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GENETIC DIFFERENTIATION OF DOUGLAS-FIR
POPULATIONS FROM THE NORTHERN ROCKY MOUNTAINS[1]

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Abstract. Growth potential, phenology, and cold hardiness were compared in progenies of 5 trees from each of 18 populations of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). Five-year-old seedlings growing in 2 nursery environments allowed assessment of population differentiation according to 6 variables related to growth and phenology. Freezing tests provided 5 variables that expressed cold hardiness in mid-September and near maximal hardiness.

Statistical analyses of 11 variables suggested a differentiation of populations into 3 provinces. One province included cool environments, regardless of geographic origin. The remaining 2 provinces included relatively warm environments from either western Montana or eastern Washington and northern Idaho. Variation among provinces, among populations within provinces, and among trees within populations contributed to the genetic variance.

Key words: cold hardiness; Douglas-fir; ecological genetics; growth potential; Idaho; northern Rocky Mountains; phenology; tree improvement.

INTRODUCTION

Adaptive differentiation of populations is conditioned by environmental heterogeneity. In the northern Rocky Mountains, environmental heterogeneity is extreme. The climate is generally controlled by westerly air masses from the Pacific Ocean, but the oceanic influence lessens each time the westerlies cross one of numerous mountain ranges shown diagrammatically in Fig. 1. Climatic gradients of decreasing oceanic influence occur from west to east and from north to south (Daubenmire and Daubenmire 1968). In addition, topographic microclimates are superimposed on the climatic gradients. Topographic effects are critical in a climate that is essentially without rain during the warm summer (Daubenmire and Daubenmire 1968).

Environmental heterogeneity is partially discernible in the mosaic of recurring climax plant communities of northern Idaho and eastern Washington (Daubenmire and Daubenmire 1968) and in western Montana (Pfister et al. 1977). Environmental complexes of sufficient contrast to produce different plant communities may control adaptive differentiation within species present in numerous plant associations. Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) has a very broad ecological amplitude. It is a successionally advanced species under relatively warm and dry conditions; it is a seral component of mesic forests; and, depending on moisture levels, it may be either seral or advanced in relatively cold environments (Daubenmire and Daubenmire 1968; Pfister et al. 1977). With a broad ecological amplitude in a region of extreme environmental

diversity, the potential for adaptive differentiation in Douglas-fir seems unlimited.

An understanding of the ecological genetics of a species is basic to evolutionary biology and necessary before developing comprehensive programs for tree improvement. In contrast to the wealth of genetic information on the coastal variety (*P. menziesii* var. *menziesii*), the ecological genetics of Douglas-fir in the inland northwest is poorly understood. Multivariate analyses of provenance tests conducted in Michigan revealed differentiation between seedlings from northern Idaho and those from western Montana (Wright et al. 1971). Based on growth responses of 2-year-old seedlings from the inland northwest, progenies from relatively cold environments were genetically differentiated from those of warm environments (Rehfeldt 1974a).

The present study of Douglas-fir was established (1) to determine whether genetic differentiation of populations was related to the recurring environments associated with particular habitat types and (2) to provide a general assessment of the genetic structure of populations.

MATERIALS AND METHODS

Eighteen populations were selected to represent the species' ecological amplitude in the inland northwest (Fig. 1). In order to minimize spurious correlations between genetic differentiation and the geographic and physiographic distribution of habitat types, an attempt was made to maximize the geographic distances and elevational differences among populations from the same habitat type. But, to gain independence of geographic, ecologic, and physiographic criteria, it was necessary to assume that populations from the *Pseudotsuga menziesii/Physocarpus malvaceus* and *Pseu-*
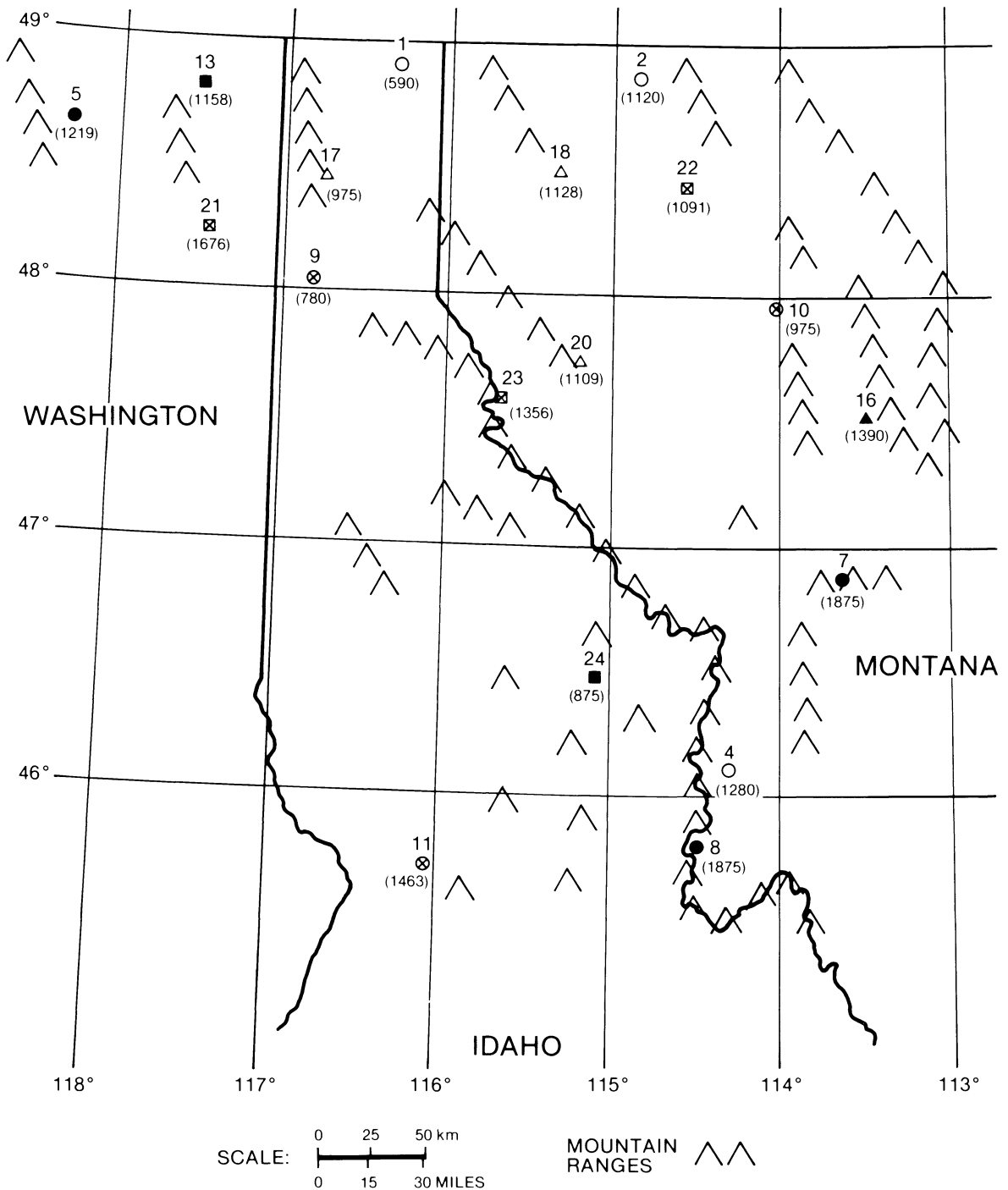
FIG. 1. Map of northern Rocky Mountains showing location of populations by their numeric code. Numbers in parentheses indicate population elevation (m), and symbols code habitat type as follows: O = *Pseudotsuga menziesii/Physocarpus malvaceus* and *Pseudotsuga menziesii/Symphoricarpos albus*; ● = *Pseudotsuga menziesii/Calamagrostis rubescens*; ⊗ = *Abies grandis/Pachistima myrsinites*; □ = *Thuja plicata/Pachistima myrsinites*; △ = *Pseudotsuga menziesii/Vaccinium globulare*; ▲ = *Tsuga heterophylla/Pachistima myrsinites*; and ⊠ = *Abies lasiocarpa/Pachistima myrsinites*.

dotsuga menziesii/Symphoricarpos albus habitat types represented the same environment; since these communities represent the warmest and driest environments in which Douglas-fir occurs within the region of study (Daubenmire and Daubenmire 1968), consolidation should introduce little bias. Five habitat types were represented by 3 populations: 1 habitat type, *Pseudotsuga menziesii/Vaccinium globulare*, was represented by a single population; and *Thuja plicata/Pachistima myrsinites* was represented by 2 populations.

Cones that had received natural wind pollinations were collected from the upper crown of 5 trees within each of the 18 populations. Seed trees selected within a given population were separated by at least 50 m and represented a random sample of those phenotypes that were bearing cones in a year of generally high cone production. Seeds were sown in unreplicated beds at a nursery at the Priest River Experimental Forest, Idaho. One-year-old seedlings were transplanted at a spacing of 30 cm into a randomized complete block design that included 2 replications at each of 2 nurseries. Each family was represented by 5 seedlings in each replication.

The nurseries included the relatively cool environment at Priest River Experimental Forest and the relatively warm environment at the Coeur d'Alene Forest Nursery located about 80 km south of Priest River. Weather records indicate that these 2 localities differ by an average of 30 days in frost-free period, 85 cm in snowfall, 1°C in summer maximum temperature, and 3°C in winter minimum temperature. Yet, both sites are at the same elevation, about 700 m.

Seedlings were grown for 5 years under optimal levels of fertilization and irrigation. During the study, the following measurements were taken on each seedling: height at ages 3 and 5; diameter at the ground at age 5; and length of a leaf from the center of the shoot produced during the 5th growing season. Dates of bud burst and bud set were scored during the 5th growing season.

Cold hardiness of each seedling was assessed in the 5th growing season soon after the 1st frost of September and after maximal levels of hardiness had been induced. For both tests, twigs (6 to 12 cm in length) were cut from the current growth of each seedling, moistened, packaged in plastic bags, and stored in a cooler at 3°C. Twigs collected in September were cooled at a rate of 4°C/h to each of 5 temperatures (−10°, −14°, −18°, −22°, and −26°C) within 48 h of collection. Twigs were exposed to particular test temperatures for one-half hour. A second set of samples was collected in late November. During a 4-week period, these twigs were slowly cooled from 3° to −10°C to promote the development of maximal hardiness (Sakai and Weiser 1973). Twigs from each seedling were cooled at a rate of 5°C/d to each of 5 temperatures: −30°, −35°, −40°, −45°, and −50°C; twigs were ex-

posed to test temperatures for 24 h. Samples from Coeur d'Alene were collected and frozen 1 week earlier than those from Priest River.

After removal from the freezer, twigs collected in September were thawed for 24 h at 8°C. Twigs collected in November were thawed 24 h at 0°C and 24 h at 8°C. After thawing, the cut end of each twig was placed in water in a plastic cup. Cups were placed on a greenhouse bench. One week later, injury to leaves, buds, and wood from freezing was scored by discoloration of tissue. As a measure of hardiness, the temperature for which injury was first observed to each tissue was recorded for each seedling represented in the nursery trials. A few seedlings exhibited no injury at the coldest temperatures and were arbitrarily assigned hardiness values of either −30 or −55 for tests of September or maximal hardiness, respectively.

Statistical analyses were made on 11 variables. Six variables reflected growth and phenology in nursery environments: 5-year height, diameter, leaf length, bud burst, bud set, and deviation from regression of 5-year height on 3-year height. Height at age 3 was subject to nonrandom environmental influences such as seed weight and transplanting shock. Because successive heights of a seedling are autocorrelated, experimental errors are perpetuated. Deviation from the height regression represents an assessment of growth rate between ages 3 and 5 that is independent of height at age 3.

Five variables were obtained from freezing tests: hardiness of leaves, buds, and wood in September and maximal hardiness of leaves and buds. A temperature of −50°C was insufficient to cause wood damage at maximal hardiness.

Statistical analyses were made after data were appropriately transformed to normalize frequency distributions of some variables (bud set and bud burst) and to eliminate relationships between means and variances for other variables (height and diameter). Analyses of variance were made to test main effects and interactions of nurseries, replications, populations and trees within populations. In addition, the main effects of populations were apportioned to the effects of habitat types and populations within habitat types. Since effects of habitat types were assumed to be fixed, a hierarchical model of mixed effects was involved. The Satterthwaite approximation (Snedecor 1955) was used for all tests of significance involving synthetic mean squares.

RESULTS

Analyses of variance are represented by intraclass correlation coefficients (Table 1) that reflect the proportion of the total variance attributable to each source of variance. Genetic differences among populations are represented by the main effects of populations. These effects were statistically significant for

all but 1 trait and accounted for as much as 19% of the total variance. Yet, for most traits, considerable genetic heterogeneity is evidenced within populations by significant main effects of trees within populations.

Main effects of the 2 nursery environments were pronounced for several traits (Table 1). In comparison to seedlings grown at Coeur d'Alene, seedlings grown at Priest River were characterized by delayed phenological events, longer leaves, and greater rate of growth since age 3. Also, since hardiness develops in response to the minimum temperature (Levitt 1972), effects of nurseries on hardiness probably reflect the cool temperatures and early frosts at Priest River.

Mean values (Table 2A) show that differences among populations can be related to habitat types. In fact, habitat types accounted for statistically significant proportions of the variance among populations for several traits (Table 3). Yet much of the effects of habitat types seems to be derived from a contrast in performance between seedlings from cool environments and those from warm environments. And further inspection of the data suggested that populations from warm environments could be divided into 2 geographic groups: western Montana or eastern Washington and northern Idaho. Consequently, variance among populations seemed to be associated with 3 provinces. Differences in mean values for these provinces were tested by the ''S'' test (Scheffé 1958) for multiple mean comparisons (Table 2B).

For all variables related to growth and hardiness in September, populations from cool environments, regardless of geographic origin, differed significantly from those of either or both of the other 2 groups. Of the populations from warm environments, those from Montana were significantly different from those of Idaho and eastern Washington for only the hardiness of leaves and buds in September. However, regardless of significance, mean values show that seedlings from cool environments were the shortest, had the smallest diameter, had the slowest growth rate from age 3 (negative deviations form regression), set buds the earliest, had the shortest leaves, and were of the greatest hardiness. On the other hand, mean values for seedlings from Idaho and eastern Washington deviated the most from those of seedlings from cool environments, and seedlings from Montana populations were intermediate.

The proportions of variance among populations that can be attributed to either the 3 provinces or the 7 habitat types are compared in Table 3. In general, classification according to province accounted for about 12% more of the population variance than classification according to habitat types. But, particularly for those traits related to hardiness, provinces accounted for substantially more variance than habitat types; and for no traits did provinces account for substantially less variance. Thus, the data suggest that populations represented in this study reflect adaptations to 3 en-

TABLE 1. Intraclass correlation coefficients derived from analyses of variance of 11 variables. Coefficients were calculated from components expected in each mean square and reflect the proportion of the total variance attributable to each source of variance.

| Source of variance | df | Nursery tests | | | | | | Freezing tests | | | | |
| | | 5-year height | Growth rate[a] | Diameter | Bud burst | Bud set | Leaf length | September hardiness | | | Maximal hardiness | |
								Leaf	Bud	Wood	Leaf	Bud
Nurseries (N)	1	0	0.17[e]	0	0.54[e]	0.43[e]	0.23[e]	0.01	0.07[e]	0.35[e]	0.30[e]	0.08[e]
Replications (R)	1	0	0	0	0	0	0.01	0.33[e]	0.05[e]	0	0.01[d]	0
Populations (P)	17	0.19[e]	0.07[e]	0.10[e]	0.08[e]	0.02[e]	0.04[e]	0.07[e]	0.07[e]	0.07[e]	0.03	0.03[d]
Trees/P (T/P)	72	0.06[e]	0.03	0.05[d]	0.07[e]	0.01	0.06[e]	0.03	0.01	0.04[e]	0.04[e]	0.06[e]
NXP	17	0.01	0.01	0.01	0.01	0.01	0.02[d]	0	0	0.01	0.06[e]	0
NXT/P	72	0	0	0.01	0	0.03[d]	0	0.02	0	0	0.04[d]	0.01
Experimental error[2]	178	0.21[e]	0.16[e]	0.15[e]	0.03[e]	0.04[d]	0.03[d]	0.15[e]	0.17[e]	0.09[e]	0.05[e]	0.05[d]
Within cells	1340	0.53	0.57	0.67	0.28	0.45	0.60	0.38	0.63	0.43	0.46	0.78
		(0.0672)[c]	(0.0281)	(0.0725)	(0.1286)	(0.0945)	(0.0234)	(4.817)	(6.694)	(9.512)	(28.00)	(9.316)

[a] Deviation from regression of 5-yr height on 3-yr height.
[b] Contains all sources of variation involving interactions with replication.
[c] Absolute value of mean square within cells.
[d] Significance of F-value at 0.05 level of probability.
[e] Significance of F-value at 0.01 level of probability.

TABLE 2. Means for 11 variables arranged according to (A) habitat types and (B) provinces. Province means (part B) that are not marked by the same letter differ significantly according to the "S" test (Scheffé 1958)

| | Nursery tests | | | | | | Freezing tests | | | | |
| | 5-year height (cm) | Growth rate[a] | Diameter (mm) | Bud burst[b] (days) | Bud set[c] (weeks) | Leaf length (mm) | September hardiness (°C) | | | Maximal hardiness (°C) | |
							Leaf	Bud	Wood	Leaf	Bud
A. HABITAT TYPE: environment[d]											
PSME/PHMA and											
PSME/SYAL: warm-dry	54.2	0.01	14.0	11.5	2.12	26.7	−14.8	−20.8	−22.8	−42.3	−49.2
ABGR/PAMY: warm-moist	63.6	.05	15.7	11.1	2.29	27.7	−14.0	−20.5	−21.7	−39.9	−48.9
THPL/PAMY: warm-moist	60.5	.03	15.6	11.7	2.29	27.4	−13.6	−20.4	−20.7	−39.3	−49.0
TSHE/PAMY: warm-moist	59.7	.03	14.9	11.9	2.12	26.2	−14.7	−21.2	−23.0	−42.2	−48.6
PSME/VAGL: cool-moist	53.1	−.03	13.8	14.9	1.93	26.5	−15.4	−21.9	−23.6	−42.4	−49.9
PSME/CARU: cool-dry	46.4	−.05	12.4	11.2	1.78	25.7	−15.4	−22.4	−24.3	−43.6	−49.3
ABLA/PAMY: cool-moist	45.4	−.08	12.7	12.0	1.77	25.6	−15.0	−22.2	−23.6	−42.0	−48.6
B. PROVINCES											
Warm Habitat Types											
Idaho and Eastern Washington	62.2 a	.05 a	15.7 a	11.6 a	2.34 a	27.8 a	−13.7 a	−20.3 a	−21.3 a	−39.4 a	−48.9 a
Montana	56.9 a	.02 ab	14.3 ab	11.4 a	2.07 a	26.3 a	−15.0 b	−21.1 b	−22.9 ab	−42.6 ab	−49.0 a
Cool Habitat Types	46.9 b	−.06 b	12.8 b	12.0 a	1.81 a	25.7 a	−15.2 b	−22.2 c	−23.9 b	−42.7 b	−49.1 a

[a] Since deviations from regression of 5-year height on 3-year height involved a difference between logarithms, mean values for growth rate are without units.

[b] Days after 1 May.

[c] Weeks after 1 August.

[d] PSME/PHMA = *Pseudotsuga menziesii/Physocarpus malvaceus*; PSMA/SYAL = *Pseudotsuga menziesii/Symphoricarpos albus*; PSME/CARU = *Pseudotsuga menziesii/Calamagrostis rubescens*; ABGR/PAMY = *Abies grandis/Pachistima myrsinites*; THPL/PAMY = *Thuja plicata/Pachistima myrsinites*; TSHE/PAMY = *Tsuga heterophylla/Pachistima myrsinites*; PSME/VAGL = *Pseudotsuga menziesii/Vaccinium globulare*; ABLA/PAMY = *Abies lasiocarpa/Pachistima myrsinites* (after Daubenmire and Daubenmire 1968; Pfister 1977).

TABLE 3. Proportions of the population variance ($\hat{\sigma}^2_P$) attributable to habitat types ($\hat{\sigma}^2_{HT}$), provinces ($\hat{\sigma}^2_{PV}$) and elevation (r^2_E)

Variable	$\dfrac{\hat{\sigma}^2_{HT}}{\hat{\sigma}^2_P}$	$\dfrac{\hat{\sigma}^2_{PV}}{\hat{\sigma}^2_P}$	r^2_E
Nursery tests			
5-year height	0.58*	0.62**	0.59**
Growth rate[a]	.57**	.54**	.54**
Diameter	.49*	.61**	.58**
Bud burst	0	0	.02
Bud set	.49	.58**	.58**
Leaf length	.20	.50**	.53**
Freezing tests			
September hardiness			
leaf	.14	.46*	.17
bud	.72**	.82**	.66**
wood	.55*	.70**	.56*
Maximal hardiness			
leaf	.33	.53**	.42*
bud	0	0	0

[a] Deviation from regression of 5-yr height on 3-yr height.
   * Significance of F-value or correlation coefficient at 0.05 level of probability.
  ** Significance of F-value or correlation coefficient at 0.01 level of probability.

vironmental provinces: 2 geographic zones and 1 ecologic zone.

Statistically significant (1% level) correlation coefficients were found between the elevation of populations and their mean values for most variables (Table 3). In fact, elevation alone accounted for as much genetic variance as habitat types and nearly as much variance as the three provinces. But provinces are not independent of elevation. In fact, provinces accounted for 51% of the total variance in elevation of those populations represented. The cool habitat types are most frequent at high elevations, and the base elevation of western Montana is higher than that of Idaho and eastern Washington. However, particularly for variables related to hardiness, provinces accounted for much more genetic variance than elevation. Thus, rather than reflecting environmental changes that regularly occur with increasing altitude, significant correlations between elevation and population performance in this study probably reflect the correlation between province classifications and elevation. It is unfortunate that the number of elevations represented within provinces is too small for accurate estimates of the independent effects of elevation on differentiation of populations.

DISCUSSION

The present results suggest that Douglas-fir populations from the northern Rocky Mountains are differentiated into 3 provinces. Populations from cool environments, regardless of geographic origin, represent one province. These populations are characterized by slow rates of growth and high levels of cold hardiness. Populations from relatively warm environments of

Montana differed from those of eastern Washington and northern Idaho. These results corroborate those of Wright et al. (1971) and Rehfeldt (1974a). Although the latter report was based solely on the height of 2-year-old seedlings, its findings are remarkably similar to present results.

The 3 provinces are intuitively appealing because they delinate environments of such contrast that adaptive differentiation is readily envisioned. That differentiation is related to cool, subalpine environments is sensible. Moreover, the boundary between Idaho and Montana roughly follows the crests of the Bitterroot and Cabinet Mountains. These mountains may sufficiently alter westerly air masses to produce regional climatic provinces that control differentiation.

Current results also contribute toward an understanding of the system of genetic variability for Douglas-fir in the northern Rockies. Three adaptive provinces accounted for only a portion of the variance among populations; considerable variation existed among populations within provinces. But variance among populations accounted for only a portion of the total genetic variance. Variation within populations was evidenced by significant effects of trees within populations which reflect genetic variance among half-sib families. Finally, analyses of variance showed that variance within cells accounted for very large proportions of the total variance. Although these variances were influenced by microenvironmental effects and the small number of observations within cells, they also include genetic variance among seedlings within families. These variances reflect heterozygosity of parental trees.

Consequently, genetic variance is evidenced at all levels of the genetic system. Adaptation to geographic and ecologic provinces provides genetic specialization to contrasting environments. Yet, heterogeneity within populations and individual trees provides flexibility. Although data on the degree of differentiation within provinces are lacking, flexibility within populations suggests that each tree, and, therefore, each population is capable of producing progenies preadapted to a range of environmental conditions.

Studies of the ecological genetics of Douglas-fir in the northern Rocky Mountains will continue. Differences among populations within provinces may represent environmental adaptations or random patterns of local differentiation (Rehfeldt 1974b). Regardless, differentiation within provinces must be assessed before seed zones are delineated and before estimates are made of gains expected from tree improvement.

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