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Jeffrey pine occurs in the mountains of California and adjacent states. In the Klamath Mountains, the northern margin of the range, it is restricted to infertile ultramafic soils, forming very open stands of low density. It occurs predominantly on more fertile soils in the Sierra Nevada and southern portions of the range, but does occur on ultramafic soils in the Sierra Nevada foothills. To contrast the Klamath Mountains with the Sierra Nevada - Southern region, I examined the mating system in five populations, and patterns of allozyme variation at 20 loci in 14 populations.

No significant difference was found in outcrossing between Klamath and Sierra populations, with both being predominantly outcrossing ($\bar{t}$ = .935). Genotype frequencies in these five populations fit Hardy-Weinberg expectations.

Eighteen of 20 loci surveyed were polymorphic. Average expected heterozygosity was lower in the Klamath than the Sierra Nevada - Southern region (.185 vs. .255). Allele frequency differences between the two regions were detected at 11 of 18 loci tested. The one Sierra population sampled on ultramafic soil was

more similar to Klamath populations in allele frequencies than were other Sierra populations, possibly suggesting genetic adaptation to ultramafic soils.

Jeffrey pine appears to be similar to most other conifers studied in having high outcrossing and high levels of genetic variability, with most of the variability occurring within populations.

Population Genetic Structure of Jeffrey Pine

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GENERAL INTRODUCTION

Jeffrey pine (Pinus jeffreyi Grev. & Balf.) is a coniferous forest tree species distributed in the mountains of California, southwestern Oregon, western Nevada, and Baja California (Critchfield and Little 1966). In the Klamath Mountains of southwestern Oregon and northwestern California, the northern limit of the species range, it is primarily restricted to sites with ultramafic soils (Jenkinson 1980a), where it occurs in nearly pure stands at wide spacing. Ultramafic soils are very infertile, having high levels of chromium, magnesium, and nickel (Walker 1954). Because of this, they often support a unique flora (Kruckeberg 1954, Whittaker 1954, 1960). Closed canopies are rarely found on these sites and the understory is composed of grasses, forbs, and sclerophytic shrubs. Moderate to large amounts of surface rock and bare soil are present in most stands, reflecting the open nature of the vegetation (Franklin and Dyrness 1973, Smith et al. 1984). Jeffrey pine also occurs on ultramafic soils in the western foothills of the central Sierra Nevada, often in association with ponderosa pine (Pinus ponderosa Dougl. ex Laws.). In contrast, Jeffrey pine grows on more fertile soils throughout the rest of its range, in both pure and mixed species stands (Jenkinson 1980a).

Southwestern Oregon and northwestern California are areas with a diversity of forest species and sites (Franklin and Dyrness 1973). The climate is mild in coastal regions, but is characterized by

extremes in the interior. Intense radiation, high temperatures, summer drought, drying winds, cold temperatures, and frost are common obstacles to reforestation efforts in the interior (Minore 1978, Stein 1981), where proper species and seed source selection are critical to reforestation success (Adams and Campbell 1982).

While ponderosa pine is often planted on the more environmentally extreme sites in this region, Jeffrey pine is not widely planted, despite some potential advantages it has for these sites. Throughout most of its range, Jeffrey pine grows in substantially harsher environments than ponderosa pine (Axelrod 1976, Haller 1959, Hallin 1957, Vasek 1978, Waring 1969), and where the species are found together on sites with short growing seasons its first year growth is actually superior to that of ponderosa pine (Fowells 1953). Relative to ponderosa pine, Jeffrey pine often completes annual top growth sooner, enters dormancy earlier, and requires longer cold exposure for rapid spring shoot elongation (Jenkinson 1980b). Because Jeffrey pine is very cold hardy (Haller 1959, 1961), adapted to short growing seasons, and drought tolerant (Stone 1957, Waring 1969, Waring and Majors 1964), it is typically dominant on cold, xeric, and infertile sites. Jeffrey pine seems well adapted to harsh conditions, and thus, is a species that should be considered when planting environmentally extreme sites in the interior region of southwestern Oregon and northwestern California.

Jeffrey and ponderosa pines are taxonomically closely related (Critchfield and Little 1966). They are indistinguishable on the basis of wood structure and are equally valued commercially (Panshin

and de Zeeuw 1970). Jeffrey pine has long been considered to be inherently slower growing than ponderosa pine, but this assumption may not be valid. In long term field studies, Jeffrey pine has indeed grown slower than ponderosa pine during the sapling stage, but has grown more rapidly during the pole stage (Callaham and Metcalf 1959, Fowells 1953, Jenkinson 1984, Oliver 1979). In a test of Sierra Nevada seed sources carried out in the North Coast Range of California, Jeffrey pine had larger mean heights and diameters at 17 years than ponderosa pine sources from comparable elevations (Callaham and Metcalf 1959). Thus, over the period of a normal rotation, these species may produce equivalent amounts of wood.

Despite the advantages of Jeffrey pine, there are some genetic factors that should be considered before widespread planting of this species in southwestern Oregon and northwestern California. In collecting wind-pollinated seed from natural stands in the Klamath Mountains, one might be concerned by the possibility of high rates of selfing in stands in which the trees are very widely spaced. Inbred seed resulting from self-fertilization would likely produce seedlings with reduced survival and growth rates (Sorensen and Miles 1982). In the first chapter of this thesis this concern is addressed by an examination the mating system in natural populations of Jeffrey pine.

Another genetic consideration in planting Jeffrey pine is the degree to which various seed sources are adapted to particular planting sites. In considering how far seed may safely be moved, one needs information on patterns of genetic variation in the

species. The best information for this purpose would come from long term field tests, in which different seed sources are grown in a range of environments and their performance is evaluated over a long period of time. Such studies are expensive and require many years before producing results. Preliminary information on the suitability of various seed transfers can be obtained from genetic mapping studies (Adams and Campbell 1982). One alternative is a short term common garden nursery test, in which growth and phenology traits are measured (Campbell 1984). Another alternative is to construct genetic maps at the single gene level using allozymes, a class of biochemical genetic markers (Conkle and Westfall 1984). Allozyme surveys do not directly examine growth and survival traits, but are less expensive and can yield results more rapidly than growth studies (Adams and Campbell 1982). Patterns of genetic variation at the single gene level may also be of value in developing gene conservation strategies (Adams 1981, Brown 1978, Brown and Moran 1981). Patterns of allozyme variation in Jeffrey pine are examined in the second chapter of this thesis.

CHAPTER 1. THE MATING SYSTEM IN NATURAL POPULATIONS OF JEFFREY PINE

Introduction

The mating system is an important determinant of plant population structure (Clegg 1980). Predominantly selfing plants have been found to be highly homozygous. In contrast, outcrossing provides the opportunity for much higher levels of recombination, often resulting in a more heterogeneous population (Brown 1979). The restriction of recombination imposed by selfing can lead to the development of multilocus gene complexes, even among loci on different chromosomes (Allard 1975).

Most plants are neither entirely selfing nor entirely outcrossing. Fyfe and Bailey (1951) described a mixed mating model in which all matings are classified as either selfs or outcrosses. They developed a method to estimate the mating system parameter t, where t (outcrossing rate) is the proportion of viable progeny resulting from outcrossing and s (= 1-t) is the proportion of viable progeny resulting from selfing. More refined statistical methods have since been developed for estimation of t (Brown and Allard 1970, Brown et al. 1975, Neale 1983, Shaw and Allard 1982).

Coniferous forest tree species are wind-pollinated, genetically highly variable (Hamrick 1979), and often display strong inbreeding depression (Franklin 1970). Despite an incomplete understanding of the mating biology of conifers, these features have led to the assumption of random mating in coniferous tree species. The mating

system of natural populations of conifers has been examined for only a small number of species (Cheliak 1983, Mitton et al. 1981, Neale 1983, Shaw and Allard 1982). Aside from the importance of such studies in understanding the population genetics of these species, this information is of practical significance for those using wind-pollinated seed for reforestation. Significant inbreeding depression, resulting in decreased survival and growth has been reported in seedling progeny of artificially selfed conifers (Sorensen and Miles 1982). Thus one wants to avoid collecting wind-pollinated seed from populations in which significant amounts of selfing occur.

In this study I examined the mating system in natural populations of Jeffrey pine (Pinus jeffreyi Grev. & Balf.), a coniferous tree species distributed in the mountains of California, southwestern Oregon, western Nevada, and Baja California (Critchfield and Little 1966). In the Klamath Mountains of southwestern Oregon and northwestern California, the northern limit of the species range, it is primarily restricted to sites with ultramafic soils (Jenkinson 1980a), where it occurs in nearly pure stands characterized by wide spacing and lack of crown closure (Franklin and Dyrness 1973, Smith et al. 1984). Ultramafic soils are very infertile, having high levels of chromium, magnesium, and nickel (Walker 1954). In contrast, Jeffrey pine grows on more fertile soils in the upper mixed conifer zone of the Sierra Nevada, in the central portion of its range. These stands generally form closed canopies comprised of a number of species, although Jeffrey

pine is the predominant species on the drier south and western aspects (Jenkinson 1980a).

Given Jeffrey pine's much more open stand structure in the Klamath Mountains, one might expect these populations to have lower outcrossing rates than those in the Sierra Nevada. The objective of this study was to estimate and compare the outcrossing rates in Klamath and Sierra Nevada populations of Jeffrey pine. I also examined the degree to which adult population structure reflected any inbreeding that might be expected from the mating system analysis.

Materials and Methods

I collected cones during 1981 from individual mother trees in three Jeffrey pine populations in the Klamath Mountains. Seed from cones collected during 1974 from individual mother trees in two central Sierra Nevada Jeffrey pine populations were obtained from the USDA Forest Service Institute of Forest Genetics at Placerville, California (Table 1.1). All sampled populations were in a natural unmanaged state. Sampling of mother trees within all populations was done randomly, with the exception that a tree was not sampled if it was less than eight meters from another sampled tree. Cones were not consciously collected from any one part of the crown. The wind-pollinated seed from these cones were extracted by hand and stored below 0°C. Seed was maintained by individual mother tree identity throughout processing and storage.

Starch gel electrophoresis was conducted on extracts of both

the megagametophyte (1N) and embryo (2N) tissue of seeds sampled from each mother tree, and a total of 16 enzyme systems were assayed. Methods for resolving aconitase (ACO), alcohol dehydrogenase (ADH), fluorescent esterase (FEST), glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT, also known as aspartate aminotransferase), glucose-6-phosphate dehydrogenase (G6P), isocitric dehydrogenase (IDH), leucine aminopeptidase (LAP), malic dehydrogenase (MDH), menadione reductase (MDR), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and 6-phosphogluconic dehydrogenase (6PG) were those of Conkle et al. (1982), with the exception that histidine-citrate (pH 8.0) gel and electrode buffers (Fildes and Harris 1966) were used for PGM and 6PG. Diaphorase (DIA) was assayed according to Yeh and O'Malley (1980), except for the use of a lithium borate (pH 8.3) gel buffer and tris-citrate - lithium borate (pH 8.3) electrode buffer (Conkle et al. 1982). Phosphomannose isomerase (PMI) was assayed according to El-Kassaby et al. (1982), except for the use of a tris-citrate (pH 8.8) gel buffer and sodium-borate (pH 8.6) electrode buffer (Adams and Joly 1980). Fluorescent hexaminase (FHEX, also known as hexoseaminidase) was assayed according to El-Kassaby et al. (1982), except for the use of morpholine citrate (pH 6.1) gel and electrode buffers (Conkle et al. 1982).

From the 16 enzyme systems assayed, 20 loci could be clearly scored in seed tissues. Sixteen of the loci (ACO, ADH, FEST, GDH, GOT1, GOT2, GOT3, G6P2, IDH, LAP1, MDH1, MDH4, PGM, PMI, 6PG1, and 6PG2) could be scored in both megagametophytes and embryos, but the

remaining four loci (DIA, FHEX, MDR, and PGI2) were consistently readable only in megagametophytes. With the exception of FHEX, inheritance of all 20 of these loci has been verified by genetic segregation tests in Jeffrey pine and the closely related ponderosa pine (Pinus ponderosa Dougl. ex Laws.) (Conkle 1981, O'Malley et al. 1979, Conkle, unpublished data, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California). While no genetic segregation data from large families was available for FHEX in Jeffrey pine, megagametophyte segregation patterns were consistent with single locus inheritance in the small families (7-20 progeny) we examined. Furthermore, FHEX in this zone has been shown to be under the control of a single gene in another conifer, Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) (El-Kassaby et al. 1982), and Conkle (1981) found great similarity between Jeffrey pine and Douglas-fir in allozyme band mobilities and appearance at the 17 loci he examined.

Based on segregation of allozymes among 7-20 (mean of 9.8) megagametophytes sampled per tree, genotypes of individual mother trees were inferred at 20 loci. The probability of incorrectly inferring the genotype of an individual tree at any one locus is less than .0156 when seven megagametophytes are sampled. In conifers, megagametophyte tissue is haploid and is genetically identical to the ovule forming the embryo in the seed. By assaying both the megagametophyte and the diploid embryo, the pollen (paternal) contribution to each embryo can be inferred. Comparison of the pollen genotype to that of the maternal tree forms the basis

for estimation of outcrossing rates.

Both single locus and multilocus methods of estimating outcrossing (t) in populations were used in this study. Both methods rely on the mixed mating model, described in detail by Shaw and Allard (1982). In this model it is assumed that each viable progeny is the result of a random outcross (with probability t) or a self-fertilization (with probability s), that the probability of observing an outcross progeny is independent of the genotype of the maternal parent, that outcross pollen pool allele frequencies are homogeneous among maternal parents, and that there is no selection between germination and census of seed progenies (Shaw et al. 1981). From this model, the conditional probabilities of observing different pollen gametes in the wind-pollinated progeny of maternal parents of known genotype can be derived. For example, the probability of observing an A_2 pollen gamete in the progeny of an A_1A_1 maternal parent is tq, where q is the frequency of the A_2 allele in the pool of pollen gametes common to all trees in the population (outcross pollen pool). The expected frequency of A_1 pollen gametes in the progeny of this A_1A_1 maternal parent is tp+s, where p is the frequency of the A_1 allele in the outcross pollen pool and s (= 1-t) is the proportion of viable progeny resulting from selfing.

For maximum likelihood estimation of t (t_s) based on single locus diallelic data, the procedure of Shaw and Allard (1982) was followed. In this procedure, t_s and p are jointly estimated from frequencies of progeny genotypes observed over all maternal

genotypic classes. For loci with three or more alleles, a triallelic extension of this procedure was used to estimate three mating system parameters (t_s and two pollen pool allele frequencies) (Neale 1983). At loci with more than three alleles, the two alleles with the highest frequencies were maintained and all remaining alleles were bulked into a synthetic allelic class. Estimation of t_s requires a minimum of two or three maternal genotypic classes for diallelic or triallelic cases, respectively. Therefore, t_s could not be estimated for all loci in all populations.

Multilocus estimation of t (t_m) was performed using the maximum likelihood estimator of Green et al. (1980). By comparing multilocus genotypes of pollen gametes to multilocus maternal parent genotypes, progeny are classified as either detectable outcrosses or ambiguous (the result of either a self or outcross). If the pollen gamete has an allele at any locus that is not carried by the maternal parent, then that progeny is classified as a detectable outcross, otherwise it is classified as ambiguous. This is done for all progenies of all maternal parents sampled in each population. The probability of observing an outcross (r_i) in the progeny of the ith maternal parent is

$$r_i = t_m G_i \quad ,$$

where G_i is the conditional probability of detecting an outcross pollen gamete given that an outcross has occurred (detection probability). The detection probability for each maternal parent was estimated by

$$\hat{G}_i = 1 - \prod_{j=1}^{k} \hat{f}_{ij} \quad ,$$

where $\hat{f}_{ij}$ is the sum of the estimated frequencies in the outcross pollen pool of alleles carried at the jth locus of the ith maternal parent and k is the number of loci. Since detection probability varies among maternal parents, the likelihood equation for the population estimate of t_m cannot be solved directly. Fisher's method of scoring (Rao 1973) was used to iteratively solve for $\hat{t}_m$.

The variance of $\hat{t}_m$ was estimated by the procedure of Green et al. (1980), which for ease of computation makes the simplifying assumption that the detection probability for each maternal parent is known and constant. Since the G_i's were estimated from the data, values of $Var(\hat{t}_m)$ given in this study should be considered minimum estimates.

In addition to the assumptions of the mixed mating model, multilocus estimation requires the assumption of independence among loci in the outcross pollen pool. Simulations by Shaw et al. (1981) have shown that potential biases in multilocus estimation are small unless deviations from random association of alleles at separate loci are large (i.e. linkage disequilibrium is strong). In predominantly outcrossing species, strong linkage disequilibrium is most likely to develop only among closely linked loci (Brown 1979). Of the loci we could clearly score in embryos, Conkle (1981) has shown that ACO and IDH are closely linked in Jeffrey pine. Since ACO was much more variable than IDH, and loci with low levels of variability provide little information about t_m (Shaw and Allard

1982), we did not use IDH in the single locus or multilocus mating system analyses. In addition, a minimum of two maternal genotypic classes were not present at some loci in some of the populations, further reducing the number of loci available for estimation of t. The number of loci used for estimation of t varied among populations from 9 to 12.

Multilocus estimation is statistically more efficient than single locus estimation because multilocus data sets contain more information about outcrossing than is available at any one single locus. As more loci are assayed, more outcrosses are detected directly and the importance of the ambiguous class decreases. Furthermore, as more detectable outcrosses are observed, multilocus estimation becomes less sensitive to violations of assumptions of the mixed mating model (Shaw et al. 1981).

All 20 loci assayed were used to assess the population genetic structure of the five sampled populations. As measures of variation in each of the populations, unweighted means over loci of observed heterozygosity (H_o) and expected heterozygosity (H_e) among parent trees were calculated. We used Nei's (1978) unbiased formula for expected heterozygosity, which is corrected for small sample size and for a single locus is estimated as

$$\hat{H}_e = (2N/2N-1)(1-\Sigma \hat{p}_i^2) \quad ,$$

where $\hat{p}_i$ is the estimated frequency of the ith allele and N is the number of trees sampled in the population.

Wright's fixation index, F_{IS}, a measure of the deviation of observed heterozygosity from that expected under Hardy-Weinberg

equilibrium, was estimated as

$$\hat{F}_{IS} = 1-(\hat{H}_o/\hat{H}_e)$$

(Kirby 1975, Wright 1951). Under Hardy-Weinberg equilibrium conditions, which include random mating, F_{IS} should have a value of 0. If the mating system is the only factor causing deviations from Hardy-Weinberg equilibrium, an expected equilibrium inbreeding coefficient, F_e, can also be calculated. This value is based on the multilocus outcrossing rate, t_m, and was estimated as

$$\hat{F}_e = (1-\hat{t}_m)/(1+\hat{t}_m)$$

(Allard et al. 1968).

Observed genotype frequencies were tested against those expected under Hardy-Weinberg equilibrium using a goodness of fit test (Sokal and Rohlf 1981). In order to account for selfing, the F_e value for each population was used to calculate a set of genotype frequencies expected under mating system equilibrium. The frequency of each homozygous class was estimated as

$$\hat{P}_{ii} = \hat{p}_i^2(1-\hat{F}_e) + \hat{p}_i\hat{F}_e \quad ,$$

and the frequency of each heterozygous class was estimated as

$$\hat{P}_{ij} = 2\hat{p}_i\hat{p}_j(1-\hat{F}_e) \quad ,$$

where $\hat{p}_i$ and $\hat{p}_j$ were the estimated frequencies of the ith and jth alleles at a locus. The same goodness of fit test (Sokal and Rohlf 1981) was used to test observed genotype frequencies against those expected under mating system equilibrium. All statistical tests of significance in this study were conducted at the $p = .05$ level.

Results

Single locus estimates of outcrossing (t_s) ranged widely over loci and populations (.493 to 1.087), and were significantly heterogeneous over loci in three of five populations (Table 1.2). Mean estimates of t_s were in all cases lower than multilocus estimates (t_m) for the same population (Table 1.3). Mean single locus and multilocus t estimates were significantly heterogeneous among populations within each region, but means for the two regions did not differ significantly.

Shaw and Allard (1982) have suggested comparing single locus and multilocus estimates of t as a means of detecting family structure in populations. This is possible because the single locus estimation procedure is more sensitive to related matings other than selfing. If mating occurs between related individuals, t_s will generally be underestimated. Ellstrand and Foster (1983) observed lower single locus outcrossing rates when family structure was introduced into experimental populations of Sorghum bicolor (L.) Moench. Examination of the spatial distribution of genotypes within Jeffrey pine populations at loci with unusually low t_s estimates indicated some clustering of genotypes. An example is the GOT2 locus in the K1 population ($\hat{t}_s$ = .493), where out of a total of 53 trees sampled, 47 trees were homozygous for allele 1 and six were heterozygous for alleles 1 and 2. Examination of the spatial distribution of the six heterozygotes revealed that they occurred in two clusters of three trees each. Similar clusters were observed at

the G6P2 locus in the K1 population and at the GOT2 locus and PGM locus in the K7 population. Such clustering could not be examined in populations S2 and S3 because information on the location of individual trees within these populations was not available. This clustering may not be unexpected in a fire-adapted species such as Jeffrey pine, in which neighboring trees may be the progeny of individual, widely spaced survivors of a past fire. Our failure to census the entire population in this study, however, makes detection of family groups difficult.

Estimates of mean observed (H_o) and expected (H_e) heterozygosities for all populations sampled were comparable to the relatively high values reported for other conifers (Hamrick et al. 1981). The range of values among the populations was great, with considerably higher values in the two Sierra Nevada populations (Table 1.4). Observed heterozygosities were very similar to those expected under Hardy-Weinberg equilibrium, which is reflected in the small deviations of F_{IS} estimates from 0 (Table 1.4). Observed genotype frequencies were not significantly different from those expected under Hardy-Weinberg equilibrium except at two loci (DIA and GOT1) in population S3 and one locus (G6P2) in population S2.

Since the mating system in Jeffrey pine includes some selfing, an excess of homozygotes would be expected in the parent trees if the populations are in mating system equilibrium. This is reflected in the F_e estimates, which are positive for all populations. Nevertheless, in all populations, estimates of F_{IS} are lower (and in four of the five populations negative) than estimates of F_e (Table

1.4), indicating an excess of heterozygotes relative to the expectation based on mating system equilibrium. Observed genotype frequencies, however, were significantly different from those expected under mating system equilibrium in only two of the five populations. Those deviations occurred at only two loci (DIA and GOT1) in population S3, but at 11 loci (ACO, ADH, DIA, FEST, FHEX, GOT1, G6P2, MDH4, MDR, PGI2, and PGM) in population S2. All deviations from mating system equilibrium in population S2 were due to an excess of heterozygotes.

Discussion

The t estimates reported in this study indicate that Jeffrey pine is a highly outcrossed species. These estimates are comparable to the high values reported for natural populations of other conifer species, including ponderosa pine (Mitton et al. 1981), jack pine (*Pinus banksiana* Lamb.) (Cheliak 1983), Douglas-fir (El-Kassaby et al. 1981, Neale 1983, Shaw and Allard 1982), and balsam fir (*Abies balsamea* [L.] Mill.) (D.B. Neale and W.T. Adams, unpublished data, Department of Forest Science, Oregon State University).

The differences in site and stand characteristics between Klamath and Sierra Nevada populations of Jeffrey pine do not appear to have an affect on the outcrossing rate. One stand characteristic that has received some study in other plant species is stand density. Outcrossing has generally been observed to be inversely related to stand density in insect pollinated species (Ellstrand et al. 1978), but for wind-pollinated species a positive relationship

might be expected. Presumably, in widely spaced stands less of the pollen cloud at any individual is outcross pollen and, hence, more is self pollen. Rudin et al. (1977), in a study of a seed tree stand of Scotch pine (Pinus sylvestris L.), presented indirect evidence to support this contention. Neale (1983), however, found no significant differences in outcrossing rate among fully stocked stands of Douglas-fir and stands thinned to 35 and 15 trees per hectare. The Klamath populations in this study ranged from 43 trees per hectare in population K7 to 17 trees per hectare in population K4, but no simple relationship between density and outcrossing is apparent. Even in these very open stands which lack crown closure, the outcrossing rate was high in the year sampled.

Sorensen (1982) has shown that when self fertility is low, as is the case in most conifers studied (Franklin 1970, Sorensen 1970), very large differences in the rate of self-fertilization are necessary to detect differences in the frequency of self progeny. This is because most self-fertilization events will not result in a viable seed, and the outcrossing rate is estimated at the viable seed stage. Results from Jeffrey pine and Douglas-fir (Neale 1983) indicate that stand density will have to become quite low before a significant reduction in outcrossing rate occurs.

Examination of adult population genetic structure in the Jeffrey pine populations sampled did not reveal any significant inbreeding effects. In fact, with the exception of GOT1 in population S3, all significant deviations of observed genotype frequencies from those expected under mating system equilibrium were

due to excesses of heterozygotes. The heterozygote excess observed at many loci in population S2 could be due to a number of causes. The most likely cause is selection moving the population out of mating system equilibrium. The outcrossing rate is estimated at the viable seed stage, yet most of the viable seed that result from self-fertilization are probably lost as young seedlings due to inbreeding depression (Franklin 1970, Sorensen and Miles 1974, 1982). This would allow very few selfs to survive to the adult stage, leaving an adult population composed almost exclusively of outcrossed individuals. This would help to explain the lack of significant deviations of observed adult genotype frequencies from those expected under Hardy-Weinberg equilibrium.

Another possible explanation for the observed excess of heterozygotes over that expected under mating system equilibrium is temporal variation in the mating system. Significant heterogeneity in outcrossing rate among seed crops collected in different years has been found in populations of alpine ash (Eucalyptus delegatensis R.T. Bak.) (Moran and Brown 1980) and small fescue (Festuca microstachys Nutt.) (Adams and Allard 1982). The test of observed genotype frequencies against those expected under mating system equilibrium assumes that the observed outcrossing rate is the same as that in the seed crop that generated the current adult S2 population. If the outcrossing rate in that seed crop was higher than that observed in this study, the test would not be valid and the significant heterozygote excess indicated by this test may not exist (Adams and Allard 1982).

The high outcrossing rate, relatively high heterozygosity, and lack of significant levels of inbreeding in adult populations of Jeffrey pine agree well with the results of studies of other conifers. This is true even for the very open stands in the Klamath Mountains. These are important components of the adaptive strategy of this species. Outcrossing is a mechanism for maintaining a high level of genetic variability, which presumably is important to survival in a forest environment that is spatially and temporally heterogeneous.

Table 1.1. Locations and designations of Jeffrey pine collection sites in the Klamath Mountains and Sierra Nevada, and number of maternal parent trees (N) sampled per population.

Region/ Population	N	Latitude	Longitude	Elevation (m)
Klamath Mountains				
K1	53	42° 35'	123° 23'	505
K4	54	41° 55'	123° 39'	1645
K7	57	41° 17'	122° 42'	1660
Sierra Nevada				
S2	17	38° 48'	120° 09'	1735
S3	17	38° 48'	120° 07'	1980

Table 1.2. Single locus population estimates of outcrossing rate, $\hat{t}_s$, for five Jeffrey pine populations.[a]

	Population				
Locus	K1[b]	K4	K7[b]	S2[b]	S3
ACO	.898[c]	.935	.899[c]	.922	.918
ADH	.921	.968	.916	.930	.983
FEST	.886[c]	1.002	.961	.847	.864[c]
GOT1	.869[c]	.961	.998	.667[c]	.961
GOT2	.493[c]	--[d]	.693[c]	.771[c]	.921
GOT3	--[d]	--[d]	--[d]	.592[c]	1.035
G6P2	.699[c]	.896	1.080	.883	.900
LAP1	1.013	.969	1.030	.734[c]	.983
MDH4	--[d]	.769[c]	1.009	.965	1.010
PGM	.922	.960	.732[c]	1.044	.792[c]
6PG1	--[d]	--[d]	1.009	.748[c]	1.087
6PG2	.845[c]	.842[c]	.985	.533[c]	.843

[a] Standard errors for $\hat{t}_s$ values ranged from .007 to .187 with a mean of .081.

[b] Significant ($p < .05$) heterogeneity of $\hat{t}_s$ over loci, based on Fisher's chi-square test (Rao 1973).

[c] Significantly ($p < .05$) different from $t_s = 1.0$ based on chi-square likelihood ratio test (Brunk 1975).

[d] Insufficient maternal genotype classes for calculation of $\hat{t}_s$.

Table 1.3. Mean single locus ($\bar{t}_s$)[a] and multilocus ($\hat{t}_m$) estimates of outcrossing (standard errors in parentheses) for three Klamath Mountains and two Sierra Nevada populations of Jeffrey pine.

Region/ Population	Loci[b]	N[c]	$\bar{t}_s$	$\hat{t}_m$
Klamath Mountains				
K1	9	422	.838(.028)	.881(.023)
K4	9	432	.922(.028)	.949(.025)
K7	11	456	.938(.019)	.971(.016)
Mean[e]			.899(.015)[d]	.933(.012)[d]
Sierra Nevada				
S2	12	316	.803(.034)	.908(.022)
S3	12	310	.946(.024)	.966(.016)
Mean[e]			.874(.021)[d]	.937(.014)[d]

[a] Unweighted mean over loci.
[b] Number of loci used in estimating $\bar{t}_s$ and t_m.
[c] Number of progeny in sample.
[d] Individual population estimates are significantly ($p < .05$) heterogeneous, based on Fisher's chi-square test (Rao 1973). All individual population t_m estimates are significantly ($p < .05$) different from $t_m = 1.0$, based on chi-square likelihood ratio test (Brunk 1975).
[e] Unweighted mean over populations.

Table 1.4. Observed (H_o) and expected (H_e) heterozygosities, Wright's fixation index (F_{IS}), and expected equilibrium inbreeding coefficient (F_e)[a] for three Klamath Mountains and two Sierra Nevada populations of Jeffrey pine.

Region/ Population	H_o	H_e[b]	F_{IS}	F_e
Klamath Mountains				
K1	.173(.038)[c]	.181(.040)[c]	.044	.063
K4	.147(.042)	.144(.040)	-.020	.026
K7	.227(.046)	.219(.042)	-.037	.015
Sierra Nevada				
S2	.325(.062)	.294(.054)	-.105	.048
S3	.329(.064)	.299(.058)	-.100	.017

[a] F_e is the inbreeding expected at mating system equilibrium, assuming the mixed mating model and outcrossing equal to t_m (Table 1.3).

[b] Unbiased expected heterozygosity (Nei 1978).

[c] Standard errors in parentheses.

CHAPTER 2. GEOGRAPHIC PATTERNS OF ALLOZYME VARIATION IN JEFFREY PINE

Introduction

Electrophoretic surveys of enzyme polymorphism have been conducted for a wide variety of plant species in an effort to characterize levels and patterns of genetic variation among and within natural populations. Forest tree species have generally been found to have higher levels of genetic variability than other plants (Hamrick 1979), but most of this variation resides within populations, with a much smaller amount due to differentiation among populations (Brown and Moran 1981, Dancik and Yeh 1983, Guries and Ledig 1982, O'Malley et al. 1979, Wheeler and Guries 1982, Yeh and El-Kassaby 1980, Yeh and O'Malley 1980).

In this study, I examined allozyme variation in Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), a coniferous tree species distributed in the mountains of California, southwestern Oregon, western Nevada, and Baja California (Figure 2.1) (Critchfield and Little 1966, Griffin and Critchfield 1972). In the Klamath Mountains of southwestern Oregon and northwestern California, the northern limit of the species' range, Jeffrey pine is primarily restricted to sites with ultramafic soils (Jenkinson 1980a), where it occurs in nearly pure stands at wide spacing (Franklin and Dyrness 1973, Smith et al. 1984). Ultramafic soils are very infertile, having high levels of chromium, magnesium, and nickel (Walker 1954). Because of this, they often support a unique flora

(Kruckeberg 1954, Whittaker 1954, 1960). The differences between ultramafic soils and more fertile nonultramafic soils are strong enough to induce in a number of plant species the formation of edaphic ecotypes genetically adapted to ultramafic soils (Kruckeberg 1967). Jeffrey pine also occurs on ultramafic soils in the western foothills of the central Sierra Nevada, often mixed with ponderosa pine (Pinus ponderosa Dougl. ex Laws.) (Figure 2.1). In contrast, Jeffrey pine grows on more fertile soils throughout the rest of its range, in both pure and mixed species stands (Jenkinson 1980a).

There recently has been increasing interest in managing Jeffrey pine in the Klamath Mountains, but there is currently no information available on patterns of genetic variation in this portion of the species' range. Such information is of practical significance in planning seed movements (Adams and Campbell 1982) and gene conservation strategies (Adams 1981, Brown 1978, Brown and Moran 1981). Given the restriction of this species to ultramafic soils in the Klamath Mountains, yielding a somewhat discontinuous distribution, one might expect large differences among populations in this region due to genetic drift. Hence, the first objective of this study was to examine patterns of allozyme variation in this region. Since the Klamath Mountains represent the northern limit of the species range and an area of restriction to ultramafic soils, our second objective was to compare allozyme variation in the Klamath Mountains to that of populations in the central part of the range. Our last objective was to at least roughly examine allozyme variation over the species' range.

Materials and Methods

Cones were collected by the author during 1981 from individual trees in seven Jeffrey pine populations in the Klamath Mountains. Seed from cones collected during 1971, 1974, and 1981 from individual trees in seven Jeffrey pine populations from the central Sierra Nevada and southern portions of the species' range were obtained from the USDA Forest Service Institute of Forest Genetics at Placerville, California (Table 2.1, Figure 2.1). All sampled populations were in a natural unmanaged state. Sampling of mother trees within all populations was done randomly, with the exception that a tree was not sampled if it was less than eight meters from another sampled tree.

In order to meet our first objective of describing patterns of variation in the Klamath Mountains, sampling in this portion of the species' range was much more intensive. For the remainder of the range we had to rely on previous cone collections, resulting in small sample sizes in these populations and large portions of the species' range that were not sampled. Despite limitations of sample size and distribution, these populations were included in the study to provide some information on genetic variation in Jeffrey pine in areas other than the Klamath. The S1 population is the only population sampled outside of the Klamath Mountains that was on ultramafic soil. The S6 population is a composite of a number of single tree collections made throughout the mountains surrounding the Los Angeles Basin. The S7 population is at the southernmost

limit of Jeffrey pine's range. The wind-pollinated seed from the collected cones were extracted by hand and stored below 0°C. Seed was maintained by individual mother tree identity throughout processing and storage.

Starch gel electrophoresis was conducted on extracts of megagametophyte (1N) tissue of seeds sampled from each mother tree, and a total of 16 enzyme systems were assayed. Methods for resolving aconitase (ACO), alcohol dehydrogenase (ADH), fluorescent esterase (FEST), glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT, also known as aspartate aminotransferase), glucose-6-phosphate dehydrogenase (G6P), isocitric dehydrogenase (IDH), leucine aminopeptidase (LAP), malic dehydrogenase (MDH), menadione reductase (MDR), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and 6-phosphogluconic dehydrogenase (6PG) were those of Conkle et al. (1982), with the exception that histidine-citrate (pH 8.0) gel and electrode buffers (Fildes and Harris 1966) were used for PGM and 6PG. Diaphorase (DIA) was assayed according to Yeh and O'Malley (1980), except for the use of a lithium borate (pH 8.3) gel buffer and tris-citrate - lithium borate (pH 8.3) electrode buffer (Conkle et al. 1982). Phosphomannose isomerase (PMI) was assayed according to El-Kassaby et al. (1982), except for the use of a tris-citrate (pH 8.8) gel buffer and sodium-borate (pH 8.6) electrode buffer (Adams and Joly 1980). Fluorescent hexaminase (FHEX, also known as hexoseaminidase) was assayed according to El-Kassaby et al. (1982), except for the use of morpholine citrate (pH 6.1) gel and electrode buffers (Conkle

et al. 1982).

From the 16 enzyme systems assayed, 20 loci could be clearly scored in seed tissue. With the exception of FHEX, inheritance of all 20 of these loci has been verified by genetic segregation tests in Jeffrey pine and the closely related ponderosa pine (Pinus ponderosa Dougl. ex Laws.) (Conkle 1981, O'Malley et al. 1979, Conkle, unpublished data, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California). While no genetic segregation data from large families was available for FHEX in Jeffrey pine, megagametophyte segregation patterns were consistent with single locus inheritance in the small families (7-20 progeny) we examined in this study. Furthermore, FHEX in this zone has been shown to be under the control of a single gene in another conifer, Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) (El-Kassaby et al. 1982), and Conkle (1981) found great similarity between Jeffrey pine and Douglas-fir in allozyme band mobilities and appearance at the 17 loci he examined.

In five of the populations (K1, K4, K7, S2, and S3), allele frequencies were calculated from genotype frequencies. Genotypes of all mother trees at all 20 loci were inferred from the segregation of isozymes in a sample of 7-20 megagametophytes per tree (mean of 9.8). The probability of incorrectly inferring the genotype of an individual tree at any one locus is less than .0156 when seven megagametophytes are sampled. In the remaining nine populations, allele frequencies were calculated from a bulk sample of megagametophytes obtained by assaying two megagametophytes per tree.

For the purpose of analysis the populations were divided into two groups, the Klamath region and the Sierra Nevada - Southern region. Heterogeneity chi-square tests were used to test for differences in allele frequencies among populations within regions and between regions (Workman and Niswander 1970). Alleles with low frequencies were bulked with the next lowest frequency allele if the expected number of individuals in a class was less than one (Snedecor and Cochran 1967). This bulking eliminated tests at a number of loci which were polymorphic only for rare alleles. An additional adjustment was required because of our sampling scheme. Morris and Spieth (1978) have shown that in the absence of inbreeding the variance of p_i (the frequency of the ith allele) is

$$\mathrm{Var}(p_i) = \{[p_i(1-p_i)]/2N\}\{2-[1-(1/2)^{k-1}]\} \quad ,$$

where N is the number of trees sampled and k is the number of megagametophytes sampled per tree. For the five populations in which 7-20 megagametophytes per individual were sampled, this is approximated very closely by

$$\mathrm{Var}(p_i) = [p_i(1-p_i)]/2N \quad .$$

For the nine populations in which two megagametophytes per individual were sampled

$$\mathrm{Var}(p_i) = [p_i(1-p_i)]/1.33N \quad ,$$

so 1.33N was used in place of 2N in Workman and Niswander's (1970) formula when performing heterogeneity chi-square calculations for these nine populations.

Genetic distance values (Nei 1978) were estimated for all pairs of populations. Association between genetic distance and geographic

distance was examined, both within regions and over the entire species' range, by correlation analysis using the product moment correlation. A t-test was used to test the significance of the correlation (Sokal and Rohlf 1981). Phenetic clustering based on genetic distance was performed using the UPGMA procedure (Sneath and Sokal 1973).

As a measure of variability, unweighted means over loci of expected heterozygosity, H_e, were estimated using Nei's (1978) unbiased formula. Unbiased expected heterozygosity is corrected for small sample size and for a single locus is

$$H_e = (2N/2N-1)(1-\Sigma p_i^2) \quad ,$$

where p_i is the frequency of the ith allele and N is the number of trees sampled in the population.

Partitioning of variation among and within populations was examined using gene diversity statistics (Nei 1973). Total gene diversity, H_T, a measure of total variation in the entire sample of populations, is given as

$$H_T = 1-\Sigma \bar{p}_i^2 \quad ,$$

where $\bar{p}_i$ is the mean frequency of the ith allele. Total gene diversity is partitioned as

$$H_T = H_S+D_{ST} \quad ,$$

where H_S and D_{ST} are average genetic diversities within and among populations, respectively. The proportion of total gene diversity due to genetic differences among populations is

$$G_{ST} = D_{ST}/H_T$$

(Nei 1973).

All statistical tests of significance in this study were conducted at the p = .05 level.

Results

Of the 20 loci assayed, 18 were polymorphic in at least one of the 14 populations surveyed, and many were highly polymorphic, with up to four alleles (Table 2.2). Much variation was also observed among populations within regions and between regions. Within the Klamath region, significant heterogeneity among populations was observed at 13 of the 14 loci tested (ACO, ADH, DIA, FEST, FHEX, GOT1, GOT2, G6P2, IDH, LAP1, MDR, PGM, and 6PG2; but not at the PGI locus). Within the Sierra Nevada - Southern region, significant heterogeneity among populations was observed at only three of the 11 loci tested (DIA, G6P2, and MDH4; but not at ACO, ADH, FEST, GOT1, LAP1, MDR, PGI, and PGM). Due to the small sample sizes in this region, however, only large allele frequency differences among the populations sampled could have been declared significant. Significant heterogeneity between regions was observed at 11 of the 18 loci tested (ACO, ADH, DIA, FEST, FHEX, GOT1, G6P2, MDH4, MDR, 6PG1, and 6PG2; but not at GDH, GOT2, GOT3, IDH, LAP1, PGI, and PGM). At some loci, the differences between regions were quite large (eg. .567 for ADH allele 1 and .384 for G6P2) (Table 2.2).

Mean genetic distance among populations within the Klamath region was 0.010, within the Sierra Nevada - Southern region was 0.027, and between the two regions was 0.060 (Table 2.3). A significant positive correlation of genetic distance with geographic

distance was observed when all populations were included in the analysis and when all the Sierra Nevada - Southern populations were analyzed separately. There was, however, no significant correlation when the Klamath populations were analyzed separately, nor when only the central Sierra Nevada populations (S1, S2, S3, S4, and S5) were analyzed. This suggests that genetic distance is related to geographic distance only over large areas and not when relatively small geographic areas (150 km) are considered. Phenetic clustering based on genetic distance clustered the populations into two distinct groups, the Klamath populations and the Sierra Nevada - Southern populations (Figure 2.2).

Mean expected heterozygosity ranged from .144 to .299 over all 14 populations sampled (Table 2.2). The values were not significantly heterogeneous among populations within either region, but the means for the two regions were significantly different, the Sierra Nevada - Southern region having almost 40% higher expected heterozygosity. In the five populations in which all trees were genotyped, goodness of fit tests (Sokal and Rohlf 1981) revealed very few significant deviations of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium, indicating that H_e is probably a good estimate of the actual amount of heterozygosity in Jeffrey pine populations.

Mean total gene diversity over all loci was 0.248, with the largest proportion of this total (86.2%) due to variation within populations (Table 2.4). Averaged over all loci, 13.8% of the total diversity was due to differences among populations, with

roughly equal amounts due to differences between regions (7.0%) and among populations within regions (6.8%). When the regions were considered individually, 4.5% of the variation in the Klamath region was due to differences among populations and 9.2% of the variation in the Sierra Nevada - Southern region was accounted for by among population differences. Considering the central Sierra Nevada populations alone, 6.8% of the variation was due to differences among populations. The amount of among population variation found in the Sierra Nevada - Southern region and the central Sierra Nevada may, however, be somewhat overestimated due to the small sample sizes.

Discussion

Levels of genetic variability found in both Klamath and Sierra Nevada - Southern populations of Jeffrey pine are within the range of values reported for other conifers (Hamrick et al. 1981). Even in the Klamath Mountains, the northern margin of the species' range, 18 of the 20 loci assayed were polymorphic and significant allele frequency heterogeneity was found among populations. The high proportion of total genic diversity observed within populations of Jeffrey pine also agrees with findings in most other conifer species (Brown and Moran 1981, Dancik and Yeh 1983, Guries and Ledig 1982, Hiebert and Hamrick 1983, O'Malley et al. 1979, Steinhoff et al. 1984, Wheeler and Guries 1982, Yeh and El-Kassaby 1980, Yeh and O'Malley 1980). It has been suggested that the high level of variability within populations of forest trees is an adaptive

response to spatial and temporal environmental heterogeneity encountered by individual populations (Campbell 1979, Silen 1982).

The genetic distance data and gene diversity analyses indicate that differentiation among Jeffrey pine populations in the Klamath Mountains is not any greater than that among populations in the central Sierra Nevada, an area of roughly comparable size. This is despite a much more discontinuous distribution of Jeffrey pine in the Klamath Mountains, a factor that might be expected to contribute to differentiation among populations due to genetic drift. Possibly, directional selection exerted by the ultramafic soils prevents dispersion of allele frequencies among the Klamath populations. Intermountain bristlecone pine (Pinus aristata var. longaeva [D.K. Bailey] Little), a conifer with a discontinuous distribution in the Great Basin, shows a level of differentiation among populations similar to that observed among Jeffrey pine populations in the Klamath Mountains (Hiebert and Hamrick 1983).

Significant differences in allele frequencies between the Klamath and Sierra Nevada - Southern regions were observed at 11 of the 20 loci examined. Gene diversity analysis revealed that half of the genic differences observed among populations in this study were due to this difference between regions. These differences were not only in allele frequencies, but also in levels of variability, with significantly higher estimates of expected heterozygosity in the Sierra Nevada - Southern populations.

Aside from being geographically distinct, the Klamath Mountains represent for Jeffrey pine an area of restriction to ultramafic

soils. Hence, the Klamath populations are both geographically and ecologically marginal. Carson (1959) has suggested that marginal populations would tend to evolve largely homozygous genotypes specialized to deal with the difficulties of marginal existence. Central populations, on the other hand, would tend to possess higher heterozygosity, allowing them to deal with their more varied environment. A different, but complementary hypothesis was proposed by da Cunha et al. (1959). They suggested that the center of a species range is the area in which the species has mastered the greatest variety of available ecological niches. In contrast, at the margin of its range a species may have mastered only a few ecological niches. If genetic diversity is the mechanism by which mastery of diverse environments is accomplished, then central populations would tend to be more variable than marginal populations. Lewontin (1957), on the other hand, has suggested that temporal instability in marginal environments could lead to selection pressures as diverse as those found in central areas.

Supporting evidence for the hypothesized pattern of lower genetic variability in marginal populations has come from studies of chromosomal polymorphisms in Drosophila species (Carson 1958, Carson and Heed 1964, da Cunha and Dobzhansky 1954, da Cunha et al. 1959). This relationship has not, however, been observed in studies of allozyme loci in these species (Ayala et al. 1971, Prakash 1973). Shumaker and Babbel (1980) did, however, find lower levels of allozyme variation in ecologically marginal populations of wild barley (Hordeum jubatum L.). Studies of allozyme loci in conifers

have found lower levels of variability in marginal populations of some species (Guries and Ledig 1982, Tigerstedt 1973), but have failed to find such a pattern in other species (Wheeler and Guries 1982).

Jeffrey pine displays lower variability in populations at its northern margin. Whether the differences in levels of variability observed between Klamath and Sierra Nevada - Southern populations are at least partially due to ecological factors is not certain, but some evidence supports this possibility. While variability is reduced in populations at the northern margin of the range, where Jeffrey pine is restricted to ultramafic soils, it is apparently not reduced at the southern margin of the range, where the species is not found on ultramafic soils. It must be cautioned, however, that we sampled only one population at the southern limit of the range (S7), and the sample size in that population was small. Babbel and Selander (1974) found lower levels of allozyme variability in edaphically restricted Lupinus species than in more widespread Lupinus species.

The differences in allele frequencies between the two regions in Jeffrey pine could be due to isolation and random genetic drift, with the two regions randomly drifting apart after they were separated. A gap exists in the species range between the Klamath Mountains and the northern Sierra Nevada (Figure 2.1). Another cause for the differentiation could be selection, possibly based on soil type. One line of evidence supporting the possibility of ecotypic differentiation in Jeffrey pine comes from examination of

genetic distance data. The average genetic distance between the Sierra Nevada ultramafic population (S1) and the Klamath populations is .027, while the average genetic distance between the remaining Sierra Nevada populations (S2, S3, S4, and S5) and the Klamath populations is .050. The S1 population is 58 km closer to the nearest Klamath population than are any other of the Sierra Nevada populations, but the magnitude of reduction in genetic distance is more than would be expected for such a reduction in geographic distance. Similarity between S1 and the Klamath populations can also be seen by comparing allele frequencies between the S1 population and means for the two regions at a number of loci, such as G6P2, MDH4, and MDR (Table 2.2), where the frequencies in the S1 population deviate strongly from the Sierra Nevada - Southern means in the direction of the Klamath means. These deviations did not, however, occur at enough loci to cause the S1 population to cluster with the Klamath populations (Figure 2.2).

The similarities in allele frequencies at some loci among the Klamath populations and the S1 population suggest the possibility of genetic adaptation to ultramafic soils in Jeffrey pine. Evidence for edaphic ecotypes adapted to ultramafic soils has been reported for other pine species. Jenkinson (1977) examined edaphic adaptation in the very closely related ponderosa pine. He planted seed from both ultramafic and nonultramafic sources on ultramafic and nonultramafic test soils. After one growing season there was no site by source interaction for growth traits, indicating an absence of edaphic adaptation. Two of the test sites have since been

maintained, however, and after three years, ultramafic sources were significantly taller than nonultramafic sources on the ultramafic test soil. The growth differences between sources on the ultramafic test soil have continued to increase through eleven year measurements on the plantation (J.L. Jenkinson, unpublished data, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California). Thus, while there is evidence for an ultramafic ecotype in ponderosa pine, it did not become apparent through differential growth response until after three years of growth in the field. Kruckeberg (1967) observed the same pattern of delayed differential growth response in a study of adaptation to ultramafic soils in lodgepole pine (Pinus contorta Dougl.). It would, therefore, not be unexpected to find an ultramafic ecotype in Jeffrey pine.

We have observed genetic differences at allozyme loci between the Klamath and Sierra Nevada - Southern populations of Jeffrey pine. Whether similar levels of differentiation between regions would be found in quantitative traits is not known. In many cases, while significant morphological differentiation has been found along environmental transects within a species' range, allele frequency differences along similar transects have not been found to be significant (Adams and Campbell 1982, Coyne et al. 1983, Jain et al. 1980, Kahler et al. 1980, Guries and Ledig 1982). Less often, the reverse is observed (Lessios 1981). Lewontin (1984) has suggested that differences among populations in quantitative traits cannot be compared to differences in allele frequencies because the power of

the tests used to detect differences for the two types of traits are very different. Therefore, they should not necessarily be expected to yield the same results. The best understanding of the genetics of a species is obtained by consideration of both types of traits.

This study has demonstrated that Jeffrey pine is a genetically highly variable species, and as is the case for most conifers, most of this allozyme variation is within populations. The discontinuous distribution of this species in the Klamath Mountains does not appear to have led to a large degree of differentiation among populations within this region. Significant genetic differences both in allele frequencies and levels of variability exist between the Klamath and Sierra Nevada - Southern regions. There are indications that one cause for these differences may be related to the presence or absence of ultramafic soils. The data on this point must, however, be interpreted with caution due to the small sample sizes in the Sierra Nevada - Southern populations. A more complete understanding of the nature of the genetic differences between Klamath and Sierra Nevada - Southern populations of Jeffrey pine must await a more extensive allozyme survey and common garden study.

Table 2.1. Locations and designations of Jeffrey pine collection sites in the Klamath Mountains (seven populations) and in the central Sierra Nevada and southern portions of the species' range (seven populations).[a]

Region/ Population	N[b]	Latitude	Longitude	Elevation (m)
Klamath Mountains				
K1	53	42° 35'	123° 23'	505
K2	49	42° 30'	123° 42'	1220
K3	50	42° 27'	123° 44'	1220
K4	54	41° 55'	123° 39'	1645
K5	41	42° 03'	122° 49'	1975
K6	46	41° 21'	122° 41'	1295
K7	57	41° 17'	122° 42'	1660
Sierra Nevada - Southern				
S1	12	39° 06'	120° 46'	1170
S2	17	38° 48'	120° 09'	1735
S3	17	38° 48'	120° 07'	1980
S4	11	38° 54'	120° 01'	1920
S5	9	38° 54'	119° 54'	2465
S6[c]	34	34° 00'	117° 00'	1525 - 2285
S7	12	30° 34'	115° 11'	--[d]

[a] Collection site locations shown in Figure 2.1.
[b] Number of trees from which cones were collected at each site.
[c] S6 is a composite of a number of single tree collections made throughout the mountain ranges north and east of the Los Angeles basin. The latitude and longitude figures represent the approximate center of the area over which these collections were made. The elevation figure represents the range of elevation over which these collections were made.
[d] Information unavailable.

Table 2.2 Estimated allele frequencies at 20 allozyme loci and mean expected heterozygosities for 14 Jeffrey pine populations, seven in the Klamath Mountains and seven in the Sierra Nevada and southern portions of the species' range.

	Region/Population[a]															
	Klamath Mountains								Sierra Nevada - Southern							
Locus/ Allele	K1	K2	K3	K4	K5	K6	K7	Mean[b]	S1	S2	S3	S4	S5	S6	S7	Mean[b]
N[c]	53	49	50	54	41	46	57		12	17	17	11	9	34	12	
ACO																
1	.651	.521	.730	.380	.464	.490	.404	.520	.416	.294	.265	.409	.334	.294	.208	.318
2	.094	.163	.120	.046	.024	.043	.114	.086	.250	.147	.176	.182	.222	.176	.417	.224
3	.104	.255	.140	.546	.378	.402	.342	.310	.292	.383	.265	.227	.222	.442	.292	.303
4	.151	.061	.010	.028	.134	.065	.140	.084	.042	.176	.294	.182	.222	.088	.083	.155
ADH																
1	.877	.796	.810	.815	.439	.728	.693	.737	.333	.147	.147	.091	.278	.029	.167	.170
2	.123	.204	.190	.176	.561	.239	.298	.256	.333	.412	.412	.409	.333	.442	.167	.358
3	0.000	0.000	0.000	.009	0.000	.033	.009	.007	.334	.441	.441	.500	.389	.529	.666	.472
DIA																
1	.792	.898	.930	.935	.854	.685	.798	.842	.417	.412	.500	.773	1.000	.956	.625	.669
2	.208	.102	.070	.065	.146	.315	.202	.158	.583	.588	.500	.227	0.000	.044	.375	.331
FEST																
1	.679	.796	.740	.926	.902	.782	.815	.806	.833	.559	.706	.909	.833	.663	.625	.733
2	.302	.204	.260	.074	.098	.185	.132	.179	.167	.412	.294	.091	.167	.279	.375	.255
3	.019	0.000	0.000	0.000	0.000	.033	.053	.015	0.000	.029	0.000	0.000	0.000	.029	0.000	.008
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	.029	0.000	.004
FHEX																
1	.915	1.000	1.000	1.000	1.000	.967	.974	.979	1.000	.853	.765	1.000	1.000	1.000	1.000	.945
2	.085	0.000	0.000	0.000	0.000	.033	.026	.021	0.000	.147	.235	0.000	0.000	0.000	0.000	.055
GDH																
1	1.000	.990	.950	1.000	1.000	1.000	1.000	.991	.958	1.000	1.000	1.000	.944	1.000	1.000	.986
2	0.000	.010	.050	0.000	0.000	0.000	0.000	.009	.042	0.000	0.000	0.000	.056	0.000	0.000	.014
GOT1																
1	.330	.429	.290	.231	.329	.163	.325	.300	.208	.147	.265	.136	.444	.221	.292	.245
2	0.000	0.000	0.000	0.000	.122	.011	0.000	.019	.083	.118	.206	.273	.056	.176	0.000	.130
3	.670	.571	.690	.769	.549	.826	.675	.678	.709	.676	.470	.591	.500	.603	.708	.608
4	0.000	0.000	.020	0.000	0.000	0.000	0.000	.003	0.000	.059	.059	0.000	0.000	0.000	0.000	.017

Table 2.2 Estimated allele frequencies at 20 allozyme loci and mean expected heterozygosities for 14 Jeffrey pine populations, seven in the Klamath Mountains and seven in the Sierra Nevada and southern portions of the species' range (continued).

	Region/Population[a]															
	Klamath Mountains								Sierra Nevada - Southern							
Locus/ Allele	K1	K2	K3	K4	K5	K6	K7	Mean[b]	S1	S2	S3	S4	S5	S6	S7	Mean[b]
N[c]	53	49	50	54	41	46	57		12	17	17	11	9	34	12	
GOT2																
1	.943	1.000	.990	1.000	1.000	1.000	.947	.983	1.000	.971	.971	1.000	1.000	1.000	1.000	.992
2	.057	0.000	.010	0.000	0.000	0.000	.053	.017	0.000	.029	.029	0.000	0.000	0.000	0.000	.008
GOT3																
1	1.000	1.000	1.000	1.000	.988	1.000	.956	.992	1.000	.971	.912	1.000	1.000	1.000	.917	.971
2	0.000	0.000	0.000	0.000	.012	0.000	.044	.008	0.000	.029	.088	0.000	0.000	0.000	.083	.029
G6P2																
1	.953	.776	.860	.870	.841	.891	.842	.862	.667	.559	.529	.545	.556	.279	.208	.478
2	.047	.224	.140	.130	.159	.109	.158	.138	.333	.441	.471	.455	.444	.721	.792	.522
IDH																
1	.896	.980	.990	1.000	1.000	.989	.947	.972	.917	1.000	1.000	1.000	1.000	1.000	1.000	.988
2	.104	.020	.010	0.000	0.000	.011	.053	.028	.083	0.000	0.000	0.000	0.000	0.000	0.000	.012
LAP1																
1	.934	.857	.930	.944	.915	.739	.807	.876	.833	.912	.941	.909	1.000	.927	.750	.896
2	.057	.143	.050	.056	.061	.250	.184	.114	.167	.029	.059	.091	0.000	.044	.167	.080
3	.009	0.000	.020	0.000	.024	.011	.009	.010	0.000	.059	0.000	0.000	0.000	.029	.083	.024
MDH1																
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH4																
1	1.000	1.000	.990	.981	1.000	.989	.982	.992	1.000	.706	.529	1.000	.611	.441	.250	.648
2	0.000	0.000	.010	.019	0.000	.011	.018	.008	0.000	.294	.471	0.000	.389	.559	.750	.352
MDR																
1	1.000	.969	.970	.981	.939	.891	.904	.950	.958	.647	.618	.819	.611	.574	.875	.729
2	0.000	.031	.030	.019	.024	.098	.096	.043	0.000	.059	.059	.045	.222	.147	.042	.082
3	0.000	0.000	0.000	0.000	.037	.011	0.000	.007	.042	.235	.235	.136	.167	.235	.083	.162
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	.059	.088	0.000	0.000	.044	0.000	.027

Table 2.2 Estimated allele frequencies at 20 allozyme loci and mean expected heterozygosities for 14 Jeffrey pine populations, seven in the Klamath Mountains and seven in the Sierra Nevada and southern portions of the species' range (continued).

Locus/ Allele	Region/Population[a]															
	Klamath Mountains								Sierra Nevada - Southern							
	K1	K2	K3	K4	K5	K6	K7	Mean[b]	S1	S2	S3	S4	S5	S6	S7	Mean[b]
N[c]	53	49	50	54	41	46	57		12	17	17	11	9	34	12	
PGI2																
1	.858	.857	.790	.750	.793	.782	.737	.795	.833	.853	.912	.864	.944	.706	.750	.838
2	.104	.143	.210	.250	.207	.196	.263	.196	.167	.147	.059	.136	.056	.294	.250	.158
3	.038	0.000	0.000	0.000	0.000	.022	0.000	.009	0.000	0.000	.029	0.000	0.000	0.000	0.000	.004
PGM																
1	.830	.827	.750	.648	.695	.913	.824	.784	.958	.853	.853	.909	.833	.721	.625	.822
2	.170	.173	.250	.352	.305	.087	.158	.213	.042	.147	.147	.091	.167	.279	.375	.178
3	0.000	0.000	0.000	0.000	0.000	0.000	.018	.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PMI																
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6PG1																
1	1.000	.959	.980	1.000	.963	.989	.974	.981	.917	.824	.941	.773	.889	.941	.958	.892
2	0.000	0.000	.020	0.000	.037	.011	.026	.013	.083	.147	.059	.227	.111	.059	.042	.104
3	0.000	.041	0.000	0.000	0.000	0.000	0.000	.006	0.000	.029	0.000	0.000	0.000	0.000	0.000	.004
6PG2																
1	.699	.724	.750	.917	.854	.761	.816	.789	.917	.941	.942	1.000	1.000	.882	1.000	.954
2	.292	.245	.200	.074	.122	.228	.140	.186	0.000	.059	.029	0.000	0.000	.044	0.000	.019
3	.009	.031	.050	.009	.024	.011	.044	.025	.083	0.000	.029	0.000	0.000	.074	0.000	.027
H_e[d]	.181	.187	.174	.144	.191	.196	.219	.185	.222	.294	.299	.214	.235	.259	.258	.255
	(.040)	(.044)	(.039)	(.040)	(.047)	(.043)	(.042)	(.016)	(.052)	(.054)	(.058)	(.055)	(.062)	(.056)	(.053)	(.021)

[a] Refer to Figure 2.1 for locations of populations.
[b] Region means are unweighted.
[c] Number of trees sampled in each population.
[d] Unbiased expected heterozygosity (Nei 1978), unweighted means over all 20 loci. Standard errors are in parentheses.

Table 2.3. Geographic distances in kilometers (above diagonal) and Nei's unbiased genetic distances (below diagonal) between fourteen Jeffrey pine populations in two regions of the species' range.[a]

Region/ Population[b]	Klamath Mountains							Sierra Nevada - Southern						
	K1	K2	K3	K4	K5	K6	K7	S1	S2	S3	S4	S5	S6	S7
Klamath Mountains														
K1		29	33	79	71	143	150	428	483	484	478	484	1074	1488
K2	.006		7	65	83	146	152	432	489	490	485	491	1079	1492
K3	.004	.003		58	81	141	147	428	485	486	481	487	1075	1487
K4	.020	.011	.011		70	97	101	379	438	439	436	442	1025	1436
K5	.023	.011	.015	.011		77	84	358	412	413	407	413	1004	1417
K6	.011	.009	.012	.013	.016		8	287	343	345	341	347	934	1346
K7	.010	.003	.008	.007	.006	.003		280	338	339	335	341	927	1340
Sierra Nevada - Southern														
S1	.035	.027	.035	.036	.022	.016	.018		63	65	69	78	647	1060
S2	.067	.060	.067	.066	.045	.045	.047	.011		3	17	25	592	1006
S3	.076	.065	.076	.075	.050	.061	.055	.025	.000		15	22	591	1005
S4	.056	.038	.045	.044	.022	.038	.031	.007	.015	.020		10	598	1012
S5	.054	.033	.042	.041	.027	.049	.033	.034	.024	.013	.010		594	1008
S6	.113	.083	.090	.083	.066	.091	.077	.064	.031	.024	.034	.012		414
S7	.128	.102	.113	.106	.100	.106	.097	.064	.033	.027	.059	.036	.017	

[a] Mean genetic distance within Klamath Mountains region = .010, within Sierra Nevada - Southern region = .027, between regions = .060.

[b] Refer to Figure 2.1 for locations of populations.

Table 2.4. Measures of genic diversity among and within 14 Jeffrey pine populations for 20 loci.[a]

Locus	H_T	D_{ST}	G_{ST}
ACO	.6928	.0482	.0696
ADH	.6428	.1684	.2620
DIA	.3700	.0737	.1992
FEST	.3615	.0224	.0619
FHEX	.0723	.0094	.1305
GDH	.0225	.0008	.0364
GOT1	.5060	.0260	.0514
GOT2	.0252	.0008	.0315
GOT3	.0362	.0019	.0513
G6P2	.4425	.1003	.2267
IDH	.0397	.0022	.0561
LAP1	.2063	.0110	.0532
MDH1	.0000	.0000	.0000
MDH4	.2954	.1259	.4262
MDR	.2840	.0359	.1265
PGI2	.3024	.0098	.0323
PGM	.3172	.0190	.0598
PMI	.0000	.0000	.0000
6PG1	.1204	.0083	.0689
6PG2	.2295	.0209	.0909
Mean	.2483	.0342	.1379

[a] H_T = total genic diversity
D_{ST} = average genic diversity among populations
G_{ST} = proportion of total genic diversity due to differences among populations.

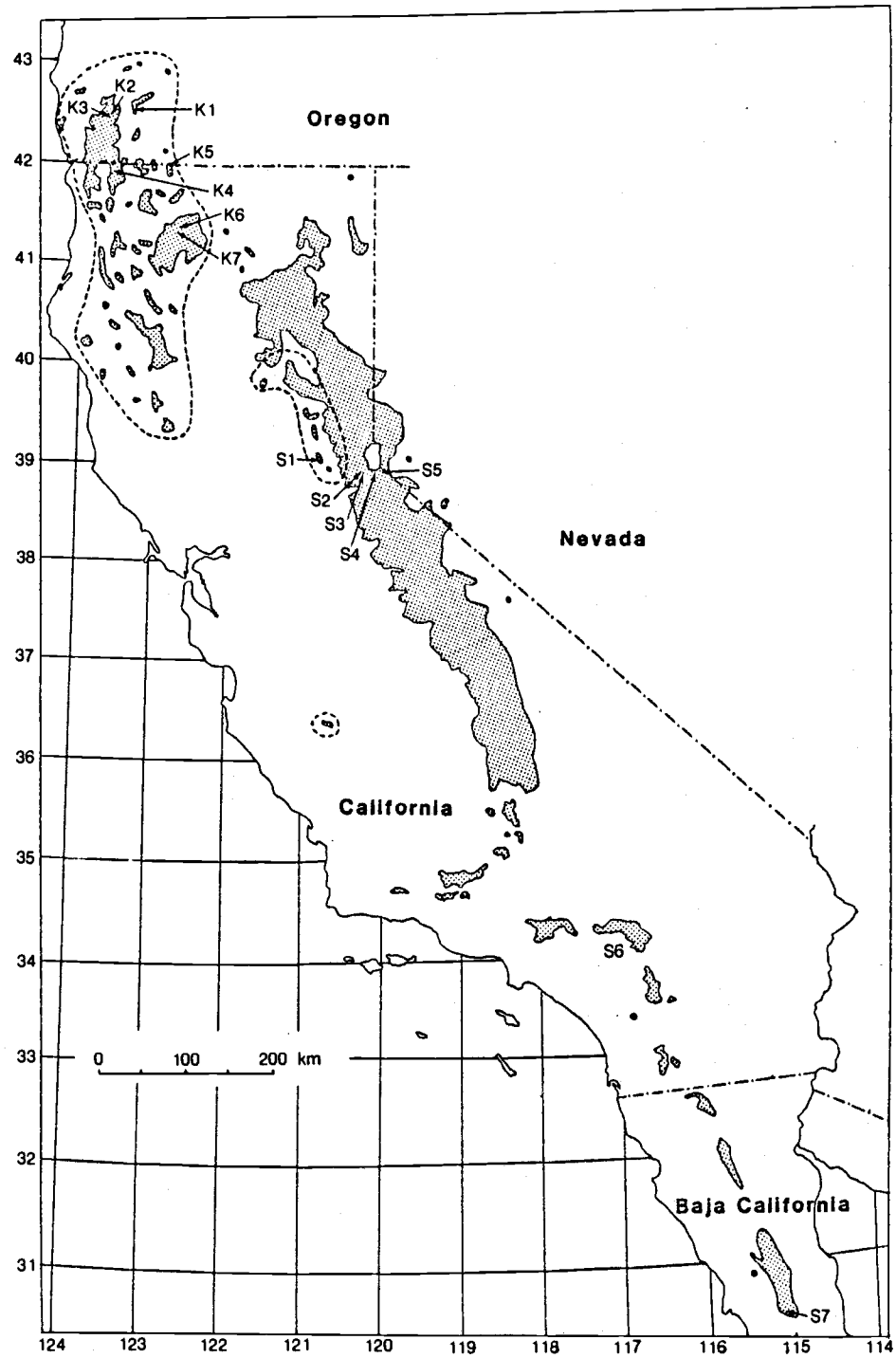

Figure 2.1. Range of Jeffrey pine (shaded areas) and locations of populations sampled in this study. Areas enclosed by dotted lines represent populations restricted to ultramafic soils. Population S6 is a composite of single tree collections from the mountains surrounding the Los Angeles basin.

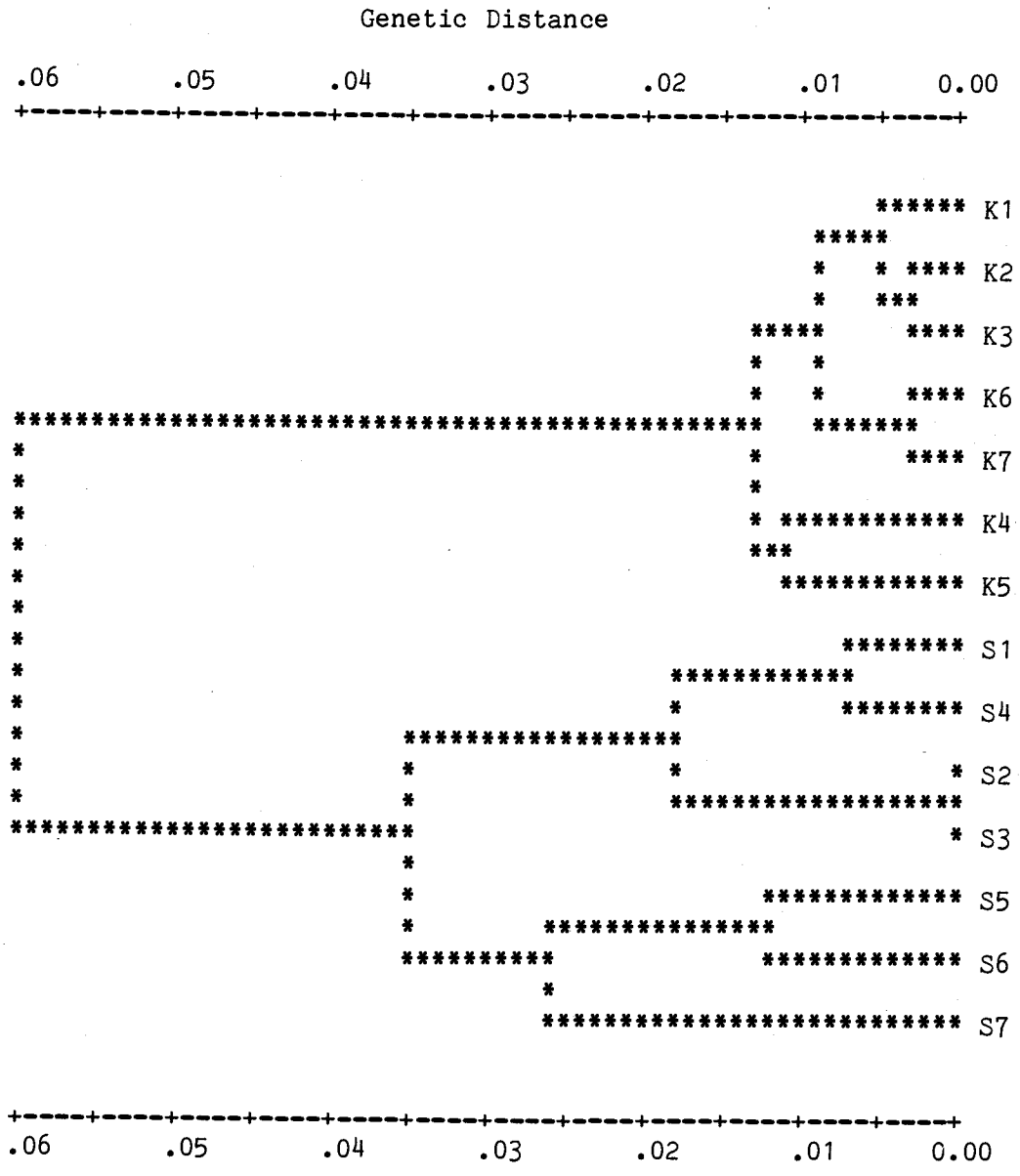

Figure 2.2. Population clustering based on Nei's unbiased genetic distance using the UPGMA method.

CONCLUSIONS FOR FOREST MANAGERS

The results of this study indicate that Jeffrey pine is a highly outcrossed species. On average, less than 10% of the filled seed sampled were selfs. This was true even for the very open, low density stands on ultramafic soils in the Klamath Mountains. This result should allay concerns that wind-pollinated seed collected from these very open stands might contain high proportions of selfed seed.

This study has demonstrated genetic differences between Klamath and Sierra Nevada - Southern populations of Jeffrey pine. The allele frequency similarities between the S1 population and the populations in the Klamath region suggest that some of the genetic differences between regions may be adaptive. This suggests that seed for reforestation in southwestern Oregon and northwestern California should be collected from populations in the Klamath Mountains, rather than from Sierra Nevada populations.

Jeffrey pine does not display a high degree of differentiation among populations within the Klamath Mountains, despite a discontinuous distribution in this region. Thus, in the absence of progeny test information, one would suspect that Jeffrey pine seed zones need not be any smaller than those used for other species in this region. Until data from common garden or field growth tests are available, it would be wise to observe current seed zone boundaries (Western Forest Tree Seed Council 1973) in planning reforestation with Jeffrey pine in the Klamath Mountains. If

Jeffrey pine is being planted in an area outside its natural distribution, one should use seed from the nearest natural populations growing in a similar climatic regime.

The existence of edaphic ecotypes in Jeffrey pine remains a distinct possibility. If the situation is similar to that in the closely related ponderosa pine, one would want to select ultramafic seed sources for planting on ultramafic sites. This should be simple enough in the Klamath Mountains, since almost all natural Jeffrey pine populations in this region occur on ultramafic soils (Jenkinson 1980a). Since stands on ultramafic soils usually have very low productivities (Smith et al. 1984) and are not often managed for timber production, the more important question is that of which seed sources to use for nonultramafic planting sites. In a study of edaphic adaptation in Sierra Nevada sources of ponderosa pine, there were no significant differences in height growth and survival after 11 years between ultramafic and nonultramafic sources planted on a nonultramafic test soil (J.L. Jenkinson, unpublished data, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California). If Jeffrey pine follows a similar growth and survival pattern, then concerns about reforesting nonultramafic sites in southwestern Oregon and northwestern California with ultramafic seed sources should be reduced.

No significant genetic obstacles to wider use of Jeffrey pine in commercial plantations in southwestern Oregon and northwestern California were revealed in this study. Thus, Jeffrey pine should

be considered a viable species option for reforesting environmentally extreme sites in this region.

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