A genetic analysis of aminopeptidase and peroxidase isoenzymes in Douglas-fir parent trees and seedling progeny

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The genetic control of isoenzymes found in bark and vegetative buds was determined for one peroxidase and one aminopeptidase locus. The segregation of alleles within full-sib progeny of a 6 × 6 tree diallel fitted expected Mendelian ratios. Eight peroxidase isoenzymes existed, but only those with relative mobilities of 0.44 and 0.49 showed allelism. Several of the other six peroxidase bands gave a false impression of segregation.


Le contrôle génétique d’isoenzymes trouvées dans l’écorce et les bourgeons a été déterminé par un locus appartenant à la peroxydase et à une aminopeptidase. La ségrégation des allèles pour une descendance biparentale d’un arbre 6 × 6 diallel confirma les rapports mendéliens. De fait, huit isoenzymes de la peroxydase étaient présentes mais ce furent seulement celles caractérisées par une mobilité relative de 0.44 et 0.49 qui manifestèrent l’allelisme. De plus, plusieurs des six autres bandes de peroxydase donnèrent une mauvaise idée de la ségrégation.

In this report, bark and vegetative bud tissues from parent trees and their 2- and 3-year-old progeny were studied. The inheritance of some of peroxidase and aminopeptidase isoenzymes was determined.

Methods

In 1973, the six trees near Corvallis, Oregon, were control pollinated in a 6 × 6 diallel design. Reciprocal crosses and self-pollinations were omitted. Seeds were obtained from 14 of the 15 families. In November 1975, about 30 seedlings of each family were planted in the field near Monmouth, Oregon.

Tissues for electrophoresis were collected in December 1975, November 1976, and December 1976. On each date, samples were gathered from 400 progeny and 6 parent trees. A lateral branch (10–12 cm in length) was cut from the current year’s growth of each seedling and parent tree. Branches were stored 1 to 2 weeks in polyethylene bags at 0-2°C. Samples (50 to 75 mg) of the dome-shaped meristem of the terminal bud and 250-mg samples of living bark were used to study aminopeptidase and peroxidases, respectively. Bud and bark tissues were macerated in one and three drops, respectively, of gel buffer solution containing 5%; soluble polyvinylpyrrolidone (PVP-40, molecular weight 40 000), absorbed on a 5 × 13 mm paper wick (Whatman No. 1 chromatography paper), wrapped in plastic, and stored at −80°C until needed for electrophoresis.

Electrophoresis apparatus used was similar to that described by Conkle (1972). Starch gel procedures used were identical to those described by Copes and Beckwith (1977). After electrophoresis, peroxidase was detected with o-dianisidine (3,3′-dimethoxybenzidine) as substrate (Brewbaker et al. 1968) and aminopeptidase activity was detected with L-leucyl-β-naphthylamide as substrate (Scandalios 1969). Isoenzymes from each gel were recorded in both diagrams and photographs. Band positions were measured to the closest

Electrophoretic techniques have been widely used since 1968 to study variation in forest trees (see review by Rudin 1976). Such techniques enabled forest geneticists to study variation in what externally appeared to be uniform populations. Knowledge has been gained in the following areas: provenance and geographic variation; clone, species, and hybrid identification; climatic adaptation; developmental changes; selection; disease resistance; differences between dwarf and normal trees; mating system analysis; and estimating levels of inbreeding.

In Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) isoenzyme studies with starch or polyacrylamide gels have been reported for acid phosphatase (Baumeister 1975; Copes 1975), peroxidase (Juo and Stotzky 1973; Mühs 1974; Copes 1975), ribonuclease and phosphodiesterase (Merjartowicz and Bergmann 1977), aminopeptidase and esterase (Copes 1975; Juo and Stotzky 1973; Yang et al. 1977), glutamate dehydrogenase and catalase (Copes 1975), and glutamate oxaloacetic transaminase (Yang et al. 1977). In these studies isoenzymes from bark, needles, bud meristems, embryos, or female gametophytes were examined. Most enzymes in Douglas-fir have multiple bands. Several enzyme systems have been analyzed for genetic control (Mühs 1974), but no studies have yet evaluated inheritance of isoenzymes in progeny from controlled pollinations.

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millimetre and transformed into relative mobility (Rm) units (the distance the isoenzyme travelled from the origin slit toward the anode, divided by the distance the bromophenyl blue marking dye moved from the origin slit toward the anode). Segregation ratios observed in progeny were compared with those expected from simple Mendelian inheritance. Tests of homogeneity were made by the chi-square 'goodness-of-fit' test.

Results
The gels stained for peroxidase revealed eight isoenzymes but only the Rm 0.44 and 0.49 bands were found suitable for genetic analyses. The Rm 0.44 and 0.49 bands were present in bark tissues from all three collection times. They formed distinct bands which
Chi-squared goodness-of-fit test between observed and expected segregation ratios among seedlings of full-sib families differing in aminopeptidase isoenzymes

<table>
<thead>
<tr>
<th>Family (9 X 1)</th>
<th>Expected ratio (Rm phenotypes)</th>
<th>No. of observed seedlings/No. of expected seedlings (Rm phenotypes)</th>
<th>Goodness-of-fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.66:0.66-0.69:0.72:0.72</td>
<td>0.66:0.66-0.69:0.66-0.72:0.69:0.72:0.72:0.75</td>
<td></td>
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<tr>
<td>1 X 2</td>
<td>0:0:0:1:1</td>
<td>0/0 0/0 0/0 (4)/0b 7/13 9/13</td>
<td>2.46° 0.12</td>
</tr>
<tr>
<td>2 X 3</td>
<td>0:0:0:0:1:1</td>
<td>0/0 0/0 0/0 0/0 6/5.5 5/5.5</td>
<td>0.09° 0.76</td>
</tr>
<tr>
<td>2 X 5</td>
<td>0:0:0:0:0:1:1</td>
<td>0/0 0/0 0/0 0/0 16/14.5 13/14.5</td>
<td>0.31° 0.58</td>
</tr>
<tr>
<td>2 X 6</td>
<td>0:0:0:0:0:1:1</td>
<td>0/0 0/0 0/0 0/0 13/16.5 20/16.5</td>
<td>1.49° 0.22</td>
</tr>
<tr>
<td>2 X 7</td>
<td>0:0:0:0:1:1</td>
<td>0/0 0/0 0/0 0/0 11/13.5 0/0</td>
<td>16/13.5 0.93° 0.34</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>0/0 0/0 0/0 0/0 42/49.5 63/53</td>
<td></td>
</tr>
<tr>
<td>Cumulative x²</td>
<td>4 df</td>
<td>5.28 0.26</td>
<td></td>
</tr>
<tr>
<td>1 X 4</td>
<td>0:1:1:1:0:1:1</td>
<td>0/0 6/6.5 6/6.5 5/5.5 7/6.5 5/6.5</td>
<td>0.77° 0.86</td>
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<tr>
<td>3 X 4</td>
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<td>0/0 7/8.25 7/8.25 5/5.5 12/8.25 8/8.25</td>
<td>2.52° 0.47</td>
</tr>
<tr>
<td>4 X 5</td>
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<td>0/0 7/7 7/7 5/5.5 7/7 3/3.5</td>
<td>1.67° 0.88</td>
</tr>
<tr>
<td>4 X 6</td>
<td>0:1:1:0:1:1:1</td>
<td>0/0 5/4.5 5/4.5 5/5.5 3/3.5</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>0/0 25/26.25 26/26.25 0/0 31/26.25 23/26.25</td>
<td>3.96° 0.91</td>
</tr>
<tr>
<td>Cumulative x²</td>
<td>9 df</td>
<td>5.28 0.26</td>
<td></td>
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<tr>
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<td>4.49° 0.11</td>
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<tr>
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<td>0:0:0:1:2:1:1</td>
<td>0/0 0/0 0/0 9/6.5 12/13 5/6.5</td>
<td>1.39° 0.50</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
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<td>9.79° 0.28</td>
</tr>
<tr>
<td>Cumulative x²</td>
<td>8 df</td>
<td>3.96 0.91</td>
<td></td>
</tr>
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</table>

*P, probability that x² will be exceeded. Null hypothesis should be rejected when value is less than 0.05.
*Numbers in parentheses denote aberrant zymograms that were deleted from x² analysis.
*Values were calculated using one degree of freedom.
*Values were calculated using three degrees of freedom.
*Values were calculated using two degrees of freedom.

stained faster and darker than other peroxidase isoenzymes at Rm 0.27, 0.35, 0.66, 0.71, 0.77, and 0.80. These six bands were omitted from genetic analysis because they were present in all trees (mono­ morphic) or were not repeatable.

Peroxidase zymograms of the six parent trees for the Rm 0.44 and 0.49 bands are shown in Fig. 1. Five parents (1, 2, 3, 5, and 6) were heterozygous (Rm 0.44–0.49), but parent tree 4 was homozygous (Rm 0.49).

The progeny phenotypes revealed Rm 0.44 and 0.49 isoenzymes to be controlled by codominant alleles located at one locus. Crosses between the five heterozygous (Rm 0.44–0.49) parents and the homozygous (Rm 0.49) parent No. 4 yielded progeny in the expected 1:1 Mendelian ratio (Table 1). No significant x² deviations were found. Crosses between the four heterozygous (Rm 0.44–0.49) parents produced progeny in the expected 1:2:1 Mendelian ratio (Table 1). Progeny from the 2 × 3 cross deviated significantly at the 0.05 level, and it was a small sample of only 11 seedlings.

Experimental error in the form of unexpected or nonparental peroxidase phenotypes was detected in 2 of 373 trees. For example, progeny from a Rm 0.44–0.49 × Rm 0.49 cross should not have yielded any trees with single-band Rm 0.44 phenotypes, yet 2 of the 133 seedlings from such crosses had aberrant zymograms. The two trees were recorded in Table 1 but were not subjected to x² analysis.

Four aminopeptidase isoenzymes were found in extracts from buds at Rm 0.62, 0.66, 0.69, and 0.72. The Rm 0.62 band was found in all six parent trees and in all the progeny. This band appeared to be controlled by a different locus from the other three bands. Three bands (Rm 0.66, 0.69, and 0.72) were polymorphic when parents and progeny were considered. The parental phenotypes are diagrammed in Fig. 1.

Progeny evaluations of the Rm 0.66, 0.69, and 0.72 isoenzymes indicate codominant inheritance (Table 2). One or two of the bands, but not all three, were present in each tree. Crosses of Rm 0.66–0.72 (parent 4) times Rm 0.69–0.72 (parents 1, 3, 5, 6) gave progeny in the expected 1:1:1:1 Mendelian ratio. Crosses of Rm 0.66–0.72 (parent 4) or Rm 0.69–0.72 (parents 1, 3, 5, 6) times Rm 0.72–0.72 (parent 2) gave progeny in the expected 1:1 ratio. Crosses of Rm 0.69–0.72 (parents 1, 3, 5, 6) times Rm 0.69–0.72 did give...
progeny in expected 1:2:1 ratio. No chi-squares deviated significantly at the 0.05 level from the expected values.

Approximately 5% (21 of 395) of the progeny contained some isoenzymes that could not be explained. Aberrant isoenzymes were found in seedlings from crosses of parent tree 4 ($R_m$ 0.66-0.72) with $R_m$ 0.69-0.72 trees (parents 1, 3, 5, 6). Fourteen progeny from these crosses had a single $R_m$ 0.69 band. Such misclassified isoenzymes were considered experimental errors and were noted in Table 2 but were not subjected to chi-square analyses.

**Discussion**

Forest geneticists should proceed cautiously when using isoenzyme data from diploid tissues to estimate levels of heterozygosity in tree populations. Consistent inheritance, persistent bands during development, and freedom from environmental modification are needed before heterozygosity can be accurately estimated. The eight peroxidase isoenzymes detected in this study illustrated this problem. Only the $R_m$ 0.44 and 0.49 isoenzymes were suitable for genetic analysis. The $R_m$ 0.44 and 0.49 peroxidase isoenzymes corresponded to the bands labeled 8 and 9 by Mühs (1974). The $R_m$ 0.77 and 0.80 bands gave a false impression of segregation because of nongenetic influences, because of developmental alteration of gene expression, or because of variability in stain visualization techniques. Classifying such isoenzymes as segregating alleles would lead to erroneous heterozygosity estimates.

The alleles of the peroxidase and the aminopeptidase loci were expressed codominantly. Both alleles were equally dark stained in heterozygotes and all bands were inherited as monomers. No 'hybrid' bands appeared in the progeny. The recombination of alleles in progeny corresponded to ratios expected from hypotheses of simple Mendelian inheritance. Only 1 of the 14 families deviated significantly from the expected ratio and that family consisted of a small sample of only 11 seedlings. Inheritance of $R_m$ 0.44 and 0.49 peroxidase isoenzymes in Douglas-fir closely resembles the two alleles—one locus peroxidase system reported in *Chamaecyparis obtusa* (Tajima et al. 1977).

The presence of nonparental isoenzymes in the progeny may have been due to experimental error. This inconsistency was small for peroxidase (0.5%) but was much larger for aminopeptidase (5.0%). Several factors may account for this difference. First, aminopeptidase bands did not stain as darkly as peroxidase bands. Very weak staining of one band in a heterozygote could result from different substrate specificities. Second, the aminopeptidase bands were more difficult to accurately identify because of similar $R_m$ values. Other workers have noted similar experimental error problems; i.e., Müller (1976) found a 2% error in aminopeptidase data from a seed and embryo study of *Pinus virginiana*, and Feret (1971) reported a 5% experimental error in peroxidase observations from *Picea glauca* needle tissues. Adequate replication and uniform experimental procedure help minimize the nongenetic effects, but it is important to recognize and include in present most isoenzyme results. Further work on or use of polyphenol-inhibiting maceration solutions, such as those reported by Kelley and Adams (1977) for juniper leaves, might help reduce within-tree variation.


