pollinated families (one family each from three blue stands B1, B2, and B4) from 1978 seed collections were soaked for 24 hours on wet filter paper in petri dishes at 4°C. Rectangular plastic trays (bottom-drained) 45 X 20 X 15 cm were filled to 10 cm depth with damp Blacklock, Caspar, or Davis soils (one container for each soil). In each container, stratified seeds were sown on 23 July 1982 in 12, 5-seed family rows, with alternate rows containing blue then green families, and each family

occupying two rows/container. The pattern of families was identical for each of the three trays. Trays were placed on greenhouse benches under intermittent mist until September 1982, when they were moved to a lathhouse. Plants were watered but not fertilized throughout the experiment.

B. Analyses. Germination was monitored regularly from the beginning of summer through early September and differences in germination between the green- and blue-origin seeds within and between trays were analyzed by chi-square. Total heights of the seedlings were measured in January 1983, July 1983, and late August 1983, at which time this experiment was ended. By the time the experiment was broken down, roots were too long and intertwined to be measured or weighed. Height differences were analyzed by two-way analyses of variance.

2. Soil Experiment II. This experiment was intended improve deficiencies of Experiment I, namely 1) to include blue and green families from the soil collection sites (stands P5 and S4), 2) to sow dry seeds rather than stratified seeds, in case soil-water uptake differed between seeds from blue and green trees, and 3) to harvest the plants in the experiment before root growth was too advanced to measure.

A. Seed Collection and Planting Design. The seeds for this experiment also came from 1982 collections: all blue seeds came from 8 wind-pollinated families at the P5 pygmy forest, and all green seeds came from 8 wind-pollinated families at the S4 collection site. Six 25 cm round, 25 cm deep sod pots were filled 22 cm deep with damp soil: two pots with Blacklock soil, two with Caspar soil, and two with Davis soil. On 29 September 1983, 56 dry seeds from green families (7 seeds/family) were sown using an 8-family mix, evenly spaced, 1 cm deep in each of three pots, each containing a different soil type. Similarly, 56 seeds of an 8-family mix of blue families were sown in each of the remaining three pots. These pots were placed on greenhouse benches under intermittent mist, and their positions on the benches rotated weekly. After 2 months, the pots were moved off the mist benches, and kept watered and unfertilized in the greenhouse, continuing to rotate their positions weekly.

B. Analyses. Germination was monitored regularly from early October through late November 1983. The experiment was stopped on 2 March 1984. Total heights of all seedlings were then taken, and several root measurements were made: 1) total length of the longest root of each seedling, 2) total number of lateral roots over 1 cm long of each seedling, and 3) oven-dry (80° C for 24 hours) root-weight of each seedling. Differences in germination between populations were analyzed by chi-square, and two-way analyses of variance were used to determine significance of differences in the other traits.

RESULTS

Plantation Tests

1. Mortality. Nursery mortality of seedlings and rooted cuttings was so low that the data were not useful for analysis of population differences in survival. Overall field mortality, scored in September 1984, differed significantly among plantation sites and between plantations at a site (Table 2). Average mortality was lower in the seedling plantations (6.8%) than in the clonal plantations (14.5%). At five of the six plantations, blue and green plants survived equally well, but in the clonal plantation at site 3, six times more blue trees

died than green trees. Mortality of transition-origin plants differed significantly from blue- and green-origin plants (seedling plantation) and from blue-origin plants (clonal plantation) at site 3.

2. Nursery and Field Heights. Before assigning rooted cuttings and seedlings to particular plantations, nursery heights of all plants were measured (Table 3). Among rooted cuttings the mean height of green-origin plants was significantly less than transition-origin rooted cuttings 9 months after the cuttings were set. Since all cuttings were the same length when set, differences among populations in early rooted-cutting height may be related to differences in rate of rooting and/or growth after rooting (Millar 1985b). Differences at other levels, stands, families, and clones, were not significant.

Nursery heights of seedlings established a consistent pattern very early: green-origin seedlings were significantly taller than transition-origin seedlings, which were significantly taller than blue-origin seedlings at all but the final measurement date (23 April 1982), at which time the transition seedlings had caught and passed the green seedlings. Differences at other levels (stands and families) were also highly significant.

Green-origin rooted cuttings and seedlings continued to grow rapidly in the field plantations (Fig. 2). Among rooted cuttings at plantation site 1 (blue region), although population means did not differ significantly, the ranking of green-origin plants changed over time from third to first. At plantations sites 2 and 3, in the transition and green regions, respectively, differences in mean heights among rooted-cutting populations were initially statistically significant, with the green population trees being the shortest. However, the slopes of the height-growth curves were greatest for green-origin plants, such that by September 1984, the green trees averaged a close second and the population means were no longer significantly different. These trends suggest that, although green rooted cuttings got a poor start in the nursery, their early plantation height growth was more rapid than that of the other two populations at all three sites.

Among seedlings, the pattern of height growth at all plantations continued the trend set in the nursery: green seedlings remained larger than transition-origin seedlings, which remained larger than blue-origin seedlings. The slope of the height-growth curves for green seedlings was also greatest at all three plantations, indicating a greater average growth rate.

Differences in mean heights at the stand, family, and clone level were not statistically significant for the clonal plantations at any of the three sites. By contrast, at the seedling plantations, there were some significant differences at these levels, especially between stands, at all plantations sites.

Foliage Counts

The frequency of sampled trees with blue stomata in all coastal stands south of the transition (S1-S6) was 0.0 and the frequency of sampled trees with blue stomata in all coastal stands north of the transition (N1-N6) was 1.0. Sampled trees in the ridgetop stands had either 100% green foliage (stands P4 and P6) or primarily (.73-.86) blue foliage (P1, P2, P3, and P5), (Table 4). The frequency of trees with blue and green foliage was not completely associated with the forest type in the ridgetop stands: one of the four "pygmy" forest stands had green foliage, and one of the two "prairie-edge" forests had mostly blue foliage. Intermediate stomatal types were in low frequency (1-7%) in four of the six ridgetop stands and absent in the others.

Isozyme Analyses

1. Genetic Variation Within Populations. Compared with most other conifers, the northern populations of bishop pine have low isozyme variability (Millar 1983). Of 46 isozyme loci studied here, 67% were polymorphic in at least one stand at the 99% frequency level; at the 95% frequency level, only 56% were polymorphic. Fifteen loci had no observed allelic variation. The number of alleles observed per stand per locus (all loci) averaged over stands was 1.74; the largest number of alleles per locus was 5 (Acp). Gene diversity, equivalent to expected Hardy-Weinberg heterozygosity (Nei 1975), varied from 0.49 for Aco in the green populations to 0.0 for monomorphic loci and averaged 0.084 over all loci.

2. Genetic Variation Among Populations. For comparison among populations, the data were partitioned into the blue-coastal, green-coastal, and ridgetop populations. The ridgetop population was further subdivided into four mostly blue stands and two all green stands, as indicated in Table 4. Isozyme variability was highest by two of three measures in the composite ridgetop population and was tied with green-coastal populations for highest in the third (Table 5). Gene diversity and percent polymorphic loci by population were greatest in the ridgetop population (0.101 and 48%, respectively), and lowest in the blue-coastal population (0.072 and 30%). The blue-coastal population also had the lowest average number of alleles per locus (1.67). Within the ridgetop population, by striking contrast, the four mostly blue stands had greater diversity by all three measures than the two all-green stands.

Tests for differences in allele frequencies among the populations for 24 of the polymorphic loci (7 of the polymorphic loci were excluded due to relatively low levels of variability, making comparisons less than usefully sensitive) showed that differences between populations at 20 loci were statistically significant (Table 6). Only 4 loci (Acp, Idh, Lap2, and Mdh2), although highly variable, did not differ significantly among the populations.

The remaining loci showed several patterns of differences in their allele frequencies (Table 7). In Group I, allele frequencies differed significantly between the blue- and green-coastal populations, but the ridgetop stands as a group did not differ significantly from either of these. For many loci of this group, the composite ridgetop population had allele frequencies intermediate to the blue- and green-coastal populations. When the ridgetop population was divided into blue and green stands, only one locus (Got3) had significantly different allele frequencies. However, in all loci of this group except Mdh3, Pgm1, and Skdh1, the differences between blue and green ridgetop stands were the same direction (greater or lesser) as the difference between the blue and green coastal populations. For example, in Got3, the blue-coastal frequency (1.000) was significantly greater than the green-coastal frequency (0.964) and the composite ridgetop frequency was intermediate (0.983); when subdivided, the frequency in blue-ridgetop stands (1.000) was significantly greater than the green-ridgetop stands (0.950).

Group II loci had frequencies that did not differ significantly between blue- and green-coastal populations, but differed significantly between the ridgetop and both coastal populations (Table 7). In this group, blue and green ridgetop stands differed significantly only in Mnr3. In all but one locus (Adh), the ridgetop stands had greater variability than the coastal stands. In fact, three loci (Mdh1, Alap2, and G6pdl) were monomorphic in the coastal population samples, but polymorphic in the ridgetop population.

Group III included four loci with frequency differences that were significant both between blue and green coastal populations and between ridgetop and both coastal populations. Two of these loci (Aco and Gotl) exemplify an extreme case of Group I loci: At these loci, the frequencies differed significantly, and the direction of difference between the blue and green ridgetop stands was the same as the direction of difference between blue and green coastal populations. The blue- and green-coastal populations had such different frequencies at these two loci that the composite ridgetop frequencies (intermediate) were also significantly different from either coastal population.

The frequencies of the other two loci of Group III (Ald2 and Mmd2) were similar in pattern to Group II, in that differences in the frequencies of the ridgetop populations differed in a way not strongly associated with the coastal populations. The allele frequencies for the subdivided stands did not differ significantly from one another, and were not intermediate to the frequencies of the coastal populations. Both of these loci were more variable in the composite ridgetop than in the coastal populations.

Genetic identity and distance statistics (Nei 1972, 1973) quantify allele frequency differences among populations (Table 8). All identity values were large, and their patterns reflect the patterns of individual loci described above: 1) Blue- and green-coastal populations were the second-most dissimilar of all pairwise population combinations with blue coastal and green ridgetop the most dissimilar; 2) the blue-coastal and blue-ridgetop, and the green-coastal and green-ridgetop pairs had greater affinities than did blue- and green-coastal or blue- and green-ridgetop; and 3) the composite ridgetop population differed less from the coastal than the blue- and green-coastal populations did from one another.

Several other patterns emerged that were not as apparent from the single-locus statistics, namely, 1) in spite of the fact that 4 of the 6 ridgetop stands were mostly blue-foliaged, the ridgetop population statistics indicate that as a whole this population was more closely related to the green-coastal than to the blue-coastal population. When subdivided, however, the blue ridgetop stands were more similar to the blue coastal population than to the green coastal population, and the green ridgetop stands were more similar to the green coastal population than to the blue coastal population, 2) the blue- and green-ridgetop stands were much more similar to one another, despite the differences noted above, than were the blue- and green-coastal populations.

A dendrogram based on the genetic distance matrix and using UPGMA (Sneath and Sokal 1973) illustrates the patterns of similarities among populations (Fig. 3). This approach clusters similar groups first (smallest genetic distance), on shorter branches, and dissimilar groups later (larger genetic distances), on longer branches. The dendrogram shows the groups clustering first by affinity related to foliage color--ie, green-ridgetop with green-coastal, and blue-ridgetop with blue-coastal. These pairwise affinities were strongest in the green-foliaged pair. Relative to these degrees of relatedness, the blue and green groups (coastal and ridgetop) were quite dissimilar.

3. Genetic Variation Partitioned Among Populations and Stands. Gene diversity statistics are another commonly used approach to summarizing allele-frequency differences in a hierarchically structured sample (Nei 1977). They are useful because diversities can be calculated at any

level within the sample and proportional statistics summarized for comparison with data from other conifers. H_T, equivalent to the expected frequency of heterozygotes in a panmictic population, is the gene diversity of the total sample, based on frequencies of alleles averaged over all stands and all populations. Similarly, H_S and H_C are the within-population and within-stand gene diversities, respectively, based on average allele frequencies of populations and average allele frequencies of stands (the subscripts "T", "S", and "C" are retained by conventional use).

Differences between the gene diversities are caused by differences in allele frequencies at these levels. The differences can be quantified by interstand and interpopulation gene diversities, where

$$D_{ST} = H_T - H_S$$

is the interpopulation gene diversity,

$$D_{CS} = H_S - H_C$$

is the interstand gene diversity, and the gene diversity of the total sample is

$$H_{Ta} = H_S + D_{ST} \text{ and}$$

$$H_T = H_C + D_{CS} + D_{ST}.$$

Proportional contributions of each level are expressed by

$$1 = H_C/H_{Ta} + G_{CT} + G_{ST}$$

where H_C/H_T is the proportion contributed to total gene diversity by variation within stands, G_{CT} is the proportion contributed to total diversity by variation of stands within populations, and G_{ST} is the proportion contributed to total diversity by variation among populations.

In addition to these general statistics, I calculated diversity statistics (D_{CS} and H_S) separately for the ridgetop, blue-coastal, and green-coastal populations (symbolized by superscripts R, B, and G, respectively in Table 9).

The relative amounts of gene diversity varied among the polymorphic loci (Table 9). The patterns described above are repeated, with certain loci (ie, Aco, Adh, Alap2, Cat1, Fdp1, Got1, Got3, G6pd1, Mdh1, Mdh3, Mmd, Mnr1, Mnr2, Mnr3, Per1, 6Pgd1, Pgi2, Pgm1, Skdh1) having high G_{ST} values that indicate large differences among populations. Remaining loci have much lower values. Overall gene diversity among populations contributed 5.04% to the total variation.

Gene diversity among stands on the average (G_{CT} = 5.94%) was about equal to the diversity among populations (Table 9). The statistics showed that ridgetop stands differed more among each other than did the green-coastal stands, although the ridgetop stand diversity was not significantly greater than the variation among the blue-coastal stands.

The total gene diversity (H_T) was quite low (0.0892) compared to other conifers (cf, 0.14 for Monterey Pine, Brown and Moran 1979), but the proportion of variation within stands (H_C/H_T) was also relatively less than other conifers (89.1%). Hence, the among-population and among-stand gene diversities stand out as rather large in bishop pine, although smaller than in Monterey Pine (Brown and Moran 1979).

Soil Tests

1. Germination. Germination rates of blue and green seedlings varied significantly among soil types and between experiments. In Experiment I (Fig. 4), stratified seeds germinated most in Davis soil (green, 93%; blue 87%), and germination rates in the Davis soil were the fastest of all soil types (plateau at 20-23 days). With the exception of one early date (21 days after sowing), germination rates of blue and green seeds

did not differ significantly at any of the dates in Experiment I, but blue-seed cumulative germination was lower than green-seed cumulative germination at all times in the Davis soil.

Stratified seeds of Experiment I germinated second-best in the Caspar soil. The rate of germination was slower than in the Davis soil, and final germination was also lower (blue, 83%; green 73%). Germination differences among populations were not statistically significant at any of the dates, but in this soil, beyond 21 days after sowing, the blue seeds had consistently higher cumulative germination than green seeds.

Stratified seed-germination was statistically poorest and slowest in the Blacklock soil (green, 66%; blue 53%). As in the Davis soil, germination of the two populations did not differ significantly, but at all dates, more green seeds had germinated than blue seeds.

When dry seeds and samples of the soil-origin populations were sown in Experiment II, germination rates differed significantly from Experiment I, and differences between population samples were more accentuated in the Caspar and Blacklock soils than in Experiment I (Fig. 5). In the Davis soil, early germination rates and final germination percents (green, 70%; blue, 63%) were lower than in Experiment I, as might be expected with dry seeds. The curves of the two experiments in Davis soils resemble each other in that differences between populations were never significant, although again, green seeds ranked higher in germination for all but one early date.

In Caspar soil, the dry seeds of the green population germinated faster in Experiment II than in Experiment I (green, 75%; blue, 66%), whereas blue seeds germinated faster but poorer. Early germination of green seeds was far ahead of blue seeds, and for most of the dates, the differences between the populations were statistically significant. The green population remained cumulatively ahead of the blue population throughout the experiment, although the final differences were not statistically significant.

Dry seeds of Experiment II in Blacklock soil germinated faster and better than in Experiment I (green, 80%; blue, 75%). Early germination of green seeds, as in Caspar soil, was rapid, and cumulative germination was significantly higher by green than by blue seeds for most of the experiment.

2. Height Growth. Total heights of Experiment I green and blue seedlings at 6.5 months, 12 months, and nearly 14 months after seed-sowing differed significantly only in the Davis soil (Fig. 6). At all times in that soil, green seedlings were taller than blue seedlings, and means of both green and blue seedlings were statistically greater in the Davis than in the Caspar or Blacklock soils.

Population means in the Caspar and Blacklock soils never differed significantly, nor did height growth on these two soils significantly differ. Except for one early date, however, the means of blue seedlings exceeded green seedlings in both of these soils.

Mean heights of seedlings in Experiment II showed a pattern similar to Experiment I (Fig. 6). Green seedlings were significantly taller than blue seedlings in Davis and Caspar soils, although the difference in means was less in the Caspar soil. In Blacklock soils, the means of the two populations did not differ significantly. Mean height growth of blue and green seedlings was significantly greater in the Davis soil than the other two soils, and green seedlings in Caspar soils were significantly taller than green seedlings in Blacklock soils.

3. Root Measurements. The patterns of significant differences between population means of root length and number of lateral roots were similar to the trends in Experiment II seedling height (Fig. 6 and 7). Performance of blue and green seedlings differed significantly among the three soils. In Davis and Caspar soils, the means of green seedlings were significantly greater for both traits than the means of blue seedlings, but in Blacklock soil, the differences were small and insignificant. Blue seedlings actually averaged slightly more lateral roots than green seedlings. Interaction F-values were significant in both analyses.

Mean dry root-weight of the blue and green seedlings differed significantly among soils, and strongly and significantly interacted among the three soils, amplifying the pattern in the other root traits (Fig. 7). In the Davis soil, green roots averaged 62 mg, whereas blue roots averaged significantly less, 35.9 mg. Mean weight of green seedling roots was also significantly higher in Caspar soil (green, 41 mg; blue, 32 mg), but in the Blacklock soil, the mean weight of blue roots (51 mg) averaged significantly higher than that of green roots (39 mg).

DISCUSSION AND CONCLUSIONS

Abrupt clinal variation in many plant and animal species has typically been related either to strong selection along environmental gradients or to historic events such as recent hybridization. The unusual genetic patterns in bishop pine seem to involve both of these evolutionary events.

The major transition at Sea Ranch between blue- and green-coastal populations has perplexed biologists because no environmental clues explained the presence of such an anomalous cline. Long-distance gene flow apparently occurs across the cline (Millar 1983), and barriers to intercrossing seem to be weak, acting at phenological and possible F_2-levels (Millar 1985a).

By contrast, the association of genetically differentiated bishop pines with pygmy forests on the ridgetops south of Sea Ranch fits the pattern of endemism and racial divergence that is typical of similar sites farther north (Jenny, Arkeley, and Schultz 1969, Westman 1975). In these areas, soil discontinuities are abrupt, and soil characteristics of the pygmy forest present severe toxicities and stresses to plants growing on them (Westman 1975). Very low pH, aluminum toxicity and low levels of macro- and micro-nutrients apparently select genotypes that are tolerant to these conditions. Many species (redwood and Douglas-fir among the conifers) appear unable to grow on these soils; by contrast, Carex californica, Cupressus pygmaea, and Arctostaphylos nummularia are endemic to the pygmy forests (Westman 1975); and lodgepole pine (Pinus contorta) has evolved a genetically distinct population (ssp. bolanderi) that is restricted to these soils (McMillan 1956). The case in P. contorta is similar to differentiation in bishop pine, since Bolander pine (P. contorta bolanderi), restricted to pygmy forests, is separated by only several kilometers from typical shore pinea(P. contorta contorta), which grows abundantly on the first coastal terrace. Bolander pine differs from shore pine in its narrower leaves, its heavier, asymmetric and serotinous cones, and by slight allozyme frequency differences (McMillan 1956, Critchfield 1957, Wheeler and Guries 1982). In the coastal region, Pinus contorta does not have abrupt genetic variation except that associated with different soil types.

The results of the current study suggest that there are primarily two genetic ecotypes of bishop pine in this region, and that the blue and green foliage is a reasonably accurate marker of these races. The ridgetop bishop pine stands differ from the coastal populations in allozyme frequencies, but the high affinities of blue-ridgetop to blue-coastal populations and green-ridgetop to green-coastal populations suggest that divergence of these ridgetop populations from the respective blue and green coastal populations has not been great.

The magnitude of genetic differences between the blue and green populations of bishop pine compares to that which distinguishes widely separated races of other conifers. Large differences in stomatal morphology, terpenes, allozymes, root-growth, and tolerance to soils suggest that blue and green populations have had separate evolutionary histories for a long time. The magnitude of relative interpopulation gene-diversity (G_{ST}) for bishop pine (.0504) falls within the upper range of values for other conifers (Brown and Moran 1979, Guries 1984). In many of these species, distant populations were studied that presumably have been long separated.

Prior impressions that the coast environments immediately north and south of the bishop pine transition area in Sea Ranch do not vary in any manner that would promote adaptive divergence are substantiated here by the results of common-garden tests. Height growth of blue and green plants over four years in six plantations showed no genotype x site interaction that might suggest a selective gradient favoring the blue population in the north and the green population in the south. Instead, the green population seemed to grow better than the blue population in the native plantation locations, as well as in the nursery (more rapid germination, greater height growth and tap-root growth). The six plantations sample areas of different site conditions, including sparse and dense forests (sites 1 and 2 respectively), meadow sites (sites 1 and 3) and different elevations. At all locations, green seedlings and rooted cuttings on average either outgrew plants from the other populations or had accelerated their relative height growth rates, which suggested that they would soon overtop blue seedlings and rooted cuttings.

Several previous plantation studies of bishop pine that included both northern blue- and green-coastal populations point to superior growth of the green relative to the blue populations. In New Zealand, growth of bishop pine saplings from northern green populations was significantly greater than growth of trees from the blue populations on nine of ten sites (Shelbourne, Bannister, and Wilcox 1982). Similarly, in two California common-garden tests (near Lafayette and in Concord), mean heights of nine-year-old bishop pines originating from wind-pollinated seed differed significantly between populations, with the Sonoma green population on average growing taller than the two blue populations (Crowley 1977). All of these plantations were on soils capable of supporting typical forest growth.

It seems, therefore, that in coastal areas, where native blue and green bishop pines establish and grow sympatrically on typical forest soils as at Sea Ranch, green plants may competitively exclude blue plants. Green-population seeds germinate more rapidly than do blue-population seeds, giving the green germinants a head-start in growth. This, coupled with superior early height growth of green trees, may be important in the meadow edges and grassy openings that are commonly the sites for seed germination and seedling establishment in the bishop pine coastal habitat. Grasses are strong competitors of the young pines until the seedlings shade out the grass foliage. Root competition with grasses is severe, and the greater elongation of taproots in green seedlings would give them a selective advantage over blue seedlings in situations where grass is competing with both.

The results from common-garden, isozyme, and soil tests are consistent with the explanation for the abrupt cline at Sea Ranch proposed before (Millar 1983) and they suggest an explanation for the ecotypic differences as well. To begin with, the evidence argues that proto-blue and proto-green populations were once isolated from one another. Fossil evidence indicates that a species with cones identical to northern bishop pine reached northern California, probably from the south, at least four million years ago (Mason 1932, Dorf 1933). Fluctuating Pleistocene climates appear to have caused populations of several species to fragment and migrate alternately north and south (Johnson 1977, Axelrod 1981). Similarly, northern populations of bishop pine were probably isolated from the rest of the species soon after their arrival in northern California.

Genetical and ecological evidence also support the reasoning that proto-blue and proto-green populations were once isolated from each other. Clinal theory suggests that a steep cline such as at Sea Ranch can evolve in continuous populations only if there is a strong selective gradient in the environment or if gene flow is weak and occurs over a short distance (Endler 1976). There is no evidence from the common-garden studies or from environmental observation for such a selective gradient at Sea Ranch, and the allozyme studies indicate that gene flow occurs over a long distance and is moderately strong. Thus, proto-blue and proto-green populations most likely were isolated from each other until recently. While isolated, the populations appear to have divergently evolved to grow best on different kinds of sites, with green trees growing on typical forest soils where grass competition is severe, and blue trees growing on pygmy forest soils.

The edaphic association of blue bishop pines with pygmy forest soils, and the low frequency of green bishop pine on these sites, suggest that blue bishop pine is better adapted to those extreme soils. Although blue bishop pine in Sonoma and Mendocino Counties currently extends onto other soils, its distribution closely parallels the extent of pygmy forests in northern California. Blue bishop pine may have evolved primarily on these soils. Gardner and Bradshaw (1954) stressed that "the most obvious characteristic of the vegetation on the Noyo soils is the almost universal occurrence of Bishop pine". Formerly, when sea levels were higher, bishop pine may have been even more restricted to these soils than at present. The pygmy-forest podsol type is described as a climax ecosystem (Gardner 1967, Jenny, Arkeley, and Schultz 1969). Since the oldest terraces date over a million years, and soil genesis of the Blacklock and Noyo soils and floristic succession to pygmy forests reaches the climax, stable state after only 100,000-250,000 years (Gardner 1967, Westman 1975), the extreme pygmy forest system has existed for at least 750,000-900,000 years. Thus, blue bishop may have contributed to the genesis of the podsols and co-evolved with the ecosystem.

The present distribution of blue bishop pine near the coast on Caspar soils might suggest that the population evolved on these more productive soils typical of the lower terraces, as well as on pygmy forest soils. However, there is evidence that bishop pine formerly was not a normal component of the Caspar soils, as it was not found mixed with other species in virgin stands on these soils (Gardner and Bradshaw 1954). Douglas-fir is the primary species of Caspar soils, and the large-scale land-clearing activities of the last two centuries on the first terrace slope probably created the opportunity for bishop pine to colonize these areas. Douglas-fir and redwood are already reclaiming the sites in many areas. In the undisturbed state on the first coastal terrace, bishop pine grows only along a narrow fringe close to the ocean, where salt spray excludes Douglas-fir. Even in this fringe habitat, bishop pine has never been the dominant conifer wherever another species grew with it. For example, in areas where extant and extinct populations of Pinus radiata and bishop pine grew together, bishop pine was the minor element (Mason 1932, 1934; Axelrod 1980). Within the distribution of blue bishop pine, shore pine (Pinus contorta contorta) also occupies the ocean-edge habitat and shares the habitat with bishop pine.

In short, several lines of evidence point to a long evolution of blue bishop pine on pygmy forests and only a recent association with other coastal soils and conditions, where green bishop pine grows. Blue bishop pine apparently has adapted to the stresses of the Blacklock and Noyo soils. The slower growth of blue trees relative to green trees on Caspar and nursery soils parallels the situation in several other species that have ecotypes adapted to extremely poor soils. For example, when grown on fertile soils, plants originating from areas of fertile soils outgrew those coming from the sterile sites (Pinus contorta, Cupressus ssp., McMillan 1956; Digger pine, Griffin 1965; and

Agrostis tenuis, McNeilly 1967). In the latter species, fitness of the heavy-metal-tolerant ecotypes was estimated to be significantly lower than non-tolerant ecotypes on fertile soils, with a selection coefficient of 0.53 against the tolerant type.

If true that blue and green bishop pine populations evolved under different environmental conditions during the Pleistocene, and acquired unique suites of traits through selection and drift, then the steep cline at Sea Ranch would be a contact zone of a recent merging. Whether the populations were in contact during earlier interglacial periods is indeterminable from the present data, although if they were, hybrid populations must have been eliminated. The abruptness of the cline may be maintained only transiently by a combination of factors, including phenological differences in flowering time, unidirectional wind patterns, and selection favoring green trees over blue trees on Caspar soils in conditions where grass competition is severe. With the blue population flowering earlier, and the predominant spring winds blowing from the northwest, the primary gene flow is from blue to green (Millar 1983). Thus, green genes can and must migrate only slowly northward against a wind/pollination gradient, and blue genes, moving in pollen across the cline into green populations, would be selected against in the seedlings produced. Female hybrid infertility at the F_2 level may further reduce introgression.

The situation in the ridgetop pygmy forests south of Sea Ranch is different. There, selection favors adaptation to the site *per se*, and abiotic factors seem to be of primary importance. Although green bishop pollen sources surround the pygmy forests, blue bishop maintains a stronghold on the pygmy forest soils, even though a low frequency of green trees and allele frequencies tending toward those of green stands were found on the sites.

The soil experiments and plantation tests showed that, although green trees significantly outgrew blue trees on Caspar and nursery soils, the differences in mean height on Blacklock soils were much smaller and not significant. Root characteristics may be even more important in explaining the differential growth of blue and green trees on native pygmy soils. Green seedlings seem able, where uninhibited by soil factors, to grow longer roots with more side roots than blue seedlings. On Blacklock soils, however, green seedlings were severely inhibited in root proliferation and growth, whereas blue seedlings actually had a larger number of lateral roots and only slightly shorter taproot lengths relative to their growth on fertile soils. The very strong interaction and pattern of differences in means of dry root-weight was due to the fact that blue seedling roots weighed much more than green seedling roots in Blacklock soils, whereas the opposite occurred on Caspar soils. Furthermore, roots of blue seedlings weighed much more when they were grown in the Blacklock than in either Caspar or Davis soils, whereas roots of green seedlings weighed the least when grown in the Blacklock soils.

These interactions suggest that the success of blue seedlings on Blacklock soils may lie more in their ability to accumulate root mass than in rapid root-extension or top height. In the highly podsolized soils of the pygmy forests, mechanical impediments (more abundant large rocks than in other soils and hardpan) would limit root penetration anyway. Competition both above and below ground may be less critical on these sites, as grass is not common and much bare ground occurs, meaning that rapid growth in roots or height may be less important than on fertile soils where grass competition is severe. Plants growing on these soils need root protection against mineral toxicities, strong acidity, high water table in the winter, and drought in the summer. The data here indicate that roots of blue seedlings are shorter and thicker than those of green seedlings when grown on Blacklock soils. These characteristics may promote water retention through low surface:volume ratios, and possibly protection against acid toxicities.

The issue of the high frequency (1.0) of trees with green foliage on pygmy site P4 and the high frequency (.86) of trees with blue foliage on prairie site P3 remains. The situation at P4 is not surprising, as this site is not actually on the coastal ridgetop, but on a slight slope with good drainage at the lowest elevation of all the ridge stands sampled. The pygmy-forest podsol ecosystem is less developed there, as determined by floristic indicators, than on the flat ridgetop sites. Noyo soils, which underlie this pygmy site as well as the P2 site, also occur at even lower elevations along the coast south of Sea Ranch, where they support normal bishop pine forests with green foliage, and are even less stunted in pygmy forest vegetation than at site P4. This suggests that green bishop pine grows on all Noyo soils except the oldest, most podsolized Noyo soils of the ridgetops, where pygmy-forest podsol development is most advanced.

The high frequency of trees with blue foliage growing on Caspar soils at prairie site P3 is less explicable. The appearance of the vegetation is not stunted, and understory vegetation is characteristic of Caspar soils. This stand may be a clue to the biogeographic question of whether at one time blue bishop extended abundantly on many soils south of Sea Ranch and was forced north and into a few ridgetop pygmy-forest pockets by northward-migrating green bishop pine. Alternatively, the ridgetop blue stands may be ancient outlying colonies of the blue population, established by long-distance migration from north of Sea Ranch. The genetic data do not shed discriminating light on this issue, except that the extremely low frequency of blue bishop pine (and all associated genetic traits) on low, coastal elevations south of Sea Ranch suggest that blue bishop pine did not extend abundantly along the coast in the recent past. By contrast, the presence along the ridgetop of blue stands such as P3, and the occasional occurrence of single blue trees on the ridge south of Sea Ranch point to a former distribution of blue bishop pine that was somewhat more extensive along the ridgetop than at present. Migrating green bishop pine may have displaced the blue bishop pine on most of the fertile sites, except occasional areas like P6.

The primary goal of the present study has been to document patterns of genetic variation of bishop pines along the north coast. The associations of certain genetic types with environmental factors point to adaptive functions and possible evolutionary histories. The associations are circumstantial evidence for these adaptations and historic events; clarification of the adaptive functions, if any, of stomatal shape, terpene composition, allozyme frequencies, phenology, and root- and height-growth traits awaits proper physiological analysis.

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TABLE 1. Origin of bishop pine cuttings and seedlings in clonal and seedling plantations at three sites near Sea Ranch, California. Collection stands from Millar 1983.

	Plantations		
	Rooted Cuttings		Seedlings
Stand	Number of families	Number of clones/family	Number of families
Blue			
B1	1	1	-
B2	2	3	9
B3	2	3	5
B4	-	-	11
B6	2	3	-
Transition			
T1	2	5	5
T2	2	4	5
T3	6	7	10
Green			
G1	3	4	7
G2	3	5	-
G3	2	2	-
G5	1	1	9
G8	-	-	7

- = no plants of these families used.

TABLE 2. Mortality for bishop pine seedling and clonal (rooted cutting) plantations at sites 1 (north of the transition), 2 (transition) and 3 (south of the transition) as of September 1984.

Plantation		Plantation Site 1			Plantation Site 2			Plantation Site 3		
Type	Pop	No. Dead	No. Alive	% Mort.	No. Dead	No. Alive	% Mort.	No. Dead	No. Alive	% Mort.
Seedl.	Blue	1	36	2.7	1	37	2.6	7	29	19.4
	Green	1	37	2.6	1	37	2.6	7	31	18.4
	Trans	1	23	4.2	0	23	0.0	1	24	4.0
Cuttg.	Blue	8	23	25.8	1	32	3.0	12	21	36.4
	Green	9	26	25.7	1	31	3.1	2	31	6.1
	Trans	10	23	30.3	2	31	6.1	3	30	9.1

TABLE 3. Nursery mean heights, 95% confidence intervals, and significance values for differences in means among populations, stands, families (seedling plantations), and clones (clonal plantations) for blue-, green- and transition-origin bishop pine seedlings and rooted cuttings. Seeds sown 21 June 1980; cuttings set 31 August 1981. 110 seedlings measured per population each date; 81 blue, 110 transition, and 110 green rooted cuttings measured.

Population	Nursery Mean Height (cm) and Confidence Interval							
	Seedlings						Rooted Cuttings	
	4 Ap 81		1 Nov 81		23 Ap 82		27 May 82	
	Mean	+ CI	Mean	+ CI	Mean	+ CI	Mean	+ CI
Blue	9.075	0.512	12.947	1.038	16.394	1.431	8.469	0.881
Transition	11.579	0.763	18.447	1.340	22.384	1.456	9.758	0.832
Green	13.601	0.573	21.688	0.938	21.935	1.088	7.189	0.507

p Values	4 Ap 81	1 Nov 81	23 Ap 82	27 May 82
Populations	.023*	.028*	.069	<.001**
Stands/Popns.	<.001**	<.001**	<.001**	>.750
Families/Stands	<.001**	<.001**	<.001**	.432
Clones/Families	NA	NA	NA	.526

NA--not applicable

p values:

* .05>p>.01

** p<.001

TABLE 4. Frequencies of native bishop pines with blue, intermediate, and green foliage in the six ridgetop stands.

Stand	Numbers of Trees			Frequency of blue-
(soil type)	Blue	Intermediate	Green	foliaged trees
P1 (pygmy)	103	2	15	0.86
P2 (pygmy)	87	6	27	0.73
P3 (prairie)	103	1	16	0.86
P4 (pygmy)	0	0	120	0.00
P5 (pygmy)	99	3	18	0.83
P6 (prairie)	0	0	120	0.00

TABLE 5. Isozyme gene diversity, average number of alleles per locus (per population), and percent polymorphic loci in the coastal-blue, coastal-green, and ridgetop bishop pine populations, and in the subdivided ridgetop stands.

Population	No. of Trees	Gene Diversity	Average No. of Alleles/Locus	% Polymorphic Loci[1]
Coastal Blue	236	.072	1.67	.30
Coastal Green	237	.079	1.78	.41
Ridgetop	119	.101	1.78	.48
Blue Stands	80	.106	1.74	.54
Green Stands	39	.085	1.50	.46

---

[1] frequency of most common allele <.99

TABLE 6. Results of chi-square tests for differences in allele frequencies among blue-coastal, green-coastal, and ridgetop bishop pine populations for 24 polymorphic loci.

Locus	X^2	df[1]	p
Acp	13.743	8	.091
Idh	0.365	2	.848
Lap2	2.814	2	.099
Mdh2	3.691	2	.086
Aco	92.918	4	<.001
Adh	55.699	6	<.001
Alap2	16.041	2	<.001
Ald2	33.112	6	<.001
Cat1	10.193	2	.006
Fdp1	112.780	4	<.001
Got1	406.913	2	<.001
Got2	6.990	2	.032
Got3	17.313	2	<.001
G6pd1	28.226	2	<.001
Lap1	8.288	2	.018
Mdh1	16.041	2	<.001
Mdh3	17.606	2	<.001
Mmd2	69.510	2	<.001
Mnr1	10.485	2	.005
Mnr3	25.122	2	<.001
6Pgd1	31.778	2	<.001
Pgi2	35.488	2	<.001
Pgm1	266.338	4	<.001
Skdh1	16.432	2	<.001

[1] degrees of freedom vary due to different number of alleles/locus.

TABLE 7. Allele frequencies of loci that differed significantly among blue-coastal, green-coastal, and composite ridgetop populations of bishop pine, for alleles with highest frequencies in green-coastal stands, grouped by pattern of differences. Allele frequencies of ridgetop stands subdivided by foliage type (blue and green) are also given.

Locus	Populations			Subdivided Ridgetop Stands		
	Bl-Coast	Gr-Coast	Ridge	Blue	Direction of Diff.[1]	Green
Group I. Allele frequencies for loci with significant differences between blue and green-coastal populations.						
Got2	.918	.951	.962	.949	same	.988
Got3	1.000	.964	.983	1.000	*same	.950
Lap1	.852	.786	.779	.795	same	.738
Mdh3	.996	.954	.953	.942	opp.	.975
Mnr1	.992	.968	.996	1.000	same	.988
6Pgd1	.951	1.000	.996	.994	same	1.000
Pgi2	.836	.956	.894	.885	same	.913
Pgm1	.884	.706	.720	.718	opp.	.725
Skdh1	.987	.936	.958	.949	opp.	.975
Group II. Allele frequencies for loci with significant differences between composite ridgetop and coastal populations.						
Adh	.871	.870	.932	.942	same	.913
Cat1	.991	.989	.962	.974	same	.938
Fdp1	1.000	.996	.873	.840	opp.	.938
Mnr3	.962	.966	.853	.833	*same	1.000
Mdh1	1.000	1.000	.977	.974	--	.988
Alap2	1.000	1.000	.977	.968	--	1.000
G6pd1	1.000	1.000	.970	.981	--	.950
Group III. Allele frequencies for loci with significant differences among ridgetop, blue-coastal, and green-coastal populations.						
Aco	.828	.565	.703	.782	*same	.550
Ald2	.831	.706	.618	.634	same	.600
Got1	.330	.956	.695	.564	*same	.950
Mmd2	.994	.959	.851	.865	same	.825

---

[1] Notes whether differences between blue and green ridgetop stands are the same or opposite direction (greater or lesser) than the direction of difference in the coastal populations. An asterisk indicates significant differences ($p < .05$) among the green and blue ridgetop stands.

TABLE 8. Nei's genetic diversity (below diagonal) and genetic distance (above diagonal) statistics for blue-coastal, green-coastal, and ridgetop bishop pine populations, and for subdivided blue and green ridgetop stands.

	Blue Coastal	Green Coastal	Ridgetop	Blue Ridgetop	Green Ridgetop
Blue-Coast	---	.014	.007	.002	.015
Green-Coast	.986	---	.003	.003	.001
Ridgetop	.993	.997	---	---	---
Blue-Ridge	.998	.997	---	---	.003
Green-Ridge	.985	.999	---	.997	---

TABLE 9. Nei's gene diversity statistics for 31 polymorphic loci in bishop pine and average diversity statistics for all 46 loci, including 15 monomorphic loci [1].

Locus	H_T	H_S	H_C	D_{CS}	D_{ST}	D_{CS}^R	D_{CS}^B	D_{CS}^G	H_S^R	H_S^B	H_S^G	G_{CT}	G_{ST}
Aco	.4240	.4000	.3850	.0120	.0240	.0300	.0080	.0080	.4210	.2880	.4910	.0283	.0566
Acp	.3786	.3778	.3480	.0298	.0008	.0200	.0462	.0220	.3595	.4112	.3622	.0787	.0021
Adh	.2003	.1977	.1777	.0200	.0026	.0050	.0220	.0340	.1290	.2330	.2310	.0999	.0130
Alap2	.0134	.0132	.0124	.0008	.0002	.0023	.0000	.0000	.0395	.0000	.0000	.0597	.0110
Ald1	.0014	.0014	.0014	.0000	.0000	.0000	.0000	.0010	.0000	.0000	.0043	.0277	.0024
Ald2	.4333	.4331	.4074	.0257	.0002	.0023	.0170	.0150	.4730	.3350	.4920	.0623	.0005
Cat1	.0331	.0327	.0318	.0009	.0004	.0020	.0004	.0001	.0730	.0170	.0080	.0272	.0120
Fdp1	.0868	.0793	.0686	.0110	.0070	.0320	.0000	.0002	.2290	.0000	.0086	.1267	.0806
Got1	.4508	.3196	.2859	.0337	.1312	.0736	.0253	.0020	.4258	.4445	.0886	.0748	.2910
Got2	.1017	.1012	.0995	.0017	.0005	.0013	.0020	.0018	.0743	.1373	.0920	.0167	.0046
Got3	.0342	.0338	.0329	.0009	.0004	.0015	.0000	.0010	.0328	.0000	.0685	.2623	.0109
G6pd1	.0193	.0189	.1177	.0012	.0004	.0035	.0000	.0000	.0566	.0000	.0000	.0622	.0184
Idh	.1302	.1301	.1219	.0082	.0001	.0056	.0147	.0044	.1461	.1218	.1224	.0630	.0008
Lap1	.3152	.3128	.3018	.0110	.0024	.0087	.0067	.0178	.3517	.2487	.3381	.0349	.0076
Lap2	.4303	.4298	.3981	.0343	.0005	.0185	.0435	.0332	.4392	.4134	.4449	.0797	.0012
Mdh1	.0137	.0135	.0131	.0004	.0002	.0013	.0000	.0000	.0405	.0000	.0000	.2920	.0146
Mdh2	.0207	.0206	.0196	.0010	.0001	.0004	.0030	.0030	.0080	.0140	.0340	.0483	.0048
Mdh3	.0610	.0603	.0577	.0026	.0007	.0040	.0001	.0036	.0841	.0083	.0884	.0426	.0117
Mmd	.1230	.1150	.1110	.0040	.0080	.0004	.0004	.0020	.2560	.0120	.0770	.0325	.0650
Mnr1	.0288	.0285	.0280	.0005	.0003	.0002	.0006	.0012	.0083	.0169	.0604	.0174	.0104
Mnr2	.0013	.0013	.0007	.0006	.0000	.0000	.0000	.0000	.0000	.0000	.0040	.4511	.0226
Mnr3	.1206	.1117	.0954	.0163	.0089	.0456	.0012	.0022	.1966	.0696	.0689	.3502	.0735
Mpi1	.0014	.0014	.0014	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0043	.0028	.0024
Per1	.0053	.0052	.0051	.0001	.0001	.0007	.0000	.0000	.0159	.0000	.0000	.0189	.0225
Per2	.0014	.0014	.0014	.0000	.0000	.0000	.0000	.0000	.0000	.0043	.0000	.0028	.0024
6Pgd1	.0375	.0366	.0355	.0011	.0009	.0002	.0028	.0000	.0165	.0932	.0000	.0293	.0238
Pgi2	.1872	.1828	.1735	.0093	.0044	.0119	.0156	.0004	.1921	.2699	.0865	.0497	.0233
Pgm1	.3618	.3497	.3340	.0157	.0121	.0290	.0078	.0072	.4226	.2082	.4183	.0434	.0333
Pgm2	.0096	.0096	.0093	.0003	.0000	.0007	.0000	.0000	.0247	.0000	.0040	.0312	.0021
Skdh1	.0794	.0785	.0767	.0018	.0009	.0023	.0004	.0029	.0796	.0250	.1310	.0027	.0113
Averages over 46 loci													
$\overline{X}$	.0892	.0847	.0794	.0053	.0045	.0066	.0063	.0035	.0999	.0732	.0810	.0594	.0504
s	.1426	.1345	.1261	.0095	.0237	.0144	.0154	.0080	.1450	.1305	.1443	NA	NA

[1] H_T is overall gene diversity; H_S is gene diversity within population; H_C is gene diversity within stands; D_{CS} is gene diversity among stands within populations; D_{ST} is gene diversity among populations within the total sample; superscripts ^{R}, ^{B}, and ^{G} in the next six columns refer to values for the ridgetop, blue-coastal, and green-coastal populations; G_{CT} is the proportion of gene diversity among stands within populations relative to the total; G_{ST} is the proportion of gene diversity among populations relative to the total.

NA not applicable: Average G_{CT} and G_{ST} values calculated from average gene diversity values.

FIGURE 1. Locations of coastal-green (S1-S6), coastal-blue (N1-N6), and ridgetop (P1-P6) bishop pine cone-collections stands used for isozyme analyses and soil experiments, and locations of three plantation sites along the north coast of California.

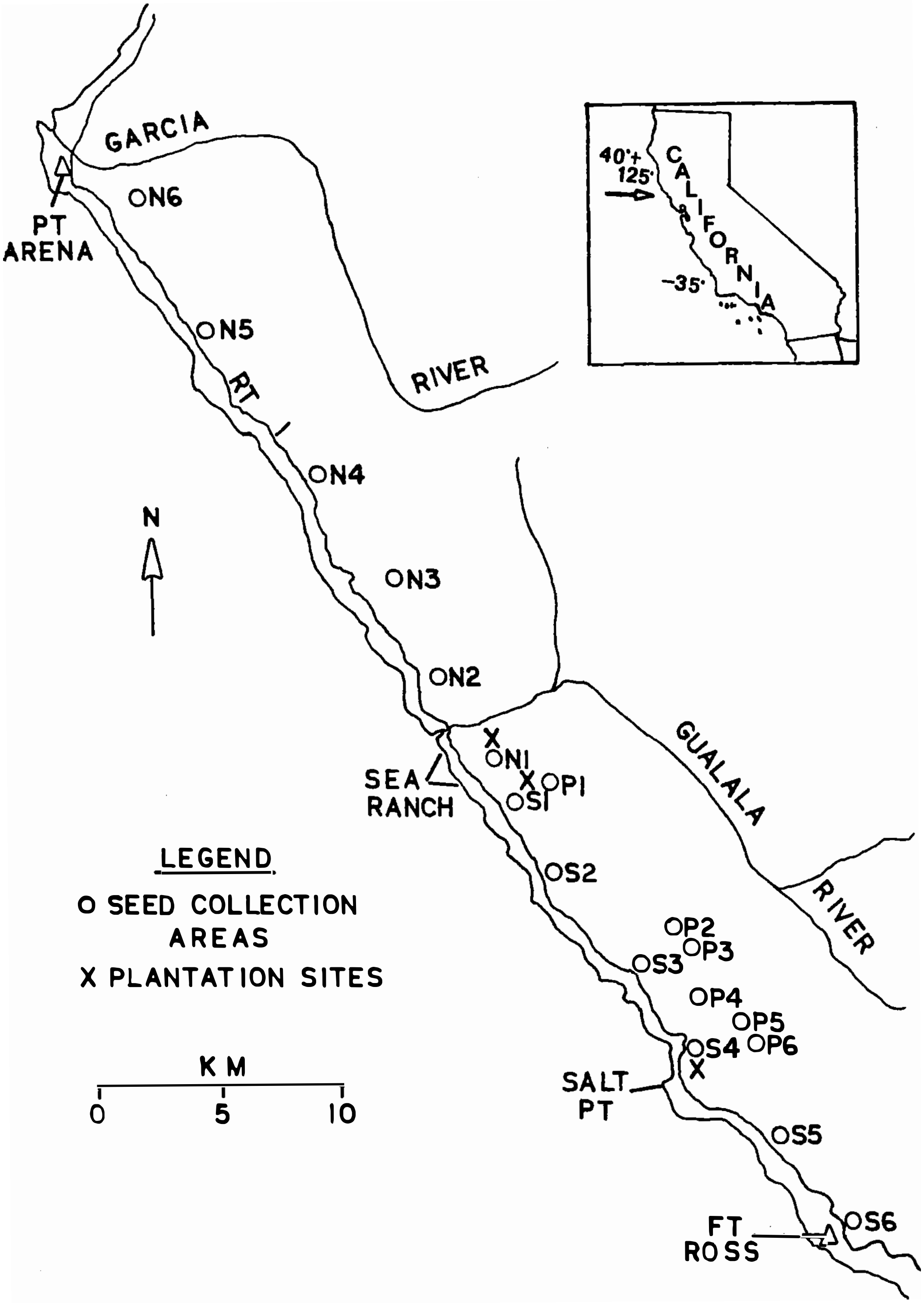
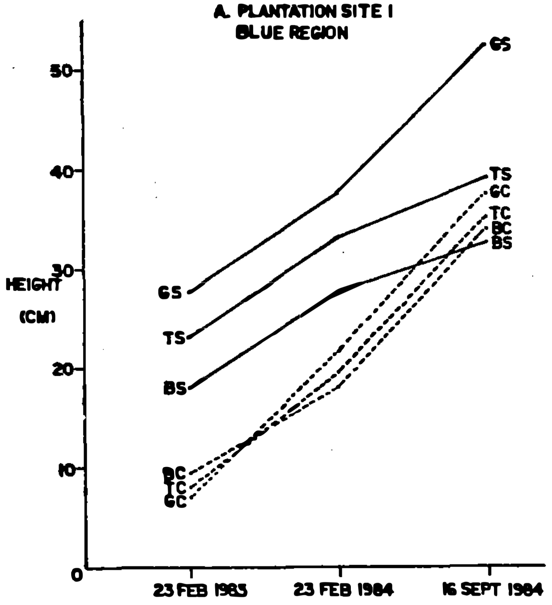
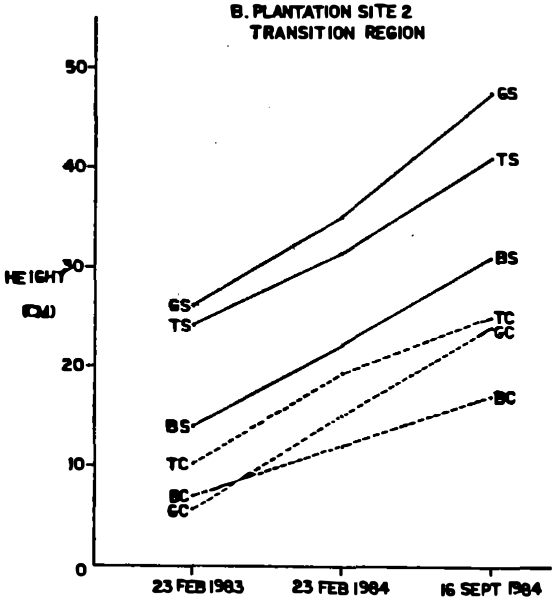

GARCIA
RIVER
PT
ARENA
ON6
ON5
RT 1
ON4
ON3
ON2
N
X
ON1
X OP1
OS1
SEA
RANCH
GUALALA
RIVER
LEGEND
O SEED COLLECTION AREAS
X PLANTATION SITES
OS2
OP2
OP3
OS3
OP4
OP5
OS4 OP6
X
SALT PT
KM
0
5
10
OS5
OS6
FT ROSS
40°+
125°
CALIFORNIA
-35°

FIGURE 2. Mean height of blue-, green-, and transition-origin bishop pine seedlings (labelled BS, GS, and TS, respectively) and rooted cuttings (labelled BC, GC, and TC, respectively) at three dates. (A) Plantation Site 1 (north of transition); (B) Plantation Site 2 (in transition); (C) Plantation Site 3 (south of transition)a Significance values for differences among populations, stands, and families (seedling plantations) and clones (clonal plantations) from ANOVA's are given below each date. NAa= not applicable.



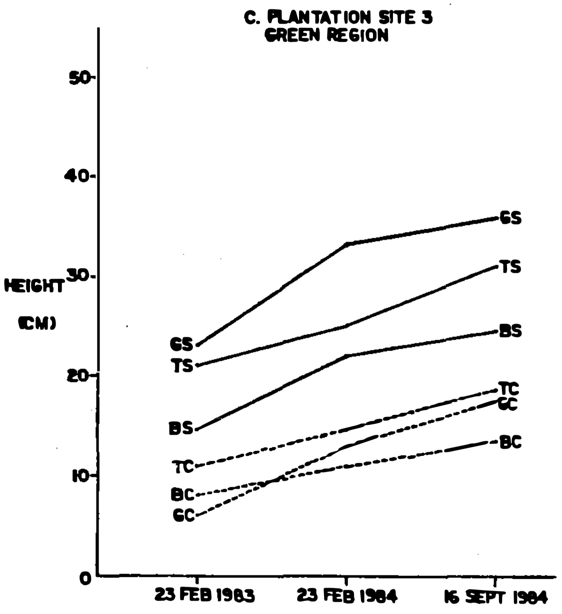
A. PLANTATION SITE I
BLUE REGION

P VALUES	23 FEB 1983 SEED.	23 FEB 1983 CUT.	23 FEB 1984 SEED.	23 FEB 1984 CUT.	16 SEPT 1984 SEED.	16 SEPT 1984 CUT.
POP.	.098	.124	.096	.511	.019	>.750
STAND	<001	>.750	.013	>.750	.069	.156
FAM.	.522	.742	.002	.396	.098	>.750
CLONE	NA	.236	NA	.416	NA	.080
N	99	83	92	74	90	70



B. PLANTATION SITE 2
TRANSITION REGION

P VALUES	23 FEB 1983 SEED.	23 FEB 1983 CUT.	23 FEB 1984 SEED.	23 FEB 1984 CUT.	16 SEPT 1984 SEED.	16 SEPT 1984 CUT.
POP.	.080	.014	.054	.020	.098	.064
STAND	.002	>.750	.010	.560	.004	.584
FAM.	.004	.250	.050	.631	.166	.574
CLONE	NA	.358	NA	.054	NA	.140
N	99	95	97	94	97	91



C. PLANTATION SITE 3
GREEN REGION

P VALUES	23 FEB 1983 SEED.	23 FEB 1983 CUT.	23 FEB 1984 SEED.	23 FEB 1984 CUT.	16 SEPT 1984 SEED.	16 SEPT 1984 CUT.
POP.	.228	.008	.155	<.001	.334	.003
STAND	.026	>.750	.004	>.750	.003	>.750
FAM.	.472	.092	>.750	.465	>.750	.267
CLONE	NA	.551	NA	.609	NA	.681
N	90	82	83	80	65	64

FIGURE 3. Dendrogram summarizing genetic distances among blue-coastal, green-coastal, blue-ridgetop, and green-ridgetop stands of bishop pine.

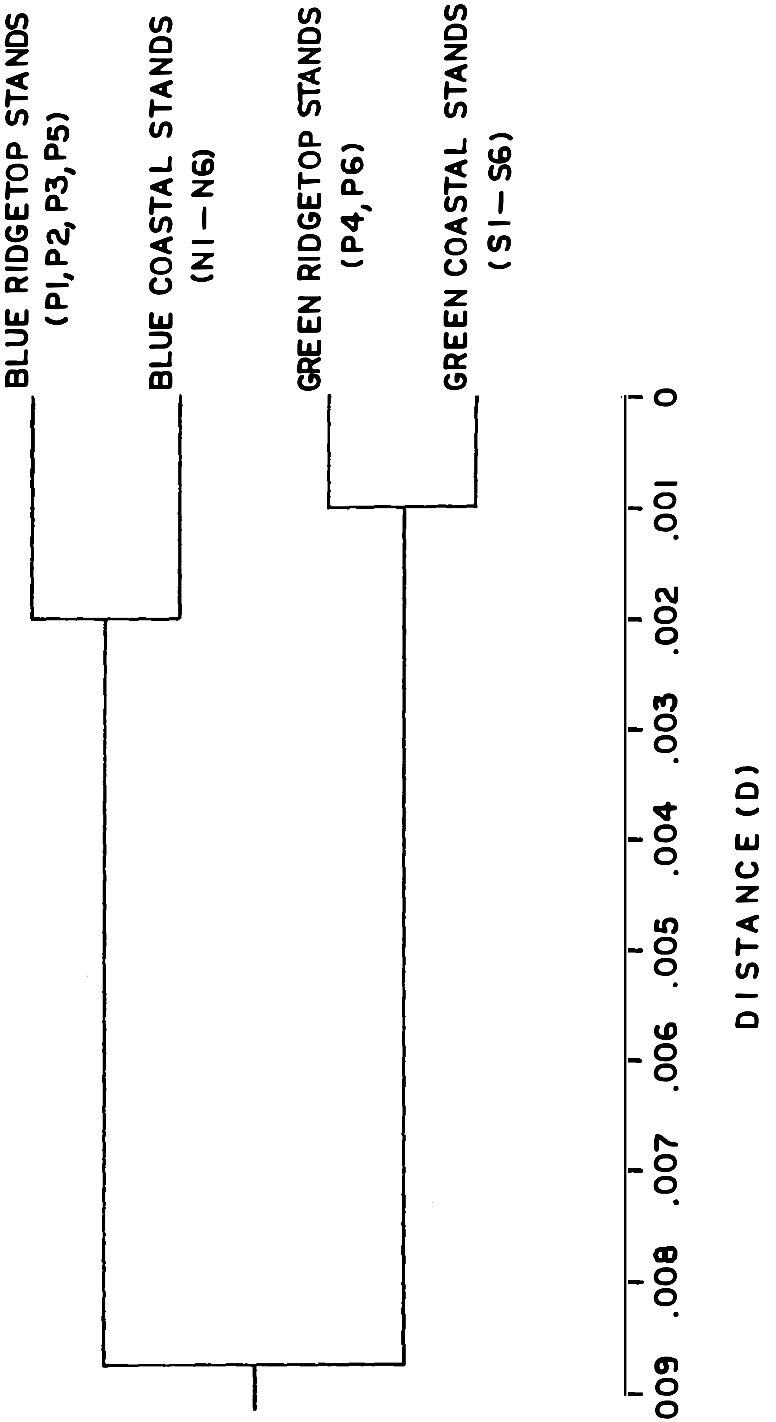
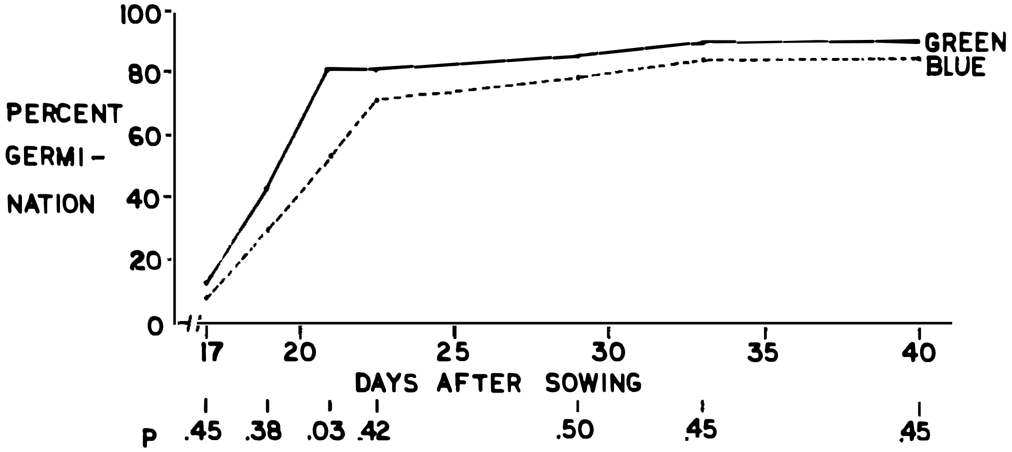
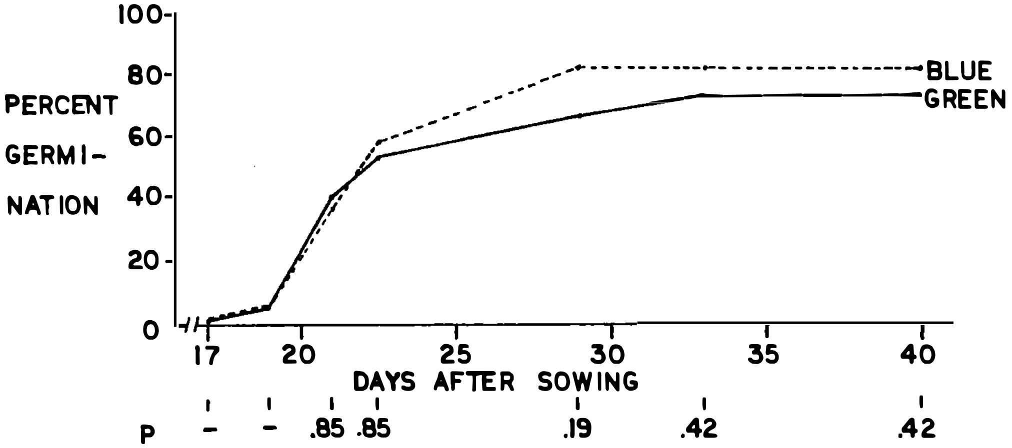

BLUE RIDGETOP STANDS
(P1, P2, P3, P5)
BLUE COASTAL STANDS
(N1 – N6)
GREEN RIDGETOP STANDS
(P4, P6)
GREEN COASTAL STANDS
(S1 – S6)
0
.001
.002
.003
.004
.005
.006
.007
.008
.009
DISTANCE (D)

FIGURE 4. Soil Experiment I. Germination percents of bishop pine seeds originating from blue and green populations for Davis, Caspar, and Blacklock soils. 30 seeds sown in each group. Significance values for differences between populations are given under each date.

1. DAVIS SOIL



2. CASPAR SOIL



3. BLACKLOCK SOIL

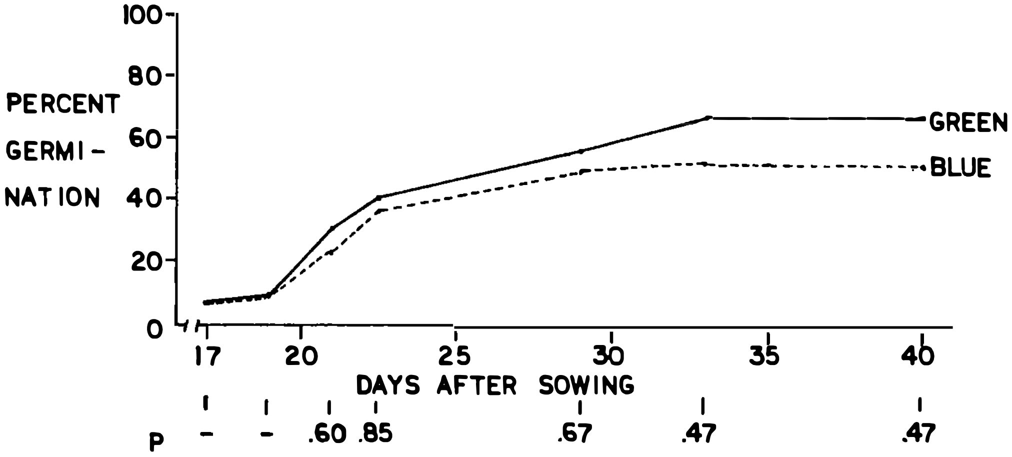
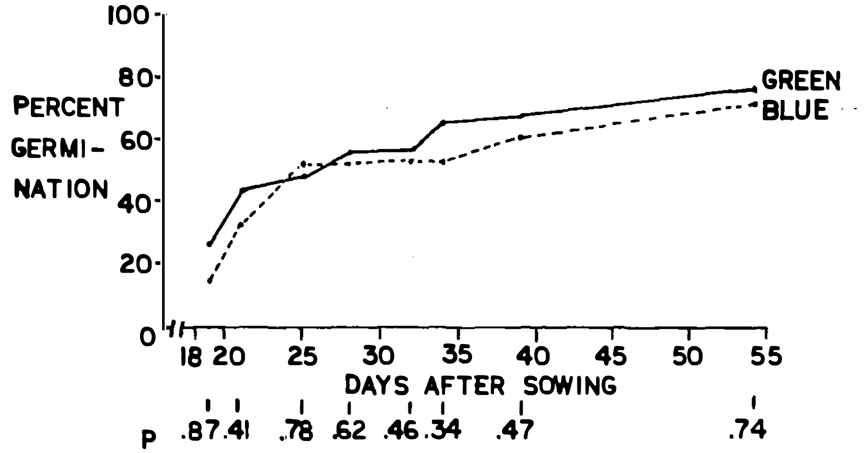
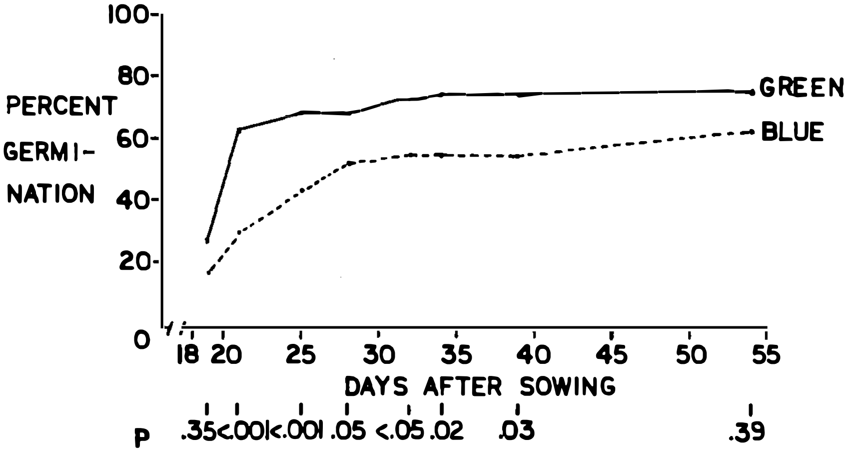


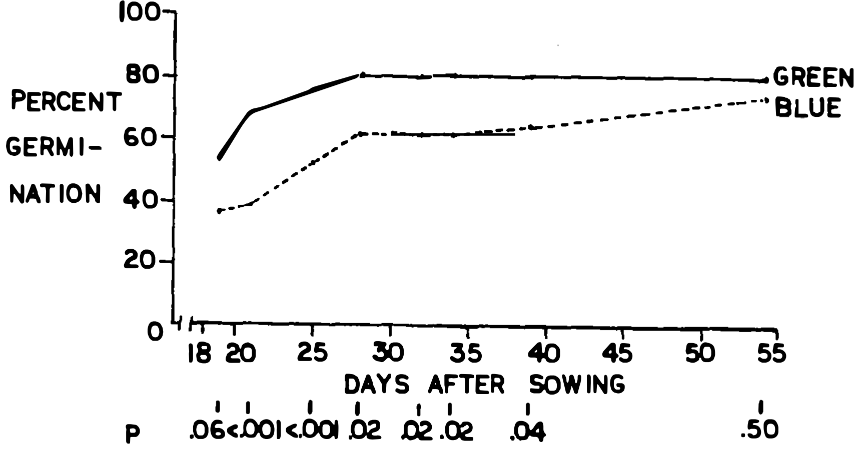
FIGURE 5. Soil Experiment II. Germination percents of bishop pine seeds originating from blue and green populations for Davis, Caspar, and Blacklock soils. 56 seeds sown in each group. Significance values for differences between populations are given under each date.



1. DAVIS SOIL



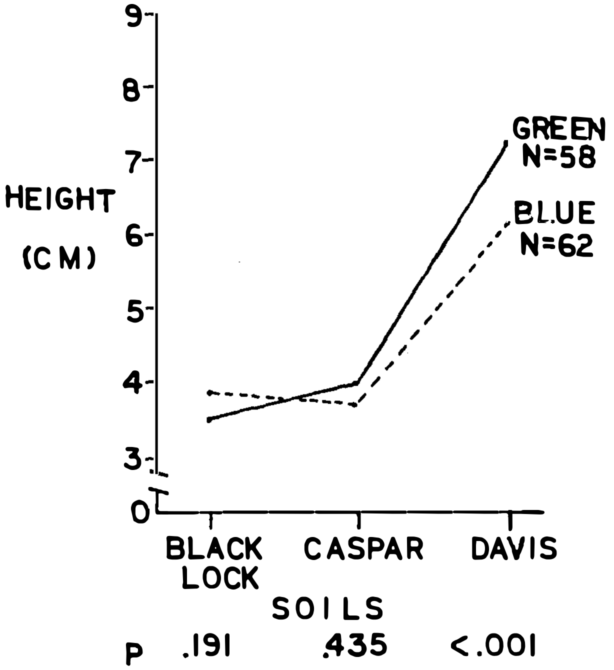
2. CASPAR SOIL



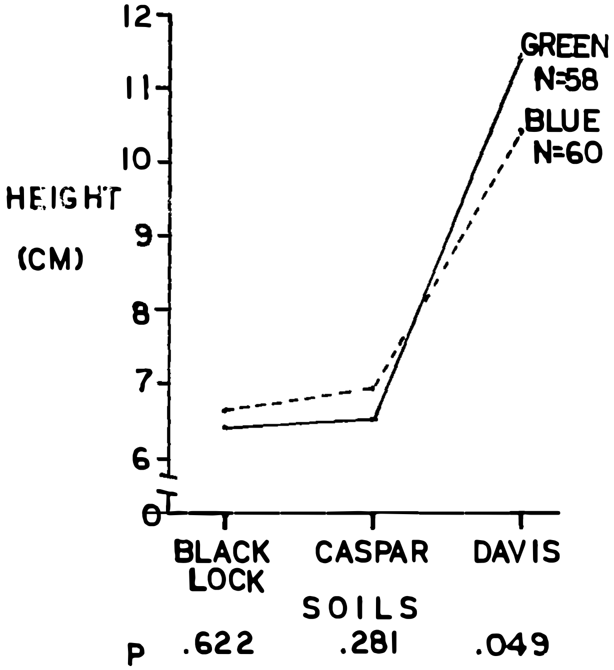
3. BLACKLOCK SOIL

FIGURE 6. Mean heights at different dates of blue- and green-origin bishop pine seedlings in soil experiments. 1. Experiment I, heights after 6.5 months (28 Jan. 1983). 2. Experiment I, heights after 12 months (8 July 1983). 3. Experiment I, heights after 13.75 months (30 Aug. 1983)a 4. Experiment II, heights after 5 months (2 Mar. 1984)a Significance values for differences between populations are given under the soil types.

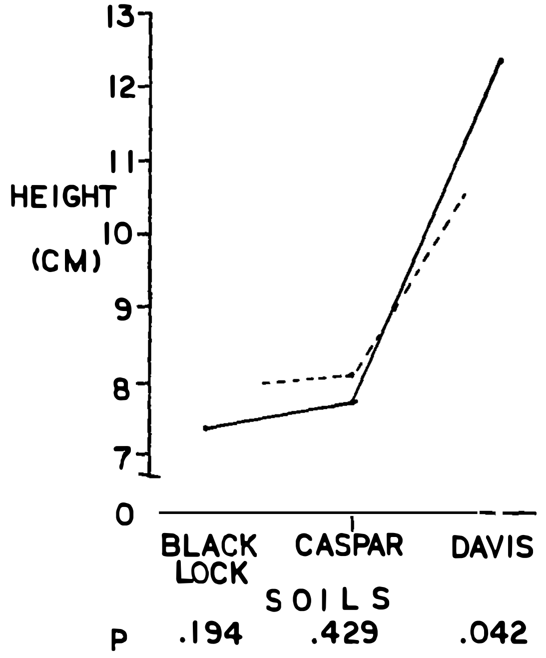
1. EXPERIMENT 1
28 JAN 1983



2. EXPERIMENT 1
8 JULY 1983



3. EXPERIMENT 1
30 AUG 1983



4. EXPERIMENT 2
2 MAR 1984

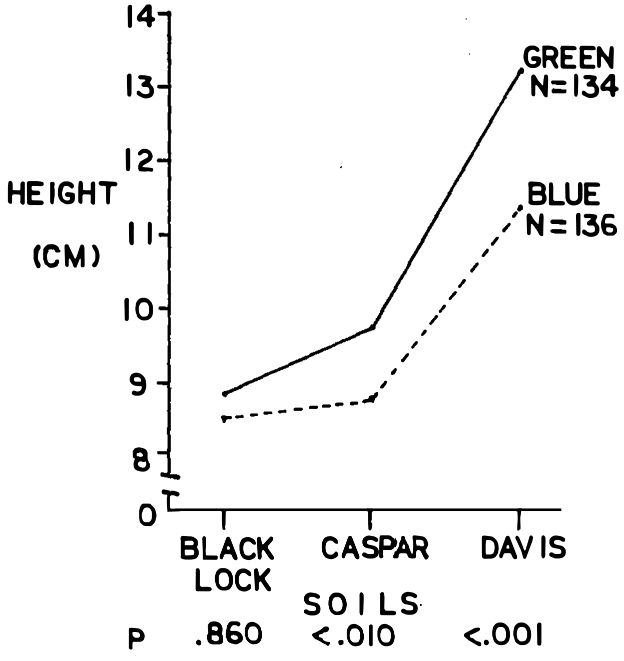
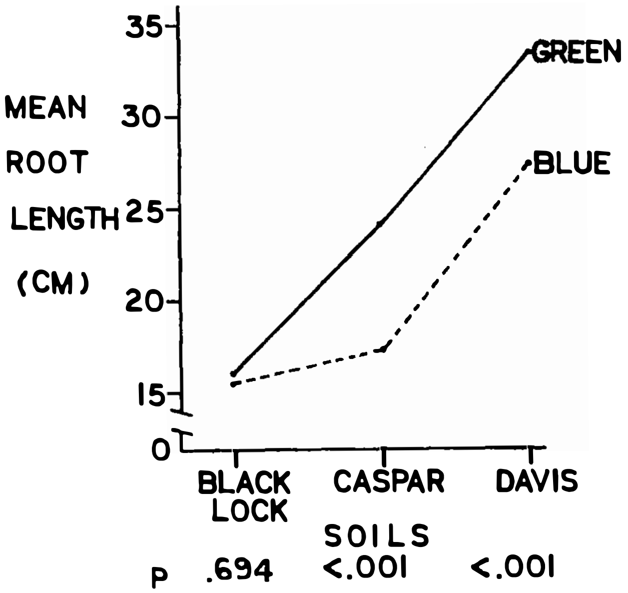
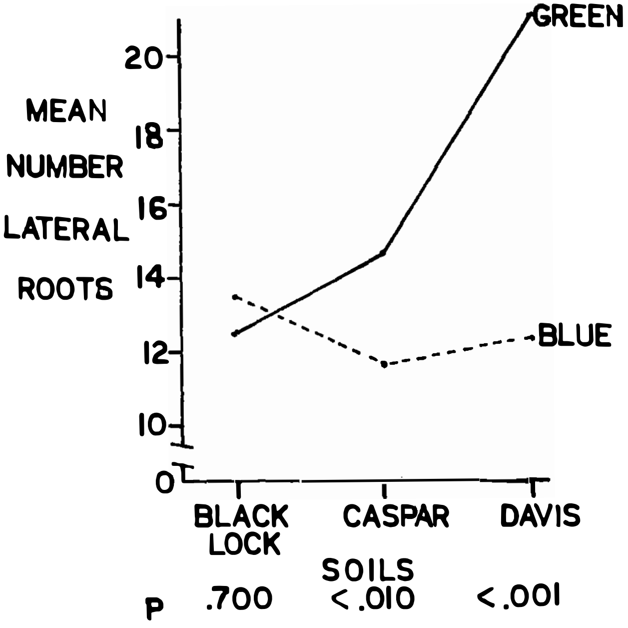


FIGURE 7. Mean root measurements in blue- and green-origin bishop pine seedlings measured 5 months after sowing. 1. Root lengths (of the longest root). 2. Number of lateral roots per seedling. 3. Dry root weights. For each trait, 44 seedlings per soil; 264 seedlings total per trait. Significance values for differences among populations are given under each soil type.

1. ROOT LENGTH



2. LATERAL ROOTS



3. DRY ROOT WEIGHT