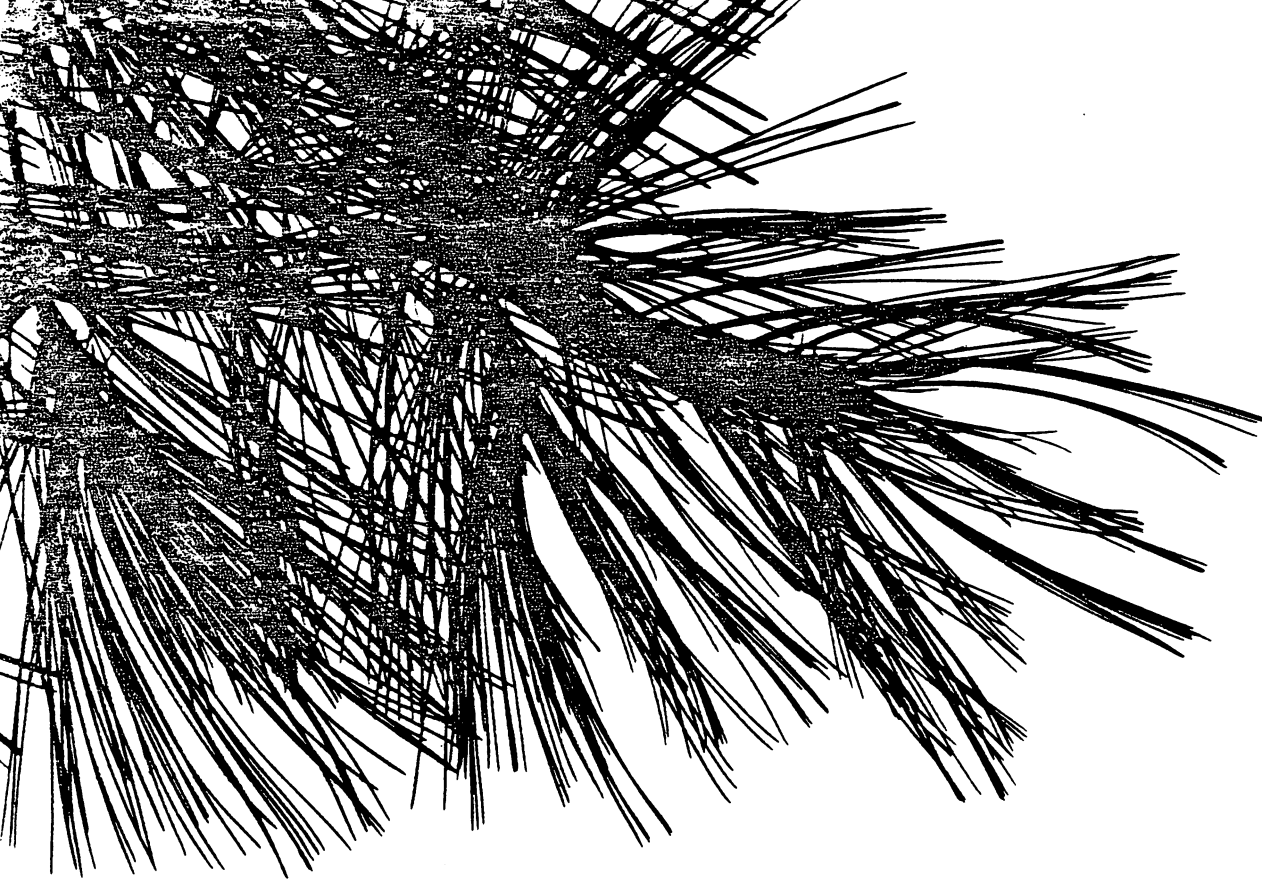
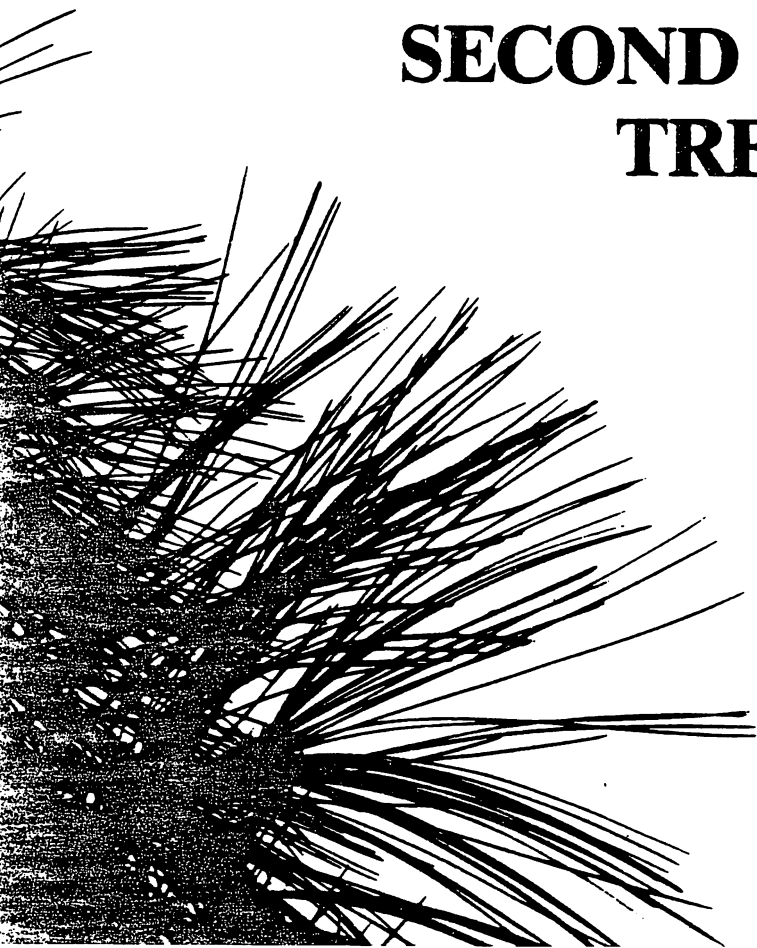




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ALLOZYMES OF LINKED LOCI SEGREGATE NORMALLY IN SEEDS
OF AN AUSTRIAN X JAPANESE RED PINE HYBRID

M. Thompson Conkle and James J. Tobolski^{1/}

Abstract.--Meiosis in an F₁ hybrid of Pinus nigra and P. densiflora, which was polymorphic for seven enzyme loci, was examined. To test recombination, 108 seeds were analyzed by using starch gel electrophoresis of extracts from the haploid tissue of female gametophytes. Three loci segregated independently: acid phosphatase 1, glutamic oxaloacetic transaminase 3, and glucose-6-phosphate dehydrogenase 1/2. The four linked loci were alcohol dehydrogenase 1, alcohol dehydrogenase 2, glucose-6-phosphate dehydrogenase 2, and leucine aminopeptidase 2, arranged in that order. The recombination percentages between linked loci were 9, 12, and 14, respectively. The order and recombination percentages of the linked loci were similar to values reported for P. sylvestris, another pine in the subsection Sylvestres, as well as for species in other subsections of the genus. The hybrid provides a means of enriching genetic variability by producing allelic combinations that do not exist in natural populations.

Additional keywords: Pinus nigra, Pinus densiflora.

Interracial and interspecific hybrids are the principal means for enriching genetic variation in forest tree populations. Fertile hybrids with gametes containing numerous new allele combinations are good materials for recurrent selection for trees with associations of traits that do not exist in natural populations.

The hybrid of Austrian pine (Pinus nigra Arn. var. austriaca (Hoess) Aschers. and Graebn.) and Japanese red pine (P. densiflora Sieb. and Zucc.) unites chromosomes from two species that are geographically separated by 5,000 miles. Crosses between these two species yielded the first control pollinated pine hybrid (Austin 1927).

Breeding barriers between these two species are difficult to assess. Sound seed set per cone from controlled pollinations of Pinus nigra with P. densiflora pollen was only 25 percent of that from P. nigra crosses (Wright and Gabriel 1958). In a southern Michigan plantation, Wright et al. (1969) found spontaneously produced hybrids from wind pollination of P. nigra trees adjacent to a plantation of P. densiflora. Those hybrids reached proportions

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as high as 92 percent of total seed set. But hybrid embryos are not favored in controlled crosses with pollen mixes. When P. nigra and P. densiflora pollen mixes in known proportions were applied to P. nigra parents, nonhybrid embryos outnumbered hybrid embryos (Tobolski and Conkle 1977).

Interspecies hybrids of Austrian and Japanese red pines planted in the Lake States Region differ in growth traits, tolerance to road salt, and susceptibility to pests (Wright et al. 1969). The F_1 hybrids grow faster than either parental species and could be improved by the addition of tolerance and resistance traits.

Selection for new combinations of desirable traits, by recurrent mass selection starting from a population of F_1 hybrids or by backcrossing to a parental species, requires fertile hybrids with normal meiosis. We have found these Austrian X Japanese red pine hybrids to be highly fertile.

This paper reports a study of meiosis in an F_1 hybrid, P. nigra X P. densiflora, by testing recombination among enzyme loci using starch gel electrophoresis of extracts of seeds.

LINKAGE TEST CONDITIONS

An earlier study determined the genotypes of trees of the two parent species and spontaneously produced hybrids (Tobolski and Conkle 1977). One of the F_1 hybrids was polymorphic for seven allozyme loci: acid phosphatase 1 (ACPH-1), alcohol dehydrogenase 1 and 2 (ADH-1, ADH-2), glucose-6-phosphate dehydrogenase 1/2 and 2 (G6PD-1/2, G6PD-2), glutamic oxaloacetic transaminase 3 (GOT-3), and leucine aminopeptidase 2 (LAP-2).

To test patterns of segregation and linkage, 108 seeds from this hybrid were analyzed by using starch gel electrophoresis of extracts from the haploid tissue of female gametophytes (Conkle 1972). This tissue, which surrounds embryos within individual seeds, develops from a haploid nucleus that is a product of meiosis. The haploid nucleus divides repeatedly, and at maturity the products of these divisions fill the ovule and produce eggs within archegonia.

Different alleles from gametophytes of a tree heterozygous for enzyme loci segregate with simple Mendelian ratios (Bergmann 1974). We tested linkage by using the frequencies of alleles in pairwise combinations. For heterozygous loci, we noted the faster allele by a 1 and the slower allele by a 2. For any two heterozygous genes, there are four allele combinations: gene one--fast, gene two--fast (1,1); gene one--fast, gene two--slow (1,2); gene one--slow, gene two--fast (2,1); gene one--slow, gene two--slow (2,2). With free recombination between two loci, the allele pairs occur with equal frequency, $f(1,1) = f(1,2) = f(2,1) = f(2,2)$. When genes are linked, two combinations have frequencies lower than those expected and two have frequencies higher than those expected (the combined frequency of 1,1 + 2,2 does not equal that of 1,2 + 2,1). When these two combined classes are unequal, the smaller of the two is assumed to be the recombinant class. The number of gametes in the recombinant class divided by the total number of gametes gives the recombination fraction. Recombination values range from 0.0 to 0.50--0.0 when two genes are totally linked and no recombinants are found, and 0.50 when the two genes recombine freely.

LINKAGE IN THE HYBRID

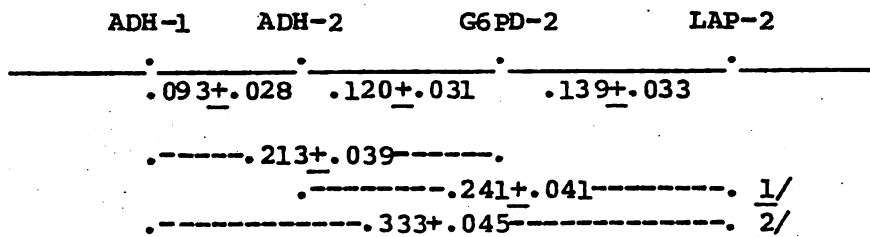
According to our gene notation for the seven polymorphic loci in the hybrid, the most anodal band was the number 1 locus and bands toward the cathodal edge of the gel were successive loci. Most pines have only two loci for G6PD, but in this test three sets of bands corresponded to three loci in the hybrid. We showed the similarity with other species' notation by naming the common zones G6PD-1 and G6PD-2, and the faintly staining middle zone G6PD-1/2.

GOT-3 was the only gene in which segregation deviated significantly from a 1:1 ratio (chi-square, 1 degree of freedom = 7.26). The observed ratio of fast to slow alleles was 40:68. This deviation did not preclude linkage tests for this locus, since recombination fractions are not disturbed when only one locus of a pair deviates from 1:1 segregation.

The seven loci provided 21 tests of linkage (table 1). GOT-3, ACPH-1, and G6PD-1/2 segregated independently. The six linkages that deviated significantly from 1:1 segregation involved ADH-1, ADH-2, G6PD-2, and LAP-2. Those genes formed a single linkage block (fig. 1).

Gene Mapping

The order of genes in the block was deduced from the recombination values (table 1). ADH-1 was .093 recombination units from ADH-2, which was .120 from G6PD-2. The order of these three genes was established on the basis of the distance of .213 between ADH-1 and G6PD-2. The position of LAP-2 in the block was determined in a similar manner.



1/ .259_±.042, including the double recombinant.

2/ .352_±.046, including the double recombinant.

Figure 1.--Four loci in an F₁ hybrid of Pinus nigra X P. densiflora formed a single linkage block.

The sum of the values for the middle (ADH-2 to G6PD-2) and right (G6PD-2 to LAP-2) segments was greater than the recombination value for the region ADH-2 to LAP-2, due to a double recombinant in the region. Double and triple recombinants are in proportion to the products of the smaller recombination fractions only when recombination in one chromosome segment is independent of events in other segments. The expectation for double recombinants in the left and middle segments was 1.2 (.093 X .120 X 108 seeds). For the left and right segments the expectation was 1.4, and for the middle and right segments, 1.8.

Table 1.--Frequencies of gametophytes in different allele pair classes and recombination proportions for 21 pairs of loci from a Pinus nigra X P. densiflora hybrid.

Loci pairs (gene 1 - gene 2)	Gametophytes in each allele pair class <u>1/</u>				Recombination fraction <u>2/</u>
	1,1	1,2	2,1	2,2	
GOT-3 - ACPH-1	18	22	34	34	.481
GOT-3 - LAP-2	23	17	36	32	.491
GOT-3 - ADH-2	15	25	30	38	.491
GOT-3 - G6PD-1/2	23	17	34	34	.472
GOT-3 - G6PD-2	15	25	31	37	.481
GOT-3 - ADH-1	19	21	30	38	.472
ACPH-1 - LAP-2	28	24	31	25	.491
ACPH-1 - ADH-2	22	30	23	33	.491
ACPH-1 - G6PD-1/2	24	28	33	23	.435
ACPH-1 - G6PD-2	22	30	24	32	.500
ACPH-1 - ADH-1	23	29	26	30	.491
LAP-2 - ADH-2	11	48	34	15	<u>.241</u>
LAP-2 - G6PD-1/2	27	32	30	19	.426
LAP-2 - G6PD-2	6	53	40	9	<u>.139</u>
LAP-2 - ADH-1	18	41	31	18	<u>.333</u>
ADH-2 - G6PD-1/2	24	21	33	30	.500
ADH-2 - G6PD-2	39	6	7	56	<u>.120</u>
ADH-2 - ADH-1	42	3	7	56	<u>.093</u>
G6PD-1/2 - G6PD-2	27	30	19	32	.454
G6PD-1/2 - ADH-1	29	28	20	31	.444
G6PD-2 - ADH-1	36	10	13	49	<u>.213</u>

1/ 1 = fast allele, 2 = slow.

2/ Recombination fractions deviating significantly from .50 are underlined.

In all, 4.4 double recombinants were expected and only one was recovered. Fewer double recombinants are observed than are theoretically expected because of chromosome interference, which restricts multiple crossovers.

Identifying Parental Chromosomes

Recombination between ADH-2 and ADH-1 showed 42 gametophytes in the 1,1 class and 56 in the 2,2 class (table 1). The sum of the gametophytes (98) in these two classes indicated that these were the parental classes, and 1,2 and 2,1 (with a sum of 10 gametophytes) were the recombinants. Therefore, the allele arrangements on the parental chromosomes were: ADH-1(1)--ADH-2(1) for one strand and ADH-1(2)--ADH-2(2) for the other. Parental classes for all combinations of ADH-1, ADH-2, and G6PD-2 were 1,1 and 2,2. The 1,2 and 2,1 classes were parental combinations for linkages involving LAP-2. Therefore, the arrangements of alleles for ADH-1, ADH-2, G6PD-2, and LAP-2 on the two parental strands were 1--1--1--2 and 2--2--2--1 (fig. 2).

ADH-1	ADH-2	G6PD-2	LAP-2	
2(59)	2(63)	2(62)	1(59)	<u>P. nigra</u>
1(49)	1(45)	1(46)	2(49)	<u>P. densiflora</u>

Figure 2.--Arrangement of fast (1) and slow (2) alleles and segregants recovered (in parentheses) for linked loci in the F_1 hybrid.

P. densiflora and P. nigra differ in alleles at the ADH-2 locus (Tobolski and Conkle 1977); this difference is useful in assigning the two chromosome strands of the hybrid to the respective parental species. All P. densiflora tested from the southern Michigan plantation were monomorphic for allele 1 of the ADH-2 locus. Three alleles in P. nigra migrated slower than allele 1 in P. densiflora. Thus, the parental strand with ADH-2(1) in the hybrid came from P. densiflora (fig. 2).

P. densiflora genes in the linkage block are underrepresented in the progeny of the hybrid (fig. 2). Segregation values for alleles from the four loci were pooled by species to determine if the alleles from each species were equally represented in the female gametes of the F_1 hybrid. Proportions for P. densiflora alleles ranged from .417 for ADH-2 to .454 for ADH-1 and LAP-2. Though the single locus segregation values did not vary significantly from expected 1:1 ratios, the mean combined value for the four linked loci was $.437 \pm .019$ for P. densiflora alleles. This deviation from equal representation, though significant, does not preclude the recovery of numerous new allele combinations.

SIGNIFICANCE OF THE RESULTS

The block of genes in the F_1 hybrid is similar to genes in Pinus sylvestris (Rudin and Ekberg 1978), which is in the same subsection of the genus (Sylvestres) as P. nigra and P. densiflora. Three loci in the hybrid, ADH-1, ADH-2, and LAP-2, are also linked in P. sylvestris (G6PD-2 has not been reported for P. sylvestris). In P. sylvestris, ADH-1 and ADH-2 are linked, with no recombination in a sample of 120 gametophytes, and the ADH loci have an average recombination fraction of $.309 \pm .025$ with LAP-2. Thus, the loci that can be compared among the three species are all in the same linkage block, both ADH loci are closely linked at similar distances from LAP-2 in each species, and GOT-3 and ACPH-1 are not linked with ADH-2 in the hybrid or in P. sylvestris.

The comparison can be extended to include species in other subsections of Pinus--P. attenuata, P. contorta, P. jeffreyi, P. lambertiana, and P. taeda (Conkle 1981). Recombination fractions between ADH-2 and LAP-2 range from .258 to .362 for these five species. The value for the Austrian X Japanese red pine hybrid is .259, within the range for the other species. ADH-1 is closely linked with ADH-2 in three of the five species. G6PD-2 is located in the middle of the segment between ADH-2 and LAP-2 in at least three of these species. Thus, the order and the recombination fractions between loci are similar for this hybrid and species in the genus. Similar gene order and recombination fractions in different pines indicate that speciation has not been accompanied by major chromosome rearrangements of this chromosome segment.

Alleles from P. nigra and P. densiflora recombined in the gametes of an F_1 hybrid to produce combinations of alleles that are not possible in pure species populations. Meiosis is normal, and the recombination of alleles at linked loci of this hybrid supports ongoing breeding efforts that are using a population of F_1 hybrids as a source of genetic variability from which to start recurrent selection for rapid growth, salt tolerance, and pest resistance.

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