Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*)[1]

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Seeds from 104 geographical locations throughout the range of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were analyzed at 20 enzyme loci to determine patterns of genetic variation and to make phylogenetic inferences. On average, the populations were polymorphic at 37% of the loci (range 5.0–65.0). Mean expected heterozygosity was 0.137 (range 0.021–0.239). Of the total genic diversity (H_T = 0.182) observed, 24% was due to differentiation among populations. One Mexican population was genetically distinct from the rest of the species, which suggests the possibility of additional *Pseudotsuga* species in Mexico. The rest of the populations clustered into two groups corresponding to the recognized coastal and interior varieties. In addition, the interior variety separated into northern and southern subgroups near 44° latitude. Transition zones between the varieties were found to be narrower and more abrupt than has been suggested previously. Populations within the coastal variety and the northern interior subgroup averaged higher expected heterozygosity than the southern interior subgroup, but in the southern interior subgroup, populations were much more highly differentiated. Allozyme variation followed a latitudinal cline in the interior variety, but only weak geographical patterning was observed in the coastal variety. In general, genetic diversity decreased towards the periphery of the species range. Range-wide patterns of allozyme variation were similar to those in terpene studies, with the major exception that the Sierra Nevada seed sources in this study were closely aligned with the coastal variety rather than with the interior variety. Range-wide patterns of genetic variation in Douglas-fir largely reflect the evolutionary history of the species as revealed by paleobotanical studies.

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Des graines provenant de 104 localités dans l'aire géographique du Sapin de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco) ont été analysées à 20 loci d'enzyme en vue de déterminer l'allure de la variation génétique et d'en tirer les conséquences phylogénétiques. En moyenne, les populations étaient polymorphiques à 37% des loci (variation de 5,0 à 65,0). L'hétérozygosité moyenne était de 0,137 (variation de 0,021 à 0,239). De la diversité génique totale (H_T = 0,182) observée, 24% étaient dus à la différentiation parmi les populations. Une population mexicaine était génétiquement distincte du reste de l'espèce, ce qui suggère la possibilité d'espèces additionnelles de *Pseudotsuga* au Mexique. Le reste des populations formait deux groupes correspondant aux variétés reconnues, côtière et de l'intérieur. La variété de l'intérieur se subdivisait en sous-groupes du nord et du sud près du 44° de latitude. Les zones de transition entre ces variétés étaient plus étroites et plus abruptes que ce qui avait été suggéré précédemment. Les populations de la variété côtière et celle du sous-groupe nord avaient en moyenne une hétérozygosité plus forte que celles du sous-groupe du sud; mais dans le sous-groupe du sud, les populations étaient beaucoup plus différentiées. Les variations allozyme suivaient un cline latitudinal dans la variété de l'intérieur, mais seule une faible tendance géographique a été observée dans la variété côtière. En général, la diversité génétique diminuait vers la périphérie de l'aire de l'espèce. Les tendances des variations allozyme dans toute l'aire étaient semblables à celles observées dans les travaux portant sur les terpènes, à l'exception importante que les sources de graines de la Sierra Nevada se sont révélées étroitement alignées avec les variétés côtières dans cette étude plutôt qu'avec la variété de l'intérieur. Les tendances de la variation génétique dans toute l'aire du Douglas reflètent l'histoire évolutive de l'espèce telle que nous l'ont révélée les travaux de paléobotanique.

[Traduit par la revue]

Introduction

Allozymes are widely used as genetic markers of forest trees in studies dealing with population genetic structure, mating systems, phylogeny, and applied breeding (e.g., Hamrick *et al.* 1981; Adams 1983; Mitton 1983; Wheeler *et al.* 1983; Neale and Adams 1985*a*). While conifers generally vary greatly at allozyme loci, most of this variation is within populations (Brown and Moran 1981). The genetic diversity among populations generally is much less than the diversity in morphological and quantitative traits (Wheeler and Guries 1982; Merkle and Adams 1987). Nevertheless, range-wide patterns of geographic differentiation based on allozymes are often similar to those based on quantitative traits (Florence and Rink 1979; Fins and Libby 1982; Wheeler and Guries 1982; Ledig and Conkle 1983; O'Malley and Guries 1983; Steinhoff *et al.* 1983). Phylogeny, as inferred from allozyme data, can offer additional insights into the evolution of tree species (Wheeler *et al.* 1983; Jacobs *et al.* 1984; Critchfield 1985).

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the most widely distributed conifers in western North America (Fig. 1), occupying many habitats (Silen 1978). Two varieties are well recognized: coastal (var. *menziesii*) and interior, or Rocky Mountain, Douglas-fir (var. *glauca*) (Fowells 1965; Hermann 1982). The coastal variety, extending from Vancouver Island and the coastal mountains of British Columbia along the Pacific slope into California, has nearly continuous distribution from sea level to eleva-

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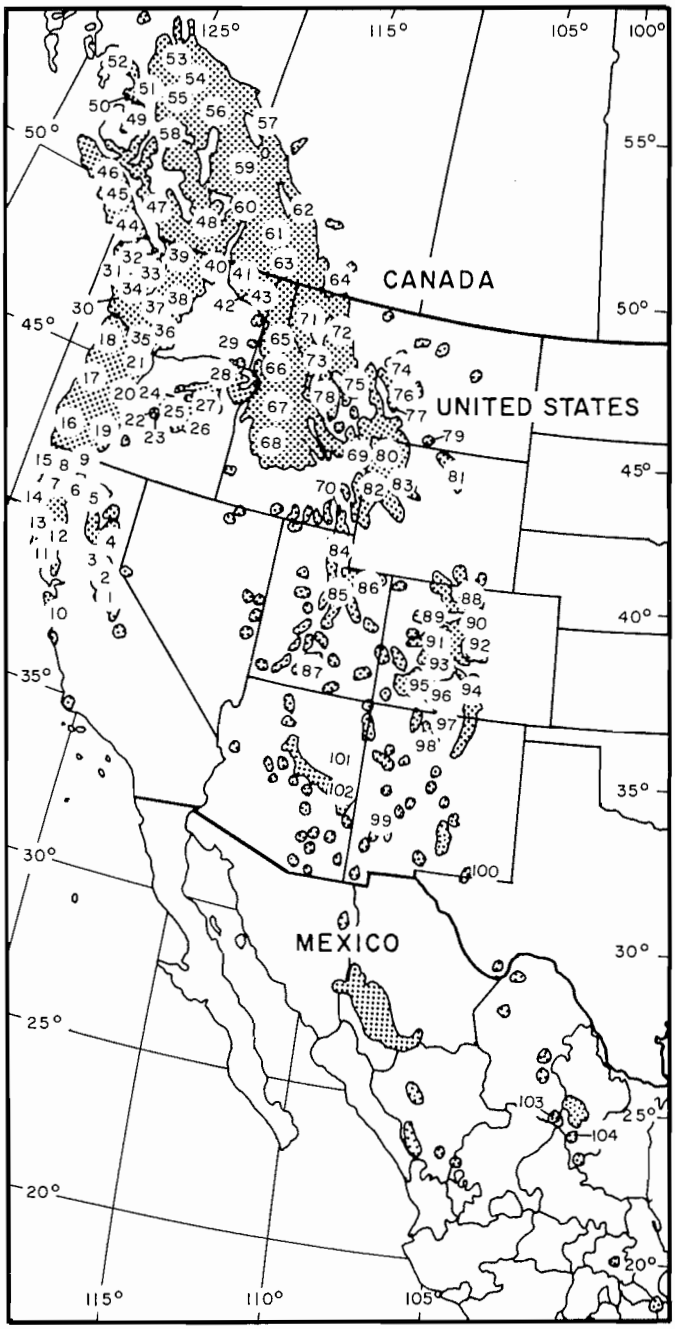

FIG. 1. Geographical distribution of Douglas-fir (shaded area) and the locations of 104 seed sources sampled in the study.

tions of 823 m at its northernmost latitude and 1830 m in the Sierra Nevada Mountains of California. The interior variety, extending along the Rocky Mountains from northern British Columbia into the southwestern United States and Mexico, has a patchier distribution, especially in the south, and a wider elevation range (580–3500 m) (Fowells 1965; Wright *et al.* 1971).

Genetic variation between and within the varieties is extensive (Ching and Hermann 1977; Sile 1978). Demarcation between them generally extends along the Fraser River near Hope, British Columbia, and north along the center of the coastal ranges, but separation between the varieties is not complete, and transitional zones have been shown in this region and elsewhere (Ching and Hermann 1977). Further genetic subdivisions of the varieties have been proposed (Kung and Wright 1972; Zavarin and Snajberk 1973). Variation among populations within varieties has been investigated primarily by measuring quantitative traits in common garden experiments. In general, these studies have revealed strong clines in traits over latitudinal, longitudinal, and elevational gradients, east–west differentiation being particularly strong in both the coastal (Griffin and Ching 1977; Campbell and Sorensen 1978) and interior varieties (Kung and Wright 1972; Rehfeldt 1978). The strong clines suggest the importance of natural selection in shaping patterns of genetic variation in this species.

Previous allozyme studies in Douglas-fir, while mostly restricted to the coastal variety, have shown that the species has great genetic diversity at enzyme loci (Muhs 1974; Yang *et al.* 1977; Yeh and O'Malley 1980; Adams 1981; Hamrick *et al.* 1981; Morris, unpublished data cited in National Council on Gene Resources 1982; Merkle and Adams 1987). As in other conifers, most variation (>95%) appears to be within populations, but some weak clines in gene frequency over environmental transects have been reported among populations (Bergmann 1975; Mejnartowicz 1976; Yang *et al.* 1977; Yeh and O'Malley 1980; Morris, unpublished data cited in National Council on Gene Resources 1982). No association between allozyme diversity and geographical variables, however, was found in an intensive sampling of Douglas-fir in southwestern Oregon, a geographically restricted but environmentally diverse region of the coastal variety (Merkle and Adams 1987).

Few studies of geographical patterns of genetic variation of Douglas-fir have been range wide, and these have not involved intensive sampling (von Rudloff 1973*a*; Zavarin and Snajberk 1973; Michaud 1987). This paper reports results of an intensive, range-wide investigation of allozyme variation in Douglas-fir. It was of particular interest to determine whether the limited allozyme differentiation among populations revealed in restricted geographical samples would hold true over the entire range of the species. It was also of interest to determine how range-wide patterns of allozyme variation compare with patterns based on other traits. Patterns of allozyme variation were used to make phylogenetic inferences in Douglas-fir.

Materials and methods

Seed sources

Douglas-fir seeds from 104 sources were obtained in 1984 from various forestry organizations in Canada, Mexico, and the United States (Fig. 1). The sources were chosen to cover as much of the natural range of the species as possible, and in most cases met the following criteria: (*i*) only naturally regenerated stands were sampled, (*ii*) seeds from at least 15 trees were included in each collection, and (*iii*) seeds had not been stored for more than 10 years. The exceptions were 17 seed sources for which seeds were obtained from less than 15 parent trees or from an unknown number of trees. For the remaining 87 seed sources, each collection was obtained from an average of 50 parent trees (range 15–275). Three types of collection were made: seeds bulked from trees in a single stand (42 sources), seeds bulked from trees in stands scattered throughout a seed zone (47 sources), and nonbulked seeds from throughout a seed zone that were kept separate by mother tree (15 sources). In this paper, seed sources will be referred to as populations.

Electrophoretic procedures

Seeds were prepared for electrophoretic assay by first soaking them in 1% hydrogen peroxide for 48 h. They were then germinated on filter paper in petri dishes in a laboratory germinator having

TABLE 1. Mean and range of frequency of the most common alleles, unbiased expected heterozygosities, and gene diversity at 20 allozyme loci over 104 Douglas-fir populations

Locus	Frequency of most common allele		Heterozygosity		Gene diversity	
	Mean	Range	Mean	Range	Total (H_T)	Among populations (D_{ST})
Aco1	0.961	0.575–1.00	0.067	0.000–0.580	0.0749	0.0063
Aco2	0.813	0.286–1.00	0.239	0.000–0.686	0.3153	0.0764
Pgm1	0.546	0.000–1.00	0.404	0.000–0.718	0.5671	0.1625
Lap1	0.616	0.024–1.00	0.373	0.000–0.699	0.5468	0.1741
Lap2	0.976	0.810–1.00	0.041	0.000–0.335	0.0466	0.0006
Pep2	1.000	—	0.000	—	0.0000	0.0000
Mpi	0.984	0.765–1.00	0.030	0.000–0.381	0.0314	0.0016
Gdh	0.996	0.925–1.00	0.007	0.000–0.146	0.0071	0.0000
Got1	0.991	0.850–1.00	0.018	0.000–0.262	0.0185	0.0010
Got2	0.935	0.650–1.00	0.116	0.000–0.511	0.1227	0.0071
Got3	0.947	0.000–1.00	0.075	0.000–0.526	0.1023	0.0257
G-6pd	0.752	0.375–1.00	0.338	0.000–0.666	0.3962	0.0629
Cat	0.848	0.220–1.00	0.199	0.000–0.586	0.2575	0.0588
Fest	0.983	0.786–1.00	0.031	0.000–0.364	0.0332	0.0020
6-Pgd	0.489	0.000–1.00	0.369	0.000–0.752	0.6203	0.2514
Idh	0.938	0.700–1.00	0.110	0.000–0.474	0.1178	0.0089
Dia	0.891	0.525–1.00	0.164	0.000–0.521	0.1949	0.0255
Mdh1	0.986	0.833–1.00	0.028	0.000–0.286	0.0288	0.0009
Mdh2	0.986	0.857–1.00	0.026	0.000–0.262	0.0275	0.0006
Mdh3	0.931	0.550–1.00	0.114	0.000–0.510	0.1287	0.0148
Mean			0.137		0.1819	0.0439

NOTE: Because of the large data set, allele frequencies and gene diversity for individual populations are not presented here. They can be obtained from W.T. Adams.

a 12-h photoperiod and alternating day and night temperatures of 24 and 22°C, respectively. Seeds were assayed electrophoretically when radicles had emerged 3–5 mm beyond the seed coat. For all seed collections from 15 or more mother trees, only megagametophytes were used. In bulked seed collections, megagametophytes from 32–50 (mean 42) randomly sampled seeds were analyzed, and in nonbulked collections, megagametophytes from 2 seeds of each mother tree (mean total 38.3, range 26–40) were analyzed. For seed collections represented by less than 15 or by an unknown number of trees, both the megagametophyte ($1N$) and embryo ($2N$) from each of 21–50 seeds (mean 39.6) were assayed. Genotypes of pollen gametes were then inferred from comparisons of the haploid genotype of the megagametophyte with the diploid genotype of the embryo (Adams and Joly 1981). In seed collections derived from few mother trees, pollen gametes probably represent genes from a much larger pool of parents, and therefore better represent the genetic composition of the population (Steinhoff *et al.* 1983).

Extracts from embryos and megagametophytes were subjected to horizontal starch-gel electrophoresis, according to methods described by Neale *et al.* (1984) and Merkle and Adams (1987). They were stained for 14 enzyme systems representing 20 loci: aconitase (*Aco1, Aco2*), phosphoglucomutase (*Pmg1*), leucine aminopeptidase (*Lap1, Lap2*), peptidase (*Pep2*), mannose-6-phosphate isomerase (*Mpi*), glutamate dehydrogenase (*Gdh*), glutamate-oxaloacetate transaminase (*Got1, Got2, Got3*), glucose-6-phosphate dehydrogenase (*G-6pd*), catalase (*Cat*), fluorescent esterase (*Fest*), 6-phosphogluconate dehydrogenase (*6-Pgd*), isocitrate dehydrogenase (*Idh*), diaphorase (*Dia*), and malate dehydrogenase (*Mdh1, Mdh2, Mdh3*). All loci scored were confirmed previously by Mendelian analysis (El-Kassaby *et al.* 1982; Neale *et al.* 1984; W.T. Adams, unpublished data).

The high resolution of haploid banding patterns allowed the extracts of two megagametophytes to be combined in each electrophoretic sample (i.e., each wick). In most cases, a single band appeared if both megagametophytes carried alleles coding the same allozyme, and two bands appeared if they differed. Some allozymes with similar migration rates could not be separated, however, and null alleles could be detected only when both megagametophytes carried the null allele. Null alleles normally occur at very low frequencies in coniferous populations (Allendorf *et al.* 1982); thus, bias in estimates from this technique should be small, although genetic variability may be somewhat underestimated. The bias in genetic resolution seemed minor in comparison with the increased sampling efficiency, which made possible the analysis of many individuals and populations in a limited time. Therefore, the technique was used in all seed collections derived from 15 or more known mother trees. In all populations, alleles were bulked to reflect only those detectable from two megagametophytes per sample. At the 20 loci surveyed, 74 alleles were scored.

Derivation of effective genome sample size

Allele frequencies in each population were estimated by counting the megagametophytes or pollen gametes carrying each allozyme and dividing them by the total number sampled. Because allele frequencies were based on random samples of gametes rather than on direct assays of parental (diploid) tissues, the effective number of alleles in each sample was less than twice the number of parent trees contributing megagametophytes or pollen gametes (Morris and Spieth 1978). Thus, an effective sample size was used for all analyses. When k megagametophytes are assayed from each of N trees, the effective sample number (N_e) can be derived from variance equations given by Morris and Spieth (1978), and when $k = 2$, $N_e = 1.33N$ (Furnier and Adams 1986*a*). When n megagametophytes (or pollen gametes) are sampled from bulked seed collections of N trees (or pollen parents), $N_e = 1/(1/n + 1/2N)$ (see Appendix). For example, if 40 seeds are sampled from 20 trees, $N_e = 1/(1/40 + 1/40) = 20$ for a bulked seed collection, and $1.33 \times 20 = 27$ for a nonbulked seed collection. When seeds came from fewer than 15 trees or from an unknown number, the number of pollen parents contributing pollen gametes was assumed to be 20. N_e averaged 24 (range 14–39) over the 104 seed sources.

For derivation of N_e for bulked collections, it was assumed that an equal number of seeds (or pollen gametes) came from each parent tree. As this is not likely, N_e is probably overestimated for these samples, but not greatly so.

Data analysis

The genetic variation within each population was quantified by calculating the percentage of polymorphic loci (frequency of most common allele ≤ 0.95), mean number of alleles per locus, and unbiased expected heterozygosity (Nei 1978). Heterogeneity of allele frequencies among populations was tested with the χ^2 procedure of Workman and Niswander (1970). Unbiased genetic distances (Nei 1978) were estimated for all population pairs. Cluster analysis of populations based on genetic distances was conducted with the UPGMA procedure (Sneath and Sokal 1973). Correlation analyses were used to examine associations between genetic and geographical patterns of variation. The degree and distribution of genetic differentiation in Douglas-fir was determined by partitioning total gene diversity into among-population and within-population components (Nei 1973; Chakraborty *et al.* 1982). All except the correlation analyses were conducted with the BIOSYS-1 computer program (Swofford and Selander 1981).

Results

Of the 20 loci examined, 19 were polymorphic in at least one population (95% criterion). Locus *Pep2* was monomorphic over all populations, although some rare variants have been previously reported in coastal Douglas-fir (Merkle and Adams 1987). On average, populations were polymorphic for 37.3% of the loci (range 5.0–65.0). The mean number of alleles per locus was 1.8 (range 1.1–2.2), and the mean expected heterozygosity was 0.137 (range 0.021–0.239).

Variation in allele frequencies

Populations differed greatly in allele frequencies, especially at four loci, *Pgm1, Lap1, Got3,* and *6-Pgd*, where the most common allele ranged in estimated frequency from zero (or near zero) to one over the 104 populations sampled (Table 1). Allelic frequencies were significantly heterogeneous ($P < 0.001$) among populations at the 19 polymorphic loci. The heterogeneity was also reflected in gene diversity (Table 1). Nei's (1973) total genic diversity (H_T) varied from 0.0000 at locus *Pep2* to 0.6203 at locus *6-Pgd* (mean 0.1819 over 20 loci). Gene diversity among populations (D_{ST}) also ranged widely among loci (0.0000–0.2514). Nevertheless, when all loci were considered together, the mean proportion of total genic diversity due to differences among populations ($G_{ST} = D_{ST}/H_T$, Nei 1973) was 24.1%, a high value compared with that for other conifers (Brown and Moran 1981; Adams 1983; Mitton 1983).

Cluster analysis based on genetic distances between all pairwise combinations of the 104 populations revealed patterns similar to those observed in earlier range-wide studies of this species (Fig. 2). With the exception of one of the two Mexican sources (103) which was genetically distinct from the rest of the species, populations clustered into two major groups corresponding to the recognized coastal and interior varieties. The interior populations further divided into a northern subgroup (British Columbia, Alberta, Idaho, Montana, and northern Wyoming) and a southern subgroup (central and southern Wyoming, Utah, Colorado, Arizona, New Mexico, Mexico) at around 44° latitude. An exception was the northernmost Colorado population, 88 (Fig. 2), which clustered with the northern subgroup.

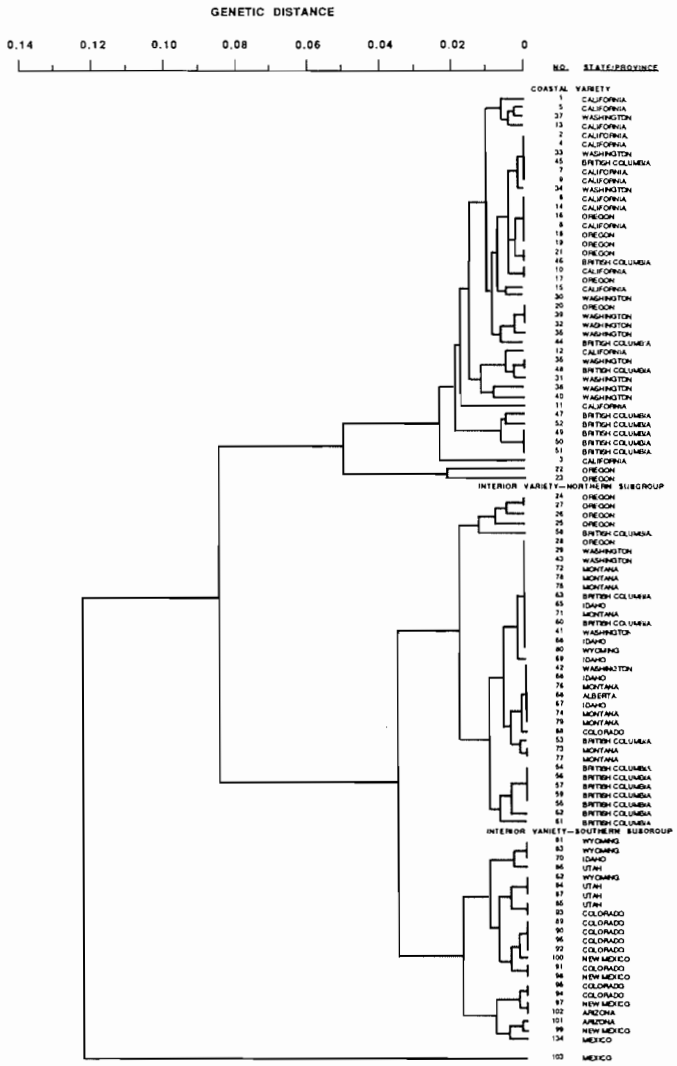

FIG. 2. Cluster diagram based on unbiased genetic distances (Nei 1978) of 104 populations throughout the natural range of Douglas-fir.

While genetic distances between population pairs over the entire range of the species (excluding seed source 103) averaged 0.051 (range 0.000–0.184), the average genetic distance between the two varieties was 0.083 (range 0.003–0.184). Average genetic distance between the coastal variety and the northern interior subgroup was 0.073 (range 0.003–0.131) and between the coastal and southern interior subgroup was 0.099 (range 0.017–0.184) (Table 2), indicating greater affinity of the northern subgroup with coastal Douglas-fir. Average genetic distance between the interior subgroups was 0.034 (range 0.000–0.115), about one-half of that between the varieties. Varieties accounted for 51.1% G_{ST} (0.231) with seed source 103 excluded. Division of the interior variety into northern and southern subgroups accounted for an additional 24.2%. Thus, 75.3% of variation among Douglas-fir populations was attributable to differences among the three major geographical subdivisions: coastal, northern interior, and southern interior.

Varieties differed in average genetic distance ($\bar{D}$) among populations. The average among interior populations (0.021) was greater than that among coastal populations ($\bar{D} = 0.015$, range 0.000–0.091). Among populations in the

TABLE 2. Mean genetic distance (with range in parentheses) between population pairs within and among the three major geographical divisions of Douglas-fir

Geographical divisions	N	Coastal variety	Interior variety	
			Northern	Southern
Coastal variety	43	0.015 (0.000–0.091)		
Interior variety				
Northern	36	0.073 (0.003–0.131)	0.008 (0.000–0.036)	
Southern	24	0.099 (0.017–0.184)	0.034 (0.000–0.115)	0.012 (0.000–0.050)

NOTE: Data are based on 20 allozyme loci (see Table 1). Geographical divisions are those identified by cluster analysis in Fig. 2. N, number of populations sampled within each division.

TABLE 3. Gene diversity statistics for the three major geographical divisions of Douglas-fir

Geographical division	N	Mean gene diversity			G_{ST}
		Total (H_T)	Within population (H_W)	Among populations (D_{ST})	
Coastal variety	43	0.1771	0.1645	0.0126	0.071
Interior variety					
Northern	36	0.1573	0.1505	0.0068	0.043
Southern	24	0.0872	0.0766	0.0106	0.122

NOTE: Data are based on 20 allozyme loci (see Table 1). Geographical divisions are those identified by the cluster analysis in Fig. 2. N, number of populations sampled within each division; G_{ST}, proportion of gene diversity due to differences among populations.

southern subgroup, $\overline{D}$ was nearly twice that of the northern subgroup (Table 2). These patterns were also reflected in the partitioning of total genic diversity. In the interior variety, G_{ST} (13.5%) was nearly twice that in the coastal variety (7.1 or 5.7%, when central Oregon populations 22 and 23 are excluded). However, 54% of G_{ST} in the interior variety was accounted for by the northern and southern subdivisions. In the southern subgroup, it was nearly three times that in the northern subgroup (Table 3).

Genetic clustering of coastal populations, except those in north-coastal British Columbia (47, 49–52; Fig. 1) and central Oregon (22, 23), appeared to have little association with geographical location (Fig. 2). For example, two Sierra Nevada sources in California (2, 4; Fig. 1) clustered with sources from Vancouver Island (45) and the Olympic Peninsula in Washington (33), as well as with two northern California sources (7, 9). This weak geographical pattern is also illustrated by the poor correlation ($r = 0.10$, $P < 0.01$, df = 901) between genetic and geographic distances in the coastal variety. The exclusion from analysis of central Oregon sources increased the correlation ($r = 0.25$, $P < 0.001$, df = 818), but geographical distance still accounted for only 6% of the variation in genetic distances among paired populations.

A stronger relationship between genetic and geographic distances appeared in cluster analysis of the northern and southern subgroups. Each subgroup further divided into groups arranged more or less north to south (Fig. 2). The northern subgroup clustered roughly into three smaller groups: northern and central interior British Columbia (54–57, 59, 61, 62); central and eastern Oregon (24–27); and the rest of the northern subgroup. The southern subgroup also clustered into three smaller groups: western Wyoming and southeastern Idaho; Utah and Colorado; and Arizona, New Mexico, and Mexico. Genetic and geographical distances correlated strongly ($r = 0.70$, $P < 0.001$, df = 1768) when all interior populations were included in the analysis. The correlation weakened somewhat when populations in the northern ($r = 0.38$, $P < 0.001$, df = 628) and southern subgroups ($r = 0.49$, $P < 0.001$, df = 274) were analyzed separately.

Variation in genic diversity

Expected heterozygosity (H_e) of populations varied significantly ($P < 0.001$) in a χ^2 test of heterogeneity (Rao 1973). Populations of the coastal variety and the northern interior subgroup had nearly equivalent genetic variability, but diversity in an average southern interior population was considerably lower (Table 4).

Geographical patterns of H_e variation among populations differed between the two varieties. In the coastal variety, $\overline{H}_e$ was highest in Oregon populations 16–21 (0.190, range 0.166–0.239), Washington populations 30–40 (0.182, range 0.151–0.216), and Vancouver Island populations 44–46 (0.183, range 0.167–0.196). $\overline{H}_e$ decreased both south, in California populations 1–15 (0.152, range 0.108–0.175), and north, in coastal British Columbia populations 47–52 (0.132, range 0.070–0.160). In Washington, populations 30–34 from the coast and Olympic Peninsula generally had lower $\overline{H}_e$ variability (0.163, range 0.151–0.176) than populations 35–40 from the nearby Cascade Mountains to the east (0.198, range 0.184–0.216). In the interior variety, H_e values showed a clinal trend from north to south, decreasing from 0.206 in interior British Columbia

TABLE 4. Mean estimate (with range in parentheses) of unbiased expected heterozygosity (H_e), percentage of polymorphic loci (P, 95% criterion), and number of alleles per locus (A) for populations within each of the three major geographical divisions of Douglas-fir

Geographical division	N	H_e	P	A
Coastal variety	43	0.165 (0.070–0.239)	45.2 (20.0–60.0)	1.8 (1.1–2.2)
Interior variety				
Northern	36	0.151 (0.102–0.206)	38.4 (20.0–65.0)	1.8 (1.5–2.2)
Southern	24	0.077 (0.033–0.116)	22.1 (5.0–45.0)	1.5 (1.2–1.8)

NOTE: Data are based on 20 allozyme loci (see Table 1). Geographical divisions are those identified by cluster analysis in Fig. 2. N, number of populations sampled within each division.

population 57 to 0.033 in Utah population 86. Expected heterozygosity was highly correlated with latitude ($r = 0.84$, $P < 0.001$, df = 54) when central Oregon populations 24–27, which clustered with the interior variety (Fig. 2), were excluded from analysis. Values of $\bar{H}_e$ for central Oregon populations 22–27 (0.178, range 0.163–0.204) were somewhat intermediate between those for coastal populations 20, 21, 35, and 36 directly to the west in the Cascade Mountains (0.203, range 0.188–0.216) and those for interior populations 28, 29, and 66–68 directly to the east (0.131, range 0.113–0.144).

In general, genic diversity of populations decreased towards the geographical periphery of the species range, H_e values decreasing northward and southward in the coastal variety and towards the southern margins in the interior variety. Although it did not decrease gradually towards the northern margin of the interior variety, the northernmost population sampled (53) had a lower H_e value (0.137) than populations 54–56 directly to the south (0.180, range 0.176–0.183). There was also evidence of a decrease towards the eastern edge of the species range; populations 74, 76, 79, and 81 from eastern Montana and Wyoming had a lower $\bar{H}_e$ value (0.109, range 0.094–0.120) than populations 73, 75, and 78 farther west (0.153, range 0.146–0.163).

Characteristics of varieties and subgroups in contact or proximity

Opportunities for gene exchange between varieties or subgroups are highest where they come close together. Indeed, characteristics of Douglas-fir in central Oregon, north central Washington, northern Idaho, and south central British Columbia have led investigators to speculate that populations in these regions are genetically transitional between the two varieties (Fowells 1965; von Rudloff 1972; Zavarin and Snajberk 1973; Ching and Hermann 1977). Results from this study provide evidence for a narrow transitional zone between the varieties in central Oregon. Late clustering of central Oregon populations 22 and 23 with the remaining coastal populations and of the four central Oregon populations 24–27 with the northern interior subgroup (Fig. 2) suggests that these sources may be somewhat intermediate to the two varieties.

The extent of the transition zone in central Oregon was examined by plotting mean genetic distances between coastal Oregon populations 16–18 and populations spanning a west–

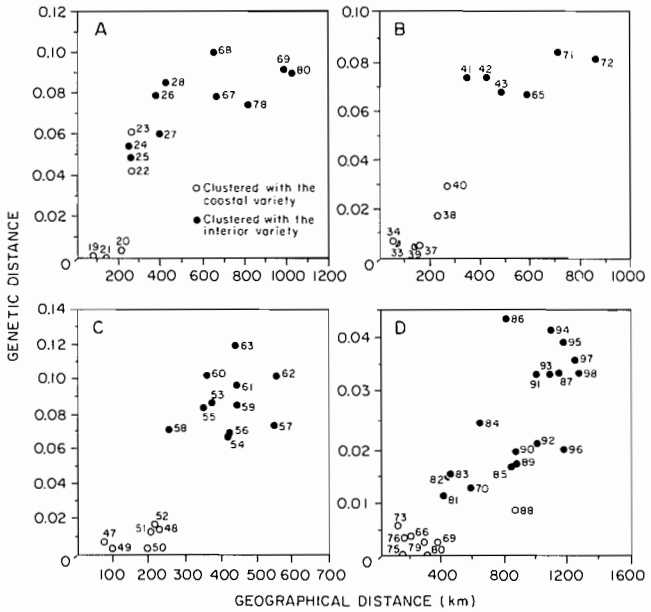

FIG. 3. Mean genetic and geographical distances between populations. (A) Distance between coastal Oregon populations 16–18 and populations 19–21 on a west–east transect through the Cascade Mountains, populations 22–28 in central-eastern Oregon, and populations 67–69, 78, and 80 in central Idaho and northeast Wyoming. Geographical distance was measured from the average longitude of populations 16–18. (B) Distance between coastal Washington populations 30–32 and populations 33, 34, and 37–40 on a west–east transect across western Washington, populations 41–43 in northeastern Washington, and populations 65, 71, and 72 in northern Idaho and Montana. Geographical distance was measured from the average longitude of populations 30–32. (C) Distance between Vancouver Island populations 44–46 and populations 47–52 on a southwest–northeast transect across mainland British Columbia, populations 53–58 in central interior British Columbia, and populations 59–63 in southeastern British Columbia. Geographical distance was measured perpendicular to a northwest–southeast line running midway through Vancouver Island. (D) Distance between northern Idaho – Montana populations 65, 71, and 72 and populations 66, 69, 73, 75, 76, and 79 on a north–south transect through central Idaho and Montana, populations 70, and 80–83 in southern Idaho and northeastern Wyoming, populations 84–96 in Utah and Colorado, and populations 97 and 98 in northern New Mexico. Geographical distance was measured from the average latitude of populations 65, 71, and 72.

east transect through Oregon into central Idaho and northwestern Wyoming against the corresponding geographical distances (Fig. 3A). Mean genetic distances from the coastal populations were very small (<0.005) for Cascade Mountain populations belonging to the coastal variety (19–21), but were large (>0.070) for central Idaho and northwest Wyoming populations belonging to the northern subgroup of the interior variety (66–67, 78, 80). As would be expected for transitional populations, the central Oregon populations (22–25, 27) generally showed intermediate genetic distances (Fig. 3A). The break in genetic distance between Cascade Mountain populations 19–21 and populations 22–25 farther east in central Oregon coincides with a break in species distribution (Fig. 1), resulting in a step cline of genetic distances from the Cascade Mountains to the eastern border of Oregon. (A steep genetic cline through central and eastern

Oregon was first reported by Sorensen in 1979 in a common garden study of Douglas-fir seedlings.)

Genetic separation of the varieties appears to be abrupt in north central Washington and central British Columbia. Mean genetic distances between coastal Washington populations and those spanning a west–east transect across Washington into northern Idaho and Montana were also plotted against corresponding geographical distances (Fig. 3B). Mean genetic distances from the coastal populations increased gradually towards the Cascade Mountains, then jumped sharply (from approximately 0.03 to 0.07) between populations 38–40 in the Cascade Mountains (Fig. 1) and populations 41–43 across a break in Douglas-fir distribution in northeastern Washington. This break also corresponded to a break in the affinity of coastal and northern interior populations (average genetic distance 0.073). Although coastal and interior varieties are not separated by distribution breaks in British Columbia, an abrupt transition was again evident (Fig. 3C). Coastal mainland populations 47–52 differed little genetically from Vancouver Island populations, but genetic distance increased sharply in central and eastern British Columbia sources 53–56.

While the analyses suggest an abrupt separation of the two varieties in north central Washington and central British Columbia, there is evidence for some gene exchange in these areas. Genetic affinity of Cascade Mountain populations 38 and 40 (Fig. 1) with the interior variety appears to be stronger than the affinity of populations farther west (Fig. 3B). Furthermore, interior British Columbia populations 53–63 appear, on average, more closely related to the coastal variety ($\bar{D}$ = 0.064) than do interior populations 28, 29, and 64–80 farther south ($\bar{D}$ = 0.086), which suggests limited gene exchange of the two varieties in northern Washington and British Columbia.

In contrast to the relatively narrow and abrupt transition zones between the varieties, the transition between the northern and southern interior subgroups was gradual (Fig. 3D). Populations 65, 71, and 72 from Montana and northern Idaho were, on average, quite distinct genetically from populations 84–96 in Utah and Colorado, but many populations were intermediate, so that a nearly continuous north–south cline in genetic distances was found over this transect.

Discussion

Racial variation and population structure

On average, genic diversity within Douglas-fir appears to be similar to that found in most conifers (Yeh and El-Kassaby 1980; Hamrick *et al.* 1981; Guries and Ledig 1982; Wheeler and Guries 1982; Steinhoff *et al.* 1983; Furnier and Adams 1986*a*). However, the extent of allozyme differentiation among Douglas-fir populations (G_{ST} = 0.241, or 0.231 with population 103 excluded) is among the highest reported for conifers having wide geographical distribution (Yeh and El-Kassaby 1980; Brown and Moran 1981; Florence 1981; Guries and Ledig 1982; Rye 1982; Wheeler and Guries 1982; Adams 1983; Nikolic and Tucic 1983; Steinhoff *et al.* 1983), and is comparable with that of some broad-leaved trees (Brown and Moran 1981; Wendel and Parks 1985). Douglas-fir is characterized by strong racial differentiation, 75% of the genic diversity among populations being due to racial differences: 51% between varieties and 24% between the northern and southern subgroups of the interior variety. Mean genetic distance (0.083) between the two varieties is greater than that between varieties of lodgepole pine (0.012, Wheeler and Guries 1982) but similar to that between lodgepole pine and other species in subsection *Contortae* (range 0.090–0.109; Dancik and Yeh 1983; Wheeler *et al.* 1983).

Mexican population 103 appeared to differ genetically from all other populations, while Mexican population 104 clustered closely with the southern subgroup of the interior variety (Fig. 2). Genetic distance between sources 103 and 104 (0.089) and between 103 and the rest of the species (0.123) is similar to that between different pine species of subsection *Contortae* (Wheeler *et al.* 1983). Mexican *Pseudotsuga*, considered to be part of var. *glauca* by American taxonomists and foresters (Little 1952, 1979; Fowells 1965; Harlow and Harrar 1969), is divided by French and Mexican taxonomists into four species: *P. flahaulti* Flous, *P. guinieri*, *P. macrolepis* Flous, and *P. rehderi* Flous (Flous 1934; Martinez 1963). Population 103 is located at General Cepeda, Coahuila (elevation 2500 m), and population 104 at La Encantada, near Zaragoza, Nuevo León (elevation 2700 m). According to the taxonomic system and distribution map of Martinez (1963), population 103 is classified as *P. flahaulti* Flous, and population 104 as either *P. macrolepis* Flous or *P. rehderi* Flous.

The large genetic distance between 103 and the remaining populations suggests that 103 may represent another *Pseudotsuga* species and supports the idea that Mexican Douglas-fir is divided into two or more species. Douglas-fir in Mexico is characterized by small isolated populations in diverse environments covering as little as 1–2 ha, conditions that may promote speciation. Although both the Mexican populations were represented by seed from more than 25 parent trees, population 103 had the lowest H_e value (0.021 vs. 0.051 for population 104) of any sampled (Table 4). This suggests that there may have been a severe bottleneck for population 103 in the recent past and that genetic drift may have been important in its differentiation. A broader sample of Douglas-fir populations in Mexico should be analyzed to clarify the taxonomy of *Pseudotsuga* there.

The racial patterns of allozyme variation found in this study conform closely to patterns determined earlier from terpenes and quantitative traits. Strong differentiation between the two varieties has been found in growth rates, frost, disease, and insect resistance, and cortical and leaf oil terpenes (von Rudloff 1972, 1973*a*; Zavarin and Snajberk 1973; Rehfeldt 1977; Michaud 1987; Stephan 1987). Provenance testing has also revealed consistent genetic differences between the northern and southern subgroups of the interior variety. Northern populations have slower growth rates, later budburst and budset, less drought resistance, and greater cold resistance than populations from the south (Kung and Wright 1972; Steiner 1979; Larson 1981). Cortical terpene analysis (Zavarin and Snajberk 1975) confirms separation of the interior variety into northern and southern subgroups, but places the break between them somewhat farther south (latitude 42°30′) than that found in this study (latitude 44°). In reality, the separation between the subgroups appears to be a gradual transition over at least 3–4° latitude, rather than an abrupt one (Fig. 3D).

The racial differentiation in Douglas-fir shown here differs in one major respect from that found by Zavarin and Snajberk (1973), which was based on cortical terpene

analysis. In addition to identifying coastal, northern interior, and southern interior races, Zavarin and Snajberk concluded that the California Sierra Nevada populations represented a fourth chemical race, with genetic affinity closer to the southern race of the interior variety than to the coastal variety. In this study, populations 1–4 of the California Sierra Nevada (Fig. 1) clustered closely with the rest of the coastal variety (Fig. 2), conforming to taxonomical treatments of the species (Fowells 1965; Harlow and Harrar 1969). This close alignment is also reflected by the average genetic distance between the Sierra Nevada populations and those remaining in the coastal variety ($\bar{D} = 0.011$), which was considerably smaller than the distance between the Sierra Nevada populations and either the northern interior ($\bar{D} = 0.088$) or southern interior ($\bar{D} = 0.101$) subgroups.

This difference in racial patterning is not necessarily conflicting, because different traits may respond differently to selection pressures. While allozyme frequencies are often unresponsive to environmental differences (Merkle and Adams 1987), terpenes might be important in the adaptation of forest trees to resist insect attack (Cates *et al.* 1983; Perry and Pitman 1983; Redak and Cates 1984). The deviation in terpene composition of the Sierra Nevada populations from the rest of the populations of the coastal variety may be related to differential selection pressure imposed by insects. In this regard it is interesting to note that Douglas-fir seed sources from north-coastal California, i.e., the intergradation zone between the Sierra Nevada and coastal terpene races (Zavarin and Snajberk 1975), were found by Stephan (1987) to be more resistant to attack by the woolly aphid than coastal seed sources farther north in Oregon, Washington, and British Columbia.

The results of the allozyme analyses agree with the earlier terpene studies in locating transition zones of the two varieties in south central British Columbia, north central Washington, and central Oregon (von Rudloff 1973*a*; Zavarin and Snajberk 1973). Interpretations differ, however, on the width of the zones and on the extent of the gene exchange, especially in British Columbia. In general, terpene analyses indicate that the zones are rather broad, but for individual terpene groups (e.g., camphene), they may be abrupt (von Rudloff 1972, 1973*a*, 1973*b*; Zavarin and Snajberk 1973; von Rudloff and Rehfeldt 1980). The results of this study suggest an abrupt transition, regardless of whether the varieties are physically separated by narrow gaps, as in central Oregon or north central Washington, or are contiguous, as in British Columbia. Perhaps the inability to detect intermediate populations in British Columbia is an artifact of lighter population sampling, especially in south central British Columbia, where sampling was heavy in the terpene studies and where the greatest intermixing of the two varieties was found (von Rudloff 1973*b*). On the other hand, it is possible that gene flow between the varieties is not as extensive in this region as was previously thought, but that genes coding terpene variants may have spread more rapidly because of selective advantage. Intensive allozyme sampling of populations in south central British Columbia might resolve this question.

Population genetic structures differ substantially among the three major geographical subdivisions. The coastal variety and northern subgroup of the interior variety are characterized by considerable genetic variation within populations but little variation between them, while populations in the southern interior subgroup are much more genetically differentiated but possess only about one-half the genetic diversity found in coastal and northern interior populations. As in earlier allozyme studies (Yang *et al.* 1977; Yeh and O'Malley 1980; Morris, unpublished data cited in National Council on Gene Resources 1982; Merkle and Adams 1987), variation of allele frequency in the coastal variety was found to be only weakly associated with geography. With the exception of the northern coastal populations in British Columbia, clustering of populations by genetic distance was not related to their physical proximity (Fig. 2). However, allozyme variation among populations in both interior subgroups appears to be arrayed more or less latitudinally. Provenance tests have also revealed strong latitudinal clines for quantitative traits in the interior variety (Wright *et al.* 1971; Kung and Wright 1972).

Genetic variation in this study decreased towards the margins of species distribution. Marginal populations of Douglas-fir also show less variability in cortical terpenes (Zavarin and Snajberk 1973). A generally decreased allozyme diversity at species margins has been reported for only a few other conifers (Guries and Ledig 1982). Often, geographically marginal populations are among the least variable sampled, but other such populations have normal or high allelic diversity (Fins and Libby 1982; Wheeler and Guries 1982; Steinhoff *et al.* 1983; Furnier and Adams 1986*a*). Perhaps the latter, although located on the geographical periphery of the species, are not growing in particularly marginal habitats, and are therefore not subject to the intense directional selection or genetic drift that may reduce variability at species margins (Stern and Roche 1974; Guries and Ledig 1982). It is possible that the most northerly of the interior populations sampled in this study fall into that category, as they are located somewhat south of the extreme northern limits of the species range.

Phylogenetic implications

The geographical patterns of genetic variation in Douglas-fir observed in this study largely comply with paleobotanical interpretation of the evolutionary history of the species. During the late Tertiary, ancestral *Pseudotsuga* occurred throughout much of its present range but extended considerably farther north than it now does (Hermann 1985). Paleobotanical evidence indicates that the two varieties may have been in existence since the Miocene, 13 million years ago (Critchfield 1984). However, some investigators have proposed that the two varieties evolved sometime in the Late Pleistocene, during the Wisconsin glaciation, 100 000 – 10 000 years ago, and that they were related to one refugium in the coastal mountains and one in the Rocky Mountains of the western United States (Halliday and Brown 1943; Heusser 1969). On the basis of a neutral gene model (Nei 1975), a rough approximation of the time since divergence between taxa can be estimated with the formula

$$t = 5 \times 10^6 \times D$$

where t is the divergence time in years, and D is the average genetic distance between taxa. Estimated time of divergence between the coastal variety and the northern and southern interior subgroups is 315 000 and 505 000 years, respectively. These approximations suggest that the two varieties have been in existence for at least half a million years and that they may have diverged during the middle Pleistocene, much

later than the Miocene, but well before the Wisconsin glaciation.

Cycles of warming and cooling during the Pleistocene may have had great impact on the genetic structure of Douglas-fir and may have reinforced its geographical differentiation (Critchfield 1984). This view is supported by the sharp genetic differentiation between the two varieties and their differences in population structure. The repeated invasions of glaciers during the Late Pleistocene eliminated Douglas-fir in southern British Columbia, where the two varieties now have contact (Hermann 1985). If recontact occurred no earlier than 7000 years ago in southern British Columbia (Tsukada 1982), and if it was even later farther north (Hansen 1955), there may have been limited opportunities for gene exchange between varieties. This would explain the abrupt transition between them in British Columbia. The existence of intermediate populations in central Oregon is striking, considering that central Oregon Douglas-fir populations are now separated from those in the Cascade Mountains by at least 50 km (Sorensen 1979). These populations may exist as a result of much earlier contact between varieties in central Oregon, or possibly of environments in central Oregon that favor intermediates.

Following the late Wisconsin glaciation (i.e., within the last 10 000 years), coastal Douglas-fir has apparently migrated from refugia northward into coastal British Columbia and east into the Cascade Mountains (Tsukada 1982; Barnosky 1985), reaching its northern limits about 5000 years ago (Heusser 1960). The high total genic diversity of the coastal variety, along with the limited differentiation among populations, suggests that it was established from one or more large refugia that must have been located south of the maximum advance of the Cordilleran ice sheet in northern Washington (Waitt and Thorson 1983) but west of the Cascade Mountains, where glaciers developed from Washington southward into northern California (Porter *et al.* 1983). From palynological analyses, Tsukada (1982) suggests that the refugia may have been located in the Willamette Valley. This view is supported by the results in this study; variability within populations was highest in coastal Oregon and Washington, but decreased northward into British Columbia and southward into California. It is interesting that many of the provenances of the coastal variety that perform best in field tests, both in the Pacific Northwest and in Europe, come from these regions of higher genetic diversity (Silen 1978; Kleinschmit *et al.* 1987; Michaud 1987).

Sharp genetic differentiation between the northern and southern interior Douglas-fir subgroups has been hypothesized to be a result of the development of physical barriers to gene flow during the Wisconsin glaciation (Zavarin and Snajberk 1973). Allozyme analyses indicate that the subgroups may have been separated for at least 170 000 years, much earlier than the Wisconsin glaciation 100 000 – 10 000 years ago. The reasons for the separation are unclear, but they are probably related to the development of deserts in southern Idaho and Wyoming and to repeated invasions of glaciers. Arid areas in southern Idaho and Wyoming, which developed during the late Tertiary, imposed a great barrier for species migration between northern and southern Rocky Mountain flora (Weber 1965), restricting gene flow between the northern and southern subgroups of the interior variety to the mountain chains in southeastern Idaho. During the Late Pleistocene, repeated invasions of mountain glaciers eliminated Douglas-fir in western Wyoming and northern Utah (Waddington and Wright 1974; Mehringer 1985), where the two subgroups now have contact, reinforcing their genetic divergence. High total genic diversity and limited genetic differentiation among populations suggest that the northern interior was repopulated with Douglas-fir from one or more large refugia after the retreat of glaciers. Tsukada (1982) has speculated that the refugia were located in the southern Rocky Mountains, from which Douglas-fir migrated northward about 11 000 years ago. Sharp genetic differentiation between the two subgroups does not support this view, but suggests that the refugia might have been located somewhere north of the Idaho–Utah border.

Mountain glaciers developed extensively in Colorado and Utah during the Wisconsin glaciation (Porter *et al.* 1983). Conifer forests in the southern Rocky Mountains responded to the full glacial environment by a southward shift of species distribution and a displacement of species to lower elevations (Spaulding *et al.* 1983; Wells 1983). It has been proposed that Douglas-fir forests in Colorado, New Mexico, and central Arizona were connected, and that they may have been linked to those in southern Utah during the maximum advance of the glaciers in the Late Pleistocene (Martin and Mehringer 1965; Hermann 1985). In the interglacial periods, however, warming climates pushed conifers northward and back to upper elevations, fragmenting their distribution (Fig. 1) and reducing population size. Repeated fractionation and shrinkage of Douglas-fir populations during interglacial periods would provide opportunities for genetic drift and would likely have been a major factor leading to low allozyme diversity within populations and high diversity among populations in this region.

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Appendix

Derivation of the "effective" genome sample size (N_e) in a sampling of gametes from a bulked seed collection was made with the following assumptions.

N parent trees are chosen at random from a population so that N_1 trees have genotype r_1r_1; N_2 trees have genotype r_1r_2; and N_3 trees have genotype r_2r_2. $(N_1, N_2, N_3) \sim$ multinomial(N,P_1,P_2,P_3).

[A1] $f(r_1r_1) = P_1 = p^2 + Fp\,(1 - p)$

[A2] $f(r_1r_2) = P_2 = 2p(1 - p)\,(1 - F)$

[A3] $f(r_2r_2) = P_3 = (1 - p)^2 + Fp(1 - p)$

where

p = frequency of the r_1 allele in the parent population
F = Wright's fixation index (Brown 1970)

Equal numbers of seeds (a) are sampled randomly from each of N trees and bulked so that the parental identities of aN seeds are lost. The relative frequency of the r_1 allele in the bulked collection is Y^*. The constants a and N and the value of the random variable Y^* are unknown to the investigator, who selects n gametes (egg or pollen) at random from the bulked seed collection for electrophoretic analysis. Of these, Y are found to carry the r_1 allele.

Formulae for E(Y) and var(Y) were sought as follows. Given that Z_2 is the number of r_1 gametes in the bulked seed collection derived from heterozygous (r_1r_2) parents, and that $(Z_2 \mid N_1,N_2,N_3) \sim \text{bin}(aN_2, 1/2)$, then

[A4] $Y^* = (aN_1 + Z_2)/aN$

and

[A5] $$\text{var } Y^* = \left(\frac{1}{aN}\right)^2 \left[a^2 \text{ var } N_1 + \text{var } Z_2 + 2a \text{ cov}(N_1,Z_2)\right]$$

Now

$$\text{var } N_1 = NP_1(1 - P_1),\ \text{var } Z_2 = \frac{aNP_2}{4}\left[1 + a(1 - P_2)\right]$$

and

$$\text{cov}(N_1,Z_2) = -\frac{aN}{2} P_1P_2$$

Substituting into eq. A5,

$$\text{var } Y^* = \left(\frac{1}{aN}\right)^2 \left\{a^2NP_1(1 - P_1) + \frac{aNP_2}{4}\left[1 + a(1 - P_2)\right] + 2a - \frac{aN}{2} P_1P_2\right\}$$

$$= \frac{1}{aN}\left\{a\left[P_1 + P_2/4 - (P_1 + P_2/2)^2\right] + P_2/4\right\}$$

Substituting eqs. A1 and A2 for P_1 and P_2,

[A6] $$\text{var } Y^* = \frac{1}{aN}\left[\frac{ap(1 - p)\,(1 + F)}{2} + \frac{p(1 - p)\,(1 - F)}{2}\right]$$

$$= \frac{p(1 - p)}{2N}\left(1 + F + \frac{1 - F}{a}\right)$$

Note, if a is large,

[A7] $$\text{var } Y^* \doteq \frac{p(1 - p)\,(1 + F)}{2N}$$

which is the maximum likelihood estimator (var p) for a large sample (Brown 1970).

As $Y \mid Y^* \sim \text{bin}(n, Y^*)$,

[A8] $\text{E}(Y \mid Y^*) = nY^*$

and

[A9] $\text{var}(Y \mid Y^*) = nY^*(1 - Y^*)$

$$\text{E}(Y) = \text{E}\,[\text{E}(Y \mid Y^*)] = n\text{E}(Y^*)$$

Substituting eq. A4,

[A10] $$\text{E}(Y^*) = \text{E}\left[(aN_1 + Z_2)/aN\right]$$

$$= \frac{1}{aN}\left[a\text{E}(N_1) + \text{E}(Z_2)\right]$$

$$= \frac{1}{aN}\left[aNP_1 + \frac{1}{2} aNP_2\right]$$

$$= P_1 + \frac{1}{2} P_2 = p$$

Therefore,

[A11] $\text{E}(Y) = np$

$$\text{var } Y = \text{var}[\text{E}(Y \mid Y^*)] + \text{E}[\text{var}(Y \mid Y^*)]$$

Substituting eqs. A8 and A9,

$$\begin{aligned}\text{var } Y &= \text{var}(nY^*) + \text{E}[nY^*(1 - Y^*)] \\ &= n^2 \text{ var } Y^* + n\,[\text{E}Y^* - \text{E}(Y^{*2})] \\ &= n\,\{n \text{ var } Y^* + \text{E}Y^* - [\text{var } Y^* + (\text{E}Y^*)^2]\} \\ &= n\,[(n - 1) \text{ var } Y^* + \text{E}Y^* - (\text{E}Y^*)^2]\end{aligned}$$

Again, assuming a is large, and substituting eqs. A7 and A10,

$$\begin{aligned}\text{var } Y &= n\left[(n - 1)\frac{p(1 - p)}{2N}(1 + F) + p - p^2\right] \\ &= np(1 - p)\left[1 + \frac{(n - 1)(1 + F)}{2N}\right]\end{aligned}$$

From eq. A11, an unbiased estimator of p is $\hat{p} = Y/n$.

[A12] $$\begin{aligned}\text{var}(\hat{p}) &= \frac{1}{n^2}\text{var } Y \\ &= \frac{p(1 - p)}{n}\left[1 + \frac{(n - 1)(1 + F)}{2N}\right]\end{aligned}$$

In a random sample of N_e gametes from the parent population,

$$\text{var } \hat{p} = \frac{\text{p}(1 - p)}{N_e}$$

Thus,

$$N_e = \frac{p(1 - p)}{\text{var } \hat{p}}$$

Substituting eq. A12 into this equation,

[A13] $$N_e = \frac{1}{\frac{1}{n} + \frac{(n - 1)(1 + F)}{2Nn}}$$

Finally, because the difference between observed and expected heterozygosities in Douglas-fir (Neale 1986) and other conifers (Yeh and Layton 1979; Guries and Ledig 1982; Woods *et al.* 1983; Neale and Adams 1985*b*; Fins and Seeb 1986; Furnier and Adams 1986*b*) is usually small (i.e., F is near zero), and because n is large in this study,

$$N_e = \frac{1}{\frac{1}{n} + \frac{1}{2N}}$$

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