# ISOENZYME GENETIC MARKERS USEFUL FOR STUDIES OF THE *PINUS RIGIDA* X *P. TAEDA* HYBRID[1]

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## ABSTRACT

Genetically determined isozyme markers useful for studies of the *Pinus rigida* x *P. taeda* (pitch x loblolly pine) hybrid are described. Seedling progeny from seven two-parent pitch x loblolly pine hybrid crosses, as well as seedling progeny from single species crosses involving each of the seven parents of these hybrids (parental families), were subjected to starch gel electrophoresis and stained for six different enzymes. Female gametophyte (1N) segregation patterns of isozymes in heterozygous parental families were used to determine the Mendelian genetics of the band patterns. Band patterns of embryos in the parental families were used to determine the expression of isozymes in diploid tissue of each species. Band patterns were strikingly similar in the two species and in hybrids were exactly those predicted from the genotypes and phenotypes expressed in the parental families. Thus, for the six enzymes studied, hybrids cannot be distinguished from individuals of non-hybrid origin on the basis of uniquely "hybrid" band types. However, individuals of hybrid origin are distinguishable when the parents of one species carry alleles leading to bands of different migration rate from those carried in the parents of the other species. Under these conditions, hybrid individuals express heterozygous phenotypes which are not possible among crosses within the species. Examples of how these markers may be used in genetic studies of this hybrid are given.

## INTRODUCTION

Several researchers have pointed out or demonstrated the usefulness of isozyme markers for genetic studies in different species of forest trees (Sakai and Miyazaki 1972, Conkle 1974, Feret 1974 and Rudin, etal. 1974). However, there have apparently been no published reports, to

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date, demonstrating the usefulness of such markers for studies of species hybrids. This paper will present some preliminary results of work on isozyme genetic markers in seedling tissue of the *Pinus rigida* x *taeda* (pitch x loblolly pine) hybrid. This hybrid is of considerable interest to forest tree breeders. It is now being commercially produced in Korea and is being developed for commercial production in the eastern United States.

## MATERIALS AND METHODS

The materials for this study were seedling progeny of: 1) seven two-parent, pitch x loblolly pine crosses (hybrid families); and 2) single species crosses involving each of the seven parents of the above hybrids (parental families). The seven parents were clones of three pitch pine (designated 62, 71 and 77) and four loblolly pine (designated 7-5, 11-9, 11-10 and 11-20) individuals. All parental families were open-pollinated, except loblolly 11-20 which was derived from a two-parent cross involving 11-20 as the seed parent.

The electrophoretic techniques were essentially identical to those described by Conkle (1972). One week to ten day old germinated seedlings (radicles emerged 1-5 mm beyond the seed coat) were used. Embryo and female gametophyte tissues were separated and then each macerated in 1-2 drops of distilled water. Juice from each squashed tissue was then absorbed into separate thick paper wicks and inserted into a slice made across the gel 5 cm from the cathode. Each gel accommodated 20-22 such wicks. The gels were of 12% hydrolyzed potato starch (Connaught Laboratories), and the gel and tray buffers were made with Lithium-borate (pH8.1, 0.2M) and Tris-citric acid (pH8.3, 0.2M) as described by Scandalious (1969). The gels were run at 250V for 15 minutes, after which the wicks were removed. Electrophoresis was then continued at 250V for 5-6 hours until the borate front had traveled 8 cm towards the anode.

Female gametophyte and embryo tissue from each seed were assayed for six enzyme systems: Phosphoglucomutase (PGM), Phosphoglucose isomerase (PGI), 6-Phosphogluconate Dehydrogenase (6-PGD), Glutamate Dehydrogenase (GDH), Leucine aminopeptidase (LAP) and Glutamate-oxaloacetate transaminase (GOT). These six enzymes were chosen because they were found to give clear banding patterns for both female gametophyte and embryo tissue in previous work with loblolly pine in our laboratory. Staining recipes for PGM, PGI, 6-PGD and GDH were modified from Brewer (1970). In addition to slight modifications in amounts of chemicals, PGM differed in that 1 ml of 0.05% glucose-1, 6-diphosphate was added to the stain and in GDH, 0.1M Tris-HCl pH8.0 was used instead of the stain buffer given. In order to get clear banding for 6-PGD, 10 mg of TPN was added to each 500 ml of molten gel before degassing and to each 300 ml of buffer in the cathodal bridge tray. The assay stain for LAP is

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[1] Little and Trew (this proceedings)

identical to that given by Scandalious (1969). The stain for GOT consisted of 5 mg of Pyridoxal-5' - phosphate, 200 mg of L-Aspartic acid, 100 mg of A-Ketoglutaric acid and 50 mg of Fast blue BB salt in each 100 ml of 0.1M Tris-HC1 pH8.0 buffer.

Female gametophyte, the nutritive seed tissue of conifers, is haploid and therefore most useful in determining the genetics of isozyme band patterns. Isozymes coded by single genes in the female gametophyte appear as single bands on the gel and segregate in a 1:1 ratio among female gametophyte progeny of heterozygous individuals. In addition, when a gene is expressed in both the female gametophyte and embryo tissues of a seed, the female gametophyte can be of use in delineating the genotype of the embryo. This is true because both tissues are derived from mitotic divisions of the same haploid nucleus. Thus, the allelic contribution of the female to a heterozygous embryo genotype is expressed in the banding pattern of the female gametophyte.

The following strategy was employed in analyzing the available seedling material for isozyme genetic markers: 1. Many seedlings from each of the three pitch pine and four loblolly pine parental families were subjected to electrophoresis and stained for the six enzyme systems indicated above. 2. Isozyme phenotypes of female gametophytes and their segregation ratios in heterozygous parental families were used to determine the genetic control of the banding patterns and to assign isozyme genotypes to each parent. 3. Embryos from these predominantly open-pollinated families were analyzed to determine band pattern phenotypes of diploid genotypes in each species. 4. Embryo progeny of the hybrid crosses were subjected to electrophoresis and their isozyme band patterns examined in terms of the phenotypes and phenotypic ratios observed in the parental families.

## RESULTS

### Genetics of banding patterns in female gametophytes

The female gametophytic banding patterns for each of the six enzymes, occurring among seedlings of the seven parental families are diagrammed as phenotypes 1 and 3 in Figure 1. In addition to the bands shown, LAP and PGI stained for bands at one other zone, and GOT in two other zones of the gel. The bands in these other zones appeared to be under genetic control but independent of the bands diagrammed in Figure 1. Little segregation data exists for these bands at present and since the exact mode of their genetic control is not known, they will be ignored in the remainder of this report.

Female gametophytic banding patterns were strikingly similar in the two species. For each enzyme system studied, bands occurred in the same zones of the gel in both species and often had overlapping migration rates

For this reason, no effort will be made, in the discussion that follows, to identify a particular banding pattern with a particular species designation.

Progeny from parental families were either fixed for phenotypes 1 or 3 (Figure 1) or found to be segregating for each of the two single-banded types. Therefore, it is postulated that each of the six enzymes is coded by a single gene, each with two allelic forms present among the seven parents. This hypothesis is supported by the observed numbers of phenotypes 1 and 3 in segregating families (Table 1). In all cases, these numbers are consistent with the expected 1:1 ratio.

The six-locus genotype of each parent, as determined from the genotypic array of its progeny (female gametophytes), is presented in Table 2. Individuals claimed to be homozygous for any one gene, bred true for one band in all individuals sampled. No less than 28 individuals were sampled per family, making the probability of error in assigning parental genotypes, under the above hypothesis, essentially zero. Alleles are denoted by a three letter abbreviation of the enzyme they code and a superscript indicating relative migrational distance of the corresponding band. For example, $Got^{26}$ is the allele coding a GOT band which migrates to R. f. 0.26 (R.f. is the migrational distance of a band measured relative to the distance traveled by the borate front) on the gel (i.e., GOT phenotype 1 in Figure 1). Identical allelic designation of bands found in the separate species does not imply they are encoded by identical genes, but only signifies that the genes in both species produce isozymes of equal migration rate.

Banding patterns in embryos

All three phenotypes shown in Figure 1, for each enzyme, are present among embryos of the parental families. Phenotype 2 of each enzyme varied in the migration distances of the component bands but was always the same in the kind of banding pattern expressed (i.e., two bands, three bands or one thick band). These band patterns are consistent with the hypothesis that for each enzyme, a single gene controls the expression of isozymes in both female gametophyte and embryo tissue, and furthermore that embryo phenotypes, 1 and 3 are expressions of homozygous genotypes and phenotype 2 of heterozygotes. This hypothesis is supported by the following observations:

1. When an individual is fixed for phenotype 1 or 3 in female gametophytes, its embryo progeny either possesses the same phenotype (homozygous for the same allele) or possesses phenotype 2 (heterozygous for an alternate allele) but never both homozygous types (never phenotypes 1 and 3).

2. When an individual embryo has phenotype 1 or 3, it always has the same phenotype in the corresponding female gametophyte of the same seed.

3. With the exception of the GDH (to be discussed below), whenever an individual has phenotype 2, the corresponding female gametophyte

expresses either the slowest or fastest migrating band in the embryo. The other band or bands must be the result of the additional allele present in the heterozygote.

4. The band patterns of postulated heterozygotes (phenotype 2) are consistent with heterozygous expression of these enzymes in other organisms.

Heterozygotes have three kinds of band patterns among the six enzymes studied: two-banded, three-banded and a single thick band of intermediate migration. LAP and PGM are both two banded and the two bands correspond in migration distance exactly to that of the single-banded phenotypes of embryos homozygous for each of the alleles present in the heterozygote. This same kind of codominant, 2-banded pattern for heterozygotes has been found for LAP in knobcone pine (Conkle, 1971) and Maize (Scandalious 1969), and for PGM in Drosophila (Trippa, etal, 1970).

Three banded heterozygotes are found in 6-PGD, PGI and GOT. This band pattern is consistent with a dimeric enzyme structure with codominant expression of alleles and has been reported for 6-PGD in Mouse (Chapman 1975), for PGI in small fescue[1] and, for GOT in Maize (Scandalious, etal. 1975) and Scotch pine (Rudin, 1975). In dimeric enzymes, two polypeptide subunit molecules must interact to produce a functional enzyme. Heterozygotes, with two polypeptides formed, are expected to produce three isozyme bands, two of which are homodimers and a third band of intermediate migration (heterodimer or hybrid enzyme) resulting from the interaction of two different polypeptide subunits.

A single diffuse band occurring approximately midway between the position of homozygous band types occurs in heterozygotes of GDH. This same unusual band pattern has been reported for Maize GDH by Pryor (1974), who suggests three possible explanations for single banded heterozygotes. The most plausible of these is that GDH is a multimeric enzyme with many molecules needed for the enzyme to be functional. Under these circumstances, homodimeric isozymes would occur very rarely. Only the many heterodimeric forms of intermediate migration would occur in sufficient quantity to produce staining on the gel, giving the single wide intermediate band in GDH heterozygotes.

Therefore, the banding patterns diagrammed in Figure 1 are all of the expected phenotypes involving alleles present in these seven families. These expectations are based on the patterns revealed among individuals in each of the parental families.

Band patterns in hybrids

The results of the seven hybrid crosses are given in Table 3. In all cases, the band patterns resulting from hybrid genotypes are identical

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[1] Adams and Allard, The effect of polyploidy on phosphoglucose isomerase diversity in *Festuca microstachys* Nutt. (Manuscript in preparation).

to those postulated for the corresponding genotypes within species and as diagrammed in Figure 1. That is, hybrid heterozygotes for LAP and PGM are two-banded, for 6-PGD, PGI and GOT three banded and for GDH single banded, and in the same positions shown in Figure 1. In addition, the phenotypes found in the progeny of each cross were as predicted from the genotypes of the parents (for examples, see Figure 2). In all cases except one, the observed numbers of progeny types in segregating familes were consistent with the 1:1 ratio expected for crosses of heterozygotes X homozygotes (Table 3). A significant X (P<0.02) was obtained only when the observed numbers from the three segregating GOT crosses were combined. In 26 this case, the proportion of homozygous progeny (Got /Got ) was higher than expected. It is interesting to note that a similar finding for GOT was obtained in controlled crosses of *Pinus sylvestris* (Rudin 1975).

## DISCUSSION

Despite the fact that *P. taeda* and *P. rigida* are separate species, they must have very similar genes coding each of the six enzymes reported in this study. This is supported by the essentially identical band patterns in both species and the fact that genes from the different genomes are capable of producing polypeptide subunits that will interact in hybrids. The heterodimeric enzymes (intermediate migrating bands) found in heterozygous hybrid phenotypes of genes coding 6-PGD, PGI GOT and GDH are all the result of such interactions.

These marker genes do not give immediately recognizable hybrid phenotypes. That is, hybrids do not possess bands or band combinations that could not be found in individuals of either of the species considered singly. This is not to say, however, that such markers are not useful for studies of this hybrid, or that hybrid individuals cannot be delineated with such markers. Once the mutilocus genotype of each parent is known, the phenotypes of the progeny of any cross can be predicted. Thus, hybrids can be distinguished from individuals of non-hybrid origin when parents of one species carry alleles coding bands of different migration rate from those carried in the parents of the other species. Under these conditions, hybrid individuals will express heterozygous banding patterns which are not possible among crosses of parents within the species. Furthermore, the ability to delineate the *specific* parents of any seedling will increase as the number of alleles at each locus and number of marker loci increases.

As an example of how these markers might be used, let us assume there is an isolated clone bank consisting of the three pitch pine parents included in this study. Furthermore, assume that we want to use a pollen mix of the four loblolly parents o mass open-pollinate the pitch clones as has been suggested previously. Now, if we want to be able to determine

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[1] Little and Trew (this proceedings)

whether any one progeny seed is of hybrid origin or produced from pitch parents crossing among themselves, only the Gdh and Pgd genes are needed as rkers. All crosses among pitch individuals will produce Gdh$^{19}$/Gdh$^{9}$ Pgd /Pgd$^{57}$ progeny (refer to Table 2 however, most hybrid progeny will be heterozygous for the Gdh gene, Gdh /Gdh$^{25}$ and those individuals which are not (only possible when fertilization is by Gdh$^{19}$ pollen from loblolly parent 11-10) must be heterozygous for the Pgd gene (Pgd$^{52}$/Pgd$^{57}$). Thus, hybrid seed can always be distinguished in this case.

In some situations, it is also possible to determine the particular pollen parent of a hybrid seed. For instance, any seed carrying Pgm$^{59}$ in its embryo must have resulted from a cross with loblolly parent 7-56. However, only 50% of the seed from this pollen parent could definitely be identified based on this one gene, since one-half of the 7-56 pollen would carry the P gm$^{61}$ allele, which is common to all the other loblolly parents. Similar probabilities could be determined for the other pollen parents and would be useful in predicting frequencies of different hybrid types under random pollination. Such predictions could then be tested in actual crosses.

## ACKNOWLEDGEMENT

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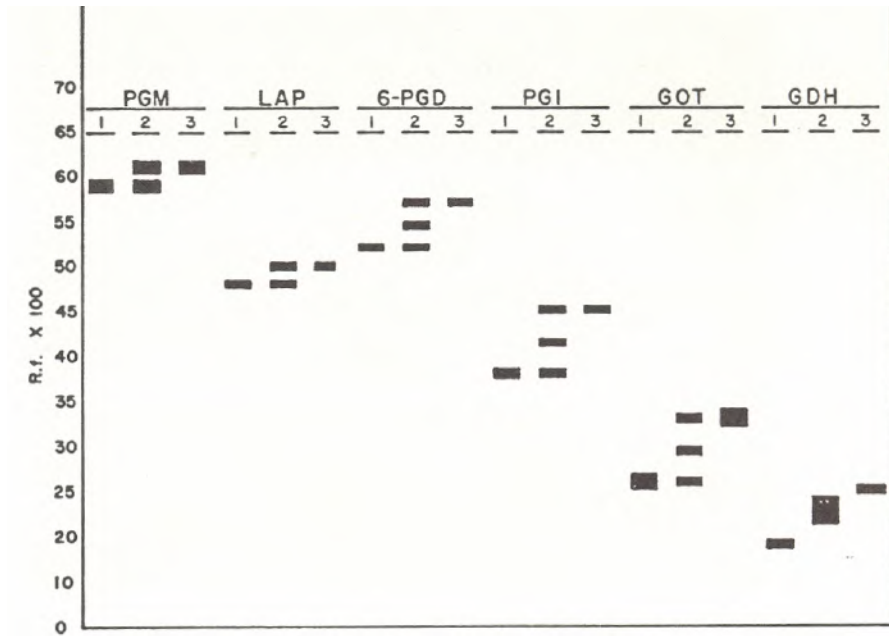


Figure 1. Banding patterns for six enzymes expressed in the seedling progeny of three _Pinus rigida_ and four _Pinus taeda_ parents. In extracts of female gametophyte tissue, only isozyme phenotypes 1 and 3 were found among the individuals sampled. In extracts of embryo tissue, all three phenotypes were found among the individuals sampled. Proposed female gametophyte genotypes (1N) corresponding to phenotypes 1 and 3, are left to right across the figure: $Pgm^{59}$, $Pgm^{61}$, $Lap^{48}$, $Lap^{50}$, $Pgd^{52}$, $Pgd^{57}$, $Pgi^{38}$ $Pgi^{45}$, $Got^{26}$, $Got^{33}$, $Gdh^{19}$, $Gdh^{25}$. Proposed embryo genotypes (2N), corresponding to phenotypes 1, 2 and 3, are left to right across the figure: $Pgm^{59}/Pgm^{59}$, $Pgm^{59}/Pgm^{61}$, $Pgm^{61}/Pgm^{61}$, $Lap^{48}/Lap^{48}$, $Lap^{48}/Lap^{50}$, $Lap^{50}/Lap^{50}$, $Pgd^{52}/Pgd^{52}$, $Pgd^{52}/Pgd^{57}$, $Pgd^{57}/Pgd^{57}$, $Pgi^{38}/Pgi^{38}$ $Pgi^{38}/Pgi^{45}$, $Pgi^{45}/Pgi^{45}$ $Got^{26}/Got^{26}$, $Got^{26}/Got^{33}$, $Got^{33}/Got^{33}$, $Gdh^{19}/Gdh^{19}$, $Gdh^{19}/Gdh^{25}$, $Gdh^{25}/Gdh^{25}$. R.f. is the migrational distance of each band measured relative to the distance covered (8.0 cm) by the borate front.

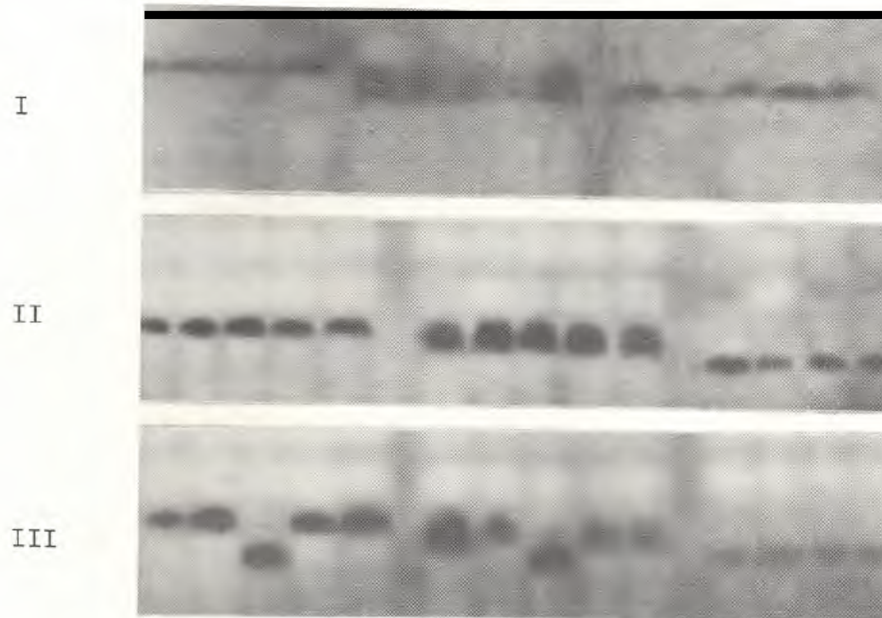


Figure 2. Photographs of three starch gels showing examples of isozyme band patterns of controlled hybrid crosses. Gel I was stained for 6-PGD and gels II and III stained for GDH. The parental designations and the seedling tissue assayed, are listed below for each of the three sets of band phenotypes, in the order they occur (from left to right), on each gel. Postulated parental genotypes are given in the parentheses. Gel I: Five female gametophyte samples of P. rigida parent 77($Pgd^{57}/Pgd^{57}$), five embryo samples of the cross 77x 11-10, and five female gametophyte samples of the P. taeda parent 11-10 ($Pgd^{52}/Pgd^{52}$). Gel II: Five female gametophyte samples of P. taeda parent 11-20 ($Gdh^{25}/Gdh^{25}$), five embryo samples of the cross 71 x 11-20, and four female gametophyte samples of P. rigida 71 ($Gdh^{19}/Gdh^{19}$). Gel III: Five female gametophyte samples of P. taeda parent 11-10 ($Gdh^{19}/Gdh^{25}$), five embryo samples of the cross 77 x 11-10, and four female gametophyte samples of the P. rigida parent 77 ($Gdh^{19}/Gdh^{19}$). Note: all bands migrated toward the anode (top of photos).

Table 2. Postulated parental genotypes.

| Parent | PGM | LAP | 6-PGD | PGI | GOT |
|---|---|---|---|---|---|
| | Enzyme gene | | | | |
| *P. taeda* | | | | | |
| 7-56 | $\frac{Pgm^{59}}{Pgm^{61}}$ | $\frac{Lap^{48}}{Lap^{50}}$ | $\frac{Pgd^{52}}{Pgd^{57}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{26}}$ |
| 11-9 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{50}}{Lap^{50}}$ | $\frac{Pgd^{52}}{Pgd^{52}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{26}}$ |
| 11-10 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{48}}{Lap^{48}}$ | $\frac{Pgd^{52}}{Pgd^{52}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{26}}$ |
| 11-20 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{48}}{Lap^{50}}$ | $\frac{Pgd^{52}}{Pgd^{57}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{33}}$ |
| *P. rigida* | | | | | |
| 62 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{50}}{Lap^{50}}$ | $\frac{Pgd^{57}}{Pgd^{57}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{26}}$ |
| 71 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{48}}{Lap^{50}}$ | $\frac{Pgd^{57}}{Pgd^{57}}$ | $\frac{Pgi^{38}}{Pgi^{45}}$ | $\frac{Got^{26}}{Got^{26}}$ |
| 77 | $\frac{Pgm^{61}}{Pgm^{61}}$ | $\frac{Lap^{50}}{Lap^{50}}$ | $\frac{Pgd^{57}}{Pgd^{57}}$ | $\frac{Pgi^{38}}{Pgi^{38}}$ | $\frac{Got^{26}}{Got^{26}}$ |

Table 2. Postulated parental genotypes.

| Parent | Enzyme gene | | | | | |
|---|---|---|---|---|---|---|
| | PGM | LAP | 6-PGD | PGI | GOT | GDH |
| *P. taeda* | | | | | | |
| 7-56 | $Pgm^{59}/Pgm^{61}$ | $Lap^{48}/Lap^{50}$ | $Pgd^{52}/Pgd^{57}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{26}$ | $Gdh^{25}/Gdh^{25}$ |
| 11-9 | $Pgm^{61}/Pgm^{61}$ | $Lap^{50}/Lap^{50}$ | $Pgd^{52}/Pgd^{52}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{26}$ | $Gdh^{25}/Gdh^{25}$ |
| 11-10 | $Pgm^{61}/Pgm^{61}$ | $Lap^{48}/Lap^{48}$ | $Pgd^{52}/Pgd^{52}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{26}$ | $Gdh^{19}/Gdh^{25}$ |
| 11-20 | $Pgm^{61}/Pgm^{61}$ | $Lap^{48}/Lap^{50}$ | $Pgd^{52}/Pgd^{57}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{33}$ | $Gdh^{25}/Gdh^{25}$ |
| *P. rigida* | | | | | | |
| 62 | $Pgm^{61}/Pgm^{61}$ | $Lap^{50}/Lap^{50}$ | $Pgd^{57}/Pgd^{57}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{26}$ | $Gdh^{19}/Gdh^{19}$ |
| 71 | $Pgm^{61}/Pgm^{61}$ | $Lap^{48}/Lap^{50}$ | $Pgd^{57}/Pgd^{57}$ | $Pgi^{38}/Pgi^{45}$ | $Got^{26}/Got^{26}$ | $Gdh^{19}/Gdh^{19}$ |
| 77 | $Pgm^{61}/Pgm^{61}$ | $Lap^{50}/Lap^{50}$ | $Pgd^{57}/Pgd^{57}$ | $Pgi^{38}/Pgi^{38}$ | $Got^{26}/Got^{26}$ | $Gdh^{19}/Gdh^{19}$ |

Table 3. Embryo segregation patterns for progeny of pitch x loblolly pine hybrid crosses.

| Parents | | Genotype and Observed Numbers | | | Deviation[1] | | Genotype and Observed Numbers | | | Deviation | | Genotype and Observed Numbers | | | Deviation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P. rigida | P. taeda | | | | $X^2$ | Prob. | | | | $X^2$ | Prob. | | | | $X^2$ | Prob. |
| | | $Pgm^{59}/Pgm^{61}$ | $Pgm^{61}/Pgm^{61}$ | N | | | $Lap^{48}/Lap^{50}$ | $Lap^{50}/Lap^{50}$ | N | | | $Pgd^{52}/Pgd^{57}$ | $Pgd^{57}/Pgd^{57}$ | N | | |
| 62 x | 7-56 | 11 | 21 | 32 | 2.53(1) | P>0.10 | 7 | 13 | 20 | 1.25(1) | P>0.20 | 14 | 18 | 32 | 0.281(1) | P>0.50 |
| 62 x | 11-9 | - | 6 | 6 | - | - | - | 16 | 16 | - | - | 15 | - | 15 | - | - |
| 62 x | 11-10 | - | 25 | 25 | - | - | 12 | - | 12 | - | - | 36 | - | 36 | - | - |
| 62 x | 11-20 | - | 6 | 6 | - | - | 5 | 6 | 11 | 0(1) | P>0.90 | 18 | 18 | 36 | 0(1) | P>0.90 |
| 71 x | 11-20 | - | 12 | 12 | - | - | - | - | - [2] | - | - | 24 | 15 | 39 | 1.64(1) | P>0.10 |
| 77 x | 11-10 | - | 14 | 14 | - | - | 11 | - | 11 | - | - | 28 | - | 28 | - | - |
| 77 x | 11-20 | - | 15 | 15 | - | - | 3 | 8 | 11 | 1.46(1) | P>0.20 | 19 | 17 | 36 | 0.028(1) | P>0.80 |
| Data combined over[3] all segregating families | | 11 | 21 | 32 | 2.53(1) | P>0.10 | 15 | 27 | 42 | 2.88(1) | P>0.05 | 75 | 68 | 143 | 0.252(1) | P>0.50 |
| | | $Pgi^{38}/Pgi^{38}$ | $Pgi^{38}/Pgi^{45}$ | N | | | $Got^{26}/Got^{26}$ | $Got^{26}/Got^{33}$ | N | | | $Gdh^{19}/Gdh^{19}$ | $Gdh^{19}/Gdh^{25}$ | N | | |
| 62 x | 7-56 | 33 | - | 33 | - | - | 33 | - | 33 | - | - | - | 33 | 33 | - | - |
| 62 x | 11-9 | 32 | - | 32 | - | - | 32 | - | 32 | - | - | - | 32 | 32 | - | - |
| 62 x | 11-10 | 42 | - | 42 | - | - | 41 | - | 41 | - | - | 16 | 25 | 41 | 1.56(1) | P>0.20 |
| 62 x | 11-20 | 35 | - | 35 | - | - | 20 | 16 | 36 | 0.250(1) | P>0.50 | - | 33 | 33 | - | - |
| 71 x | 11-20 | 14 | 25 | 39 | 2.56 | P>0.10 | 26 | 13 | 39 | 3.69(1) | P>0.05 | - | 38 | 38 | - | - |
| 77 x | 11-10 | 30 | - | 30 | - | - | 30 | - | 30 | - | - | 15 | 15 | 30 | 0(1) | P>0.90 |
| 77 x | 11-20 | 42 | - | 42 | - | - | 26 | 16 | 42 | 1.93(1) | P>0.10 | - | 42 | 42 | - | - |
| Data combined over all segregating | | 14 | 25 | 39 | 2.56(1) | P>0.10 | 72 | 45 | 117 | 5.78(1) | P<0.02 | 31 | 40 | 71 | 0.901(1) | P>0.30 |

1. All deviation $X^2$'s calculated with Yates correction for continuity (Steele and Torrie, 1960).
2. No data on Lap available for this cross (71 x 11-20).
3. In all cases where data is combined over families, the heterogeneity $X^2$ has a probability greater than 0.20.