Distinction of Douglas-fir Provenances Using Peroxidase-Isoenzyme-Patterns of Needles*)

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Introduction

Douglas-fir is a species which does not only show great variation of the morphological and physiological characters between provenances of its natural range but also within a small region of origin. Up to now the classification and characterization of provenances has been done using only morphological and physiological characters (ALLEN, 1960, 1961; TUSKO, 1963; SZIKLAI 1969; YAO, 1971). Additionally other characters has been investigated: Terpene content (HANOVER and FURNISS, 1966; RUDLOFF, 1972) and DNA content (EL-LAKANY and SZIKLAI, 1971, 1972). All methods showed differences between the provenances.

In this investigations peroxidase isoenzymes are used as characters. Isoenzymes are strictly genetically determined and therefore they particularly suited for characterization of provenances. Needles were used as investigation material because needles are available for each age of the tree. Even by repeated removal of needles the tree is neither influenced in its viability nor destroyed. In detail the following problems are investigated: 1.) The development of a method to identify provenances with the aid of the isoenzyme technique, 2.) the development of a statistical method to test the distinction of provenances, 3.) a first estimation of the variability of the iso-peroxidase bands between and within provenances.

Material and Method

Provenances: 14 provenances of the IUFRO-collection (1966167) were studied (table 1). These provenances can be parted in three groups. Group (1) consists of four provenances originating from British Columbia from approximately the same geographical latitude of about 50° N. Among them the provenance with the IUFRO no. 1028 stems from the region east of the Fraser river, no. 1027 from the Canadian coastal mountains, no. 1030 from the coastal region, and no. 1032 from the eastern coast of Vancouver Island.

Group (2) includes 7 provenances from British Columbia and Washington also of the same geographical latitude (about 48° 40' N). 3 provenances, no. 1041, 1042 and 1043, were collected in the southern part of Vancouver Island, provenance no. 1051 from a region near the coast, no. 1047 and 1049 from the Cascades and no. 1048 from the Upland east of the Cascades. The provenances 1048 and 1028 belong to the Interior variety according to the classification of SZIKLAI (1969).

Group (3) consists of 3 provenances. Two of them originated from the South of Washington, no. 1075 from the Cascades and no. 1078 from the same geographical latitude but east of the Cascades. Provenance no. 1102 is from the Cascades as well but from Oregon.

Seedlings *and* sampling: Seedlings of all provenances were grown in the same greenhouse. They stood in boxes and had not yet been transplanted. Only seedlings which were at least 9 to 10 cm high were selected. All seedlings belonged to a provenance trial and any damaged seedlings were avoided.

96 two years old seedlings of each provenance were investigated. The samples (6 X 16) were taken at random.

Isoenzyme technique: About 10 needles of each seedling were homogenised in a mortar in some drops of gel buffer and some Polyclar AT and after that immediately absorbed through a single layer of Kleenex with a small piece of chromatography paper (Whatman no. 3). The extracts were directly brought into the gel. In no case were

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Table 1. — Douglas Fir Provenances Collected 196167 by the I.U.F.R.O.

NO.	County	Name	Latitude N.	Longitude W	Altitude feet
Canada-Br. Columbia					
1027		Alta	50° 11' 30''	122° 52' 30''	2100
1028		Merritt	50° 04' 20''	120° 51' 00''	2700—3000
1030		Squamish	49° 46' 40''	123° 09' 00''	50
1032		Courtenay	49° 41' 45''	125° 03' 30''	220
1041		Caycuse	48° 55' 25''	124° 26' 00''	700
1042		Duncan	48° 45' 00''	123° 45' 00''	200
1043		San Juan	48° 34' 50''	124° 04' 48''	700
U.S.A. Washington (W) — Oregon *(O)*.					
1047	W Whatcom	Concrete	48° 39'	121° 07	1300—1800
1048	W Ferry	Republic	48° 36'	118° 44'	2400
1049	W Skagit	Bacon Point	48° 36'	121° 23'	1500—1800
1051	W Skagit	Sedro Woolley	48° 32'	122° 19'	200
1075	W King	Enumclaw	47° 16'	121° 56'	800
1078	W Kittitas	Cle Elum	47° 13'	121° 07'	2100
1102	O Linn	Upper Soda	44° 23'	122° 12'	3000—3500

the extracts frozen. This excluded any possible influence of the freeze on the isoenzyme pattern.

The procedure of micro starch gel electrophoresis was already described: micro-method on slides according to MUHS (1974) with 10% (w/v) starch and the buffer system according to POULIK (1957). The gels were sliced horizontally and laid in petri dishes. The staining solution per petri dish consisted of 15 ml 1% H_2O_2 in distilled water and 15 ml of 2% (w/v) benzidine solved in 20% acetic acid in distilled water.

The frequency of a character (single isoenzyme band) of each provenance was ascertained.

Statistical methods: The data were analysed according to three methods: (1) χ^2test, (2) measure of distinctiveness, and (3) analysis of variance and intraclass correlation.

(1) The χ^2-test is applied as heterogeneity or homogeneity test. In the test all characters and all provenances were included. Gradually the provenances which strongly increase the χ^2-value are then discarded until homogeneity is reached.

Further the χ^2-test is used to test the distribution of existence or non-existence of a character in all provenances. This happens at best if the data are given in form of a contingency table (14×2) for each character and the χ^2-value is calculated according to the formula of Brandt-Snedecor (from WEBER, 1967).

At least each single provenance is tested against each other provenance taking all characters into account. The procedure of calculation is the same as above. The comparison of these χ^2-values with those of the measure of distinctiveness is of special interest.

(2) The measure of distinctiveness is developed and calculated according to the method set out by GREWAL (1962) and BERRY (1963). The authors calculated the measure of distinctiveness (or divergence) but they did not use any test for testing significance at given levels. Here the mean measure of distinctiveness is calculated and a simple but valid test is developed *(see appendix)*.

(3) For the analyis of variance and the intraclass correlation 6 samples each of 16 trees have been investigated of every provenance. The observed numbers of each sample were transformed into angular values according to the equation

$$\Theta = \sin^{-1} (1 - 2p)$$

(see also the measure of distinctiveness). This experimental design allows an analysis of variance with simple classification and random effects. The component of variance between provenances was tested with the F-test. Beyond that the intraclass correlation was calculated in order to get a measure for likelihood within provenances according to the model of KEMPTHORNE (1957).

Results

Isoenzymes: The peroxidase isoenzymes patterns are shown in *figure 1*. Altogether 11 different bands were observed which are marked according to the decreasing electrophoretical mobility (MUHS, 1973). The bands were divided into three groups. Group (1) includes the bands no. 1 to 4. They are strongly coloured and are situated at the top of the zymogram. The bands no. 2 and 3 occur most frequently, while the bands no. 1 and 4 were scarcely found and therefore discarded.

Group (2) consists of the bands no. 5, 6 and 7 which are coloured more weakly. Reproducibility was not high, thus they were not taken into account in this investigation.

Group (3) comprises the bands no. 8, 9, and 10. These bands are coloured strongly and had a high reproducibility. A little weaker band no. 11 appears below band no. 10. This band occurs in almost every zymogram and therefore it was omitted here (see *figure 1*).

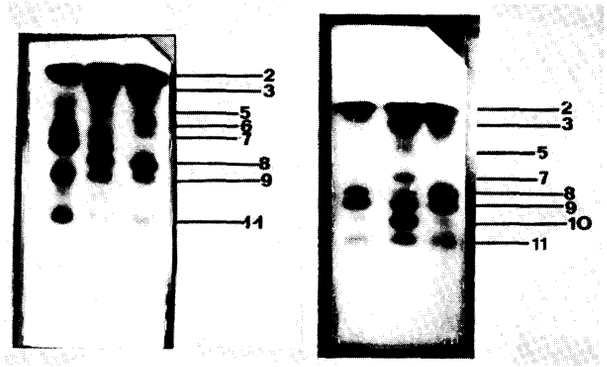

Figure 1. — Isoenzyme patterns of peroxidases from needle extracts. Each of the pictures shows the patterns derived from samples of two years old seedlings of provenance 1049 (left and middle patterns) compared with one sample of the reference tree (right pattern). The longer lines connecting the band numbers with the pictures distinguish the bands investigated from the discarded ones marked by short connecting lines.

During the investigation from August to January the investigated bands no. 2, 3, 8, 9 and 10 showed only small variations in the staining intensity. This had been tested by choosing a single reference tree. So an influence of the vegetation period at these bands was not observed.

Isoenzyme frequencies: Since 96 trees of each provenance have been investigated the maximum frequency of an isoenzyme band (character) is 96. Band no. 2 appears in almost all trees *(table 2)*. Of the total of 1344 trees only in 6 trees band no. 2 was lacking. The distribution of these trees on the provenances seems to be at random.

Table 2. — Isoenzyme frequencies of needle extract peroxidases (96 trees of each provenance were investigated)

Provenance no.	No. of character (isoenzyme band)				
	2	3	8	9	10
1027	96	55	30	91	44
1028	95	28	11	95	23
1030	94	20	37	89	52
1032	96	56	16	89	20
1041	95	44	17	95	13
1042	96	34	9	96	20
1043	96	48	6	95	34
1047	95	41	—	96	30
1048	96	25	3	96	65
1049	96	22	33	91	22
1051	96	52	10	87	36
1075	96	33	50	95	36
1078	96	45	25	92	41
1102	95	47	28	95	44

Band no. 3 occurs more rarely than band no. 2. The frequencies are between 20 (at provenance no. 1030) and 56 (at provenance no. 1032). Variation between provenances is high. A trend is not recognizable in the distribution of this band.

Band no. 8 is most rare, but is shows the highest variation of all bands. 50 were observed in the provenance no. 1075 and none in the provenance 1047. In spite of this a clear trend in the distribution cannot be stated.

Band no. 9 occurs very frequently in all provenances. In the provenances no. 1042, 1047 and 1048 it appears even in all investigated trees. The variation is small. The lowest frequency was found in provenance no. 1051 with 87. Thus this band as well as band no. 2 contributed only little to the distinction of the provenances.

Band no. 10 was observed in approximately the same frequencies as band no. 3 but its variation is higher. The lowest frequency of 13 was found in the provenance no. 1041, the highest of 65 in provenance no. 1048. There cannot be recognized any striking trend in the distribution. Together with the bands no. 3 and 8 band no. 10 is suitable to characterize provenances.

Homogeneity test: The homogeneity test gives information wether the distribution of the characters (isoenzyme bands) between the provenances are homogeneous or not. In test (1) (see *table 3)* all of the 14 provenances were used and all characters were included. The computed χ^2-value of 227,8 is much higher than the value in the table at the 1% level. That means that the distribution of the characters is not homogeneous.

Table 3. — Homogeneity Tests. — All characters were included

no.	included provenance		d.f.
(1)	all 14 provenances	227,8**	52
(2)	provenance no. 1027, 1028, 1032, 1041, 1042, 1043, 1047, 1051, 1078, 1102.	79,5**	36
(3)	provenance no. 1028, 1032, 1042, 1043, 1047, 1051, 1078.	43,6**	24
(4)	provenance no. 1028, 1032, 1042, 1043, 1047, 1051.	28,6	20

** represents significance at the 1% level.

The provenances no. 1030, 1048, 1049 and 1075 contribute a high χ^2-value to the total value and were eliminated. These provenances were collected in different geographical regions and have different isoenzyme frequencies with the exception of the provenances no. 1030 and 1075 which show rather similar frequencies. The χ^2-test (2) with the remaining 10 provenances yields a value which is still higher than the corresponding table value at the 1% level. Thus these provenances cannot be regarded as homogeneous.

Now the provenances with the highest χ^2-values were discarded: no. 1027, 1041 and 1102. These three provenances have different origin. The provenances no. 1027 and 1102 are very similar in their isoenzymes frequencies but differ from that of provenance no. 1041. In test (3) a χ^2-value of 43,6 was calculated including 7 provenances. This value is significant at the 1% level. This means that these provenances are not homogeneous.

If the provenance no. 1078 was omitted, the obtained χ^2-value indicates a homogeneity of the remaining provenances: 1028, 1032, 1042, 1043, 1047 and 1051. Nearly all provenances come from the same geographical latitude (Vancouver Island up to the Cascades) with the exception of the provenances no. 1028 and 1032 which stem from more northern latitudes.

This coarse grouping with the homogeneity test leads to the following results: (a) The distribution of the characters between the provenances is only homogeneous in a small group of 6 provenances. (b) A dependence of the isoenzyme frequencies of the geographical latitude does not seem to exist or this correlation is rather weak. (c) the heterogeneity between the provenances is high and can be used for the characterization of the provenances.

Variation of characters: Each character was tested with the χ^2-test in a contingency (2×14) table (existence and non existence of the character). The character 2 varied only little, its χ^2-value is not significant. This character occured in nearly all of the trees. The characters no. 3, 8, 9, and 10 show significant χ^2-values *(table 4).* This means that the differences of their frequencies between provenances are high and can be used to distinguish the provenances.

Table 4. — Variation of characters. — All provenances were included

character no.	χ^2	d.f.
2	12,7	13
3	84,0**	13
8	176,5**	13
9	42,7**	13
10	84,1**	13

** represents significance at the 1% level.

Distinction of provenances by the χ^2-test: The provenances were tested two by two referring to all characters (see *table 5).* 69 of the total of 91 tests show significant values at the 5% level and 52 tests even show significant values at the 1% level. The remaining 22 tests had no significant values.

Only the provenance no. 1048 differs significantly from all the other provenances. It is remarkable that often just adjacent provenances are different from one another significantly, i.e. no. 1027/1030 or no. 1047/1049. It is also striking that provenances being far distant from one another cannot be distinguished, i.e. no. 1027 from 1051, 1078 and 1102, and 1078/1102 (see *table 5).*

In detail the following 22 pairs of provenances do not differ significantly: 1027/1051, 1027/1078, 1027/1102, 1028/1041, 1028/1042, 1028/1043, 1028/1051, 1030/1075, 1032/1041, 1032/1042, 1032/1043, 1032/1051, 1041/1042, 1042/1043, 1042/1051, 1043/1047, 1043/1051, 1049/1075, 1051/1078, 1051/1102, 1075/1102, and 1078/1102 (see *table 5).*

Measure of distinctiveness: Tests with the measure of distinctiveness are more reliable than the χ^2-test with non transformed values. *Table 6* shows the values of the measure of distinctiveness ϑ. The corresponding standard deviations are put in parenthesis. The measure of distinctiveness was calculated for the provenances two by two including all 5 characters (isoenzyme bands) as for the χ^2-test.

Nearly all ϑ-values show significant values at the 1% levels except the ϑ-values of the following 6 provenance pairs which could not be differentiated significantly: no. 1027/1078, 1027/1102, 1028/1041, 1028/1042, 1041/1042 and 1078/1102.

16 provenance pairs which could not be distinguished by the χ^2-test showed significant differences with the measure of distinctiveness. Exactness of the measure of distinctiveness is much higher than the χ^2-test. Therefore it is especially suitable for the discrimination of provenances.

Analysis of variance and intraclass correlation: The sources of variance can only be determined by a variance

Table 5. — χ^2-Test for Distinction of Provenances Two by Two. — All characters were included

Provenance no.	1028	1030	1032	1041	1042	1043	1047	1048	1049	1051	1075	1078	1102	Provenance no.
1027	17.26**	16.85**	10.77*	17.24**	19.09**	15.60**	30.04**	36.01**	17.09**	8.95	11.32*	1.09	0.70	1027
1028		24.01**	9.50*	7.34	0.98	7.50	14.18**	22.85**	11.56*	8.91	22.46**	10.58*	13.14*	1028
1030			39.24**	38.69**	32.82**	37.58**	48.92**	31.10**	11.14*	32.48**	7.72	13.22*	12.52*	1030
1032				2.84	6.70	8.97	20.18**	44.74**	20.53**	6.09	26.45**	9.62*	11.54*	1032
1041					5.08	14.41**	22.48**	48.97**	14.85**	29.17**	25.10**	13.98**	16.21**	1041
1042						5.55	11.56*	26.61**	16.36**	7.59	27.94**	12.96*	15.82**	1042
1043							6.28	17.89**	30.61**	1.56	35.87**	11.76*	14.03**	1043
1047								18.84**	40.09**	11.64*	47.72**	24.64**	27.37**	1047
1048									45.84**	21.97**	50.15**	28.19**	30.01**	1048
1049										30.42**	5.49	12.60*	13.44**	1049
1051											29.91**	6.84	8.62	1051
1075												10.35*	9.45	1075
1078													0.20	1078

* represents significance at the 5% level,** at the 1% level

Table 6. — Measure of distinctiveness ϑ between provenances and its standard deviations. — All characters were included

Provenance no.	1028	1030	1032	1041	1042	1043	1047	1048	1049	1051	1075	1078	1102	Provenance no.
1027	0.1608** (0.0517)	0.1204** (0.0448)	0.9851** (0.1284)	0.0921** (0.0392)	2.4412** (0.2016)	0.4259** (0.0842)	3.9077** (0.2571)	2.6163** (0.2090)	0.2249** (0.0612)	0.3334** (0.0745)	0.0658** (0.0329)	-0.0097	0.0067 (0.0121)	1027
1028		(0.1757** (0.0541)	0.0649** (0.0330)	0.0216 (0.0190)	0.0028 (0.0069)	0.0456** (0.0276)	0.0342* (0.0240)	0.1736** (0.0537)	0.0706** (0.0343)	1.1263** (0.1370)	0.1783** (0.0545)	0.0884** (0.0384)	0.1002** (0.0408)	1028
1030			0.2546** (0.0651)	0.2617** (0.0661)	0.2700** (0.0670)	0.2591** (0.0658)	0.4851** (0.0899)	0.3032** (0.0711)	0.8256** (0.0371)	0.9543** (0.1262)	0.0707** (0.0343)	0.0851** (0.0377)	0.0827** (0.0372)	1030
1032				0.0744** (0.0351)	0.0862** (0.0379)	0.0543** (0.0301)	0.2184** (0.0603)	0.3532** (0.0767)	0.1173** (0.0442)	0.8209** (0.1170)	0.1885** (0.0557)	0.4620** (0.0277)	1.2320** (0.1435)	1032
1041					0.0219 (0.0605)	0.0692** (0.0339)	0.1749** (0.0540)	0.3468** (0.0760)	0.0895** (0.0386)	1.1570** (0.1387)	0.1691** (0.0531)	0.0928** (0.0393)	0.1030** (0.0414)	1041
1042						0.0270* (0.0212)	0.0797** (0.0364)	0.1862** (0.0557)	0.1186** (0.0444)	1.2987** (0.1470)	0.2048** (0.0584)	0.0834** (0.0372)	0.1203** (0.0447)	1042
1043							0.0507** (0.0290)	0.0743** (0.0352)	0.1841** (0.0553)	1.0823** (0.1345)	0.2421** (0.0635)	0.0593** (0.0314)	0.0785** (0.0361)	1043
1047								0.1443** (0.0490)	0.3642** (0.0779)	1.3588** (0.1760)	0.5200** (0.0930)	0.2670** (0.0667)	0.2733** (0.0674)	1047
1048									0.3462** (0.0759)	1.3850** (0.1519)	0.3172** (0.0727)	0.1701** (0.0532)	0.1069** (0.0572)	1048
1049										0.9880** (0.1283)	0.0492** (0.0286)	0.0741** (0.0351)	0.1091** (0.0426)	1049
1051											1.2736** (0.1607)	0.9307** (0.1245)	1.1419** (0.1379)	1051
1075												0.0594** (0.0314)	0.0739** (0.0351)	1075
1078													-0.0021	1078

The values in parantheses are the standard deviations of the measure of distinctiveness ϑ.

For negative ϑ-values no standard deviations were calculated. ** represents significance at the 1% level.

analysis. For each of four characters (isoenzyme bands no. 3, 8, 9 and 10) which showed great variation between provenances (see χ^2-test, variation of characters) the analysis of variance was applied.

The mean squares and the expected mean squares are given in *table 7*. The mean squares are very high for the source of variance "between provenances". The F-tests of the component of variance "between provenances" shows significant values at the 1% level for three characters. Only for the character no. 9 the variances are small and the F-value is low. With the exception of character no. 9 the component of variance "between provenances" represents the main source of variance, so these characters are qualified for identification.

In order to examine the strength of the correlation within the provenances the intraclass correlation was calculated. High positive values between 0,807 and 0,930 are given for three characters, except for character no. 9, i.e. the samples of the same provenances have very similar frequencies of the characters. We can conclude that the frequencies of characters are very appropriate for an identification of genetically different populations.

Table 7. — Analysis of variance, F-test and intraclass correlation for three characters. — All provenances were included

character no.	source of variance	degree of freedom	mean square	expected mean square	F-value	intraclass correlation
3	between prov.	13	4,5856	$\sigma_e^2 + 6\sigma_g^2$	4,186**	0,807
	within prov.	70	0,1756	σ_e^2		
8	between prov.	13	25,6629	$\sigma_e^2 + 6\sigma_g^2$	13,373**	0,930
	within prov.	70	0,3159	σ_e^2		
9	between prov.	13	0,0020	$\sigma_e^2 + 6\sigma_g^2$	0,259	0,206
	within prov.	70	0,0008	σ_e^2		
10	between prov.	13	7,6631	$\sigma_e^2 + 6\sigma_g^2$	6,062**	0,858
	within prov.	70	0,2050	σ_e^2		

** represents significance at the 1% level.

Discussion

Isoenzyme bands of the peroxidases: The micromethod used here gave reliable and reproducible results (MUHS, 1974). Therefore, it was applied here with only few modifications.

An important factor was the preparation of the needle extracts which also allowed reproducible results. Extracts of a reference tree showed no variation during the time of investigation, so the method was acceptable, especially since only one extraction method had been used in all series of experiments.

It can be assumed, that some of the observed isoenzyme bands may be so-called conformational bands or dissoziation products of the enzyme which are still able to react with the artificial substrate. This must be kept in mind and could modify the interpretation, that each band represents one isoenzyme. Additionally, determination of the genetical basis of peroxidase variation is needed. For this reason we have begun to plan investigations on the offspring of controlled crosses, this will be the only way to raise the precision of interpretation.

Distinction of provenances: The frequencies of the bands no. 3, 8 and 10 varied in a wide range, those of the bands no. 2 and 9 do not. The latter two contribute only little to the distinction of the provenances. So they can be discarded in further investigations.

The χ^2-test of homogeneity shows that a small group of 6 provenances can be regarded as homogeneous. It is remarkable that this group consists of provenances coming nearly from the same region of the southern part of Vancouver Island and the adjacent coastal region of Washington.

The pairwise comparison of the provenances using the χ^2-test showed that of 91 comparisons only 22 do not reach significant values. The measure of distinctiveness allows distinction of all except 6 provenance pairs. So the measure of distinctiveness delivers better results than the χ^2-test.

Distribution of characters: The question for the sources of variance is of great interest for breeders. The components of variance "between provenances" have high values for three included characters (no. 3, 8, and 10). Compared with the componentes of variance "within provenances" they are significantly higher. So the main source of variance lies between provenances. These results, however, may be influenced by other factors:

(1) Seed had been collected from a few trees per stand only (about 15—20) (BARNER, 1973). Thus the entire variability was not included.

(2) These trees were dominating in the stand. Possibly they have same or similar characters, i. e. certain bands of peroxidase isoenzymes.

(3) In the investigation only plants of a minimum height of about 8 to 10 cm were used. Thus a sampling error cannot be excluded which restricted the variance "within provenances" systematically.

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Summary

(1) 14 provenances from British Columbia, Washington and Oregon collected by the IUFRO (1966/67) were investigated with the help of electrophoresis of peroxidases by using needle extracts of two years old seedlings.

(2) Of 11 isoenzyme bands, 5 bands (no. 2, 3, 8, 9 and 10) showed reproducible results. Their frequencies were estimated with a sample size of 96 per provenance.

(3) The frequencies of three bands (no. 3, 8, and 10) varied strongly between provenances. Bands no. 2 and 9 were found in almost any plant. A trend could not be ascertained for the distribution of the bands.

(4) The provenances were tested two by two including all characters (bands) with the χ^2-test. Of 91 provenances pairs, 69 showed significant values at the 5% level, 22 could not be distinguished.

(5) The measure of distinctiveness was defined and a test was developed for it. In 85 provenance pairs the measure of distinctiveness reaches significant values, only 6 pairs were not significant at the 5% level. Therefore the measure of distinctiveness is more reliable than the χ^2-test with non transformed values.

(6) Isoenzyme frequencies are best qualified for distinction of provenances. Even closely adjacent provenances can be distinguished.

(7) The distribution of the isoenzyme bands was investigated by an analysis of variance. The component of variance "between provenances" was much higher than "within provenances". The F-values for the characters no. 3, 8 and 10 suggest a homogeneous distribution within provenances and heterogeneity between provenances.

Key words: Douglas-fir provenances, isoenzyme distinction, needle peroxidases.

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Appendix

The observed numbers representing the existence of a character are reduced to percentages for each provenance. Hence follows a table with the percentage incidences for all characters and all provenances. Then the percentage incidence of each character is transformed into angular values. The angular value Θ measured in radians and being a function of the percentage incidence p is defined by

$$\Theta = \sin^{-1}(1 - 2p).$$

The variance of Θ at a sample size of n is approximately 1/n. This transformation has the advantage that the variance of Θ has a rather simple expression (BERRY, 1963).

Suppose, two populations with percentage incidences have the expected values Θ_1^* and Θ_2^*. Then the square of the difference of two corresponding angular values is called measure of distinctiveness:

$$D^{*2} = (\Theta_1^* - \Theta_2^*)^2.$$

This measure of distinctiveness D^{*2} is estimated according to the observed numbers of the samples by subtracting the variance of $(\Theta_1 - \Theta_2)$ (BERRY, 1963). This variance is

$$V\{(\Theta_1 - \Theta_2)\} = \frac{1}{m_1} + \frac{1}{m_2} = V.$$

Herewith Θ_1 and Θ_2 are the angular values of the sample of population 1 resp. population 2, m_1 and m_2 are the corresponding sample sizes. This variance will be denoted by V. Now we can compute the measure of distinctiveness of two populations by the estimation formula:

$$D^2 = (\Theta_1 - \Theta_2)^2 - \left(\frac{1}{m_1} + \frac{1}{m_2}\right),$$

which is basing on the two samples drawn from the populations.

Presupposing the 0-hypothesis (H_0: $\Theta_1^* = \Theta_2^*$) the difference $(\Theta_1 - \Theta_2)$ is a random variable which is distributed almost normally with the mean zero and the variance V. Under validity of the 0-hypothesis the measure of distinctiveness D^2 is distributed according to $V(\chi^2_{1df} - 1)$. For this reason it becomes significant at the 5% level if it is equal or greater than 2,84V, and at the 1% level if it is equal or greater than 5,64V.

Up to now only one character was regarded. If all characters are taken into account we sum upon all characters i from 1 to k and divide by the numbers of characters k. The mean measure of distinctiveness

$\frac{\sum_{i=1}^{k} (D^*)_i^2}{k}$ can be estimated by $\frac{\sum_{i=1}^{k} \{[(\Theta_1)_i - (\Theta_2)_i]^2 - V\}}{k} = \vartheta$

which is denoted by ϑ. The variance of this estimate value for the mean measure of distinctiveness is calculated by simplification and taking into account of the tenet according to LU (1961). Hence we get

$$\mathrm{Var}(\vartheta) = \frac{4}{k^2} V \cdot \sum_{i=1}^{k} \{[(\Theta_1)_i - (\Theta_2)_i]^2 - V\}$$

The standard deviation is the square root of the variance. The computation of the mean measure of distinctiveness and its variance is rather simple. This is a great advantage.

A significance test is necessary for testing the mean measure of distinctiveness between two populations taking into account all characters. If the 0-hypothesis (H_0: $\Theta_1^* = \Theta_2^*$) is valid we can write the following equation for each i, i = 1, 2, ... k:

$$[(\Theta_1)_i - (\Theta_2)_i]^2 - V = V \cdot (\chi^2_{1df} - 1)$$

After summing up upon all i and dividing by the number of the characters k the following simple relation is given:

$$\vartheta = \left(\frac{1}{k} \chi^2_{kdf} - 1\right) \cdot V$$

In the case of k = 5 the table value for $\chi^2_{5df} = 11{,}1$ at the 5% level and 15,1 at the 1% level. From that the following expressions are computed:

ϑ significant at the 5% level if equal or greater than 1,22V,
ϑ significant at the 1% level if equal or greater than 2,02V.

In the case here the sample size for all provenances is $m_1 = m_2 = 96$, then we get the following values:

$$1{,}22V = 0{,}0254$$
$$2{,}02V = 0{,}0421.$$

The mean measure of distinctiveness between two populations is a quantitative expression of the distinction or divergence. Prerequisite for the measure of distinctiveness is the independence of the characters from each other.