Inheritance of Peroxidase Isozymes in Needles of Loblolly and Longleaf Pines

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Abstract

Progeny from diallel matings were examined by electrophoresis. Eleven different isozymes were observed, nine of which were common to both species. Diallel matings facilitated determining inheritance. The isozyrne segregation frequencies approximated Mendelian expectations as did intensity segregants for one loblolly isozyme. Segregation frequencies for three isozymees varied according to the genetic background of certain parents. The segregation of three loblolly and five longleaf isozymes could be attributed to polymorphic loci.

Key words: Pinus palustris, Pinus taeda, Isozymes, Needles, Inheritance.

Zusammenfassung

Die Vererbung von Paroxidase Isoenzymen in Nadeln von *Pinus taeda L.* und *Pinus palustris* MILL.

Nachkommenschaften aus Diallelkreuzungen wurden mit Hilfe der Elektrophorese untersucht. 11 verschiedene Isoenzyme der Peroxidasen wurden beobachtet, hiervon konnten 9 Isoenzyme in beiden Arten gefunden werden. Durch die diallelen Kreuzungen kannte der Vererbungsmodus analysiert werden. Die Segregationshäufigkeiten der Isoenzyme entsprachen den Erwartungswerten nach Mendel. Dies traf auch zu für ein Isoenzym von *Pinus taeda,* das zwei unterschiedliche Färbeintensitäten aufwies. Die Segregationshäufigkeiten von drei Isoenzymen variierten entsprechend den genetischen Konstitutionen der bestimmten Eltern. Insgesamt konnte die Segregation von drei Isoenzymen bei *Pinus taeda* und 5 Isoenzymen bei *Pinus palustris* auf polymorphe loci zurückgeführt werden.

Introduction

Electrophoxasis is an efficient tool for large-scale genetic investigations. It is particularly valuable to the forest geneticist since more tree genes can be readily identified with it than by any other technique (HAMAKER and SNYDER 1973, FERET and BERGMANN 1976). Once the inheritance of isoenzymes is determined by the study of segregating material (FERET 1971, CONKLE 1971), the technique can be applied to a wide variety of basic and applied research problems such as: identifying ramets, populations, or specias hybrids (BROWN and ALLARD 1969, HARE and SWITZER 1969, MUHS 1974, COPES 1975); determining the effect of inbreeding and psllen migration an the genetic structure of population and seed orchards (RUDIN *et al.* 1974, ERIKSSON 1972); or aiding breeding programs through indirect selection ar through development of breeding procedures (BROWN and ALLARD 1971, BROWN 1975). In this study we examined the inheritance of needle peroxidase isozymes in control-pollinated progeny of loblolly *(Pinus taeda* L.) and longleaf *(P. palustris* MILL.) pine parents.

Materials and Methods

For each species six parents (ortets or ramets) and their crassed and selfed progeny were analyzed. All were growing in southern Mississippi. Ortets were batween 30 and 50 years old. The loblolly ortets, natives of Texas, were represented by ramets randomized within the planting of control-pollinated progeny. The longleaf progeny were 15 years old and the loblolly were 4 years old. Not all of the expected 21 diallel progeny families per species were available *(Table* 1). For thase that were available, the number sf trees/family varied from four to 20.

Mature needles of the current growing season were collected during a 1-week period in September from branches in the center of the crown. Until they could be frozen the needles were temporarily held on ice. Twenty frozen needles from each tree were finely chopped. A 0.5 g subsample was grsund with a mortar and pestle in 5 ml of an aqueous buffer extracting solution containing 60 percent (VIV) 0.1 M

Table 1. — Number of trees sampled for each family. Loblolly farnilies, (parents A—F), appear in upper triangle, and longleaf families (parents G—L) in lower triangle.

Loblolly Parent Tree

	A	B	C	D	E	F	
	13	0	20	20	20	20	A
G	20	20	20	20	0	20	B
H	20	8	16	20	20	20	C
I	20	20	17	20	20	20	D
J	20	20	20	4	16	0	E
K	0	20	0	0	8	20	F
L	20	20	0	0	20	6	
	G	H	T	J	K	L	

Longleaf Parent Tree

Tris-HCl[2]) buffer pH 8, 16 percent (W/V) sucrose, 0.1 percent (W/V) L-Cysteine hydrochloride, 1 percent (VIV) Tween 80[2]) and 2 percent (W/V) ascorbic acid, readjusted to pH 8 with NaOH. All equipment and solutions used in this and subsequent operations were chilled prior to and during use.

Macerated tissue was filtered through Miracloth[2]) and the solute volume tripled with acetone (to approximately 12 ml). After 1 hour the solution was centrifuged, the supernatant removed, and the remaining pellet suspended in 0.25 ml of the buffer solution. After centrifuging again the supernatant was collected for assay.

Tric electrode-buffers were made up according to HARE (1970). The aqueous gel, pH 9.2, contained 6.0 percent (W/V) polyacrylamide and 0.3 percent (W/V) bis-acrylamide (Cyanogum 41[2]), 0.1 percent (VIV) TEMED[2]), 0.1 percent (W/V) potassium persulfate, 0.0005 percent (W/V) riboflavin, 1 percent (W/V) Tris-HCl, 0.1 percent (W/V) EDTA, and 0.038 percent (W/V) borate. Next, a 3 mm, 30-slot, vertical slab gel was "precharged" for 30 minutes at 100 mA of current. Then 2 μl of the supernatant was injected into each slot, and the current was applied again for 90 minutes. At the end of this time the dye front migrated 14 cm. Staining with a benzidine dihydrochloride substrate followed procedures of HAMAKER and SNYDER (1973). Starting with anodal isozymes nearest the dye front for loblolly pine, isozymes

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[2]) Use of trade names is solely for identification and does not imply indorsement by U.S. Forest Service or the U.S. Dept. of Agriculture.

were sequentially numbered. Similarly positioned isozymes in longleaf, if present, were assigned corresponding numbers. The relative stain intensity of an isozyme was estimated visually if isozyme intensity. varied appreciably from tree to tree.

Three conventional assumptions were used in formulating the genetic hypotheses. They were that single genes control single isozymes, that these genes are co-dominant multiple alleles at one or more loci, and that it is appropriate to postulate recessive null alleles. The genotypic notation used in this paper can be illustrated by supposing that segregation at a locus is for isozymes 1 and 3. The alleles at the locus would be designated as 1/3 or, if for isozyme 2 and no isozyme, they would be designated as 2/0.

Parental genotypes were assigned on the basis of parent phenotypes and the segregation observed in their progeny. Expected ratios from crossing the genotypes were compared with the observed ratios. Chi-square statistics were used to test the goodness-of-fit. Two-class chi-square formulas included a correction for discontinuity. Chi-square values small enough to exceed the 0.05 probability level were considered to indicate a good fit.

Results and Discussion

The analysis of the 619 trees revealed 11 peroxidase bands. Loblolly and longleaf pines were similar in that loci for bands 11, 10, 9, 6, and 2 were polymorphic (bands present or absent) and those for 7, 5, 4, and 3 were monomorphic (present). Band 9 was a dark, broad band in both species. The two species differed in bands 8 and 1. Band 8 was faint or absent in longleaf but never present in loblolly; whereas, band 1 was faint or absent in loblolly but never present in longleaf *(Figure 1)*.

Inheritance in loblolly pine

Among the 350 trees studied, 34 segregation patterns were found for bands 11, 10, 9, 6, 2, and 1. Large Chi-square values indicated that observed ratios for bands 9, 2, and 1 did not fit Mendelian expectations. Mendelian ratios such as 1 : 3 approximated the frequencies of the absence and presence of bands 11, 10, and 6, and all chi-square values for these indicated a good fit.

The genes for isozymes 11, 10, and a null appeared to be alleles at two independent duplicate loci *(Table 2)*. For example, parent A is hypothesized to have a genotype at one locus of 11/0 and at the duplicate locus of 11/10, and gametes 11/0, 11/11, 10/0, 11/10 while parent F has genotype 11/0, 10/0 and gametes 0/0, 11/0, 10/0, 11/10. When crossed, progeny are expected to segregate in the following phenotypic proportions: isozyme 11—5; isozyme 10—2.5; and isozymes 11 and 10—12.5. The observed proportions of 7 : 2 : 11 fit this expectation satisfactorily.

The staining intensity of isozyme 10 also varied. It was dark when carried by parents B and C and either light or absent for other parents. Segregations of intensity approximated monofactorial expectations *(Table 3)*. To relate the gene action for the presence and absence of isozyme 10

Fig. 1. — Photograph of peroxidase bands 2 to 11 in crosses of longleaf pine. Patterns for 17 different trees are shown. In loblolly pine a band 1 may appear but band 8 is absent.

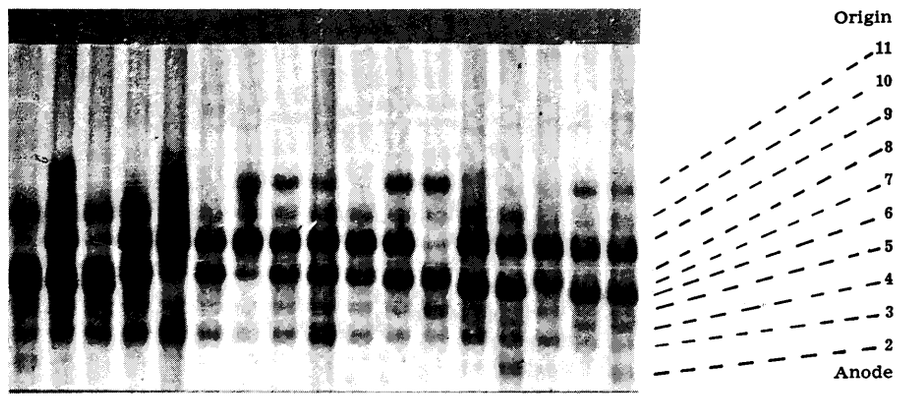

Table 2. — Inheritance of bands 10 and 11, loblolly pine. Duplicate loci inheritance hypothesized.

Parents crossed or selfed	Genotypes	Number Tested		Phenotypic Frequencies for bands 0	11	10	10&11	Chi Square
Expected Ratio of 1:3								
AxD, AxE	11/0, 11/10 x 11/11, 11/10	40	OBS		35		81	
DxD, ExE, DxE	11/11,11/10 x 11/11, 11/10	56	EXP		29.0		87.0	1.39
DxF	11/11,11/10 x 11/0, 10/0	20						
Expected Ratio of 1:7								
BxD, CxD, CxE	11/10,10/0 x 11/11,11/10	60	OBS		9		51	
			EXP		7.5		52.5	0.15
Expected Ratios of 1:4:11[2]								
AxA	11/0 ,11/10 x 11/0, 11/10	13	OBS		1	12	56	
BxB, BxC, CxC	11/10,10/0 x 11/11, 11/10	56	EXP		4.3	17.2	47.4	5.66
Expected Ratios of 1:2:5[2]								
AxF	11/0, 10/0 x 11/0, 10/0	20	OBS		5	17	38	
BxF, CxF	11/10,10/0 x 11/0, 10/0	40	EXP		7.5	15.0	37.5	1.11
Expected Ratio of 1:1:6								
AxC	11/0, 11/10 x 11/10, 10/0	20	OBS		2	2	16	
			EXP		2.5	2.5	15.0	0.27
Expected Ratio of 1:3:3:9								
FxF	11/0, 10/0 x 11/0, 10/0	20	OBS	0	7	4	9	
			EXP	1.2	3.8	3.8	11.2	4.34

[1]) All chi square values and heterogeneity chi square values (not shown) have probabilities exceeding the .05 level.

[2]) Includes ratios of 4 : 1 : 11 or 2 : 1 : 5.

to that for its staining intensity, the genotypes of parents B and C, which had been 11/10, 10/0 *(Table 2)* were reassigned as 11/10L, 10I/0 where the letters indicate whether isozyme 10 stained lightly or intensely. The observed ratios (not shown here) all had small chi-square values when compared with the expected ratios.

The isozyme 6 segregations require postulation of a monofactorial system modified by suppressor genes *(Table 3)*. That is, undisturbed segregations appear in all families of parent F which, therefore, shows no suppressor. How-

Table 3. — Inheritance of the staining intensity of band 10 and of the presence and absence of band 6, loblolly. Single locus inheritance without modification hypothesized for band 10 and with modification[1]) for band 6.

Parents crossed or selfed	Genotypes	Number Tested		Phenotypic Band Frequencies: Absent or Light[2]/	Present or Dark	Chi Square[3]/
Band 10 Intensity, 1:3 expected						
BxB, CxC, BxC	I/L x I/L[4]/	56	OBS	8	48	
			EXP	14.0	42.0	2.85
Band 10 Intensity, 1:1 expected						
AxC, BxD, BxF CxD, CxE, CxF	L/L x I/L	120	OBS	67	53	
			EXP	60.0	60.0	1.39
Band 10 intensity, No intense band expected						
AxA, DxD, ExE FxF, AxD, AxE AxF, DxE, DxF	L/L x L/L	167	OBS	165	2	
			EXP	167.0	0.0	--
Band 6, 1:3 expected						
DxD, DxF, FxF	6/0 x 6/0	60	OBS	18	42	
			EXP	15.0	45.0	0.54
Band 6, 1:1 expected						
AxF, BxF, CxF	0/0 x 6/0	60	OBS	30	30	
			EXP	30.0	30.0	0.00
Band 6, No band expected						
AxA, BxB, CxC, ExE AxC, AxE, BxC, CxE	0/0 x 0/0	145	OBS	221	4	
			EXP	225.0	0.0	--
AxD, BxD, CxD, ExD	0/0 x 6/0[1]/	80				

[1]) Parents A, B, C, E postulated to carry complementary inhibitor genes which prevent expression of band 6 in crosses with parent D.

[2]) Band 6 classified as present vs absent. Band 10 classified as absent or light vs present & intensively stained.

[3]) All chi square values and heterogeneity chi square values (not shown) have probabilities exceeding the .05 level.

[4]) L denotes gene for absent or lightly stained. I denotes gene for present & intensely stained.

Table 4. — Inheritance of bands 11, 9 and 2, longleaf pine. Single locus inheritance hypothesized.

Parents crossed or selfed	Genotypes	Number Tested		Phenotypic Band Frequencies: Absent	Present	Chi Square[1]/
Band 11, 1:3 expected						
JxJ, KxK, LxL, KxL	11/0 x 11/0	38	OBS	11	27	
			EXP	9.5	28.5	.13
Band 11, 1:1 expected						
GxJ, GxL, HxJ, HxK HxL, IxJ	0/0 x 11/0	120	OBS	52	68	
			EXP	60.0	60.0	1.87
Band 11, no band expected						
GxG, HxH, IxI, GxH GxI, HxI	0/0 x 0/0	105	OBS	96	9	
			EXP	105.0	0.0	--
Band 9, 1:3 expected						
LxL	9/0 x 9/0	6	OBS	0	6	
			EXP	1.5	4.5	0.89
Band 9, 1:1 expected						
HxL, KxL	0/0 x 9/0	40	OBS	16	24	
			EXP	20.0	20.0	1.21
Band 9, no band expected						
HxH, KxK, HxK	0/0 x 0/0	36	OBS	34	2	
			EXP	36.0	0.0	---
Band 9, only band 9 expected						
GxG, IxI, JxJ, GxH GxI, GxJ, GxL, IxH JxH, IxJ	3/3 x any	181	OBS	8	173	
			EXP	0.0	181.0	---
Band 2, 1:3 expected						
HxH	2/0 x 2/0	8	OBS	2	6	
			EXP	2.0	6.0	0.00
Band 2, 1:1 expected						
GxH, HxI, HxJ, HxK HxL	0/0 x 10/0	100	OBS	46	54	
			EXP	50.0	50.0	0.49
Band 2, no band expected						
GxG, IxI, JxJ, KxK LxL, GxI, GxJ, GxL IxJ, KxL	0/0 x 0/0	155	OBS	155	0	
			EXP	155.0	0.0	---

[1]) All chi square values and heterogeneity chi square values (not shown) have probabilities exceeding the .05 level.

Table 5. — Inheritance of bands 8 and 6, longleaf pine. Single locus inheritance with modifications[1],[2] hypothesized.

Parents crossed or selfed	Genotypes	Number Tested	Phenotypic Band Frequencies	Absent	Present	Chi Square[3]
Band 8, 1:3 expected						
IxI, IxJ, JxJ	8/0 x 8/0	41	OBS	7	34	
			EXP	10.2	30.8	0.98
Band 8, 1:1 expected						
GxI, GxJ	0/0 x 8/0	40	OBS	22	18	
			EXP	20.0	20.0	0.22
Band 8, no band expected						
HxI, HxJ	0/0 x 8/0[1]	40	OBS	179	3	
			EXP	182.0	0.0	--
GxG, HxH, KxK, LxL GxH, GxL, HxK, HxL KxL	0/0 x 0/0	142				
Band 6, 3:1 expected						
GxG, IxI, JxJ, GxI, GxJ, IxJ, HxH	6/0 x 6/0[2]	109	OBS	83	26	
			EXP	81.8	27.2	0.03
Band 6, 1:3 expected						
HxG, HxI, HxJ	6/0 x 6/0	60	OBS	13	47	
			EXP	15.0	45.0	0.20
Band 6, 1:1 expected						
HxL	6/0 x 0/0	20	OBS	13	7	
			EXP	10.0	10.0	1.25
Band 6, no band expected						
LxL, LxG, LxK	0/0 x any[2]	46	OBS	33	13	
			EXP	46.0	0.0	--
Band 6, only band 6 expected						
HxK	6/0 x 6/6	20	OBS	5	23	
			EXP	0.0	28.0	---
KxK	6/6 x 6/6	8				

[1]) The modification hypothesized is that band 8 is not expressed in crosses involving tree H.
[2]) Modification is that a 6/0 genotype does not express band 6 and that a 6/6 genotype is required — holds for crossed families except those involving tree H.
[3]) All chi square values and heterogeneity chi square values (not shown) have probabilities exceeding the .05 level.

ever, parent D, the other carrier, is able to express this gene only in its selfed family or when crossed with parent F. That it will not express isozyme 6 in crosses with A, B, C, and E suggests complementary suppressor gene action.

Two trees with dark band 10 occurred in the selfed and supposedly nonsegregating family of parent D when none were expected. We ignore such deviations as long as they occur in 2% or less of the trees of the nonsegregating class. They could be due to contamination during pollination.

Researchers intending to use the isozyme technique in future applied research on loblolly pine should confine their efforts to peroxidase bands 11 and 10. The inheritance of these did not vary with the genetic backgrounds of individual parents.

Inheritance in longleaf pine

Among the 269 trees studied, 35 segregation patterns were found for bands 11, 10, 9, 8, 6, and 2. Except for band 10, whose segregation could not be explained the observed ratios approximated monofactorial Mendelian ratios. All chi-square values were small. Bands 11, 9, and 2 segregated as expected according to the simple Mendelian hypotheses, but there was an excessive number of segregants in supposedly nonsegregating families for bands 11 and 9 *(Table 4)*.

Bands 8 and 6 appeared less frequently than expected, and we were able to explain their inheritance only after modifying the Mendelian hypothesis *(Table 5)*. The modification for band 8 is that tree H genes suppress band 8 appearance. In most cases segregation frequencies of band 8 realized expectations. But whenever tree H, which carried no band 8 allele, appeared in crosses with trees that did (H × I, H × J), no band 8 appeared. Similar difficulties were encountered by Feret (1971). The phenomenon is similar to the replacement of a parental band by a hybrid band (Endo, 1973), but in this case the replacement is a "null band". Such a replacement may be an exception to the usual assumption of dominance of a band allele over a null allele.

The necessary modifying hypothesis for band 6 is that the effect of the 06 genotype is insufficient for expression of band 6 in crossed families, except those of family H. That is, in crosses among the parents G, I, J, K, and L, expression of band 6 requires a 66 dosage. Thus for both bands 8 and 6, expression appears to depend on the genetic background of the parent. Although the modifying hypothesis for band 6 satisfactorily results in only small deviations among the 11 segregating families, it fails for three supposedly nonsegregating families — G × L, K × L, and H × K. The unexpected appearance of segregates suggests that penetrance is somewhat unpredictable and that the development of a wholly satisfactory hypothesis for band 6 may be difficult.

A recommended marker for applied research is the normally segregating peroxidase band 2. Bands 11 and 9 might be useful if the unexpected segregates can be tolerated or their frequency somehow reduced.

Conclusions

We recommend longleaf band 2 and loblolly bands 11 and 10 as suitable for applied research. Some of the other bands are not recommended because their inheritance is too complicated or because expected and observed frequcies do not agree sufficiently. Failure in these cases can not be attributed to varying developmental stages since we noted no instability of expression in other tests involving different ages, seasons, and parts of the crown; cf. Miyazaki and Sakai (1969) and Feret (1971). Furthermore, in this test we sampled only needles during one part of the season and from one part of the crown. Similar problems have been

noted for other gymnosperms by FERET (1971) and by RUDIN and RASMUSON (1973). That the complicated inheritance occurs more frequently in forest trees than in cultivated crops is expected since wild heterozygous organisms carry more modifying genes than do inbred ones that have been cultivated longer.

Further research on the problem of excessive segregates in supposedly nonsegregating families would be worthwhile. Also, future sampling should compare geographic sources to determine the generality of our findings.

The gene actions underlying band patterns were not obscured even though appearance of some bands was complicated or marred. None of the 84 chi-square tests indicated poor fits. We attribute this result to the resolving power of the diallel mating design. Too few unrelated crosses would probably have resulted in failure to reach working genetic hypotheses.

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Inheritance of glutamate oxalo-acetate transaminase isozymes in virginia pine megagametophytes[1])

By M. S. WITTER and P. P. FERET[2])

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Summary

Polyacrylamide gel electrophoresis was used to examine inheritance of GOT isozymes in megagametophytes of Virginia pine. Two unlinked loci were found to code for GOT: Locus A with 5 alleles, and Locus B with 3 alleles. Irregular segregation patterns were found among megagametophyte populations of some trees. These results suggest the possibility of non-random degeneration of 3 of 4 megaspores produced in meiosis.

Four populations of Virginia pine were compared on the basis of allelic frequency and occurrence. Little or no significant differences were found.

Key words: Isozymes, Virginia pine, megagametophyte, electrophoresis, glutamate oxalo-acetate transaminase.

Zusammenfassung

In Megagametophyten von *Pinus virginiana* MILL. wurde die Vererbung von GOT Isoenzymen elektrophoretisch untersucht. Zwei nicht gekoppelte Loci codieren für GOT: Locus A mit 5 Allelen und Locus B mit 3 Allelen. Bei Megagametophytenpopulationen einiger Bäume wurden unregelmäßige Segregationsmuster gefunden. Diese Ergebnisse sprechen für die Möglichkeit nicht zufälliger Degeneration von 3 der 4 Megasporen der Meiose.

Vier Populationen von *Pinus virginiana* MILL. wurden hinsichtlich Vorkommen und Frequenz von Allelen verglichen. Unterschiede waren gering oder nicht signifikant.

Introduction

The development of seed orchards is an established aspect of forest tree improvement programs. The direct determination of genetic diversity in domesticated populations may become mandatory as breeding programs progress. In particular, the significant decrease or increase in the genetic base of selected seed orchard populations relative to that of natural populations of a given species begs exploration. This problem is relevant for several reasons. Drastic decreases in population size can lead to allelic losses creating largely monomorphic populations (BONNELL and SELANDER, 1974). Populations with disproportionately fewer alleles per locus may be more susceptible to insect and disease epidemics than populations composed of widely divergent genotypes (NAS, 1972). The dangers of a narrow genetic base have been clearly demonstrated with inbred crop species and could be even more pronounced in a long-lived tree species. Of long term importance is the possibility

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