Inheritance and Linkage Relationships of Some Isozymes of Larix laricina in New Brunswick, Canada

By L. YING and E. K. MORGENSTERN

Dept. of Forest Resources, University of New Brunswick,
Fredericton, N.B., E3B 6C2, Canada

(Received 9thNovember 1989)

Summary

Eight natural populations of tamarack (*Larix laricina* [DU ROI] K. KOCH) were sampled in southern and central New Brunswick. Analyses were based on the haploid megagametophytic tissue from the seeds of 388 trees. Fifteen enzyme systems coding for 27 loci were assayed. Sixteen loci were polymorphic and 15 of these exhibited regular Mendelian inheritance, *Acp-1* being the exception. Linkage was detected among five loci in two groups: (1) *Mdr-1--Acp-1--Acp-2*, and (2) *Lap-1--Me-2*.

Key words: eastern larch, isozymes, allozymes, polymorphism, linkage groups.

Zusammenfassung

Acht natürliche Populationen von *Larix laricina* [DU ROI] K. KOCH im südlichen und mittleren New Brunswick wurden untersucht. Analysiert wurden Samen von 388 Bäumen, wobei in den haploiden Megagametophyten 15 Enzymsysteme mit 27 Genloci identifiziert werden konnten. Von 16 polymorphen Loci folgten 15 einer typischen Mendelspaltung; die Ausnahme war *Acp-1*. Kopplung zwischen 5 Loci wurde festgestellt, die in 2 Gruppen auftraten: (1) *Mdr-1--Acp-1--Acp-2;* und (2) *Lap-1--Me-2*.

Introduction

The use of isozymes as gene markers to study genetic variation in forest trees has led to the rapid accumulation of information on levels of enzymatic variation in a wide variety of species (EL-KASSABY and WHITE, 1985). However, the advantages of isozyme analysis for population genetic studies of a given species can be fully realized only if its Mendelian inheritance and linkage relationships are confirmed (BARTELS, 1971).

Table 1. — Enzyme and buffer systems for electrophoretic analyses of tamarack.

Enzyme system	Abbreviation	Enzyme commission code	Buffer system
Aspartate amino-transferase	AAT	E.C.2.6.1.1	B
Aconitase	ACO	E.C.4.2.1.3	H
Acid phosphatase	ACP	E.C.3.1.3.2	H
Fumarase	FUM	E.C.4.2.1.2	H
Glucose-6-phosphate dehydrogenase	G6PD	E.C.1.1.1.49	H
Glutamate dehydrogenase	GDH	E.C.1.4.1.3	H
Leucine-amino peptidase	LAP	E.C.3.4.11.1	B
Malate dehydrogenase	MDH	E.C.1.1.1.37	H
Malic enzyme	ME	E.C.1.1.1.40	H
Menadione reductase	MDR	E.C.1.6.99.2	H
6-phosphogluconate dehydrogenase	6PGD	E.C.1.1.1.44	H
Phosphoglucose isomerase	PGI	E.C.5.3.1.9	B
Phosphoglucomutase	PGM	E.C.2.7.5.1	H
Shikimic acid dehydrogenase	SDH	E.C.1.1.1.25	H
Superoxide dismutase	SOD	E.C.1.15.1.1	B

Larix laricina (Du Roi) K. Koch, i. e., tamarack or eastern larch ,is one of the most rapidly growing North American conifers (Mead, 1978) and has recently been given much attention in reforestation, genetic studies and breeding programs (Fowler, 1986). Based on a range-wide sample and the analysis of 12 enzyme systems, Cheliak and Pitel (1985) first described inheritance and linkage in this species. In this paper we report on inheritance and linkage in 15 enzyme systems of natural stands sampled in a much smaller area in New Brunswick.

Materials and Methods

Stands Sampled

The area sampled in southern and central New Brunswick is part of the Acadian Forest Region (Rowe, 1972) where tamarack is a frequent pioneer on recent forest clearings and abandoned fields. Eight natural stands with on average 48 trees each were included, with mean tree ages ranging from 7 to 21 years and heights from 5 m to 11 m. The area sampled ranges from 45°24' to 47°00' latitude N and 65°32' to 67°30' longitude W. Trees were selected in early May solely on the basis of flowering, and wind-pollinated cones were collected in late August 1986. All cones were kept separate by parent tree and stand and seed was extracted by hand and stored at 4 °C.

Laboratory and Electrophoretic Procedure

Alcohol flotation was used to separate empty or light seeds. Seeds were allowed to imbibe distilled water for 48 hours. Seed coats and embryos were then removed. Individual well developed megagametophytes were crushed in a scintillation cup (0.5 ml) with one drop of the extraction buffer (Cheliak and Pitel, 1984). Gel and electrode buffers, electrophoretic conditions and starch were slight-

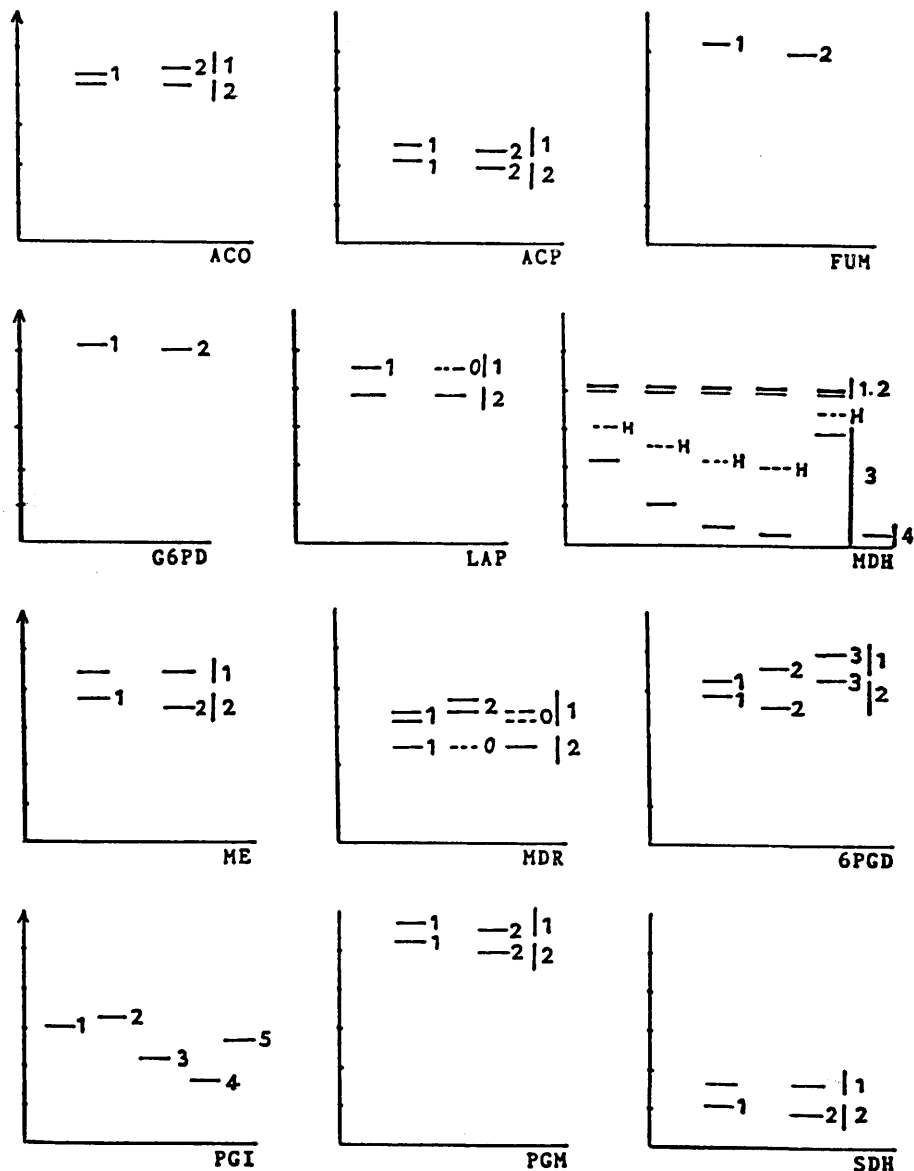


Figure 1. — Megagametophytic banding patterns, locus designations, and allozyme numbers for 12 enzyme systems with 16 variable loci. The vertical scale is the relative migration distance of the allozymes (R_f). Numbers at vertical lines on the right indicate the zone of activity (nominally a locus) and numbers to the right of the horizontal line the allozyme. Bands shown by dashed lines are either null alleles or inter-locus dimerization products.

ly modified from CHELIAK and PITEL (1984). A constant current of 30 mA for the B buffer system, and 60 mA for the H system were supplied. Enzyme systems, their abbreviations, enzyme commission codes, and gel buffers upon which they were run are listed in *Table 1*.

At least 8 seeds from each tree were used for initial genotyping of maternal trees. Maternal trees polymorphic at two or more loci were chosen, and about 30 to 54 seeds from each of these trees were used for the study of linkage.

Data Analyses

Inheritance of allozyme polymorphisms was detected by testing observed segregation ratios against expected ratios (assuming simple Mendelian inheritance), by means of a maximum-likelihood G-test (SOKAL and ROHLF, 1981, p.722—724). Two parameters are computed: a pooled G value (G_p) with one degree of freedom which tests for an overall 1:1 ratio in the data of each locus, and a heterogeneity G value (G_h) based on heterogeneity in the observed ratio in gametophyte arrays from different mother trees where the degrees of freedom are one less than the number of mother trees tested.A non-significant deviation from the expected ratio was taken as evidence for single-locus control and regular Mendelian inheritance (BARTELS, 1971).

Linkage between loci was detected by a similar three-way maximum likelihood G-test (SOKAL and ROHLF, 1981, p. 722—724). A 1:1:1:1 segregation ratio was taken as evidence for jointly independent inheritance, or an absence of linkage at a pair of loci (HEDRICK, 1985). Recombination frequencies (R) were calculated as: $R = r/n$, where r is the number of observations in the smaller class (coupling or repulsion) and n is the number of total observations. The standard error of this estimate is given by $\sqrt{R(1-R)/n}$ (RUDIN and EKBERG, 1978). The recombination values were converted to map distances in centiMorgans (cM) (KOSAMBI, 1944) as:

$$X = 1/4 \ln ((1+2R)/(1-2R)),$$

where X is the map distance in cM,
ln is the natural logarithm (base e) and
R is the recombination frequency.

Results

Banding Patterns and Inheritance

For the 15 enzyme systems, 27 presumed loci were identified. Eleven of these were monomorphic loci and 16 were polymorphic in at least four populations. Zymogram phenotypes for polymorphic loci in 12 enzyme systems are illustrated in *Figure 1*.

The interpretation of electrophoretic banding patterns was as follows: I. If an enzyme system was controlled by multiple loci, the isozymes and the corresponding loci were identified by the symbol of the enzyme and a hyphenated numeral; the most anodal migrating isozyme was designated as locus 1, the next as locus 2, and so on.

2. Within each locus, the most common allozyme was designated as allele 1, the next as allele 2, and so on. For easier comparison with the study of the same species by other authors, alleles of the same enzyme were named as before (CHELIAK and PITEL, 1985).

Inheritance of allozymes will be presented separately for each enzyme system as follows, and values of the G-test are given in *Table 2*.

Aspartate amino-transferase (AAT)

Three zones of activity were found on the gels stained for AAT. The most cathodal zone (*Aat-3*) had two bands which showed the same variation, so was considered to be a double-banded variant. CHELIAK and PITEL (1985) found it to be single banded. Resolution of *Aat-2 was* difficult and of Aat-3 was inconsistent, and therefore no attempt was made to score these two loci. Only *Aat-1*, a monomorphic locus, was included in this study.

Aconitase (ACO)

Two zones of activity were evident on gels stained for ACO. Only the more anodal zone (*Aco-1*) showed single-banded variants which segregated according to a 1:1 ratio.

Acid phosphatase (ACP)

Four bands appeared on gels stained for ACP, but only two zones of activity could be scored reliably. A significant deviation from 1:1 segregation was observed for the more anodal zone (*Acp-1*), with an excess of allele 1 (*Table 2*).

Fumarase (FUM)

A single zone of activity was observed in gels stained for FUM, with two variants that segregatet in the expected 1:1 ratio.

Glucose-6-phosphate dehydrogenase (G6PD)

There was only one zone of enzyme activity on gels stained for G6PD with two variants which segregated in the 1:1 expected ratio. The null allele reported by CHELIAK and PITEL (1985) could not be found here.

Leucine aminopeptidase (LAP)

Two zones of activity were evident on gels stained for LAP. Only the more anodal zone (*Lap-1*) showed variants, the alternate allele being null.

The observed numbers of segregations in Lap-1 were not significantly different from the expected 1:1 segregation. A significant heterogeneity among segregation ratios of heterozygous mother trees was observed (*Table 2*).

Malate dehydrogenase (MDH)

Four zones of activity were observed on the gels stained for MDH. The two most anodal zones, *Mdh-1*, *Mdh-2* and the most cathodal zone *Mdh-4* were monomorphic.

Mdh-3 was highly polymorphic with five alleles as compared to seven observed by CHELIAK and PITEL (1985). There was a heterodimeric band between *Mdh-2* and *Mdh-3*, resulting in a three-banded pattern for these two loci (*Figure 1*). Three genotypes of heterozygous trees (12, 14, 15) were observed. There were no deviations from the expected 1:1 segregation ratio (*Table 2*).

Menadione reductase (MDR)

Three zones of activity were evident on gels stained for MDR, but the most cathodal zone *Mdr-3* was not consistent enough to score and was not included in the study.

The loci *Mdr-1* and *Mdr-2* showed double-banded variation with three alleles including a null allele. There was no deviation from the 1:1 ratio. No significant heterogeneity was found among segregation ratios of heterozygous mother trees for both loci (*Table 2*).

Malic enzyme (ME)

Two zones of activity were observed on gels stained for ME. Variation was only detected in the more cathodal

Table 2. — G-test of observed and expected 1:1 segregation ratio for polymorphic loci (G), and G value indicating heterogeneity in the observed ratio among trees (G_h).

Locus	Pair	#1	#2	G (1)[a]	G_h	(df)[b]
Aco-1	1 : 2	181 :	188	0.133	17.333	(13)
Acp-1	1 : 2	361 :	277	11.092***	23.520	(20)
Acp-2	1 : 2	539 :	505	1.108	41.371	(31)
Fum	1 : 2	27 :	22	0.511	0.000	(0)
G6pd	1 : 2	449 :	445	0.019	34.933	(28)
Lap-1	1 : 0	637 :	695	2.525	81.482***	(41)
Mdh-3	1 : 2	297 :	332	1.010	16.566	(19)
	1 : 3	72 :	60	1.092	0.000	(0)
	1 : 4	34 :	32	0.000	0.064	(1)
	1 : 5	48 :	41	0.551	0.553	(2)
	2 : 4	43 :	41	0.048	0.000	(0)
Me-2	1 : 2	187 :	173	0.544	14.906	(11)
Mdr-1	1 : 2	171 :	178	0.140	12.532	(10)
	1 : 0	93 :	70	3.256	0.000	(0)
	2 : 0	14 :	13	0.037	0.000	(0)
Mdr-2	1 : 0	372 :	365	0.067	14.282	(23)
Pgi-2	1 : 3	319 :	368	3.498	25.260	(20)
	1 : 5	179 :	189	0.272	9.030	(10)
	2 : 5	23 :	21	0.091	0.000	(0)
	3 : 5	68 :	78	0.686	1.926	(4)
Pgm-1	1 : 2	26 :	33	0.832	0.874	(1)
Pgm-2	1 : 2	46 :	35	1.498	0.685	(1)
6Pgd-1	1 : 2	265 :	259	0.069	10.513	(16)
	1 : 3	71 :	73	0.028	5.246	(3)
	2 : 3	13 :	17	0.535	0.000	(0)
6Pgd-2	1 : 2	11 :	5	2.310	0.000	(0)
	1 : 3	18 :	12	1.208	0.000	(0)
Sdh-2	1 : 2	193 :	178	0.607	0.000	(0)

Significance levels: ***), 0.1%.
[a]) G-test with one degree of freedom.
[b]) Heterogeneity G-test (degrees of freedom).

zone (*Me-2*). The variants appeared as single-banded phenotypes and segregation ratios did not deviate significantly from the expected distribution. No heterogeneity was observed among segregation ratios of heterozygous mother trees (*Table 2*).

Phosphoglucose isomerase (PGI)

Two zones of activity were observed for PGI, with a monomorphic locus, *Pgi-1*. The second locus, *Pgi-2*, was in agreement with previous description (CHELIAK and PITEL, 1985). No significant deviations from the expected 1:1 segregation ratio were observed (*Table 2*).

Phosphoglucomutase (PGM)

One zone of activity was reported by CHELIAK and PITEL (1985) for the same species. However, in the present study, two zones of activity were evident on gels stained for PGM. *Pgm-1* stained much more intensely than *Pgm-2*. Both zones (*Pgm-1* and *Pgm-2*) segregated in heterozygous mother trees as single bands with observed segregation ratios not significantly different from the expected 1:1 ratio. No significant heterogeneity was observed among segregation ratios of hereozygous mother trees at either locus (*Table 2*).

6-Phosphogluconate dehydrogenase (6PGD)

Two zones of activity were evident on the gels stained for 6 PGD with three allozymes in each zone. All pairs of combinations were not significantly different from the expected 1:1 segregation ratio (*Table 2*).

Shikimic acid dehydrogenase (SDH)

Two zones of activity were observed in the gels stained for SHD. The more anodal zone (*Sdh-1*) was monomorphic, and the more cathodal zone (*Sdh-2*) was polymorphic with two variants. The segregation ratio in *Sdh-2* indicated agreement with single-gene inheritance.

Glutamate degydrogenase (DGH) and superoxide dismutase (SOD)

These two enzyme systems produced only monomorphic loci with will not be considered here.

Aldolase (ALD)

Only ten trees showed enzyme bands in the gels stained for ALD. Two zones of activity were detected, both of which were without variation. CHELIAK and PITEL (1985) also found two loci encoding this enzyme in tamarack, and variation was detected in only one tree in the more cathodal zone (*Ald-2*). This indicates extremely low variation at this locus. The sample size in this study was considered to be too small to detect any variation, therefore, this enzyme was not included in further analysis.

Diaphorase (DIA)

Two zones of activity appeared on the gels stained for DIA, with three allozymes in both zones. However, it could not be scored reliably, so was not included in further study.

Linkage Study

For the 16 polymorphic loci in this study, only 82 of the 120 possible pairs of loci were represented by at least one doubly heterozygous mother tree. Two combinations (*Acp-1--Me-2* and *Acp-1--Pgi-2*) showed significant deviation at both loci and were excluded from the linkage analysis.

The remaining 80 combinations were grouped as follows for analysis:

A. Pairs with only one tree value. There were 19 pairs which were based on only one tree. Linkage for these pairs of loci could not be confidently demonstrated.

B. Pairs with more than one tree. A total of 61 different combinations were included in this class.

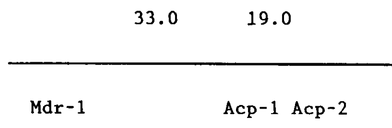
B. 1 Fifty-seven pairs showed nonsignificance in the G-heterogeneity test. This means that joint segregation ratios were homogeneous among doubly heterozygous mother trees. G-pooled data were then used to check the total deviation for a specific pair of loci. All of these loci showed nonsignificant deviation and, therefore, no linkage was detected in these loci.

B. 2 Four pairs (*Acp-1--Acp-2, Lap-1--Me-2, Acp-1--Mdr-1, Acp-2--Mdr-1*) showed significant deviation in G-heterogeneity value (*Table 3*). This means that joint segregation ratios differed among these doubly heterozygous mother trees. Unfortunately, the G-pooled value will be insensitive because different directions of segregation were pooled. Therefore individual G-values were examined and more than 80% of all individuals in these combinations exhibited highly significant deviations. This suggests that there could be linkage between these pairs. Linked loci, their recombination values and standard errors are presented in *Table 4*.

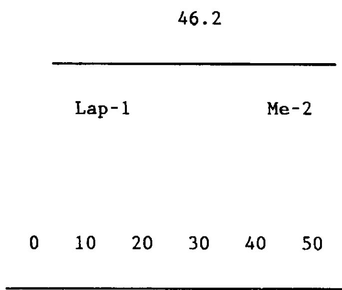
Table 4. — The recombination values (R) and standard errors (S.E.) for the linked loci.

Pair name		R	S.E.
Acp-1	Acp-2	0.181	0.016
Mdr-1	Acp-1	0.289	0.038
Mdr-1	Acp-2	0.324	0.032
Lap-1	Me-2	0.364	0.039

Group 1.



Group 2.



centiMorgan scale for diagrams

Figure 2. — Linkage groups in tamarack. The number indicates the distance in centiMorgans (cM).

Table 3. — G-test for deviation at A and B loci, pooled G value (G_p), and heterogeneity G value (G_h) for the pairs of loci which showed significant non-random joint segregation.

Pair of loci		No. of trees	A1B1	A1B2	A2B1	A2B2	Deviation[a] A	Deviation[a] B	$G_p(3)$	$G_h(df)$[b]
Acp-1	Acp-2	20	273	71	39	225	10.6**	0.4	265.3***	160.5(57)***
Acp-1	Mdr-1	7	24	61	40	17	5.6*	1.4	23.9***	41.1(18)**
Acp-2	Mdr-1	9	36	75	67	32	0.7	0.1	26.5***	90.5(24)***
Lap-1	Me-2	5	30	43	55	26	0.4	1.7	11.2*	38.8(12)***

a) Significance levels: *), 5%; **), 1%; ***), 0.1%.
b) Numbers in parenthesis are degrees of freedom.

A linkage map for loci *Acp-1*, *Acp-2*, *Mdr-1*, *Lap-1* and *Me-2* is shown in *Figure 2*, with the map distance calculated from KOSAMBI's (1944) formula. The linkage groups were found to be in the following order:

Group 1. *Mdr-1--Acp-1--Acp-2*

Group 2. *Lap-1--Me-2*

Whether or not these two linkage groups occupy the same chromosome is not known.

Discussion

Inheritance

Mendelian inheritance was confirmed for 15 polymorphic loci, but a significant deviation from the 1:1 expected segregation was detected in the 16th locus, *Acp-1* (*Table 2*). This could possibly be a case of segregation distortion which may be attributed to non-random sampling. Due to the difficulty of separating full and empty seed in this species by ordinary methods, alcohol flotation was used. In addition, megagametophytes which were not well developed or not healthy, were excluded from analysis. Thus, alloymes which code for poorly developed phenotypes could have been removed, leading to the distortion observed here. Many authors have found segregation distortion in conifers, and have attributed it to meiotic drive and/or lethal or semi-lethal mediated allelism from inbreeding (LUNDKVIST, 1974; RUDIN, 1975; SIMONSEN and WELLENDORF, 1975; NEALE, 1978; WITTER and FERET, 1978; ADAMS and JOLY, 1980; ECKERT *et al.*, 1981; EL-KASSABY *et al.*, 1982; CHELIAK *et al.*, 1984; CHELIAK and PITEL, 1985; BOYLE and MORGENSTERN, 1985).

Significant heterogeneity was found in the *Lap-1* locus (*Table 2*). Individual trees segregated differently in the ratio of the two alleles, with some trees showing an excess of one allele, and other trees an excess of another allele. After individual G-values were pooled, the segregation ratio in different alleles met the 1:1 expectation.

Heterogeneity among the individuals could be due to natural selection, such that individuals carry different allele combinations in variable environments. NAMKOONG (1980) stated that the adaptability of plants to particular environments is controlled by many physiological functions, which are in turn governed by environmentally affected substrates and by enzymes which are influenced by structural genes, and by genes which affect the rate or timing of structural gene actions. Different adaptations have evolved when allelic variation exists such that some alleles favour performance in certain environments while alternate alleles convey advantages in other environments. ADAMS and JOLY (1980) found that in several cases, e.g., in loblolly pine, *Pinus taeda* L., the deficiency of a particular allele of a heterozygous genotype is consistent over several parents, indicating that the allozyme gene itself, or at least a small part of the chromosome marked by that gene, is the target of selection.

Our findings generally agree with the results of CHELIAK and PITEL (1985); only minor differences were found. These include the discovery of two loci for PGM in this study vs. one locus by CHELIAK and PITEL (1985) and a monomorphic locus for SOD which was polymorphic in their study (see Results). Results on five enzyme systems (ACP, ME, MDR, SDH, and FUM) have not previously been reported and are being presented here for the first time for this species.

Major reasons for these differences are probably different sampling and analytical procedures. CHELIAK and PITEL (1985) based their study on seed collections made by cooperators across the range of the species over several years, while we collected our seed all in one year and in a much smaller area. CHELIAK and PITEL (1985) report on a possible case of segregation distortion in MDH resulting from errors in seed collection or labelling (seed from two trees pooled and labelled as one tree). They also detected the presence of a null allele in G6PD which may be related to seed aging (CHELIAK and PITEL, 1985). The possible effect of methods of seed extraction and cleaning used in our study has already been mentioned. Finally, geographic variation in gene frequencies and polymorphism is to be expected and has been detected in several other species (BERGMANN, 1975; RUDIN and EKBERG, 1978).

Linkage Analysis

The four linked loci pairs are reported here for the first time for tamarack (*Figure 1, Tabelle 4*). Although they all showed highly significant heterogeneity values, their pooled G-values and most individual G-values were also very high (*Table 3*). Therefore, linkage was confirmed for these pairs.

Some of the results are supported by studies in other species. The most tightly linked pair, *Acp-1* and *Acp-2*, was also found to be linked in loblolly pine (CONKLE, 1981). Another enzyme linked to ACP in that study was DIA, which appeared in the same linkage group in both lodgepole pine (*Pinus contorta* DOUGL.) and Jeffrey pine (*Pinus jeffreyi* GREV. and BALF.) (CONKLE, 1981). It would have been interesting to compare these results with tamarack but unfortunately DIA could not be reliably scored here. Another linked pair, *Lap-2* and *Me-1*, was discovered in Japanese black pine (*Pinus thunbergii* PARL.) by SHIRAISHI (1988).

We could not confirm the linkage of 6Pgd-1 and Pgi-2 reported by CHELIAK and PITEL (1985). In our study seed from 13 trees was used to examine this pair but no significant deviation from the expected 1:1:1:1 ratio was found. The remaining linkage groups proposed by CHELIAK and PITEL (1985) were based on specific AAT and SOD loci which could not be reliably visualized in our laboratory.

The differences between results of the present study and those of CHELIAK and PITEL (1985) are not easily explained. In other genera, there was considerable consistency in linkage groups within and even among species within genera especially in the genus *Pinus* L. (CONKLE, 1981; EL-KASSABY and WHITE, 1985), but the number of studies in the genus *Larix* MILL. is still too small for such general comparisons. There is also a problem with the distinction of some enzyme phenotypes, for example, ERNST *et al.* (1987) pointed out that menadione reductase (MDR) is the equivalent of diaphorase (DIA). In addition, differences in sample size of studies of the same species and the statistical inability to detect loosely linked loci could be reasons why differences in the results exist. It is clear that further studies are required.

Since linked loci do not give independent estimates of parameters used in the analysis of population structure, one locus of a linked pair will be excluded from further analysis.

Acknowledgements

Support by the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. Dr. T. J. B. BOYLE

gave advice on laboratory techniques and Dr. F. C. YEH made computer programs available. Two anonymus reviewers provided constructive comments.

Literature Cited

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. 71: 33—40 (1980). —— BARTELS, H.: Genetic control of multiple esterases from needles and macrogametophytes of *Picea abies*. Planta (Berl.) 99: 283—289 (1971). —— BERGMANN, F.: Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genet. 24: 175—177 (1975). —— BOYLE, T. J. B. and MORGENSTERN, E. K:. Inheritance and linkage relationships of some isozymes of black spruce in New Brunswick. Can. J. For. Res. 15: 992—996 (1985). —— CHELIAK, W. M., MORGAN, K., DANCIK, B. P., STROBECK, C. and YEH, F. C.: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* LAMB. Theor. Appl. Genet. 69: 145—151 (1984). —— CHELAK, 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 (1984). —— CHELIAK, W. M. and PITEL, J. A.: Inheritance and linkage of allozymes in *Larix laricina*. Silvae Genet. 34: 142—148 (1985). —— CONKLE, M. T.: Isozyme variation and linkage in six conifer species. *In*: Isozymes of North American forest trees and forest insects. U.S. For. Serv., Gen. Tech. Rep. PSW-48. pp. 11—17 (1981). —— EL-KASSABY, Y. A., YEH, F. C. and SZIKLAI, O.: Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. 24: 325—335 (1982). —— EL-KASSABY, Y. A. and WHITE, E. E.: Isozymes and forest trees: an annotated bibliography. Can. For. Serv. Inf. Rep. BC-X-267 (1985). —— ECKERT, R. T., JOLY, R. J. and NEALE, D. B.: Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For. Res. 11: 573—579 (1981). —— ERNST, S. G., KEATHLEY, D. E. and HANOVER, J. W.: Inheritance of isozymes in seed and bud tissue of blue and Engelmann spruce. Genome 29: 239—246 (1987). —— FOWLER, D. P.: Strategies for the genetic improvement of important tree species in the Maritimes. Can. For. Serv. Inf. Rep. M-X-156 (1986). —— HEDRICK, P. W.: Genetics of populations. Jones and Bartlett Publishers, Inc., Boston (1985). —— KOSAMBI, D. D.: The estimation of map distance from recombination values Ann. Eugenics. 12: 172—175 (1944). —— LUNDKVIST, K.: Inheritance of leucine aminopeptidase isozymes in *Picea abies* K. Hereditas 76: 91—96 (1974). —— MEAD, D. A.: Comparative height growth of eastern larch and black spruce in northwestern Ontario. For. Chron. 54: 296—297 (1978). —— NAMKOONG, G.: Breeding for variable environments. Forest Industry Lecture Series, No. 6. University of Alberta, Edmonton, Alta. (1980). —— NEALE, D. B.: Allozyme studies in balsam fir. M. Sc. thesis, University of New Hampshire, Durham, N.H. (1978). —— ROWE, J. S.: Forest regions of Canada. Can. For. Serv. Pub., No. 1300 (1972). —— RUDIN, D.: Inheritance of glutamate-transaminases (GOT) from needles and endosperms of Pinus sylvestris. Hereditas 80: 296—300 (1975). — RUDIN D. and EKBERG, I: Linkage studies in *Pinus sylvestris* L. using macrogametophyte allozymes. Silvae Genet. 27: 1—12 (1978). —— SHIRAISHI, S.: Linkage relationship among allozyme loci in Japanese black pine *Pinus thunbergii* PARL.. Silvae Genet. 32: 60—66 (1988). —— SIMONSEN, V. and WELLENDORF, H.: Some polymorphic isoenzymes in the seed endosperm of Sitka spruce (*Picea sitchensis*) (BONG.) CARR.). For. Tree Improv. 9: 5—20 (1975). —— SOKAL, R. R. and ROHLF, F. J.: Biometry. Second ed. W. H. Freeman, San Francisco (1981). —— WITTER, M. S. and FERET, P. P.: Inheritance of glutamate oxaloacetate transaminase isozymes in Virginia pine megagametophytes. Silvae Genet. 27: 129—134 (1978).

Pressemitteilungen

Actual Problems of the Legislation of Forest Reproductive Material and the Need for Harmonization of Rules at an International Level

(June 10 to 14, 1991)

The next meeting of the IUFRO Working Party S2.02—21 — Legislation of Forest Reproductive Material — will be held at the Forstliche Ausbildungsstätte Ort near Gmunden, Austria. The meeting will be held in a Castle at the shore of the marvellous Traunsee (from Monday June 10 to Thursday June 13). On June 14, the meeting will be held in Vienna, Austria. The following topics will be discussed at this meeting:

- Principles for classification of selected reproductive material;
- Principles of testing;
- Categories of in reproductive material;
- Clonal forestry;
- Forest policy and law.

For further information please contact: Dr. HANS J. MUHS, Institute of Forest Genetics and Forest Tree Breeding, Federal Research Centre for Forestry and Forest Products, Sieker Landstraße 2, D W-2070 Grosshansdorf, Germany. Telephone: (04102) 61079; Fax: 040 — 73962480.

Genetics of Oaks Species

(September 2 to 6, 1991)

The first meeting of the IUFRO Working Party S2. 02-22 — on 'Genetics of Oaks Species' will be held on the campus of the National School of Forest Ingenieurs (ENITEF) located at the National Arboretum des Barres France. Les Barres is situated 120 km south of Paris near the town of Nogent sur Vernission. The following topics will be covered in this meeting:

- Taxonomy;
- Hybridization and sexual reproduction;
- Geographic variation and progeny testing;
- Breeding techniques and biotechnology;
- Tree Improvement.

For further information contact: A. KREMER, INRA Laboratoire de Genetique et Amelioration des arbres forestiers, BP Gazinet, F — 33610 Cestas, France. Telephone: 56 68 03 03; Fax 56 68 02 23.

Buchbesprechungen

Natürliche und künstliche vegetative Vermehrung von Fichten der subalpinen Kampfzone (Picea abies Karst.). Dissertationen der Universität für Bodenkultur in Wien, 32. Von H. TIEFENBACHER. 1989. 95 Seiten mit 4 Faltblättern, 16 Photos, 48 Abbildungen und 4 Anhängen. ISBN 3-85369-731-3. Broschiert öS 132,—/DM 20,—.

Am Alpenostrand, wo Wald- und Baumgrenze durch starken Wind und Frosttrocknis erheblich gedrückt werden, gibt es im Stuhleck- und Hochwechselgebiet auf hunderten Hektar an der aktuellen Waldgrenze Fichten-Kampfgemeinschaften ("Rotten"), die sich durch Ableger (bewurzelte Äste) erweitern. Diese in ihrer Ausdehnung bisher nicht bekannte vegetative Verjüngung wurde nach Verbreitung, Wachstum und Entwicklungsdynamik untersucht, um waldbauliche Schlußfolgerungen zur Gewinnung von Stecklingspflanzen für Hochlagen zu erhalten, wenn Saatgut nicht ausreichend zur Verfügung steht.

Das Wachstum innerhalb der Rotten und die Entwicklung der Ableger wurden analysiert, genetische Einheitlichkeit bzw. Un-