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Summary

An intergraded population of Abies is reported in central Idaho between Abies grandis and Abies *concolor*. Its presence probably reflects a history of introgression and subsequent back crossing to parental grand fir. Analysis of 1100 trees for bark periderm color and decay by increment boring and 56 trees for foliar characteristics indicates an intermediate population. Periderm color tends to vary independently from foliar traits. Trees with yellow periderm have less incidence of decay than those with reddish periderm. The most common fungus isolated from decayed wood was Echinodontium tinctorium E. and E.

Key words: Abies grandis, Abies *concolor*, Echinodontium tinctorium, hybridization.

Zusammenfassung

Es wird über eine Hybridpopulation zwischen Abies grandis und Abies concolor in Zentral-Idaho berichtet. Ihre Existenz ist vermutlich auf Introgression mit nachfolgender Rückkreuzung zurückzuführen. Die Untersuchung von 1100 Individuen auf Peridermfarbe sowie andere Rinden- und Blattmerkmale hin läßt den Schluß auf eine Zwischenpopulation zu. Peridermfarbe und Blattmerkmale korrelieren nicht. Bäume mit gelbem Periderm sind gegenüber Echinodontium tinctorium E.a.E. resistenter als solche mit rötlichem Periderm.

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Isoenzyme Variation of Coastal Douglas-fir I. A Study of Geographic Variation in Three Enzyme Systems[1]

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Introduction

Genetic polymorphism or individual variation in morphological, phenological, physiological, and biochemical characters in natural populations of Douglas-fir (Pseudotsuga menziesii (MIRB.) FRANCO) indicates fairly high heterozygosity in the gene pool of this species. By review of papers published by CHING et al. (1965) and others, STERN (1968) postulated the existence of genetic polymorphism of strobilus color as well as needle color in Douglas-fir. These characters were thought to be under the control of "complex loci" or "supergenes". The controlling mechanisms, however, could not be effectively explored and analyzed by traditional methods such as provenance trial, study of inheritable quantitative traits, investigation of rare recessive mutants, or cytogenetic studies.

Information on the distribution of allelic variation in natural populations can be obtained with the aid of electrophoretic analysis of isoenzymes. This is because enzymes are composed of polypeptides synthesized by the action of one or more structural genes. The electrophoretic variations of enzymes can be directly related to changes in gene structure or codon sequence and always follow Mendelian segregation in ideal populations. The objectives of this study were to reveal the existenee and patterns of polymorphism in three enzyme systems, leucine aminopeptidase (LAP), esterase (EST), and glutamate oxaloacetate transaminase (GOT), and to analyze the extent of genetic differentiation among Douglas-fir provenances in terms of changes in allele frequency.

Literature Review

The applicability of electrophoretic separation of enzymes and proteins to the study of geographic variation in forest trees has been evidenced by several reports. LEWIS and CECH (1969) found a high uniformity within a geographic area in acid phosphatase, leucine aminopeptidase, and peroxidase among trees of black cherry (Prunus serotina EHRH.). FERET and STAIRS (1971) indicated that, of the eight electrophoretic variants of peroxidase in *Ulmus* pumila L., three were seed-source specific and five exhibited varying frequencies in most seed sources.

HARE and SWITZER (1969) reported that the electrophoretic patterns of seed proteins from western sources of loblolly pine (Pinus taeda L.) are more similar to those of shortleaf pine (P. echinata MILL.) than are the eastern sources. Esterase zymograms of needles of clonal grafts representing 16

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mother trees of Scotch pine (*P. sylvestris* L.) from northern Sweden exhibited considerable variation among trees (RASMUSON and RUDIN 1971). Another test on genetic variation of esterases in needles from progeny of different types of crosses of Scotch pine from central Sweden revealed the presence of at least six independent two-allele systems (RUDIN and RASMUSON 1973). Further investigation of Scotch pine was undertaken by RUDIN *et al.* (1974) of allelic frequencies in three isozyme loci of three Swedish populations. These populations were also tested for homogeneity. Work by FERET (1974) on peroxidase and esterase of needles and macrogametophytes in *P. pungens* LAMB. indicated that significant differentiation among three small stands was observed in parent trees but not from progeny.

TIGERSTEDT (1973) analyzed two delimited populations of Norway spruce (*Picea abies* (L.) KARST.) in Finland with regard to three different enzyme systems, esterase, leucine-aminopeptidase, and acid phosphatase. Much genetic variability of allozymes was apparent even at the species margin.

In a study on geographical variation in esterase and leucine aminopeptidase of Norway spruce in Sweden and Finland, BERGMANN (1973 a and b) observed north-south clinal variations of allele frequencies. He found that genetic identity and genetic distance values showed increasing genetic differentiation with increasing distance between provenances. Marked genetic divergence between closely located Finnish and Swedish provenances was further noted, however, probably because of isolating barriers (BERGMANN 1974). The same author (1975), working with seeds of Norway spruce and Douglas-fir, identified a gene locus coding for acid phosphatase that showed a marked environmentally-dependent variation. The inheritance of leucine aminopeptidase and acid phosphatase isozymes in *Picea abies* has also been reported by LUNDKVIST (1974, 1975). MUHS (1974) found that the component of variance in frequencies of peroxidase isoenzyme bands was much higher *between* provenances of Douglas-fir than *within* provenances. Isoenzymes of 26 abnormal and 13 normal Douglas-fir trees were compared for seven enzymes by COPES (1975), and he found that many zymograms of abnormal trees were significantly different from those of normal trees.

Material and Methods

Cones were collected from at least ten dominant or co-dominant trees in each of nine provenances ranging from Vancouver Island to California (*Table 1*). Seeds from each tree were kept separate except those from the area of Rockport, Washington, which were bulked as a single commercial lot. A total of 107 seedlots was obtained for this study.

Seeds were germinated on vermiculite in plastic boxes at 86 F during the 8-hour photoperiod and 68 F in the dark. Each germinant with fully elongated cotyledons was homogenized in 1.3 ml cold Tris-HCl buffer, pH 7.4 (0.05 M Tris, 3 mM EDTA and 10 mM mercaptoethanol), and the homogenate was centrifuged at 18,000 rpm for 10 minutes at 0 C. The supernatant containing soluble proteins and enzymes was dialyzed against Tris-HCl buffer, pH 7.5 (0.05 M Tris and 1.0 mM mercaptoethanol), overnight at 0 C.

The technique used for isoenzyme separation was similar to that of DAVIS (1964), except for the addition of 0.5 percent electrostarch to the acrylamide gel to improve the resolution of LAP and GOT isoenzymes. The dialyzed extract of each seedling was loaded on one gel, and electrophoresis was conducted at 0—4 C with Tris-glycine, pH 8.3 (0.05 M Tris and 0.38 M glycine), as reservoir buffer solution. Five mA direct current was applied to each gel. The electrophoretic process was terminated when the bromophenol blue marker had migrated to about 0.6 cm from the bottom of the gels. The gels were then removed from the glass tubes and stained according to BREWBAKER *et al.* (1968) for LAP and EST and according to GOTTLIEB (1973) for GOT.

Migration distances of isoenzyme bands and bromophenol blue front were measured, and the ratios of migration distance of enzyme bands to those of the front (Rf values) were calculated. The Rf values represent the relative positions of various isoenzyme bands.

Statistical Analysis

The fit of the observed phenotypic frequencies to those predicted by the Hardy-Weinberg Law was subjected to the chi-square goodness-of-fit test. Differences in allelic frequencies and proportions of estimated heterozygotes among provenances were studied by the chi-square test of independence and the test for homogeneity of variance for binomial distributions, respectively (SNEDECOR and COCHRAN 1967). The relation between heterozygosity levels and latitude or altidude of locations of nine provenances was investigated by linear regression analysis. The genetic identity and genetic distance between provenances were measured by methods developed by NEI (1972).

Table 1. — Geographic locations of Douglas-fir provenances in isoenzyme study.

Region	Provenance	Latitude, north		Longitude, west		Elevation	Range in elevation	Location	Trees sampled
		Deg	Min	Deg	Min	Ft	Ft		No.
Vancouver Island, B. C.	A	50	15	125	43	900	800—1,00	White River Valley Big Tree Creek	13
Washington	C	49	29	121	32	1,750	1,500—2,000	Rockport area	—[1]
Oregon	D	45	10	122	19	3,600	3,400—3,800	Clackamas Tree Farm, Molalla	10
Oregon	E	44	30	123	12	1,900	1,800—2,000	McDonald Forest, Corvallis	10
Oregon	F	42	28	122	25	3,000	2,700—3,300	Butte Falls, Medford	20
California	G	41	56	122	47	2,693	1,700—3,800	Mt. Ashland	14
California	H	40	14	124	00	886	850—1,540	Humboldt Redwoods State Park	13
California	I	40	23	123	23	3,581	2,400—4,650	Forest Glen	13
California	J	37	08	122	11	568	100—830	Santa Cruz	14

[1] Bulked seedlot.

Results and Discussion

Assay of Zymogram

A total of four isoenzymes of LAP, five of EST, and five of GOT were observed in the nine provenances. The relative positions of the isoenzymes as represented by Rf values are shown in *Figures 1, 2, and 3.* Without exception, one deeply stained band appeared in each of the enzyme system studied: Rf 39 for LAP, Rf 21 for EST, and Rf 36 for GOT. Such consistent bands were called monomorphic. The others were called polymorphic bands because they showed individual variation in appearance or disappearance on the zymograms. SHAW (1965) has noted that if electrophoretic variants show alteration in one of the two bands, they likely are controlled by different genetic loci. Therefore, the three monomorphic bands probably are controlled by three monomorphic loci other than those controlling the adjacent polymorphic bands. The monomorphic loci, each with a single allele, were named LAP-B, EST-A, and GOT-C.

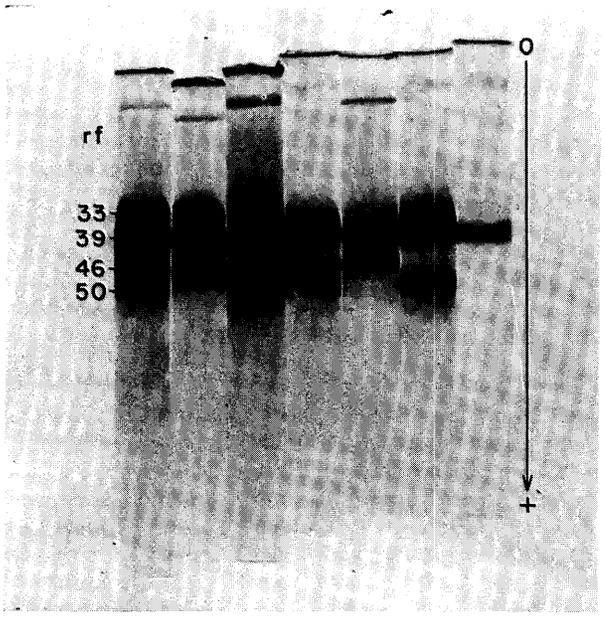

Figure 1. — Photographic zymogram of leucine aminopeptidase. The origin is indicated by 0. The arrow shows the direction of migration toward the anode.

Because meaningful analysis of electrophoretic variations of enzymes requires scoring of alleles at loci encoding particular enzymes rather than mere comparison of numbers and positions of bands on gels (WAGNER and SELANDER 1974), we attempted to assign the isoenzyme bands to the corresponding alleles by conducting a Hardy-Weinberg equilibrium analysis. That is, the allele system of every locus can be rationalized genetically by first postulating the genotype corresponding to each phenotype and then testing the hypothesis by comparing the observed frequencies with those predicted by a Hardy-Weinberg equilibrium in the population. As a result, four polymorphic loci, namely, LAP-C, EST-B, EST-C, and GOT-B, were sufficiently identified *(Figures 4, 5, and 6)* because the observed proportions of various phenotypes agree fairly well with the expected ones in most provenances. Some exceptions to significant x^2 values were also seen, however: provenance J for LAP-C; provenances A, C, and J for EST-B; provenances A, D, and J for EST-C; and provenances C and I for GOT-B. In spite of such deviation from equilibrium, the generally good correspondence between the expected and observed results indicated that the assumption of three-allele system at each of four polymorphic loci was correct. They are LAP-C^{46}, LAP-C^{50}, and LAP-C^{-} in the LAP-C locus; EST-B^{28}, EST-B^{34}, and EST-B^{-} in the EST-B locus; EST-C^{52}, EST-C^{64}, and EST-C^{-} in the EST-C locus; and GOT-B^{27}, GOT-B^{30}, and GOT-B^{-} in the GOT-B locus. LAP-C^{46} and LAP-C^{50} represent the two productive codominant alleles that are responsible for the appearance of the two bands, Rf's 46 and 50. LAP-C^{-} represents the null recessive allele that is responsible for the inactivation of LAP synthesis. The same phenomena also exist at EST-B, EST-C, and GOT-B loci. The phenotypes, their corresponding genotypes, and the frequencies of occurrence at each of four polymorphic loci are shown in *Table 2.*

Because of the faint stain and infrequent occurrence of LAP at Rf 33 and GOT at Rf 13 and 21, these bands can not be assigned to any loci. Probably a locus, designated as LAP-A *(Figure 4),* with two alleles — one null and one productive — can account for the banding variation at LAP Rf 33 zone. The most likely genotypes associated with the observed phenotypes are as follows: germinants exhibiting no band are homozygous for the recessive null allele; among the germinants exhibiting a single band are both heterozygotes and homozygotes for the dominant pro-

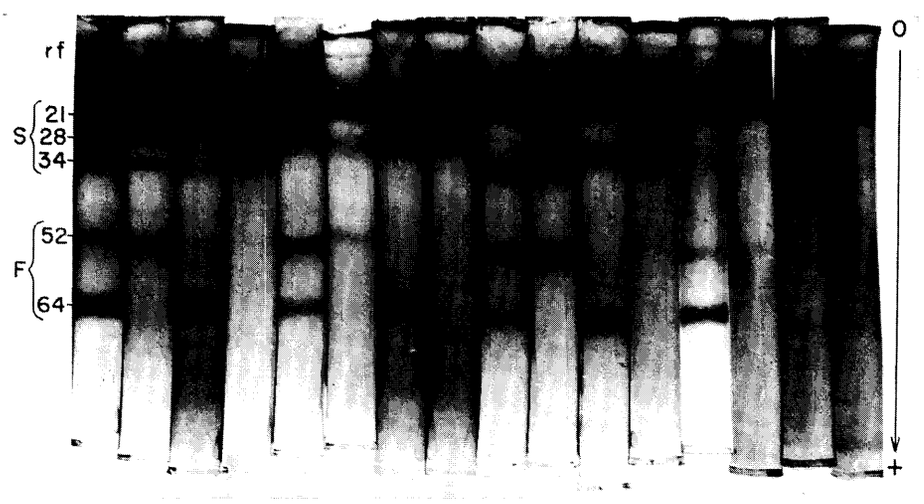

Figure 2. — Photographic zymogram of esterase. The orgin is indicated by 0. The arrow shows the direction of migration toward the anode.

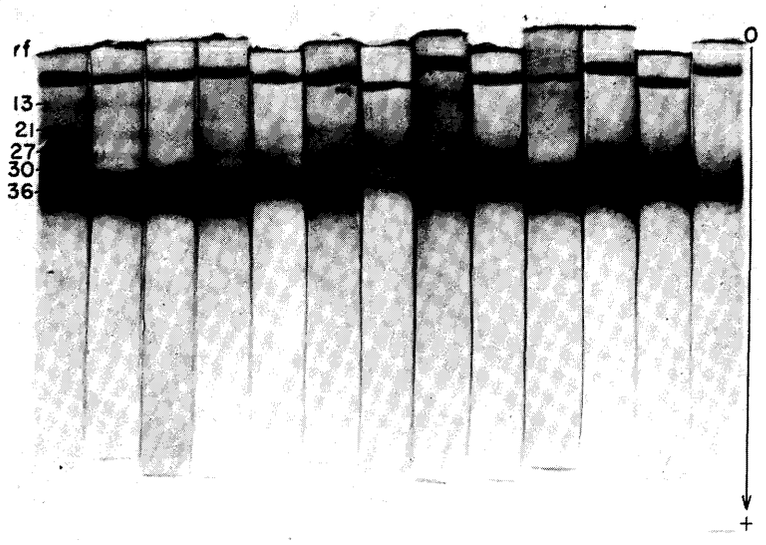

Figure 3. — Photographic zymogram of glutamate oxaloacetate transaminase. The origin is indicated by 0. The arrow shows the direction of migration toward the anode.

ductive allele. Because we can not distinguish visually between the homo- and heterozygotes on the gels, the proposed genotypes can not be verified effectively in a Hardy-Weinberg equilibrium analysis. Thus, for the purpose of conservatism, the allelic variability at this locus is not sufficiently reliable for the population study. Consequently, no attempt was made to evaluate critically the isoenzyme variation at LAP-A locus. Similarly, no allelic relation could be found between GOT Rf's 13 and 21 bands or between these two bands and others by chi-square analysis, so these bands also were excluded in the subsequent study on geographic variation of GOT isoenzymes. Such deviant bands probably arise as a result of allelic interactions, genic regulators, environmental alteration of enzyme expression, or extraction artifacts. All these have been discussed by FERET (1974) as the limiting factors for the broad use of isoenzyme variation in population studies.

Geographic Variation of Allele Frequencies and Genic Heterozygosity

Following the method described by FALCONER (1960), the frequencies of alleles *(Table 3)* were determined from the observed frequencies of phenotypes *(Table 2)* in a sample size of 78 to 136 germinants from nine provenances each under the Hardy-Weinberg assumption. Although the three estimated allele frequencies at each locus usually did not add up to one, they were close to one if large numbers of samples were analyzed. Adjusting the estimated frequencies so that they add up to one is desirable, and is done by a simple device described by LI (1955):

$p' = p(1 + 1/2\,d)$, $q' = q(1 + 1/2\,d)$, $r' = (r + 1/2\,d)(1 + 1/2\,d)$, where p, q, and r are original frequencies of two codominant and one recessive allele, respectively; p', q', and r' are adjusted frequencies; and $d = 1 - (p + q + r)$. Adjustment was usually made once, but sometimes a repeat was needed, using the new deviation $d' = 1 - (p' + q' + r')$. The results are given in *Table 3.*

Chi-square tests of independence of allele frequencies at each of four polymorphic loci mostly give highly significant chi-square values ($p < 0.005$), indicating that considerable differences in allele frequencies of four loci exist among as well as within natural populations of Douglas-fir. Different loci exhibit different geographic patterns of allelic variation, however. The predominance of some alleles over others, in frequency of occurrence, was observed at LAP-C and EST-B, but no predominant allele occurs at EST-C and GOT-B. At LAP-C locus, LAP-C^{46} predominates over LAP-C^{50} and LAP-C^{-} in five of the nine provenances examined, that is, provenances A, C, D, G, and I, which are either in northern latitudes or at high elevations. Similarly, EST-B^{28} predominates over the other two alleles, EST-B^{34} and EST-B^{-}, at EST-B locus in all provenances studies. It is most frequent from high elevations such as D, F, and I. LAP-C^{-}, the null recessive allele, occurs rarely in all the provenances, and the null EST-B^{-} allele has somewhat higher frequencies that equal those of EST-B^{34}, a productive codominant allele, in most provenances. At EST-C locus, two common alleles, EST-C^{52} and EST-C^{64}, have moderate

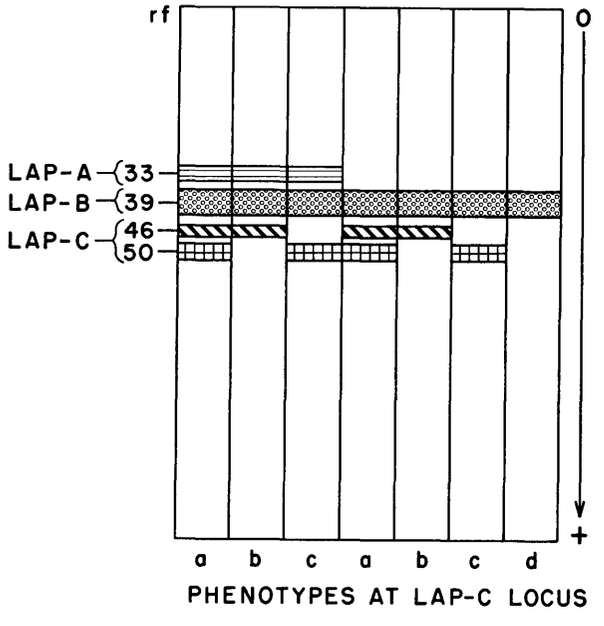

Figure 4. — Diagram of LAP zymogram. Phenotypes at LAP-C locus are indicated as: a (46 50), b (46 —), c (— 50), and d (— —).

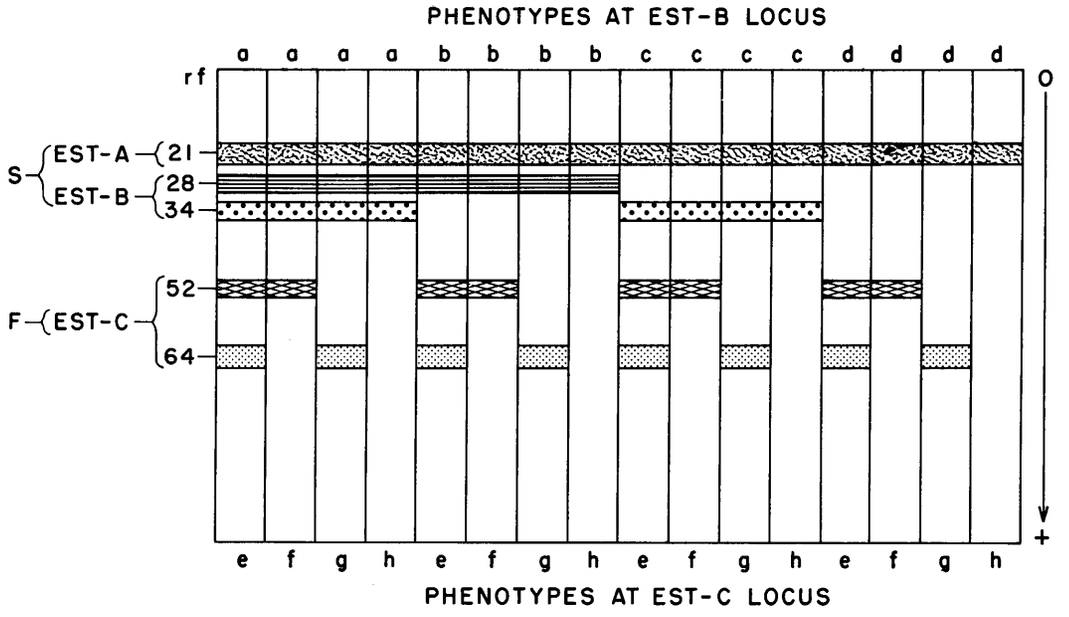

Figure 5. — Diagram of EST zymogram. Phenotypes at EST-B locus are indicated as: a (28 34), b (28 —), c (— 34), and d (— —). Phenotypes at EST-C locus are indicated as e (52 64), f (52 —), g (— 64), and h (— —).

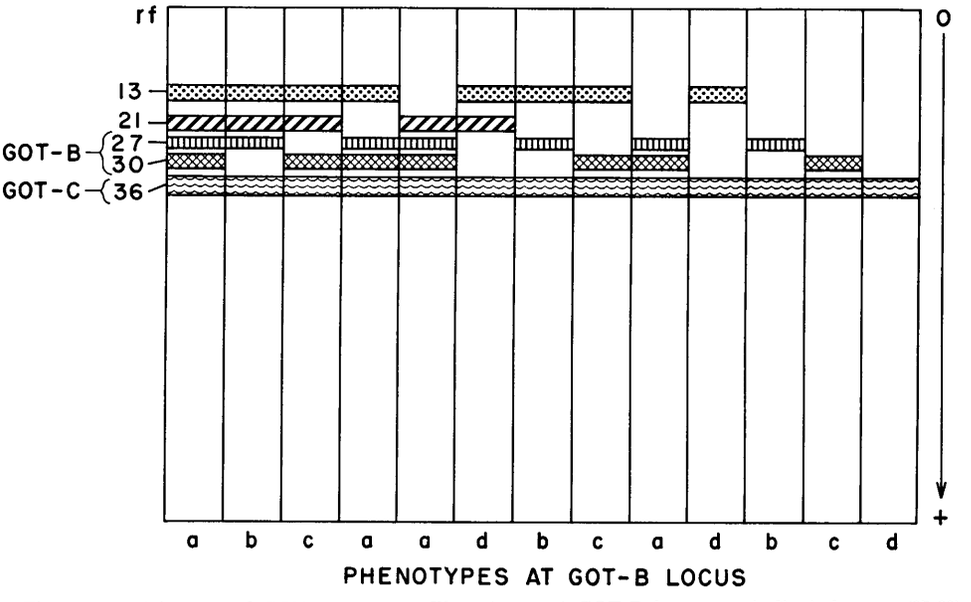

Figure 6. — Diagram of GOT zymogram. Phenotypes at GOT-B locus are indicated as: a (27 30), b (27 —), c (— 30), and d (— —).

and nearly equal frequencies, and the null allele, EST-C$^-$, has low frequency in most provenances except the extreme northern and southern populations, A and J, in which three alleles are more or less equal in frequency. The frequencies of GOT-B^{27} and GOT-B^{30} are moderately low to moderate in nine provenances examined. In provenances A, C, and J, the frequency of GOT-B$^-$ is slightly higher than that of either GOT-B^{27} or GOT-B^{30}. Thus, the null GOT-B$^-$ is not a rare allele like LAP-C$^-$ but a frequent one at GOT-B locus.

The geographic variations of genic heterozygosity at four polymorphic loci *(Table 3)* are more definite than allelic frequency patterns, and hence provide an appropriate index to the degree of genic variation in Douglas-fir provenances. Heterozygosity is defined here as the percentage of heterozygotes in a population. Because of the null alleles, LAP-C$^-$, EST-B$^-$, EST-C$^-$, and GOT-B$^-$, we could not score the proportion of heterozygotes directly from the zymograms on which the heterozygotes are represented by both two-band and parts of one-band phenotypes. Under these circumstances, heterozygosity was estimated from our data by taking the frequencies of all alleles at a locus in a population, and calculating the expected frequencies of heterozygotes from the Hardy-Weinberg proportions for each provenance separately, $H(\%) = 2\,(p'q' + p'r' + q'r')$.

The heterogeneity in proportions of heterozygotes found in different Douglas-fir provenances was also examined by using the chi-square test. As shown in *Table 3*, the geographic differences among provenances are not statistically significant at LAP-C, EST-C, and GOT-B, but they are at EST-B. The geographic patterns of heterozygote variations at four loci are different from one another. A more or less clinal increase in proportions of heterozygotes from the north to the south was observed at LAP-C locus. TIGER-

Table 2. — Phenotypes, genotypes, and their observed frequencies (%) at four polymorphic loci.

Provenance	Polymorphic loci: LAP-C				EST-B			EST-C				GOT-B			
	46 — LAP-C^{46} — LAP-C^{46} & LAP-C^{46} — LAP-C —	46 50 — LAP-C^{46} — LAP-C^{50} & LAP-C — LAP-C^{50}	— — LAP-C — LAP-C^{50} & LAP-C^{50} — LAP-C^{50}	28 — EST-B^{38} — EST-B^{38} & EST-B^{38} — EST-B —	28 34 EST-B^{38} — EST-B^{34} & EST-B^{34} — EST-B —	— 34 EST-B^{31} — EST-B^{34} & EST-B — EST-B^{34}	— — EST-B — EST-B — EST-B —	52 — EST-C^{52} — EST-C^{52} & EST-C^{52} — EST-C —	52 64 EST-C^{52} — EST-C^{44} & EST-C^{44} — EST-C^{44}	— — EST-C — EST-C — EST-C —	27 — GOT-B^{27} — GOT-B^{27} & GOT-B^{27} — GOT-B —	27 30 GOT-B^{27} — GOT-B^{30} & GOT-B^{30} — GOT-B^{30}	— 30 GOT-B^{30} — GOT-B — GOT-B^{30}	— — GOT-B — GOT-B — GOT-B —
A	43.52	43.52	12.04	0.92	55.88	8.82	33.83	1.47	18.38	38.97	25.74	16.91	41.67	17.59	29.63	11.11
C	31.03	41.38	27.59	0	64.76	14.29	19.05	1.90	28.57	42.86	24.76	3.81	19.54	29.89	32.18	18.39
D	31.25	38.54	30.21	0	66.32	16.81	14.74	2.10	23.16	46.32	25.26	5.26	31.25	40.63	21.87	6.25
E	24.14	35.63	40.23	0	66.15	11.54	12.31	10.00	26.93	36.92	28.46	7.69	26.14	37.50	30.68	5.68
F	27.18	33.98	38.84	0	72.65	17.95	7.69	1.71	30.77	36.75	25.64	6.84	18.10	39.05	37.14	5.71
G	43.59	29.49	26.92	0	47.67	26.75	24.42	1.16	27.91	45.35	23.25	3.49	20.51	34.61	41.03	3.85
H	29.79	29.79	40.42	0	62.74	17.65	17.65	1.96	23.53	45.10	27.45	3.92	26.60	42.55	25.55	5.32
I	53.00	30.00	17.00	0	77.88	17.31	4.81	0	25.96	48.08	24.04	1.92	26.00	47.00	22.00	5.00
J	28.57	17.35	53.06	1.02	61.07	6.11	32.06	0.76	25.95	39.70	18.32	16.03	19.39	21.43	44.90	14.28

STEDT (1973) also found, in LAP, lower heterozygosity and higher homozygosity at the species margin. The percentage of heterozygotes (H) was regressed on latitude (L), $H = 0.8082 - 0.5795L$, with a correlation coefficient of —0.686 ($p < 0.05$). In contrast to the LAP-C locus, heterozygosity at EST-B did not demonstrate a macrogeographically clinal trend, but showed some relation to elevation. A linear regression analysis revealed a statistically significant ($p < 0.05$) negative relation between percentage of heterozygotes and the elevation of the provenances; $H = 0.6814 - 0.0064E$, with $r = -0.710$ and $F = 7.11$ ($p < 0.05$). About half of the interprovenance variation in heterozygosity may be accounted for by linear regression on elevation. Similarly, we found slightly lowered heterozygosity in populations from high elevation at the GOT-B locus. The correlation between percentage of heterozygotes and elevation of provenances is $r = -0.664$, which is close to the significant level at 5 percent ($r_{0.05} = 0.666$). For EST-C, no clinal changes or relation to elevation appears in heterozygosity.

The nonsignificant chi-square values at LAP-C, EST-C, and GOT-B indicated some geographic uniformity in genic heterozygosity. Similar uniformity was found in studies on several protein or enzyme polymorphisms over the distributional range of certain rodents, fishes, and *Drosophila* (SELANDER *et al.* 1969; PRAKASH et al. 1969; AVISE and SMITH 1974; WAGNER and SELANDER 1974). The cause of this uniform pattern has been described mostly as effects of migration or gene flow through a series of subpopulations in many generations, and these patterns are then maintained by some form of directional or balancing selection. This explanation seems also to apply to variation in heterozygosity in Douglas-fir. Large amounts of Douglas-fir pollen can be dispersed widely from source points in a large continuous stand, particularly during a year of heavy production (SILEN 1962). Apparently, potential for gene flow exists through a series of subpopulations, and hence the neighborhood size or panmictic unit of Douglas-fir is large. Geographic uniformity in genic heterozygosity would be expected. One point should be clear, however: "uniform" does not mean "identical" pattern, because directional selection related to geographic gradients of environments would be responsible for subtle changes in degree of heterozygosity. For instance, the north-south cline of increase in heterozygote proportions suggests that natural selection with some latitude-associated environmental gradients, perhaps temperature or photoperiod, is responsible for the maintenance of genic variability at LAP-C locus.

An increase in elevation associated with significant decrease in heterozygotes at EST-B locus and slight decrease of GOT-B heterozygotes indicates a negative effect of some selective pressures imposed by edaphic or climatic factors on the maintenance of high heterozygosity in Douglas-fir. The drastic changes of edaphic and climatic factors, particularly soil type and summer moisture stress, in mountainous areas would result in lessening the panmictic unit and barring gene influx somewhat. As a consequence, the degree of heterozygosity in high-elevation provenances would be decreased.

In general, heterozygosity decreases with increasing altitude and, to a lesser extent, latitude. This trend is paralleled by general physiological functions of Douglas-fir observed by GRIFFIN (1974) who concluded, after reviewing several references, that growth decreases and tolerance to drought and cold increases as one proceeds from coast inland, and to a lesser extent from south to north — a trend paralleled by increasing severity of climate. Increasing heterozygosity in the gene pool of Douglas-fir might strengthen the capacity of trees to stimulate active development and growth. Increasing homozygosity could help attain more

Table 3. — Allele frequencies and estimated heterozygosity (H) at LAP-C, EST-B, EST-C and GOT-B loci in Douglas-fir provenances.

Provenance	LAP-C				EST-B					EST-C			GOT-B			
	46	50	— —	H (%)	28	34	— —	H (%)	52	64	— —	H (%)	27	30	— —	H (%)
A	0.618	0.322	0.060	51.1	0.456	0.272	0.272	64.4	0.320	0.374	0.306	66.4	0.367	0.278	0.355	66.2
C	.495	.461	.044	54.0	.581	.196	.223	57.5	.444	.412	.144	61.2	.275	.365	.360	66.1
D	.475	.466	.059	55.4	.618	.181	.201	54.5	.417	.434	.149	61.6	.445	.367	.188	63.2
E	.389	.542	.069	55.0	.535	.129	.336	58.4	.382	.394	.224	64.9	.383	.421	.196	63.8
F	.405	.515	.080	56.5	.707	.140	.153	45.7	.414	.372	.214	64.5	.329	.488	.183	62.0
G	.526	.372	.102	57.4	.519	.316	.165	60.4	.457	.416	.127	60.2	.325	.498	.177	61.5
H	.399	.497	.104	58.3	.588	.206	.206	57.0	.415	.450	.135	60.7	.421	.412	.167	62.5
I	.630	.292	.078	51.1	.821	.124	.055	30.7	.466	.449	.085	57.4	.446	.412	.142	61.1
J	.289	.499	.212	62.3	.490	.245	.265	63.0	.381	.324	.295	66.3	.227	.414	.359	64.8
χ^2		56.8		8.0[1]		67.4		78.4		37.5		7.5[1]		48.6		2.4[1]
P (16 d. f.)		<0.005				<0.005				<0.005				<0.005		
P (8 d. f.)				0.45				<0.005				0.49				0.96

[1] Not significant.

Table 4. — Coefficients of genetic identity and genetic distance between Douglas-fir provenances based on four polymorphic and three monomorphic loci of isoenzymes.

Provenance		Provenance								
		A	C	D	E	F	G	H	I	J
		Genetic Identity								
A			0.987	0.983	0.970	0.971	0.982	0.977	0.964	0.977
C	Genetic Distance	0.013		.993	.990	.990	.990	.992	.977	.986
D		.017	.007		.994	.994	.992	.998	.987	.978
E		.021	.010	.006		.992	.985	.995	.967	.987
F		.029	.010	.006	.008		.988	.995	.983	.985
G		.018	.010	.008	.015	.012		.992	.981	.979
H		.023	.008	.002	.005	.005	.008		.981	.983
I		.036	.023	.013	.033	.017	.019	.019		.947
J		.023	.014	.022	.013	.015	.021	.017	.053	

specific genetic adaptability for survival under severe climatic selection pressures. More research work will be needed to confirm these trends by statistical correlations between isoenzyme variation and plant growth.

Genetic Differentiation in Douglas-fir at the Enzymatic Level

Geographic variation of allele frequencies at the four enzymic loci, as described above, implies significant differentiation among Douglas-fir provenances. A simple and integrated feature of genetic differentiation among populations can be synthesized by constructing a matrix of coefficients of genetic identity and genetic distance (Nei 1972). These coefficients were calculated from allele frequencies of four polymorphic loci (LAP-C, EST-B, EST-C, and GOT-B) and three monomorphic ones (LAP-B, EST-A, and GOT-C). As given in *Table 4*, values for genetic identity between populations are high and, as a consequence, genetic distance is small. This is because the indices are complements of each other to unity. A similar finding also was noted in Swedish populations of Norway spruce by Bergmann (1973 a). In general, the shorter the geographic distance between two provenances from Vancouver Island to Oregon, the smaller the distance values or the larger similarity values, except those among California populations.

Results reveal that the pairs of provenances from Vancouver Island down to Oregon are obviously differentiated

parallel to latitude, which indicates that genes did not transfer over a great distance in a short time, but moved gradually through a series of subpopulations. The association with latitude may again reflect the important role of macrogeographic environmental gradients on modulating the genetic differences of Douglas-fir populations.

The irregular changes of values of genetic identity and genetic distance among Californian provenances may imply that the effect of migration has been obscured by other limiting factors, including external environmental variables and, to a lesser extent, internal genetic constitution. The most striking distinctions were evidenced between two provenances of almost the same latitude (H and I).

Provenance H, at the coast, is separated from inland provenance I by only 26 miles. But drastic changes in elevation and climate — particularly frost-free growing season and the mean January temperature — have created quite different sites. Differentiation between these two provenances is extensive and reflects the adaptation of various genotypes to different ecological conditions. Even though migration may exert some influence, strong differential climatic selection could reduce its effect. GRIFFIN (1974) showed significant differences in seed characteristics, early growth, and phenology between coastal and inland seedlings and concluded that complex microgeographic differentiation among Douglas-fir populations in northern California might have occurred largely because of vigorous differential selection pressures imposed by variable topography and climate. In general, local differentiation is more pronounced in California populations than in other geographic locations as evidenced by our isoenzyme data and GRIFFIN's study.

On the average, California provenances demonstrated more divergence from the Vancouver Island population than did Oregon ones because of higher values of genetic distance. With time, genetic differences would gradually accumulate in such a way that the greatest differences could be found between the most northern and most southern populations of Douglas-fir.

Summary

Genetic variability in natural populations of Douglas-fir was studied at the enzymatic level. By the techniques of disc gel electrophoresis and biochemical staining, the isoenzyme patterns of leucine aminopeptidase (LAP), esterase (EST), and glutamate oxaloacetate transaminase (GOT) were characterized in young seedlings at the stage when cotyledons were completely elongated. Samples of 107 seedlots were collected from nine provenances located at Vancouver Island, Washington, Oregon, and California.

Allelic frequency differences are statistically significant among the provenances examined, but appear to be rather complex. By contrast, variation in genic heterozygosity exhibited some geographic uniformity or clinal changes. The combined effect of gene flow and some form of balancing or directional selection was considered as the probable mechanism that maintains uniformity or clinal variation of allelic heterozygosity.

The decreased proportions of heterozygotes at EST-B and GOT-B loci were considered to be associated with negative effects of some selective pressures imposed by edaphic or climatic factors, such as soil type and moisture stress in the summer.

Genetic differentiation in terms of genetic identity and genetic distance between provenances of Douglas-fir is more or less parallel to the geographic distance.

Key words: Pseudotsuga menziesii, geographic variation, isoenzyme studies, leucine aminopeptidase, esterase, glutamate oxaloacetate transaminase, allele frequencies, genic, heterozygosity.

Zusammenfassung

Mit Hilfe der Disc-Gelelektrophorese und biochemischer Färbung wurde an Sämlingen aus 9 Douglasien-Provenienzen von 107 Standorten auf Vancouver Island, in Washington, Oregon und Kalifornien die genetische Variabilität untersucht. Dabei wurde die Isoenzymverteilung von Leucin-Aminopeptidase (LAP), Esterase (EST) und Glutamat-Oxaloacetat-Transaminase (GOT) im Zeitpunkt der völligen Elongation der Kotyledonen charakterisiert.

Die allelen Frequenzen scheinen ziemlich komplex zu sein, zeigen jedoch statistisch signifikante Differenzen zwischen den untersuchten Provenienzen. Im Gegensatz dazu zeigt die Variation der Gen-Heterozygosität eine gewisse geographische Einheitlichkeit oder allenfalls klinale Wechsel. Der kombinierte Effekt von Genfluss und einer gewissen Form ausgleichender oder gelenkter Selektion wird als der wahrscheinliche Mechanismus für die Aufrechterhaltung der Einheitlichkeit oder des klinalen Wechsels der allelen Heterozygosität angesehen.

Die verringerten Anteile von Heterozygoten an EST-B und GOT-B Loci werden mit den negativen Auswirkungen gewisser Selektionsdrucke, herbeigeführt durch edaphische oder klimatische Faktoren, wie zum Beispiel Bodentyp und Trockenheitsbelastung im Sommer, in Verbindung gebracht.

Die genetische Differenzierung, ausgedrückt als genetische Identität und genetische Entfernung zwischen Provenienzen von Douglasien, ist mehr oder weniger identisch mit der geographischen Entfernung.

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Genotype x Environment Interaction and Genotpic Stability in loblolly pine

I. General introduction and description of the experiment*

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General introduction and description of the experiment

Genotype × environment interactions — the differential response of genotypes to varied environments, when present, introduce biases in genetic and environmental improvement predictions. There are two ways by which a breeder can circumvent the undesirable consequences of such biases: (1) by grouping the genotypes according to their regions of optimal adaptation and (2) by changing the testing design to minimize such interaction effects through manipulation of plot size, plot shape, number of replications and number of test environments. Tree breeders favor selection and breeding for wide adaptation partly because of the great variation in site classes for which they breed and partly to avoid working with narrowly adapted populations with restricted genetic bases that might prove vulnerable to pests and adverse future environments. Also, there are practical limitations as to how far testing designs can be manipulated to reduce the impact of genotype × environment interaction in tree breeding; the long duration of the tests and the high hazards of the forest environment often determine the lower limit of the number of replications and the number of trees per plot. Furthermore, difficulties of obtaining seed of all the families for testing at any one time is a well known constraint on the number of test evironments possible.

While selecting and testing genotypes for broad adaptability, it is important to obtain estimates of the amount of bias in genetic gain prediction due to genotype × environment interaction effects. Of particular importance in the widely adopted population improvement breeding strategies is the ratio of additive genetic variance × environment interaction to additive genetic variance. To date, tree breeding experiments reported in the literature have not been big enough and well designed to provide the type of information referred to here because of (a) insufficient specification of genotypes studied, (b) insufficient numbers of genotypes studied or (c) insufficient numbers of test environments studied.

The experiment reported here was designed to provide information on the relative size and importance of genotype × environment interaction and genotypic stability in a genetic improvement program with loblolly pine. The experiment had its origin in the controlled crosses made by Woessner in 1964 and 1965 to explore the possibilities of heterotic combinations from wide crosses. The original experimental objective of value assessment for wide crosses was included in the reported investigation for completeness. The investigation was designed to answer the following questions:

1. Do open-pollinated progeny, local single crosses, and wide crosses differ in genotypic stability?
2. What is the most suitable genotypic stability parameter for use in loblolly pine family comparisons?
3. Do wide crosses show heterosis and, if so, how consistent is the heterosis over several environments?
4. To what extent can the genotype × environments interaction observed reduce the repeatability of family means over environments?
5. To what extent can genotype × environment interaction bias estimates of components of genetic variance and consequently genetic gain predictions?
6. Is there evidence of genetic differentiation within the loblolly pine range which could be utilized to broaden the gene base of breeding populations?

* This work was carried out in partial fulfilment of the requirements for the Ph. D. degree at North Carolina State University, 1975.

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