Enzyme Variations in Natural Populations of Douglas-fir, Pseudotsuga Menziesii (Mirb.) Franco, from British Columbia. 1. Genetic Variation Patterns in Coastal Populations[1])

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Summary

We have studied genic variation patterns at 21 loci coding for 15 enzymes in eleven natural populations of Coastal Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. The average proportion of polymorphic loci per population was 68.83 ± 1.86% and 51.52 ± 1.78% at the 99% and 95% criterion for polymorphism, respectively. The average number of alleles per locus per population was 2.19 ± 0.21. The average proportion of heterozygous loci per individual was 15.46 ± 3.70%. A high degree of inter-locus variation in heterozygosity within populations was revealed. Spatial pattern of allelic frequencies and heterozygosities departed from random at eight loci. The observed patterns were clinal with respect to latitude, longitude and elevation.

Our measurement of gene diversity for the 21 loci showed a 3% significant effect of interpopulation differentiation. Ninety-seven percent of the total gene diversity resided within populations.

The mean genetic distance over all pairs of Coastal Douglas-fir was 0.0037. A product-moment correlation between the genetic distance of populations and the geographic distance between them yielded a significant value, $r = 0.3116$ ($P \leq 0.05$).

Key words: Megagametophytes, population genetics, isozyme polymorphisms, genic variation, spatial patterns.

Zusammenfassung

In elf autochthonen Beständen der Küstendouglasie (*Pseudotsuga menziesii* [Mirb.] Franco) in British Columbien wurden genische Variationsmuster von 21 Loci, die 15 Enzyme codieren, untersucht. Der durchschnittliche Anteil polymorpher Loci pro Population betrug 68,83 ± 1,86% und 51,52 ± 1,78% hinsichtlich des 99% bzw. 95% Kriteriums für Polymorphismus. Die durchschnittliche Anzahl der Allele je Locus und Population betrug 2,19 ± 0,21. Der durchschnittliche Anteil an heterozygoten Loci je Individuum betrug 15,46 ± 3,70. In bezug auf Heterozygotie gab es große Variation zwischen Loci innerhalb von Populationen. Die räumlichen Muster der Allel- und Heterozygotenhäufigkeiten wichen bei 8 Loci von der Zufallsverteilung ab. Die beobachteten Muster standen in klinaler Beziehung zu Breiten- und Längengrad sowie zur Höhe. Das Maß für genische Vielfalt für die 21 Loci zeigte einen signifikanten Effekt (3%) hinsichtlich der Differenzierung zwischen Populationen. 97% der gesamten Genunterschiede liegen innerhalb der Populationen. Der mittlere genetische Abstand über alle Paare der Küstendouglasie betrug 0,0037. Eine Produkt-Moment Korrelation zwischen dem genetischen Abstand der Populationen und der geographischen Entfernung ergibt einen signifikanten Wert, $r = 0{,}3116$ ($P \leq 0{,}05$).

Introduction

There has been an immense accumulation of data on electrophoretically detectable isozyme polymorphisms in haploid and diploid tissues of various coniferous tree species (e.g., Lewis and Cech, 1969; Bergmann, 1971; Conkle, 1971; Tigerstedt, 1973; Muhs, 1974; Rudin et al., 1974; Simonsen and Wellendorf, 1975; Yang et al., 1977). Reasons for the growing interest in such information are without doubt numerous. One ist the strong need to acquire information pertaining to the structure of natural conifer populations and to the distribution of genic variation within and between populations. In addition, isozymes as gene markers have facilitated the solving of pratical problems encountered in forest tree breeding and genetics research (Rudin, 1976 for review). Beyond this, it would be very desirable to demonstrate the spatial distribution of isozyme polymorphisms in long-lived perennials in order to shed light on the current controversy in basic population genetics concerning the hypothesis of selection (e.g., Clarke, 1970) and neutrality (e.g., Kimura, 1968) for the maintenance of isozyme polymorphisms in natural populations.

With the exception of the findings of Fowler and Morris (1977) on red pine, *Pinus resinosa* Ait. there is universal occurrence of isozyme polymorphisms in natural populations of coniferous tree species. A striking feature of the more extensive surveys has been the demonstration of a high degree of interlocus variation in heterozygosity within populations (Lundkvist and Rudin, 1977; Yang et al., 1977). It is important then, that a large and random sample of loci be studied when assessing genic variation patterns in coniferous tree species.

The present paper contains a description of genic variation patterns in Coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco from British Columbia, Canada. This species is a wind-pollinated tree with a natural range spanning the west coast of British Columbia, from Kemano to California (Fig. 1). Twenty-one randomly selected gene loci, coding for fifteen enzyme systems, have been studied in samples from eleven natural populations.

We think that the study of geographic variation patterns at the single gene level will provide valuable insight for an understanding of the evolutionary history of Coastal Douglas-fir in British Columbia. Specifically, we wish to answer three questions: (1) How much genic variation exists within populations? (2) Are there differences in genic variation among populations? (3) If so, are such differences associated with geographic variables (longitude, latitude and elevation) in linear fashion?

Materials and Methods

Megagametophytes of Coastal Douglas-fir were used as the enzyme source material. The advantage of assaying of this material is that it is haploid and therefore allows direct analysis of genetically determined enzyme mobility differences. Inheritance of allelic variation can be inferred

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[1]) Contribution No. 4 from the Population Genetics Laboratory, Research Branch, British Columbia Ministry of Forests, Victoria, Canada V8W 3E7. This article is dedicated to A. L. Orr-Ewing, on the occasion of his retirement, who established the foundation of forest genetics in British Columbia.

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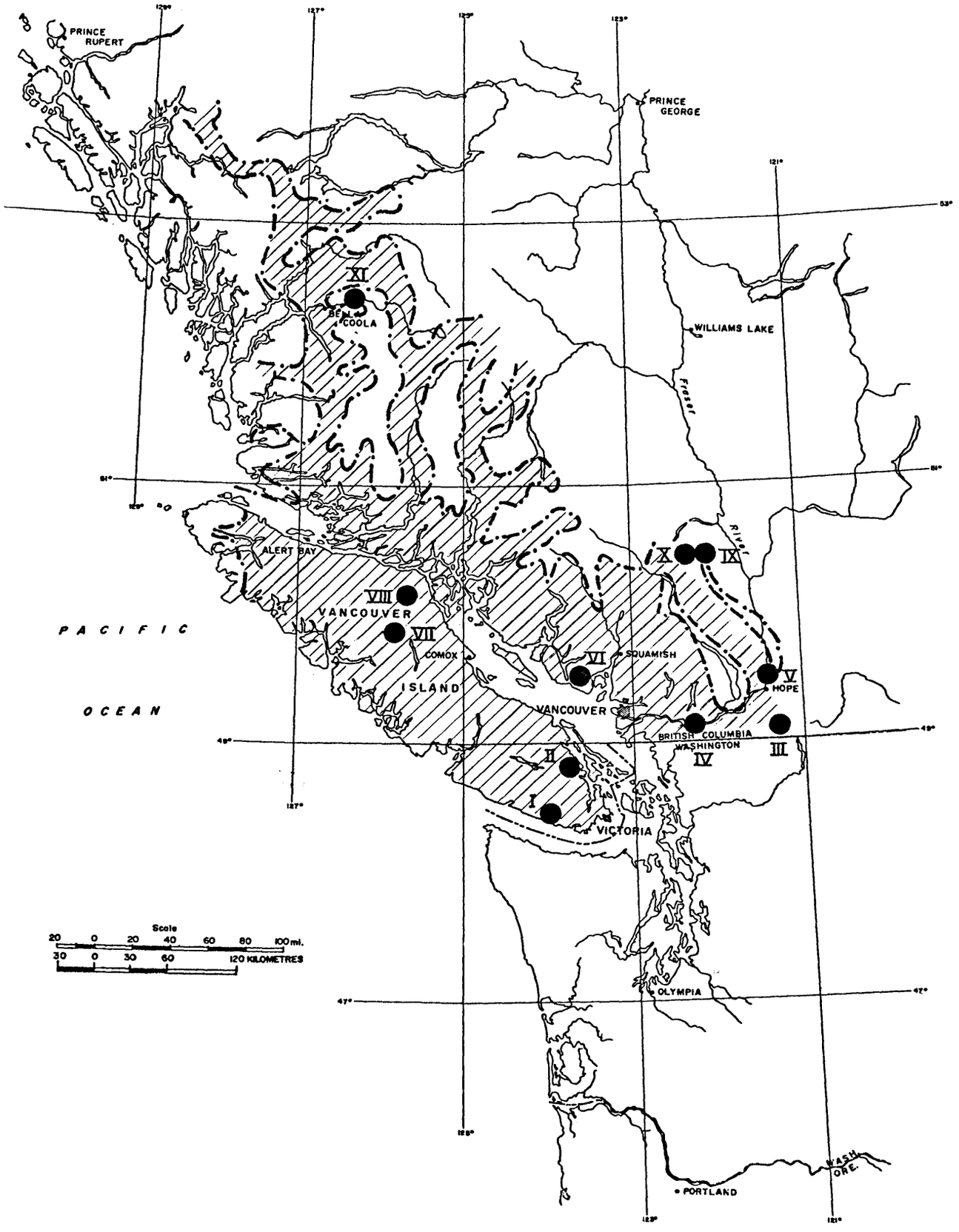

Figure 1. — Species range and population location

from Mendelian (1:1) segregation of alternative enzyme phenotypes when scoring numerous megagametophytes from a single tree heterozygous at a locus (BARTELS, 1971; RUDIN, 1975).

The inheritance patterns of isozymes reported in this study have been determined (EL-KASSABY, unpublished). A minimum of sixty megagametophytes per tree were scored in segregation analyses of polymorphic loci. In all cases, chi-square values were non-significant at the 5% level of probability.

Seeds used in this study were selected from bulk stand (population) collections made within the natural range of Coastal Douglas-fir in British Columbia *(Fig. 1)*. The seed trees were randomly chosen from a 10 to 24 hectare area within each stand. Collections were made by the Silviculture Branch of the Ministry of Forests. The exact number of seed trees sampled from each stand was unknown but was probably greater than 100 trees. The origins of the seeds and the numbers of megagametophytes analysed are presented in *Table 1*.

Since the number of trees sampled per population could not be determined, a second, single tree collection, was made in one of the stands to compare with the calculated allelic frequencies in the bulked collection. Sixty seed trees were sampled and genotyped from Population II. The allelic frequency estimates from both samples were in excellent agreement with each other. This suggests that the bulk collections used were representative of the variation presented in *Tab. 1*.

Seeds were hydrated for 24 hours and then dissected, or surface dried and refrigerated for later use. Megagametophytes were individually homogenized in 0.5 ml autoanalyse cups (Elkay Products, Worcester, Massachusetts) containing three drops of extraction buffer, pH 7.5 (see below). Each megagametophyte yielded sufficient sample to assay for 15 enzyme systems. Within these 15 systems we were able to resolve 21 loci using only 3 starch gel buffer systems and 1 cellulose acetate buffer system.

A minute quantity of the crude homogenate was applied to a cellulose acetate strip (2.5 cm × 15 cm, Gelman Co., Ann Arbor, Michigan) and then run in a Gelman electrophoretic chamber (O'MALLEY and YEH, 1978). Additional technical details can be found in BREWER (1970). The remainder of the sample was absorbed onto 3 mm × 10 mm filter paper wicks (Absorbent Paper, Gelman Co., or Whatman 3 mm) and these inserted along a cut 1.5 cm from the edge of the gel. A 12.5% (w/v) starch gel (Electrostarch Co., Madison, Wisconsin), 9.5 cm (w) × 21 cm (l) × 1 cm (thick) would accommodate 40 samples along the longitudinal axis. After loading the gel, the appropriate current or voltage was applied until a tracking dye (dilute red food colouring) had migrated 5-10 mm (~ 15 minutes). The wicks were then removed, icepacks applied, and electrophoresis was continued.

Upon completion of electrophoresis the starch gel was sliced horizontally into 5-6 slices 1-1½ mm thick. Each gel slice or cellulose acetate strip was placed in a tray containing a specific enzyme-staining solution. Details of enzyme assays, including the extraction, gel and electrode buffers and composition of each of the enzyme staining solutions, are listed below.

Extraction Buffer: 10 ml electrode buffer (C), 50 mg NADP, 50 mg NAD, 0.018 g ascorbic acid (.001 M), .034 g EDTA (.001 M), 100 mg bovine serum albumin, H_2O to 100 ml, pH adjusted to 7.5, 5 drops 2-mercapto-ethanol.

Gel and Electrode Buffers: four buffer systems were used:

A. electrode buffer: 0.04 M citric acid (anhydrous), adjust pH to 6.1 with N-(3-aminopropyl)-morpholine; gel buffer: 1:20 dilution of electrode buffer; power: 200 V (~ 50 mA) until tracking dye has migrated 6 cm (CLAYTON and TRETIAK, 1972).
B. electrode buffer: 0.06 M lithium hydroxide and 0.3 M boric acid, pH 8.1; gel buffer: 0.03 M Tris, 0.005 M citric acid (anhydrous) and 1% electrode buffer, pH 8.5; power: 250 V (~60 mA) until tracking dye has migrated 5 cm (RIDGEWAY et al., 1970).
C. electrode buffer: 0.13 M Tris and 0.043 M citric acid (anhydrous), pH 7.0; gel buffer: 1:15 dilution of electrode buffer; power: 60 mA (~160 V) until tracking dye has migrated 5 cm (SICILIANO and SHAW, 1976).
D. electrode buffer: 1:10 dilution of 0.214 M Na_2HPO_4 and 0.027 M citric acid (anhydrous), pH 7.0; power: 1.5 mA per cellulose acetate strip for 40 minutes (adopted from SHAW and PRASAD, 1970).

Enzyme Staining Solutions:

Aconitase (ACO; E. C. 4. 2. 1. 3): buffer system A; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 5 ml 5% cis-aconitic acid

Table 1. — The origins of the seeds and the average number of genomes analysed per locus in natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (MIRB.) FRANCO from British Columbia.

Population	Latitude °N	Longitude °W	Elevation M	Seed Zones[1]	Average number of genomes analysed per locus[2]
I	48.55	124.08	244	1020	118±1.22
II	48.87	123.75	457	1020	117±1.03
III	49.23	121.23	930	1060	116±1.43
IV	49.25	122.35	152	1050	116±0.97
V	49.50	121.35	884	1060	114±1.34
VI	49.50	123.87	175	1040	119±1.05
VII	49.87	125.83	250	1020	116±1.23
VIII	50.25	125.73	137	1020	116±1.22
IX	50.53	122.47	457	1070	115±0.98
X	50.57	122.53	1067	1070	118±1.71
XI	52.42	126.25	244	1090	118±1.57

[1]) Seed zones have been established within broad biogeoclimatic regions as defined by KRAJINA (1972). Seed should be used within the zone from which it was collected and within 152.4 m of its original elevation.

[2]) 120 megagametophytes are analysed per population. However, variations exist in the numbers of genomes scorable per locus.

(w/v), 40 units isocitric dehydrogenase, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg NBT, 5 mg PMS.

Aldolase (ALD; E. C. 4. 1. 2. 13): buffer system A; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 250 mg fructose-1, 6-diphosphate, 75 mg arsenic acid, 300 units glyceraldehyde-3-phosphate dehydrogenase, 10 mg NAD, 10 mg MTT, 5 mg PMS.

Aspartate aminotransferase (AAT; E. C. 2. 6. 1. 1): buffer system B; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 1 mg pyridoxal-5'-phosphate, 200 mg L-aspartic acid, 100 mg α-ketoglutaric acid, 200 mg Fast Blue BB salt.

Diaphorase (DIA; E. C. 1. 6. 4. 3): buffer system C; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 1 mg 2, 6-dichlorophenol -indophenol, 25 mg β-NADH, 10 mg MTT.

Esterase (EST; E. C. 3. 1. 1. 1): buffer system C; stain: 50 ml 0.2 M phosphate buffer pH 6.4, 50 mg each of α- and β- naphthyl acetate in 5 ml acetone; 100 mg Fast Blue RR salt.

Glutamate dehydrogenase (GDH; E. C. 1. 4. 1. 3): buffer system B; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 400 mg L-glutamic acid, 10 mg NAD, 10 mg NBT, 5 mg PMS.

Glucose-6-phosphate dehydrogenase (G6P; E. C. 1. 1. 1. 49): buffer system C; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 200 mg glucose-6-phosphate, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg MTT, 5 mg PMS.

Isocitrate dehydrogenase (IDH; E. C. 1. 1. 1. 42): buffer system C; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 200 mg DL-isocitric acid, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg NBT, 5 mg PMS.

Malate dehydrogenase (MDH; E.C.1.1.1.37): buffer system D; stain: 25 ml 0.2 M Tris — HCl pH 8.0, 25 ml 0.5 M DL-malic acid pH 7.0, 10 mg NAD, 10 mg NBT, 5 mg PMS.

Malic enzyme (ME; E.C.1.1.1.40): buffer system A; stain: 25 ml electrode buffer A, 25 ml 0.5 M DL-malic acid pH 7.0, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg MTT, 5 mg PMS.

Peptidase (PEP; E.C.3.4.13.1): buffer system B; stain: 50 ml Tris — HCl pH 8.0, 60 mg L-leucyl-L-alanine, 60 mg L-leucyl-glycyl-glycine, 60 mg glycyl-L-leucine, 10 mg crude peroxidase, 10 mg snake venom, 50 mg 0-dianisidine DiHCL, 5 ml 1% $MgCl_2$ (w/v).

Phosphoglucose isomerase (PGI; E.C.5.3.1.9): buffer system C; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 25 mg fructose-6-phosphate, 10 units glucose-6-phosphate dehydrogenase, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg NBT, 5 mg PMS.

Phosphoglucomutase (PGM; E.C.2.7.5.1): buffer system C; stain: 50 ml 0.2 M Tris — HCl pH 8.0, 300 mg glucose-1-phosphate, 0.5 ml glucose-1, 6-diphosphate solution (0.1 mg/ml H_2O), 50 units glucose-6-phosphate dehydrogenase, 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg MTT, 5 mg PMS.

6-Phosphogluconic dehydrogenase (6PG; E.C.1.1.1.44): buffer system C; stain: 5 ml 0.2 M Tris — HCl pH 8.0, 10 mg phosphogluconic acid (Na_3salt), 1 ml 1% $MgCl_2$ (w/v), 10 mg NADP, 10 mg MTT, 2.5 mg PMS (add stain dropwise to gel).

Superoxide dismutase (SOD; E.C.1.15.1.1): scored on gels stained for GDH activity; SOD appeared as white bands against a blue background.

When an enzyme system was controlled by multiple loci, those isozymes and the corresponding loci were identified by the symbol of the enzyme and a hyphenated numeral. The locus specifying the most anodally migrating isozyme was designated as 1, the next as 2, and so on. Within each locus, the most common allozyme was given the arbitrary value of 100. The other allozymes were numbered according to their migration relative to the common allozyme (i. e. the 100 form) and were designated by superscripts to the locus notion.

Allelic frequencies at each locus in individual populations were obtained by direct count since haploid megagametophytes were used. The genic variation within individual populations was quantified by measuring the proportion of polymorphic loci, the average number of alleles per locus and the expected proportion of heterozygous loci per individual (Lewontin, 1967). Investigations of population differentiation were accomplished by two methods of analysis of the genic variation between populations. The first was to consider the amount and apportionment of genic diversity within Coastal Douglas-fir following Nei's (1973) application of the gene diversity concept. The second procedure was to analyse the relationships between populations using Nei's (1972) genetic distance measures.

Results and Discussion

Genic Variation Within Coastal Douglas-fir

In our survey 21 isozyme loci were resolved with sufficient consistency and clarity to permit us to say that they were electrophoretically monomorphic or polymorphic, if the latter, to score for allelic variants in our population samples. *Table 2* with its population samples arranged in increasing latitudes, gives the frequency at which each allele is found in each population. The table also gives the proportion of individuals expected to be heterozygous in each population at each locus. With the exception of three loci, the AAT-1, the PEP-2 and the PEP-3, we have found variant alleles at every locus, although not in every population.

For convenience of discussion, polymorphic loci are considered under the following arbitrary categories according to their degree of average heterozygosity.

1. Polymorphism with more than 45 percent heterozygosity:

Two loci, the EST-1 and the G6P fall into this category. At these two loci, no one allele is present in highest frequency in all populations. Heterozygosities at a given locus vary moderately from population to population yet conspicuous changes in allelic frequencies between populations are found. At the EST-1 locus, allele 85 occurs in Populations VI and X at a 0.025 and 0.035 frequency, respectively, but it is absent or present at very low frequencies in all other populations; frequencies of alleles 92, 100 and 110 vary sometimes over wide limits. At the G6P locus, allele 80 occurs in Populations IV, VI and XI at an approximate 0.042 frequency, but it is absent or present at very low frequencies in all other populations; frequencies of alleles 90 and 100 have wide ranges between 0.383-0.681 and 0.277-0.617, respectively.

2. Polymorphism with 25-45 percent heterozygosity:

Three loci, the AAT-2, the IDH and the MDH-1 fall into this category. At each of these loci one allele, (i. e. allele 100) is the most common allele in all populations. Heterozygosities and allelic frequencies at a given locus vary considerably between populations. At the AAT-2 locus, heterozygosities have a range between 0.120-0.375; allele 82 occurs in Population I at a 0.075 frequency but it is absent or present at very low frequencies in all other populations; frequencies of alleles 100 and 112 fluctuate widely between 0.750-0.937 and 0.036-0.250, respectively. At the IDH locus, heterozygosities vary between 0.113-0.369; allele 65 is absent in Populations III and IX; alleles 90, 100 and 122 fluctuate sometimes over wide limits. At the MDH-1 locus, we have not observed all four alleles within any

Table 2. — Allelic variation of 21 loci in natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco from British Columbia

Locus	Allele	Populations										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
AAT-2	82	.075	.017	.035	.026	.027	.008	.017	.000	.027	.000	.000
	100	.792	.847	.861	.802	.937	.891	.808	.815	.784	.828	.750
	112	.133	.136	.104	.172	.036	.101	.175	.185	.189	.172	.250
	Heterozygosity	.349	.264	.247	.327	.120	.196	.316	.302	.349	.285	.375
AAT-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Heterozygosity	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
ACO-1	77	.025	.047	.106	.134	.088	.000	.010	.101	.059	.110	.008
	100	.975	.953	.894	.866	.912	1.000	.990	.899	.941	.890	.992
	Heterozygosity	.049	.090	.190	.232	.161	.000	.020	.182	.111	.196	.016
ALD_1	80	.026	.000	.033	.009	.030	.009	.009	.000	.000	.017	.017
	100	.957	.983	.917	.955	.950	.973	.982	1.000	.953	.957	.983
	110	.017	.017	.050	.036	.020	.018	.009	.000	.047	.026	.000
	Heterozygosity	.083	.033	.156	.087	.096	.053	.036	.000	.090	.083	.033
DIA-2	65	.000	.000	.000	.008	.000	.000	.033	.000	.000	.008	.000
	100	1.000	1.000	1.000	.992	1.000	1.000	.967	1.000	1.000	.992	.992
	144	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.008
	Heterozygosity	.000	.000	.000	.016	.000	.000	.064	.000	.000	.016	.016
EST-1	85	.009	.008	.009	.009	.000	.025	.000	.000	.000	.035	.000
	92	.219	.126	.364	.250	.292	.275	.166	.296	.359	.377	.281
	100	.544	.622	.455	.353	.485	.475	.617	.546	.342	.333	.412
	110	.228	.244	.137	.388	.223	.225	.217	.158	.299	.254	.307
	Heterozygosity	.604	.538	.631	.662	.630	.648	.545	.589	.665	.681	.657
GDH	88	.000	.017	.008	.000	.000	.000	.000	.000	.000	.000	.000
	100	1.000	.983	.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Heterozygosity	.000	.033	.016	.000	.000	.000	.000	.000	.000	.000	.000
G6P	80	.000	.017	.000	.042	.035	.042	.000	.017	.000	.009	.041
	90	.454	.538	.658	.471	.427	.681	.383	.525	.608	.581	.442
	100	.546	.445	.342	.487	.538	.277	.617	.458	.392	.410	.517
	Heterozygosity	.496	.512	.450	.539	.527	.458	.473	.514	.477	.494	.536
IDH	65	.101	.050	.000	.009	.009	.034	.017	.034	.000	.009	.008
	90	.025	.025	.009	.059	.026	.059	.009	.026	.075	.034	.017
	100	.782	.825	.879	.847	.896	.814	.871	.888	.792	.803	.941
	122	.092	.100	.112	.085	.069	.093	.103	.052	.133	.154	.034
	Heterozygosity	.369	.306	.215	.272	.192	.324	.230	.207	.349	.330	.113
MDH-4	75	.000	.035	.000	.000	.000	.000	.000	.000	.000	.000	.000
	100	1.000	.965	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Heterozygosity	.000	.068	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-3	84	.158	.112	.058	.084	.080	.084	.085	.059	.079	.067	.033
	100	.800	.879	.925	.916	.920	.908	.915	.924	.921	.925	.967
	130	.042	.009	.017	.000	.000	.008	.000	.017	.000	.008	.000
	Heterozygosity	.333	.215	.141	.154	.147	.168	.156	.143	.146	.140	.064
MDH-2	90	.100	.129	.008	.000	.000	.025	.059	.110	.053	.008	.117
	100	.850	.836	.992	1.000	1.000	.975	.941	.881	.947	.992	.883
	110	.050	.034	.000	.000	.000	.000	.000	.009	.000	.000	.000
	Heterozygosity	.265	.283	.016	.000	.000	.049	.111	.212	.100	.016	.207
MDH-1	90	.017	.000	.000	.017	.000	.000	.000	.017	.000	.009	.000
	95	.067	.086	.158	.218	.165	.143	.153	.109	.281	.203	.133
	100	.916	.905	.834	.765	.835	.849	.814	.874	.719	.788	.792
	105	.000	.009	.008	.000	.000	.008	.034	.000	.000	.000	.075
	Heterozygosity	.156	.174	.279	.367	.276	.259	.313	.224	.404	.338	.349
ME-2	85	.043	.085	.104	.062	.088	.089	.059	.000	.094	.144	.053
	100	.923	.873	.754	.903	.858	.895	.911	.982	.843	.838	.894
	130	.034	.042	.142	.035	.054	.016	.030	.018	.063	.018	.053
	Heterozygosity	.145	.229	.401	.180	.253	.191	.166	.035	.277	.277	.195
ME-1	80	.034	.000	.008	.017	.000	.000	.000	.017	.000	.008	.000
	100	.966	1.000	.992	.983	1.000	1.000	1.000	.983	1.000	.992	1.000
	Heterozygosity	.066	.000	.016	.033	.000	.000	.000	.033	.000	.016	.000
PEP-3	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Heterozygosity	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEP-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Heterozygosity	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGI-2	70	.008	.000	.000	.000	.017	.000	.000	.000	.000	.000	.000
	100	.975	.941	.967	.984	.932	.947	.967	.958	.908	.873	1.000
	125	.017	.045	.033	.008	.051	.036	.033	.042	.092	.127	.000
	154	.000	.000	.000	.008	.000	.017	.000	.000	.000	.000	.000
	Heterozygosity	.049	.111	.064	.032	.129	.102	.064	.081	.167	.222	.000
PGM-1	94	.069	.050	.086	.059	.102	.042	.042	.094	.050	.068	.042
	100	.871	.874	.845	.882	.833	.881	.858	.872	.917	.840	.941
	105	.060	.076	.069	.059	.065	.076	.100	.034	.033	.092	.017
	Heterozygosity	.233	.228	.274	.215	.292	.216	.252	.230	.156	.281	.113
SOD	68	.017	.008	.024	.043	.017	.017	.008	.000	.000	.059	.067
	100	.983	.992	.968	.957	.983	.983	.992	1.000	1.000	.941	.933
	120	.000	.000	.008	.000	.000	.000	.000	.000	.000	.000	.000
	Heterozygosity	.033	.016	.062	.082	.033	.033	.016	.000	.000	.111	.125
6PG-1	85	.008	.085	.126	.076	.085	.059	.092	.090	.117	.119	.060
	100	.975	.907	.773	.898	.847	.882	.883	.892	.875	.780	.931
	107	.017	.008	.101	.026	.068	.059	.025	.018	.008	.101	.009
	Heterozygosity	.049	.170	.376	.187	.271	.215	.211	.196	.221	.367	.130

single population; heterozygosities vary between 0.156-0.404; alleles 90 and 105 are present in some populations and at low frequencies; alleles 95 and 100 fluctuate sometimes over wide limits.

3. Polymorphism with 10-25 percent heterozygosity:

Six loci, the ACO-1, the MDH-2, the MDH-3, the PGM-1 and the 6PG-1 fall into this category. At each of these loci, allele 100 occurs with the highest frequency in all populations. Heterozygosities and allelic frequencies at a given locus vary widely between populations, and geographic variation patterns are apparent at most loci. At the ACO-1 locus, Population VI is monomorphic; frequency of the allele 77 and heterozygosities are higher in the eastern populations, while the frequency of allele 100 is correspondingly lower. At the MDH-2 locus, Populations IV and V are monomorphic; frequency of allele 90 and heterozygosities are lower in the eastern populations while the opposite trend is observed for allele 100. Allele 110 occurs only in the Vancouver Island Populations I, II and VIII. At the MDH-3 locus, north-south clinal decreases are observed in the frequency of allele 84 and heterozygosity, while allele 100 shows a gradual increase from 80% in Population I to 96.7% in Population XI. Allele 130 occurs at a 0.042 frequency in Populations I, but it is absent or present at very low frequencies in all other populations. The ME-2 locus show clinal variation in both allelic frequencies and levels of heterozygosity along longitudinal and elevational gradients. Allele 85 is absent in Population VIII. The frequency of the 100 allele is higher in the western and lower elevation populations, while the opposite trends are observed for allele 130 and heterozygosity. The PGM-1 locus shows only little differentiation in allelic frequencies and heterozygosities in different populations.

However, Populations IX and XI seem to differ from the rest of the populations both in heterozygosity and the frequency of allele 100. At the 6PG-1 locus, the frequency of alleles 85 and 107, and heterozygosity show increases along an altitudinal gradient while the opposite trend holds true for allele 100.

4. Polymorphism with 5-10 percent heterozygosity:

Two loci, the ALD-1 and the PGI-2 belong to this category. Again at each of these loci, allele 100 is the most common allele in all populations. Geographic variation patterns are apparent. At the ALD-1 locus, clinal changes in allele frequency and heterozygosity are observed along an east-west transect, and associated with elevation. Population VIII is monomorphic. The frequency of allele 100 is higher in the eastern and lower elevation populations, while the opposite trends are observed for allele 110 and heterozygosity. At the PGI-2 locus, we have not observed all four alleles in any single population. Allele 70 occurs only in Populations IV and VI. The frequency of allele 100 decreases along an altitudinal gradient whereas the opposite trend holds true for allele 125 and heterozygosity.

5. Polymorphism with less than 5 percent heterozygosity:

The remaining five loci fall into this category. At all of these loci the 100 allele is fixed or nearly fixed in all populations and locally endemic alleles are present. Only Population I is polymorphic at the MDH-4 locus. Both Populations II and III are polymorphic at the GDH locus. Polymorphism is observed only in five populations at the ME-1 locus. At the DIA-2 locus, allele 144 is present only in Population XI; whereas allele 65 is observed only in Populations IV, VII and X. At the SOD locus, we have not observed polymorphism in Populations VII and IX; allele 120 is present only in Population III.

The data of *table 2* are summarized in *table 3* for each population. The overall mean of the percentage of loci polymorphic per population, loci heterozygous per individual, and the number of alleles per locus per population, are presented in this *table*. Since two criteria for polymorphic loci are often found in the literature (AYALA et al., 1971), two percentages of loci polymorphic per population are given in *Table 3*.

Table 3 shows that an average population of Coastal Douglas-fir is polymorphic for some 51.52-68.83 percent of its loci depending upon the criterion of polymorphism used. These figures are in close agreement with that of 57.14 percent obtained for Coastal Douglas-fir populations from Washington, Oregon and British Columbia (YANG et al., 1977). However, these estimates are not comparable since they depend upon the extent to which the samples of isozymes are biased in the two studies. Although the observed percentages at the 5 percent criterion of polymor-

Table 3. — Genetic variation in natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (MIRB.) FRANCO from British Columbia

Population	Percentage of loci polymorphic [1])		Average number of alleles per locus	Percentage of heterozygous loci per individual
	Criterion 1	Criterion 2		
I	42.86	71.43	2.33 ± .22	15.61 ± 4.08
II	57.14	76.19	2.33 ± .21	15.57 ± 3.53
III	52.38	76.19	2.29 ± .20	16.82 ± 3.96
IV	47.62	71.43	2.29 ± .22	16.12 ± 4.10
V	57.14	61.91	2.10 ± .22	14.88 ± 3.88
VI	47.62	61.91	2.34 ± .24	13.87 ± 3.82
VII	47.62	71.43	2.14 ± .20	14.15 ± 3.56
VIII	47.62	61.91	2.05 ± .22	14.04 ± 3.72
IX	61.91	61.91	1.91 ± .18	16.72 ± 4.14
X	57.14	76.19	2.29 ± .21	18.35 ± 4.15
XI	47.62	66.67	2.05 ± .20	13.95 ± 4.13
Average	51.52 ± 1.78	68.83 ± 1.86	2.19 ± .21	15.46 ± 3.70

[1]) Criterion 1: the frequency of the most common allele is $\leq .95$;
Criterion 2: the frequency of the most common allele is $\leq .99$.

phism are linearly dependent upon the elevation from which populations are sampled ($r = 0.659$; $P \leq 0\ 05$), there is no apparent clinal variation with elevation at the 1 percent criterion of polymorphism. The disagreement between the two criteria of polymorphism suggests that percentage of polymorphic loci is clearly an arbitrary definition and is not a good measure of genic variation. Nevertheless, interpopulation differences in percentage of polymorphic loci are not significant at both the 1 and 5 percent criteria of polymorphism, using the statistical procedure of GLICK (1935).

The average number of alleles per locus per population ranges from 1.91 in Population IX to 2.34 in Population VI. Interpopulation differences are not significant due to large standard errors *(Table 3)*. Therefore, we may conclude that an average population of Coastal Douglas-fir has 2.19 alleles per locus.

Mean percentage of heterozygous loci per individual, (H), in the eleven populations listed in *Table 3* ranges from 13.95 in Population XI to 18.35 in Population X. Visual inspection of the geographic variation pattern at the H values reveals that the mainland or high elevation populations tend to be genetically more heterogeneous. This departure of the variation pattern from a random one at H is in part due to a east-west cline ($r = -0.678$; $P \leq 0.05$) and an elevational trend ($r = 0.698$; $P \leq 0.05$). This is in variance to the conclusion that in general, heterozygosity decreased with increasing altitude for Coastal Douglas-fir (YANG et al., 1977). It is clear that although Population XI which is at the species margin seems to be genetically less heterogeneous, interpopulation differences in H are not statistically significant. Thus, the mean percentage of heterozygous loci per individual is about 15.5 in all eleven populations of Coastal Douglas-fir. Although we cannot discount the possibility that marginal populations in Coastal Douglas-fir experience less gene exchange or more severe selection limiting the number of genotypes than do central populations, the data at hand do not argue in favor of the hypothesis. The absence of a genic heterozygosity differential between marginal and central populations of Coastal Douglas-fir is consistent with observations on other natural populations of plants (TIGERSTEDT, 1973; GOTTLIEB, 1975; LEVIN, 1977) and animals (PRAKASH, 1973; AVISE et al., 1974; DESSAUER et al., 1975). Our estimate of H for Coastal Douglas-fir is comparable to that of 10 percent obtained for outbreeding organisms (NEI, 1975) but is considerably lower than the 33.8 percent previously reported for Coastal Douglas-fir (YANG et al., 1977). We feel our estimate is low not because of population differences or methodological reasons, but to the extent to which samples of isozymes are biased in the two studies. The average heterozygosity at each locus observed by YANG et al. (1977) was either zero or about 60 percent. Whereas, we observe a continuum of values from a low of zero at the AAT-1, PEP-2 and PEP-3 locus to a high of 60 percent at the EST -1 locus, although there is a gap between MDH-1 (28.53%) and G6P (49.78%) *(Table 2)*.

Several generalizations emerge from the data in *Tables 2* and 3. First, in every locus expect EST-1 and G9P, there is a most common allele (allele 100), not only with respect to its frequency in the populations, but also with respect to how many populations contain it. This similarity in the allelic composition at most loci over the main contiguous distribution of the species has also been found in *Limulus* (SELANDER et al., 1970), *Drosophila* (RICHMOND, 1972; AYALA et al., 1974), *E. Coli* (MILKMAN, 1973) and Coastal Douglas-fir (YANG et al., 1977), and argues strongly for the effect of gene flow or balancing selection as a basis for the maintenance of isozyme polymorphisms in natural populations. There is no general agreement about the extent of effective gene flow in conifers. Although the majority of pollen dispersal from a point source occurs over a short distance (LIBBY et al., 1969), clouds of pollen have been reported to travel remarkable distance (KOSKI, 1970). Apparently, large amounts of Douglas-fir pollen can be dispersed widely from a pollen source in large continuous stands (SILEN, 1962; EBELL and SCHMIDT, 1964); hence, the potential for gene flow through a series of subpopulations exists.

Second, there is an enormous amount of inter-locus variation in heterozygosity within population samples. Certain isozymes such as those from categories 1 to 4 are universally polymorphic in every population, but to varying degrees. Others, such as AAT-1, PEP-3, are completely monomorphic in the population samples. Still others, such as those from category 5, tend to be largely monomorphic in the population samples. Thus, the isozymes surveyed do not seem to be equivalent in their contribution to the overall mean heterozygosity of the populations. This suggests that many isozyme loci should be surveyed to reliably estimate genetic variation patterns in Coastal Douglas-fir. This high degree of inter-locus variation can be engendered by the difference in mutation rate or natural selection among loci, and is theoretically expected if each locus undergoes gene substitution independently at a low rate. More precise information concerning mutation rates and how environments influence isozyme functions is needed to distinguish among the different hypotheses.

A third aspect of the data suggests that the species is highly heterozygous, the mean for the genome as a whole over all populations being 15.5 percent. If we consider that about 25 to 30 percent of codon differences are detectable by electrophoresis (KING and WILSON, 1975) and make the correction for this factor, an individual is expected to be heterozygous for about 46.5 to 62 percent of its total genes. This rich gene pool within local populations is not surprising considering that Coastal Douglas-fir is exceedingly variable in morphology, both across its native range and from tree to tree within stands.

It is possible that the optimum evolutionary strategy for a pioneer species such as Coastal Douglas-fir is to sacrifice specialization to the most frequently encountered sets of conditions for general adaptability to a variety of environments (LEVINS, 1968; REHFELDT and LESTER, 1969). Thus, genetic variation is required for Coastal Douglas-fir to exploit the varied opportunities throughout its wide range of climates and site conditions, and heterozygosity within populations should be substantial. VAN VALEN (1965) has hypothesized that variation within a population is positively related to the niche width of that population; i e., it is adaptive. SLOBODKIN (1968) has pointed out that population response to environmental heterogeneity depends upon the length of cycles of environmental variation relative to generation time. If this rich gene pool of local populations in Coastal Douglas-fir has an adaptive component, it is most likely in response to microsite differences, which are

often critical for the survival of young seedlings (NAMKOONG et al., 1972; MITTON et al., 1977).

The data further suggests that heterozygosities and frequencies of alleles at loci in categories 1 to 4 fluctuate sometimes over wide limits in non-random fashion (*Table 2*). Thus, attempts were made to correlate heterozygosity and allelic frequency patterns with geographic variables for these loci. The results are striking. Eight out of thirteen or 62 percent of the loci display significant correlations with geographic variables (*Tables 4* and *5*). Neutral alleles with migration and hybridization of certain genotypes could produce the observed patterns, but for many loci chosen at random such correlations should not predominate. The thirteen loci investigated cannot be considered a random sample of loci, but certainly there was a random element in the way they were selected. While these correlations do not prove that the eight isozymes are selectively important, they are suggestive. Whether these loci are markers of adaptive gene complexes (ALLARD et al., 1972) and/or are locally adapted to the environment and respond to selection, is unknown. More evidence is required. From the patterns so far found, predictions as to what genotypes should be found in other areas of the distribution of Coastal Douglas-fir can be made. This is of practical importance in the certification of seed sources for reforestation purposes.

Table 4. — Linear correlations[1]) between heterozygosities and scalars (latitude, longitude and elevation) of polymorphic loci[2]) in natural populations of Coastal Douglas fir *Pseudotsuga menziesii* (MIRB.) FRANCO from British Columbia.

	Scalars		
Locus	Latitude	Longitude	Elevation
AAT-2	+0.383	+0.513	-0.467
ACO-1	-0.175	-0.613*	+0.469
ALD-1	-0.325	-0.856**	+0.639*
EST-1	+0.409	-0.425	+0.282
G6P	+0.258	+0.174	-0.204
IDH	-0.537	-0.301	+0.008
MDH-3	-0.767**	-0.074	-0.164
MDH-2	+0.024	+0.661*	-0.490
MDH-1	+0.598	-0.173	+0.131
ME-2	-0.060	-0.731*	+0.772**
PGI-2	-0.034	-0.455	+0.622*
PGM-1	-0.617*	-0.487	+0.581
6PG-1	+0.044	-0.595	+0.801**

[1]) * $P \leq 0.05$
** $P \leq 0.01$
[2]) The average frequency of the most common allele is ≤ 0.99

Table 5. — Linear correlations[1]) between allele 100 and scalars (latitude, longitude and elevation) of polymorphic loci[2]) in natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (MIRB.) FRANCO from British Columbia.

	Scalars		
Locus	Latitude	Longitude	Elevation
AAT-2	-0.502	-0.498	+0.467
ACO-1	+0.137	+0.657*	-0.453
ALD-1	+0.316	+0.834**	-0.642*
EST-1	-0.458	+0.489	-0.311
G6P	+0.069	+0.358	-0.213
IDH	+0.308	+0.290	-0.033
MDH-3	+0.754**	+0.043	+0.175
MDH-2	+0.010	-0.670*	+0.480
MDH-1	-0.572	+0.277	-0.138
ME-2	+0.075	+0.673*	-0.785**
PGI-2	+0.025	+0.372	-0.629*
PGM-1	+0.608*	+0.446	-0.595
6PG-1	-0.027	+0.553	-0.818**

[1]) * $P \leq 0.05$
** $P \leq 0.01$
[2]) The average frequency of the most common allele is ≤ 0.99

Genetic Differentiation Between Coastal Douglas-fir

Natural populations of Coastal Douglas-fir that are geographically separated tend to accumulate different genes due to the genetic processes of mutation, recombination, isolation, selection and random drift. The combined effects of all genetic processes lead to the development of variation patterns and population subdivisions. The extent of subdivision of the species can be investigated by partitioning the gene diversity (as measured by heterozygosities) of the total population (H_T) into its components, i.e., the gene diversity within (H_S) and between (D_{ST}) populations (NEI, 1973) The relative measure of genetic differentiation between populations (G_{ST}) is defined by

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

and its sampling variance (V $[G_{ST}]$) can be used to study the significance of the effect of population subdivisions (CHAKRABORTY, 1974).

The analysis of gene diversity in Coastal Douglas-fir *(Table 6)* is given for each locus separately and combined over all loci. Both H_T and H_S vary considerably with locus. The values of G_{ST} also vary, ranging between 0.000 and 0.051 for the 21 loci investigated. The mean value of G_{ST} is 0.0259, so that about three percent of genic variation in the total population is due to interpopulational gene differences. The small value of G_{ST} in Coastal Douglas-fir is, however, due to the large value of H_S in this species. Nevertheless, we get a standard error of 0.003 for G_{ST} given by the square of the expression in (V$[G_{ST}]$) which shows a significant effect of subdivision in the present sample of Coastal Douglas-fir.

The apportionment of total gene diversity in Coastal Douglas-fir is similar to that in man (NEI and ROYCHOUDHURY, 1972) and the horseshoe crab (SELANDER et al., 1970) where over 90 percent of the total gene diversity resided within local populations. We have also extended the analysis of gene diversity to the findings of YANG et al. (1977) on isozyme variation in Coastal Douglas-fir. The results are strikingly similar. The mean value of G_{ST} calculated over seven loci is 0.026, with individual values of G_{ST} between a high of 0.083 at the glutamate oxaloacetate transaminase -B locus to lows of 0.000 at the monomorphic loci. That the majority of genic variation in Coastal Douglas-fir is maintained within populations is, perhaps, a reflection of its ecological amplitude (BRADSHAW, 1972), its breeding system, and the lack of effective barriers to gene flow between sub-populations.

The relative amounts of genic variation between individuals within populations and between populations should influence selection methods in an applied tree improvement program. For electrophoretically detectable genic variation, there was little variation between populations. This is con-

Table 6. — Analysis of gene diversity and degree of differentiation at 21 loci among natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco from British Columbia[1])

Locus	Total gene diversity (H_T)	Gene diversity within populations (H_S)	Proportion of inter-population gene differentation (G_{ST})
AAT-2	.2903	.2844	.0203
AAT-1	.0000	.0000	.0000
ACO-1	.1173	.1131	.0354
ALD-1	.0690	.0681	.0124
DIA-2	.0103	.0101	.0164
EST-1	.6422	.6226	.0305
GDH	.0045	.0045	.0042
G6P	.5157	.4978	.0347
IDH	.2689	.2643	.0171
MDH-4	.0063	.0061	.0319
MDH-3	.1668	.1641	.0162
MDH-2	.1206	.1144	.0514
MDH-1	.2925	.2853	.0246
ME-2	.2189	.2134	.0251
ME-1	.0152	.0149	.0002
PEP-3	.0000	.0000	.0000
PEP-2	.0000	.0000	.0000
PGI-2	.0955	.0921	.0359
PGM-1	.2282	.2262	.0087
SOD	.0476	.0466	.0203
6PG-1	.2230	.2176	.0240
Combining all 21 loci	.1587	.1546	.0259
Standard Error	.0382	.0370	.0025

[1]) The tabulated gene diversity measures are calculated following the conventions used by Nei (1973) and Chakraborty (1974).

Table 7. — Pair wise geographic distance[1]) (above the diagonal) and genetic distance[2]) (below the diagonal) measures between natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco from British Columbia.[1])

Populations	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I		33.88	162.35	110.12	172.24	94.59	159.53	173.65	201.88	194.82	367.06
II	0.0007		141.18	88.94	154.41	62.12	146.82	156.71	169.41	160.94	341.65
III	0.0082	0.0056		56.47	35.29	138.35	262.59	262.59	127.06	132.71	400.94
IV	0.0048	0.0050	0.0050		57.88	83.29	207.53	208.94	114.35	114.35	360.00
V	0.0037	0.0033	0.0026	0.0018		125.65	248.47	244.24	93.18	98.82	369.88
VI	0.0050	0.0027	0.0017	0.0041	0.0035		124.24	124.24	117.18	105.88	290.82
VII	0.0017	0.0015	0.0076	0.0038	0.0019	0.0061		26.82	211.77	197.65	223.06
VIII	0.0018	0.0012	0.0040	0.0034	0.0022	0.0029	0.0021		197.65	183.53	200.47
IX	0.0071	0.0060	0.0028	0.0013	0.0041	0.0022	0.0066	0.0042		14.12	285.18
X	0.0078	0.0065	0.0012	0.0018	0.0024	0.0023	0.0067	0.0042	0.0002		273.88
XI	0.0037	0.0040	0.0070	0.0019	0.0040	0.0055	0.0025	0.0023	0.0036	0.0052	

[1]) Geographic distance measures between populations are expressed by the straight-line distances (km) between them.

[2]) Tabulated genetic distance measures (D) are calculated using the relationship $D = -\log_e I$. I is the measure of genetic identity of Nei (1972) calculated using the data for the 21 loci described in this manuscript.

sistent with the belief of many tree breeders that an intra-population selection scheme should be effective for Coastal Douglas-fir in British Columbia.

To investigate the causative factor(s) and to further quantify the genetic differentiation between Coastal Douglas-fir populations, we have computed the genetic distance statistics proposed by Nei (1972). This technique measures the accumulated number of gene differences per locus between populations and expresses them in a single index. If population differentiation is largely the result of isolation by distance, then geographic (spatial) distance and genetic distance are expected to be positively correlated. The pair-wise comparisons for geographic distance and genetic distance between Coastal Douglis-firs are presented in *Table 7*.. The genetic distance estimates between populations are small, ranging from a low of 0.0002 between pop-

ulations IX and X to a high of 0.0082 between populations I and III. The mean genetic distance over all pairs of Coastal Douglas-fir is 0.0037. The magnitude of genetic distance between Coastal Douglas-fir populations compares favorably with values obtained between populations within species in conifers (LUNDKVIST and RUDIN, 1977; YANG et al., 1977).

Generally, lower values of genetic distance are found between populations of Coastal Douglas-fir located in the same reforestation seed zone (e.g., populations I, II, VII and VIII in seed zone 1020; populations IX and X in seed zone 1070). A product-moment correlation between the genetic distance of populations and the geographic distance between them yield a significant value, $r = 0.3116$ ($P \leq 0.05$). Thus as geographic distance between population increases, genetic similarity decreases. This suggests that isolation by distance, may be an important factor in population differentiation of Coastal Douglas-fir.

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