conclusive, support this principle but, based on the definitions of HALL (1984) the HAM shoots do not represent an ativistic development.

References

ABBE, E. C.: Studies on the phylogeny of the *Betulaceae*. II. Extremes in the range of variation of floral and inflorescence morphology. Bot. Gaz. **99**: 431—469 (1938). — ABBE, E. C.: Flowers and inflorencenses of the *'amentiferae'*. Bot. Rev. **40** (2): 159—261 (1974). — BLOCH, R.: In Brookhaven Symposia in Biology No. 6. Abnormal and pathological plant growth. Biology Dept. Brookhaven Nat. Laboratory (1953). — BROWN, I. R., KENNEDY, D. and WILLIAMS, D. A.: The occurrence of natural hybrids between *Betula pendula* ROTH. and *B. pubescens* EHRH. Watsonia **14**: 133—145 (1982). VON GOETHE, J. W.: Translation by E. M. COX 1863. Essay on the metamorphosis of plants. Jour. Bot. **1**: 327—345, 360—374 (1970). — HALL, B. K.: Developmental mechanisms underlying the formation of atavisms. Biol. Rev. **59**: 89—124 (1984). — HESLOP-HARRISON, J.: The experimental modification of sex expression in flowering plants. Biol. Rev. **32**: 38—90 (1957). — MASTERS, M. T.: Vegetable Teratology. Ray Society (1968). — MEYER, V. G.: Flower abnormalities. Bot. Rev. **32**: 165—218 (1966). — PEACE, T. R.: Pathology of trees and shrubs. Oxford, Clarendon Press (1962). — PHILLIPS, D. H. and BURDEKIN, D. A.: Diseases of Forest and Ornamental Trees. The Macmillan Press Ltd (1982). — TOLSTOPYATCHENKO, A. I.: Bisexual inflorescences in *Betula* species. Botanicheskii Zhurnal 59, **12**: 1834—1844 (1974). — WARDLAW, C. W.: The floral meristem as a reaction system. Proc. Roy. Soc. Edinb. Sec. B. **66**: 394—408 (1957). — WORSDELL, W. C.: The Principles of Plant Teratology 2 vols. The Ray Society, London (1915—16).

Inheritance of Allozymes in a Black Spruce Diallel Cross

By J. A. PITEL[1]), W. M. CHELIAK[1, 3]) and J. BARRETT[2])
Petawawa National Forestry Institute,
Chalk River, Ontario K0J 1J0,
Canada

(Received 30th October 1985)

Summary

Methods developed in our laboratory for extracting and characterizing electrophoretic variants of enzymes from needle tissues were used in this study for the analysis of the genetic control of allozyme variants of a 7 × 7 black spruce diallel cross. From this controlled mating scheme 1,066 progeny were analyzed. Of the 16 enzymes tested, seven (AAT, ACO, GDH, LAP, MDH, PGM, and SDH) produced phenotypes that were variable and had banding patterns that possessed good activity and high resolution. For one of these enzymes (AAT), two variable loci were present.

Key words: allozymes, inheritance, *Picea mariana*, pedigree certification, electrophoresis, somatotyping.

Zusammenfassung

In dieser Studie wurden in unserem Labor entwickelte Methoden zur Gewinnung und Charakterisierung elektrophoretischer Enzymvarianten aus Nadelgeweben zur Analyse der genetischen Kontrolle von Allozymvarianten eines 7 × 7 Kreuzungs-Diallels mit *Picea mariana* angewandt. Aus diesem kontrollierten Kreuzungsschema wurden 1066 Nachkommen analysiert. Von den 16 getesteten Systemen produzierten 7 (AAT, ACO, GDH, LAP, MDH, PGM und SDH) Phänotypen, die variabel waren und Bandmuster hatten, die eine gute Aktivität und eine hohe Festigkeit besaßen. Für eines dieser Enzymsysteme (AAT) waren 2 variable Loci vorhanden.

Introduction

Black spruce (*Picea mariana* [MILL.] B.S.P.) grows in all 10 provinces and two territories of Canada. It is a commercially important species, as the long fibres make this tree highly desirable to the pulpwood industry. A specific mating design, the diallel cross, was initiated by MORGENSTERN (1974) at the Petawawa National Forestry Institute for the purpose of evaluating variance components such as those due to selfing, maternal, and reciprocal effects. In this report we examine the isozyme composition of progeny from a 7 × 7 black spruce diallel cross that was established at Petawawa in 1973. We used techniques that were developed in our laboratory for the extraction of enzymes from vegetative tissues of conifers (PITEL and CHELIAK, 1984; CHELIAK and PITEL, 1984a). The purpose of the study was to demonstrate the genetic control of allozyme polymorphisms at seven loci of black spruce. In addition, we describe other enzyme systems of black spruce that either lacked variation or whose starch gel phenotypes were difficult to interpret. Isozyme analysis also indicated that some progeny of a cross did not have the expected genotype compositions, suggesting contamination. The use of isozymes as simple ge-

FEMALE PARENT

MALE PARENT	29	52	59	60	62	63	65
29	M	14	M	16	17	18	19
52	M	21	22	23	24	25	26
59	27	28	29	30	31	32	33
60	34	35	36	37	38	39	40
62	41	42	43	44	45	46	47
63	48	49	50	51	52	53	54
65	55	56	57	58	59	M	61

Figure 1. — Design of the controlled mating scheme used for seven black spruce trees and the cross numbers allocated to each cross. "M" indicates that the cross was missing.

[1]) Canadian Forestry Service, Petawawa National Forestry Institute, Chalk River, Ontario K0J 1J0, Canada

[2]) Lakehead University, School of Forestry, Thunder Bay, Ontario, P7B 5E1, Canada

[3]) Associate Professor, University of Alberta, Edmonton, Alberta, Canada, T6G 2H1

netic markers may thus play a role in maintaining the identity and genetic integrity of breeding stocks.

Materials and Methods

Diallel cross design

Controlled matings, involving seven black spruce trees growing at the Petawawa National Forestry Institute (Lat. 45°59'N and Long. 77°21'W), were performed in the spring of 1970 (*Figure 1*). Of the possible 49 two-way combinations, 45 were successfully crossed. Self-pollinations and biparental crosses were made inside standard pollination bags.

Mature control-pollinated cones were harvested in the fall of 1970 and the seeds extracted. Seed was stored at −18° C until used for sowing in the greenhouse in March, 1971. In July, 1971, seedlings were transferred to the nursery. Seedlings were then planted in spring, 1973, in the field at the Petawawa National Forestry Institute. Planting was by a random block design involving 27 trees for each cross. Each cross consisted of three separate blocks with nine trees within each block.

Tissue collection and preparation

Needles were collected from each tree in March 1984, and stored at 4° C in plastic bags for up to three months until completion of electrophoretic analyses. Gametophytes were also examined from seeds of each of the seven parental trees.

Needle tissue was cut into small pieces and 100 mg were homogenized with 0.5 ml of the needle extraction buffer as described by Cheliak and Pitel (1984a, b). Fine purified sand was added to the chilled pestle and mortar to ensure optimal tissue extraction. Each extract was then placed in a 0.5 ml polystyrene sample cup. For, gametophytes, these were placed individually in the cups, together with 50 μl of seed extraction buffer (Yeh and O'Malley, 1980) and then homogenized using a teflon head fitted into a motorized grinder. After homogenization, crude seed and needle homogenates were absorbed onto 11 × 1.5 mm wicks cut from Whatman 3MM filter paper.

Electrophoresis and enzyme detection

The composition of gel and electrode buffers, the electrophoretic conditions, and the type and concentration of starch used were as described by Cheliak and Pitel (1984b). The following enzymes displayed isozyme variation, and were routinely assayed: aconitase (ACO, EC 4.2.1.3), aspartate aminotransferase (AAT, EC 2.6.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.3), leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), and phosphoglucomutase (PGM, EC 2.7.5.1). Although shikimate dehydrogenase (SDH, EC 1.1.1.25) displayed isozyme variability, technical difficulties precluded assay of all progeny. ACO, MDH, and SDH were run using the histidine/citrate gel and electrode buffer systems as decribed by Cheliak and Pitel (1984a, b), while the other five were run using the lithium/borate buffer systems. The composition of the staining mixtures for each enzyme was as decribed by Cheliak and Pitel (1984a).

Statistical analysis

The maximum likelihood log-linear G test (Sokal and Rohlf, 1969) was used to test for agreement of the observed to expected segregation ratios in the progeny of the controlled crosses. A heterogeneity $G(G_{II})$ was calculated from the sum of each cross and the pooled distribution of all crosses ($G_H = G_T - G_p$, where G_p is the pooled test statistic, and G_T the total test statistic given by $G_T = \Sigma_i G_i$, i = 1, . . . , n; where n is the number of distributions tested) for a particular expected distribution.

Results and Discussion

Monomorphic enzymes

Enzymes with no observed variations in banding patterns among the seven parental trees, or any of the progeny, included isocitrate dehydrogenase (IDH, EC 1.1.1.42) and phosphoenolpyruvate carboxylase (PEP-C, EC 4.1.1.31).

Polymorphic enzyme zones

Among the seven parental trees or their progeny, eight enzyme systems were inferred to be variable and easy to score. These were AAT-2, AAT-3, ACO, GDH, LAP-1, MDH-3, PGM, and SDH. In addition, variation was observed for aldolase (ALD, EC 4.1.2.13), MDH-4, and 6-phosphogluconate dehydrogenase (loci 1 and 2) (6PGD, EC 1.1.1.44), but these were difficult to score consistently. Some variability among the progeny was detected for phosphoglucose isomerase (locus-2) (PGI, EC 5.3.1.9) but this variability did not appear to be heritable.

Aspartate aminotransferase

Three zones of activity were observed on gels stained for AAT, with variation among parent trees being present for the two slowest zones, AAT-2 and AAT-3 (*Figure 2*). These results differ from that of a 5 × 5 white spruce, dial-

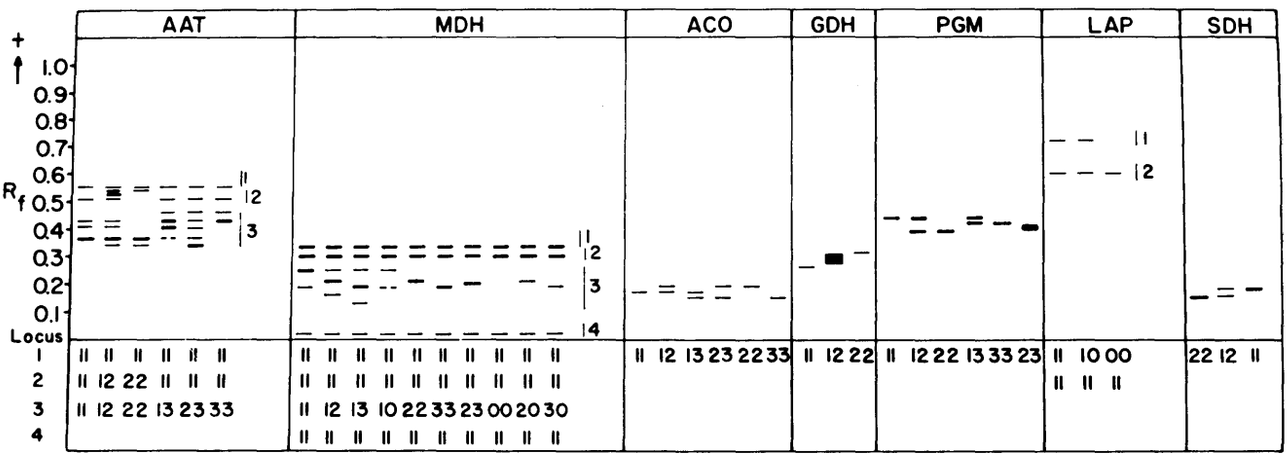

Figure 2. — Representative banding patterns for seven variable enzyme systems in black spruce and their genotypes.

lel (Cheliak and Pitel, 1984b) in which variation among parent trees was observed only in the fastest zone (AAT-1). In another study with black spruce (Boyle and Morgenstern, 1985) only two loci were present for AAT. For AAT-2, six of the parent trees displayed the same band (*Table 2, Figure 2*), while for the seventh tree (62) three bands were present. This last tree was inferred to be heterozygous. Crosses among trees with a single band resulted in progeny with only a single band. Crosses among trees with a single band and the inferred heterozygote produced progeny with either single- or triple-banded phenotypes in exactly equal proportions (*Table 2*). Heterogeneity G values were not significant. The one cross (41) that indicated a significant departure from that expected is likely due to chance. The intermediate band had approximately double the staining intensity of the upper and lower single bands, indicating a dimeric subunit structure for AAT-2. Selfing of the heterozygous tree (62) resulted in three classes of progeny in approximately a 1:2:1 ratio (*Table 2*). These results suggest that this region is under the control of a single locus.

For AAT-3, three different gel phenotypes were observed among the seven parental trees (*Figure 2, Table 2*). Five of the seven parent trees carried the same three-banded phenotypes. It was hypothesized that these trees were homozygous for AAT-3. Crosses among these trees, as well as the selfed lines, produced progeny with the same three-banded phenotypes. Crosses of parental trees of the three-banded phenotype with trees of either of the parental four-banded phenotypes (trees 59 or 62) resulted in close agreement of observed and expected segregation in the progeny (*Table 2*). The two trees with four-banded phenotypes are inferred to be heterozygous. Crosses of these two parental trees (59 and 62) resulted in four classes of progeny in approximately a 1:1:1:1 ratio (*Table 2*). The progeny from the selfing of these two parental trees conformed to Mendelian expectations and resulted in three types with a 1:2:1 ratio (*Table 2*). These patterns suggest that this region is controlled by a single gene, AAT-3.

Table 1. — Inferred genotypes for seven black spruce parental trees.

ENZYME LOCUS	Parent tree*)						
	29	52	59	60	62	63	65
Aat-2	11	11	11	11	12	11	11
Aat-3	11	11	13	11	12	11	11
Aco	11	11	13	12	11	11	11
Gdh	11	11	11	11	11	11	12
Lap-1	11	11	10	10	10	10	11
Mdh-3	11	11	11	11	12	13	10
Pgm	11	13	11	13	12	11	11

*) The representative banding patterns for each inferred parental genotype are shown in *Figure 2.*

Aconitase

One zone of activity was observed on gels stained for ACO (*Figure 2*). Five parental trees (29, 52, 62, 63, and 65) stained for one single band and were thus inferred to be homozygotes (*Table 1*). One parental tree (60) had two bands, one of which had the same mobility as the above trees and another band of faster mobility. The last parental tree (59) also had two bands, one of which was common to the above five trees, but with the other band being of slower mobility. These last two parental trees (59 and 60) are inferred to be heterozygotes. Crosses among homozygous trees produced progeny with only one band coincident with the mobility of

Table 2. — Goodness-of-fit tests for single-locus segregation patterns at seven loci in black spruce.

ENZYME LOCUS	CROSS TYPE	NO. CROSSES	OFFSPRING GENOTYPES																			G-TESTS	
			OBSERVED										EXPECTED									LACK OF FIT	OVERALL HETEROGENEITY
			11	12	22	13	33	23	10	00	20	30	11	12	22	13	33	23	10	00	20	30	
Aat-2	11x12	12	154	154	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0.00(1)	10.15(11)*)
	12x12	1	4	7	7	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	1.73(2)	-
Aat-3	11x12	10	137	117	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1.58(1)	2.33(9)
	12x12	1	4	11	3	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	1.04(2)	-
	11x13	9	121	0	0	99	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2.20(1)	5.58(8)
	13x13	1	3	0	0	10	8	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	2.41(2)	-
	12x13	2	15	12	0	10	0	17	0	0	0	0	1	1	0	1	0	1	0	0	0	0	2.17(3)	5.00(3)
Aco	11x12	9	121	107	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0.86(1)	5.33(8)
	12x12	1	5	16	2	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	4.95(2)	-
	11x13	9	115	0	0	107	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.29(1)	5.35(8)
	13x13	1	6	0	0	12	3	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	1.45(2)	-
	12x13	2	12	15	0	9	0	15	0	0	0	0	1	1	0	1	0	1	0	0	0	0	2.03(3)	1.97(3)
Gdh	11x12	10	132	113	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1.48(1)	10.52(9)
	12x12	1	2	12	6	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	2.90(2)	-
Lap-1	10x10	16	307	0	0	0	0	0	0	88	0	0	3	0	0	0	0	0	0	1	0	0	1.60(1)	19.87(15)
Mdh-3	11x12	8	87	119	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	4.99(1)	2.33(7)
	12x12	1	5	9	4	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	0.11(2)	-
	11x13	8	105	0	0	91	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1.00(1)	1.60(7)
	13x13	1	4	0	0	16	3	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	3.76(2)	-
	11x10	7	77	0	0	0	0	0	93	0	0	0	1	0	0	0	0	0	1	0	0	0	1.51(1)	2.21(6)
	10x10	1	7	0	0	0	0	0	9	4	0	0	1	0	0	0	0	0	2	1	0	0	1.03(2)	-
	12x13	2	12	13	0	17	0	11	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1.51(3)	3.83(3)
	12x10	2	15	14	0	0	0	0	12	0	8	0	1	1	0	0	0	0	1	0	1	0	2.50(3)	1.17(3)
	13x10	1	7	0	0	5	0	0	9	0	0	5	1	0	0	1	0	0	1	0	0	1	1.65(3)	-
Pgm	11x12	8	108	97	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0.59(1)	7.55(7)
	12x12	1	2	15	1	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	0	9.07(2)	-
	11x13	14	176	0	0	167	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.24(1)	14.39(13)
	13x13(a)	2	13	0	0	26	10	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0.58(2)	2.32(2)
	13x13(b)	2	7	0	0	21	7	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	1.41(2)	1.94(2)
	12x13(c)	2	12	18	0	9	0	11	0	0	0	0	1	1	0	1	0	1	0	0	0	0	3.42(3)	1.93(3)
	12x13(d)	2	12	18	0	14	0	6	0	0	0	0	1	1	0	1	0	1	0	0	0	0	6.51(3)	1.52(3)

*) Degrees of freedom associated with the test are in parentheses.

(a) Represents crosses for 52 × 60 *(Table 1)*

(b) Represents selfs for 60 and 52 *(Table 1)*

(c) Represents crosses for 52 × 62 *(Table 1)*

(d) Represents crosses for 60 × 62 *(Table 1)*

the parental band. Crosses between the homozygous trees and either of the two heterozygous trees (59 or 60) produced progeny with either single or double-banded phenotypes in approximately equal proportions (*Table 2*). Heterogeneity G values were non-significant. Progeny from the selfing of the two heterozygous trees produced three classes of phenotypes in approximately a 1:2:1 ratio (*Figure 2, Table 2*). Crosses between the two heterozygous trees produced four classes of progeny in an approximate 1:1:1:1 ratio (*Figure 2, Table 2*). No significant levels of heterogeneity were observed among progeny of the above crosses. The above patterns indicate that ACO in black spruce is under the control of a single gene which codes a functionally monomeric enzyme.

Glutamate dehydrogenase

One zone of activity on the gels was observed for GDH (*Figure 2*). Six of the seven parental trees produced a single sharp band and were inferred to be homozygotes (*Table 2*), while the seventh parental tree (65) had a faster and a more diffuse zone of activity and was inferred to be a heterozygote. Crosses among inferred homozygotes produced only one class of progeny, all having a single band of activity that migrated coincident with the parental band. Crosses between trees inferred to be homozygous and the heterozygous tree produced two types of progeny, one with a sharp band and the other with a diffuse band, representing many individual bands as a result of multimeric subunit structure (Mitton *et al.* 1979; Adams and Joly, 1980). Segregation ratios did not differ significantly from expected (*Table 2*). The one cross (55) not conforming to a 1:1 expectation is likely due to chance. The selfed cross of the heterozygous tree (65) produced three classes of progeny in approximately a 1:2:1 ratio (*Table 2*). The above results indicate that GDH in black spruce is under the control of a single locus, as in white spruce (Cheliak and Pitel, 1984b).

Leucine aminopeptidase

Gels stained for LAP had two zones of activity, only one of which (LAP-1) was variable among the plants surveyed (*Figure 2*). Two loci have also been observed for other species, such as Norway spruce (*Picea abies* K.) (Lundkvist and Rudin, 1977) and pitch pine (*Pinus rigida* Mill.) (Guries and Ledig, 1978). However, three LAP loci have been reported in knobcone pine (*Pinus attenuata* Lemm.) (Conkle, 1971). A monomer structure of the enzyme was proposed (Lundkvist and Rudin, 1977). For black spruce, three of the seven parental plants had a single band of activity for LAP-1 and were inferred to be homozygous for the same allele (*Table 1*). Crosses among these plants resulted in progeny with the same single band. Although four parental trees (59, 60, 62, and 63) had the same banding pattern as the other three trees (although with somewhat reduced activity), they were inferred to be heterozygotes based on the results of crosses among each other and also with the homozygous trees (*Table 1, Figure 2*). Crosses between inferred homozygous and heterozygous trees resulted in progeny with the same banding patterns as the parental trees. However, crosses among the four inferred heterozygous trees, as well as for the selfs of each, resulted in progeny displaying two different types of phenotypes in approximately a 3:1 ratio (*Table 2*). The one cross (36) that indicated a significant departure from expected is likely due to chance. One phenotype had the common single band for LAP-1, while for the others no band was present. The segregation patterns suggest that these four inferred heterozygous trees have a "null" or "silent" allele which, although it cannot be detected in heterozygotes ("10" form), will display itself in the homozygotes ("00" form). Unlike most codominant isozyme systems, this null system must necessarily appear as a classical Mendelian dominant/recessive gene. Null alleles for LAP have also been observed in Norway spruce (Lundkvist and Rudin, 1977) and trembling aspen (*Populus tremuloides* Michx.) (Cheliak and Pitel, 1984c). However, Guries and Ledig (1978) have suggested that some null alleles may be artifacts as a result of using systems with synthetic substrates which are not likely to occur *in vivo*.

Malate dehydrogenase

Four zones of activity were observed on gels stained for MDH (*Figure 2*). Two of these zones (inferred to be loci 1 and 2) were invariant among the plants surveyed. The slowest zone (MDH-4) was variable, but difficult to score. The other zone (MDH-3) displayed several different phenotypes (*Table 1*). The most heavily stained band for MDH-3 is thought to be an interlocus heterodimer, formed between the loci encoding Mdh-2 and Mdh-3. A similar phenomenon has been observed for other conifers (e.g. El-Kassaby, 1981; Cheliak *et al.*, 1985; Cheliak and Pitel, 1985). The MDH-3 locus was weakly stained, and in some of the crosses only the heterodimer was observed. Four of the parent trees (29, 52, 59, and 60) were inferred to be homozygotes while the other three trees (62, 63, and 65) were inferred to be heterozygotes. For tree 65, the banding pattern was similar to that of the homozygous trees except that the banding was not as strong. This tree was inferred to be a heterozygote that contained a "null" allele. Analyses of gametophytes from the seeds of this tree produced two types of patterns in approximately a 1:1 ratio; one type having the same banding pattern as the gametophytes of the homozygous trees, and the other with the absence of any bands for MDH-3.

Crosses among inferred homozygous trees produced progeny with the same banding patterns as for the parental trees. Crosses between inferred homozygotes and all three types of heterozygotes resulted in progeny that conformed to Mendelian expectations by segregating in approximately a 1:1 ratio (*Table 2*). Only with the pooled data for crosses of the four homozygotes with tree 62 was there a departure from the expected frequency (*Table 2*). Progeny from the selfing of each of the three heterozygous trees resulted in three classes of progeny in approximately a 1:2:1 ratio (*Table 2*). Crosses between plants inferred to be heterozygotes produced four classes of progeny in approximately a 1:1:1:1 ratio.

Phosphoglucomutase

PGM appeared as a single zone of activity with three different gel phenotypes observed among the seven parental trees (*Table 1, Figure 2*). Four of the seven trees (29, 59, 63, and 65) stained for one band and were inferred to be homozygotes. One parental tree (62) displayed two bands: one band with the same mobility as the four homozygous trees and the second band with reduced mobility. The last two trees (52 and 60) also had two bands, one with the same mobility as the homozygous trees and the other with a mobility intermediate between this band and the slow band of the other heterozygous tree (62). These last three trees (52, 60, and 62) were inferred to be heterozygotes.

Crosses among the inferred homozygous trees produced progeny with a single band coincident with that of the

parental bands. Crosses between the homozygous trees and either of the two types of heterozygous parental trees produced one- and two-banded progeny in approximately a 1:1 ratio (*Table 2*). Heterogeneity G values were not significant. Progeny from the selfs of these two types of heterozygous trees produced three classes of phenotypes in approximately a 1:2:1 ratio (*Table 2*). The cross between the two heterozygous trees having the same slow allele (trees 52 and 60) also produced progeny in a 1:2:1 ratio as expected (*Tables 1* and *2*). Crosses between the two types of heterozygous trees (52 or 60 crossed with 62) produced progeny in approximately a 1:1:1:1 ratio. The above crosses conform to Mendelian expectations and indicate that PGM in black spruce is under the control of a single gene that codes for a functionally monomeric protein. The presence of only one locus of PGM for black spruce observed in this study is similar to the results of CHELIAK and PITEL (1984b) and KING and DANCIK (1983) for white spruce, and agrees with those of BOYLE and MORGENSTERN (1985) in another study of black spruce. Under different electrophoretic conditions, Stewart and SCHOER (1986) have observed two PGM loci in white spruce.

Shikimate dehydrogenase

A single zone of activity containing three different phenotypes were observed on gels stained for SDH (*Figure 2*). Although insufficient trees were analyzed to obtain proper segregation data for parental crosses, our results suggest that the two-banded phenotype represents heterozygous trees while the two single bands represent trees that are homozygous for alternate alleles. In studies with needles of Douglas-fir (*Pseudotsuga menziesii* var. [MIRB.] FRANCO), NEALE *et al.* (1984) observed one zone of activity and indicated that SDH was functionally monomeric. Also, results by LINHART *et al.* (1981) for ponderosa pine (*Pinus ponderosa* LAWS.) indicated that SDH was a monomer. However, LINHART *et al.* observed the presence of three alleles, rather than the two present for black spruce.

Occurrence of mislabelled trees

Results for the above black spruce 7 × 7 diallel indicated progeny in the selfed lines crossed as expected with no detectable contamination (i.e., outcrossed trees). For example, a parent homozygous for one enzyme locus, e.g. "11" for AAT-2, produced progeny that were only of the homozygous "11" genotype. The presence of a "12" genotype among the progeny would be an indication of outcrossing. These results are unlike those of a recent study dealing with the genetic control of allozyme variants of a 5 × 5 white spruce diallel (CHELIAK and PITEL, 1984b) in which many plants from the selfed lines had been detectably outcrossed for several of the parents. However, in the black spruce study, one cross (cross 58, putatively of 60 × 65) produced phenotypes for LAP and MDH-3 that should not have been present. For MDH-3, one allele (3), detected in the progeny in all three blocks, was not present in either of the supposed parents. The progeny contained the expected "11" and "10" genotypes for MDH-3 (*Table 1*), as well as eight "13" genotypes. If we assume that tree 60 is the female parent, then the observed progeny fit a distribution predicted where the pollen both of trees 63 and 65 was contributed to produce cross 58 (*Table 1*). In addition, for LAP, the presence of progeny with "00" genotypes also indicates that pollen from tree 63 could have been present. The data from this cross was not used in this report because of the obvious contamination.

In addition, data from a few trees in other crosses were not used because the observed gel phenotypes were not as expected (e.g. having double bands when only one should be present). Evidence of errors in controlled crosses, as determined by the use of isozyme markers, has also been reported by ADAMS (1981), who suggested that the isozyme technique can detect most errors in seed-handling and control-crossing experiments. In another example (CHELIAK and PITEL, 1984c), electrophoretic techniques were useful in demonstrating that putative clones of trembling aspen were actually composed of several distinct genotypes. Therefore, the use of isozymes as genetic markers allows for the certification of progeny from control-pollinated families as well as ramets of clones to be placed in seed orchards, breeding archives, or clone banks.

Literature Cited

ADAMS, W. T.: Applying isozyme analysis in tree breeding programs. *In:* Isozymes of North American forest trees and forest insects. T. M. CONKLE, Ed. USDA Forest Serv., Gen. Tech. Rep. PSW-48. p. 60—64 (1981). — ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. **71**: 33—40 (1980). — BOYLE, T. J. B. and MORGENSTERN, E. K.: Inheritance and linkage relationships of some isozymes of black spruce in New Brunswick. Can. J. Forest Res. **15**: 992—996 (1985). — CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Can. For. Serv., Inf. Rep. PI-X-42. 49 pp. ISBN 0-662-13418-4. (1984a). — CHELIAK, W. M. and PITEL, J. A.: Genetic control of allozyme variants in mature tissue of white spruce trees. J. Hered. **75**: 34—40 (1984b). — CHELIAK, W. M. and PITEL, J. A.: Electrophoretic identification of clones in trembling aspen. Can. J. Forest Res. **14**: 740—743 (1984c). — CHELIAK, W. M. and PITEL, J. A.: Inheritance and linkage of allozymes in *Larix laricina*. Silvae Genet. **34**: 142—148 (1985). — CHELIAK, W. M., PITEL, J. A. and MURRAY, G.: Population structure and the mating system of white spruce. Can. J. Forest Res. **15**: 301—308 (1985). — CONKLE, M. T.: Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. Forest Sci. **17**: 190—194 (1971). — EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Hered. **72**: 451—452 (1981). — GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). Heredity **40**: 27—32 (1978). — KING, J. N. and DANCIK, B. P.: Inheritance and linkage of isozymes in white spruce (*Picea glauca*). Can. J. Genet. Cytol. **25**: 430—436 (1983). — LINHART, Y. B., DAVIS, M. L. and MITTON, J. B.: Genetic control of allozymes of shikimate dehydrogenase in ponderosa pine. Biochem. Genet. **19**: 641—646 (1981). — LUNDKVIST, K. and RUDIN, D.: Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas **85**: 67—74 (1977). — MITTON, J. B., LINHART, Y. B., STURGEON, K. B. and HAMRICK, J. L.: Allozyme polymorphisms detected in mature needle tissues of ponderosa pine. J. Hered. **70**: 86—89 (1979). — MORGENSTERN, E. K.: A diallel cross in black spruce, *Picea mariana* (MILL.) B.S.P. Silvae Genet. **23**: 1—3 (1974). — NEALE, D. B., WEBER, J. C. and ADAMS, W. T.: Inheritance of needle tissue isozymes in Douglas-fir. Can. J. Genet. Cytol. **26**: 459—468 (1984). — PITEL, J. A. and CHELIAK, W. M.: Effect of extraction buffers on characterization of isozymes from vegetative tissues of five conifer species: a user's manual. Can. For. Serv., Inf. Rep. PI-X-34. 64 pp. ISBN 0-662-13417-6. (1984). — STEWART, S. C. and SCHOER, D. J.: Segregation at enzyme loci in megagametophytes of white spruce, *Picea glauca*. Can. J. Genet. Cytol. **28**: 149—153 (1986). — SOKAL, R. R. and ROHLF, F. J.: Biometry. W. H. Freeman, San Francisco (1969). — YEH, F. C. H. and O'MALLEY, D.: Enzyme variations in natural populations of Douglas-fir, (*Pseudotsuga menziesii* [MIRB.] FRANCO) from British Columbia. 1. Genetic variation patterns in coastal populations. Silvae Genet. **29**: 83—92 (1980).