ANALYSIS OF ISOENZYMES AND INBREEDING IN A NATURAL WHITE SPRUCE STAND

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Abstract. --The distribution of isoenzymes in a natural stand of white spruce was studied using seed collected from 47 trees during a year of abundant seed production. Enzymes extracted from megagametophytes were separated electrophoretically on starch gels and treated with enzyme-specific stains to screen them for activity in 19 different enzyme systems. Only MDH (malate dehydrogenase) and GDH (glutamate dehydrogenase) consistently produced zymograms that were sufficiently clear to permit genetic interpretation of the results. Observed frequencies of MDH and GDH genotypes did not differ significantly from those expected in a panmictic population. Since these data contain no evidence of an inbreeding effect, seed collected in this stand should be suitable for use in reforestation.

Additional key words: starch gel electrophoresis.

INTRODUCTION

The degree of coancestry of the trees in any stand used for the production of white spruce (Picea glauca (Moench) Voss) seeds is important in view of the inbreeding depression known to occur in the progeny of crosses between related individuals (Ying 1978). Coles and Fowler (1976) showed that there were family relationships between near neighbours in a white spruce stand, but trees separated by distances greater than 100 metres were shown to be unrelated. These estimates of the level of inbreeding were based upon variation in proportionate yields of filled and empty seed, and on variation in epicotyl length. Similar methods were used by Morgenstern (1972), who found evidence of inbreeding in natural stands of black spruce (Picea mariana (Mill.) B.S.P.). Rudin et al (1974) used isoenzyme analysis to demonstrate the occurrence of inbreeding in three populations of Scots pine (Pinus sylvestris L.). In contrast, analysis of isoenzymes in two populations of Norway spruce (Picea abies (L.) Karst) indicated complete randomness of gene distribution within stands (Tigerstedt 1973). Contrasting results such as these led to the inclusion of this investigation of inbreeding in a natural stand of white spruce as part of work on the development of isoenzyme techniques that was initiated at Petawawa National Forestry Institute in 1978.

METHODS

The white spruce seed used in this study were collected from trees in a natural stand, approximately 19 hectares in area, containing scattered

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white spruce in mixture with hardwoods. In 1974, a year of abundant seed production, collections were taken from 47 trees that were generally well spread throughout the stand, but it was the presence of a heavy cone crop rather than the distance from another seed-tree that determined whether or not a collection was made. No written record was kept of the location of the 47 trees in relation to each other, or in relation to the location of other white spruce in the stand.

Samples of at least 16 seeds from each tree were soaked in distilled water at room temperature for 24 hours before being spread on wet filter paper in a petri dish and incubated at 5 C for 72 hours. These hydrated seeds were dissected and enzymes in each megagametophyte were extracted in a cold room, separated electrophoretically on starch gels, and stained using procedures that were the same as those described by Yeh and O'Malley (1980) except for minor modifications in gel buffer concentration and running current (Table 1).

Table 1.--Buffer solutions and electrical current used in starch gel electrophoresis

Buffer system	Buffer reagents		Buffer pH	Current per gel
	Name	Amount per litre		
A				
electrode	Citric acid (anhydrous)	7.684 g	6.1	
	Morpholine solution	To adjust pH		30mA
gel	Electrode buffer	40 ml	6.1	300V
B				
electrode	Lithium hydroxide	2.518 g	8.1	
	Boric acid	18.552 g		
				30mA
gel	Tris	3.634 g	8.5	250-300V
	Citric acid	0.961 g		
	Electrode buffer B	10 ml		
C				
electrode	Tris	15.743 g	7.0	
	Citric acid (anhydrous)	8.262 g		30mA
				150-160V
gel	Electrode buffer C	50 ml	7.0	
D				
electrode	Citric acid (anhydrous)	78.773 g	7.0	
	Sodium hydroxide	To adjust pH		
				30mA
gel	L-Histidine	0.776 g	7.0	150-300V
	Sodium hydroxide	To adjust pH		

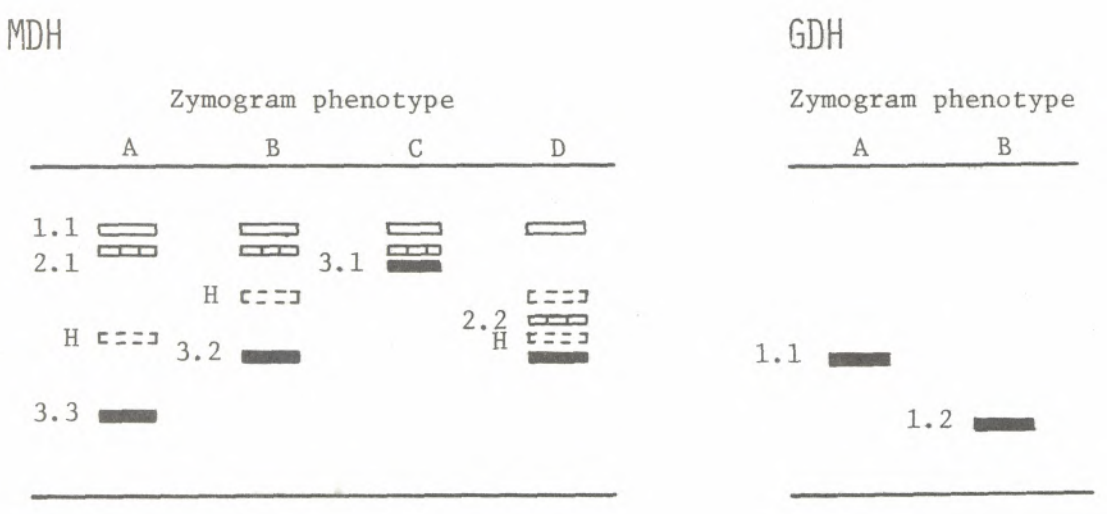
RESULTS

Bands of enzyme activity were identified on the zymograms produced by many of the 19 different enzymes examined (Table 2). However, only two enzyme systems, MDH (malate dehydrogenase), and GDH (glutamate dehydrogenase), consistently produced band patterns that were sufficiently clear to permit genetic interpretation of the results.

Table 2.--Bands of enzyme activity detected in zymograms of extracts from white spruce megagametophyte tissue following electrophoresis in the given buffer systems

Enzyme	Maximum no. of bands detected in one zymogram	Buffer system
Acid phosphatase	4	A
Aconitase	0	C
Adenylate kinase	1	D
Alcohol dehydrogenase	1	C
Aldolase	2	A
Aspartate aminotransferase	4	B
Diaphorase	3	C
Esterase	1	C
Glucose-6-phosphate dehydrogenase	1	C
Glutamate dehydrogenase	2	A, B
Glyceraldehyde-3-phosphate dehydrogenase	2	A
Isocitrate dehydrogenase	2	C
Leucine aminopeptidase	0	B
Malate dehydrogenase	5	A, C
Malic enzyme	0	A
Peptidase	0	B
Phosphoglucomutase	1	C
6-phosphogluconate dehydrogenase	2	C
Phosphoglucose isomerase	2	C

The occurrence of the four MDH zymogram phenotypes observed (Figure 1) was characteristic of the behaviour of an enzyme produced at three independent loci with one, two and three alleles, respectively. These isoenzymes also formed heterodimers that were clearly visible in three of the four zymograms. Two single-banded GDH phenotypes (Figure 1) were identified as the patterns produced by an enzyme at a single locus with two alleles.



Locus	Allele	R_f
1	1	0.27
2	1	0.25
	2	0.18
3	1	0.24
	2	0.14
	3	0.08

Locus	Allele	R_f
1	1	0.14
	2	0.08

(H represents heterodimer)

Figure 1.--Schematic diagrams of MDH and GDH zymogram phenotypes, and relative mobilities of megagametophyte isoenzymes separated in buffer system A.

The identities of the alleles occurring at each of the two polymorphic MDH loci and at the single GDH locus were determined by inspection of the set of 16 zymograms prepared for each of the 47 trees. Individual trees were then identified as particular MDH and GDH genotypes. Differences between the observed frequencies of individual genotypes and the frequencies expected in a population conforming to the Hardy-Weinberg Law were found to be non-significant in a chi-squared test (Table 3).

Table 3.--Comparison of observed and expected numbers of MDH and GDH diploid genotypes in a 47-tree sample from a natural white spruce stand

Enzyme	Locus	Genotype[1/]	Number of Trees		Chi Square
			Observed	Expected	
MDH	2	1 and 1	46	46	
		1 and 2	1	1	
		2 and 2	0	>0	
MDH	3	1 and 1	1	0.19	
		2 and 2	38	37.50	
		3 and 3	0	0.09	4.19
		1 and 2	4	5.36	(0.500>P>0.250)
		1 and 3	0	0.25	
		2 and 3	4	3.58	
GDH	1	1 and 1	4	3.88	
		1 and 2	19	19.24	0.007
		2 and 2	24	23.88	(1>P>0.995)

[1/] Combination of two of the numbered alleles at each locus

DISCUSSION

The fact that the observed and expected frequencies of MDH and GDH genotypes did not differ significantly leads to the conclusions that the trees from which the seeds were collected were the result of random mating in the previous stand, and that these 47 trees were not closely related. Since these trees were spread throughout an area of 19 hectares, a simple calculation gives an average distance between trees of 63 metres, which is much less than the 100-metre separation between unrelated trees reported by Coles and Fowler (1976). However, the validity of this comparison is weakened by lack of information about the actual spatial distribution of the 47 trees. Moreover, the isoenzyme data provide only weak evidence to support the above conclusions about the mating system, as only three polymorphic loci were involved in the analysis, and one of these was monomorphic except for a single tree.

Additional evidence supporting the above conclusions is found in a report that the cones collected from the 47 trees in 1974 produced high yields of filled seeds (Wang and Haddon 1976). If these trees and any other flowering trees in this stand had been closely related, a much higher proportion of empty seed would have been expected as a consequence of inbreeding.

CONCLUSIONS

Analysis of isoenzymes in megagametophytes from 47 different white spruce trees indicated that the trees were not closely related to each other, but satisfactory analytical procedures must be developed for additional enzymes before the degree of coancestry of trees in forest stands can be evaluated with confidence.

The data collected in this study, supplemented by reports of high yields of filled seed per cone, suggest that seed collected from this stand during a year of abundant seed production could be used for reforestation without fear of creating plantations in which growth would be depressed as a result of inbreeding.

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