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Inheritance of Allozymes in Larix decidua Mill.

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Summary

The inheritance of eleven enzyme systems at 19 loci was investigated using both macrogametophyte and embryo tissues of open pollinated seeds of *Larix decidua*. Eleven polymorphic loci were tested for deviations from the Mendelian segregation. Four loci (Fdh, Mdh4, Mnr2, Sod1) were monomorphic and another four loci (Gdh, Idh, Sod2, Srdh) possessed rare alleles observed only in bulked seed collections from 11 populations throughout the natural range of *L. decidua*.

Key words: Larix decidua, inheritance, allozymes.

Zusammenfassung

Die Vererbung von 11 Enzymsystemen wurde an 19 Enzymloci von *Larix decidua* in Macrogametophyten und Embryogeweben anhand von Einzelbaumsaatgut analysiert. Die Abweichungen von der Mendelspaltung wurden an 11 polymorphen Loci geprüft. Vier Loci (Fdh, Mdh4, Mnr2, Sod1) waren monomorph, an vier anderen (Gdh, Idh, Sod2, Srdh) wurden seltene Allele nachgewiesen, die nur in der Mischprobe von 11 Populationen aus dem natürlichen Verbreitungsgebiet von *L. decidua* auftraten.

Introduction

The number of enzyme gene markers known in *Larix decidua* is still small compared with other conifers. So far only few enzymes have been analysed in macrogametophyte tissue of this species (MEJNARTOWICZ and BERGMANN, 1975; KOSINSKI and SZMIDT, 1984; BERGMANN and RUETZ, 1987). The aim of our report was to present electrophoretical patterns for 11 enzyme systems in *L. decidua* and to determine the inheritance of allozymes controlled by 11 polymorphic loci. These investigations are a first part of a study which also includes the genetic structure and mating system of this species.

Material and Methods

Cones were collected from 70 trees growing in four natural populations of *L. decidua* in Poland and 25 clones growing in a clonal seed orchard near Kórnik.

Open pollinated seeds were extracted from cones for each tree separatly.

For the isozyme study initialy 6 seeds were analysed from each tree. Afterwards, 18 trees with polymorphic loci were chosen to complete the study.

Additionaly, bulked seed collections, from 11 populations throughout the natural range of *L. decidua*, were used to identify possible other allozymes of the investigated enzymes.

Macrogametophyte tissue and embryo were isolated separately trom the seeds and homogenized in 35 μl and 15 μl of Tris-HCl buffer (pH 7.2) respectively. A 0.15% 2-mercaptoetanol was added to the homogenate buffer as an antioxidant. Homogenates were subjected to horizontal (12%) starch gel electropharesis.

Two different buffer systems were used. System I according to RIDGEWAY *et al.* (1970): electrode buffer — 0.06 M lithium hydroxide and 0.3 M boric acid, pH 8.1 Gel buffer: 0.03 M Tris, 0.005 M citric acid and 1 % electrode buffer, pH 8.5. System II after SICILIANO and SHAW (1976): electrode buffer — 0.13 M Tris and 0.043 M citric acid, pH 7.0. Gel buffer: 1:10 dilution of electrode buffer or 1:6 dilution of electrode buffer designated as system IIa.

Gel silices were stained for the activity of 11 different

Table 1. — Allozyme variants for 11 assayed systems.

Enzyme	Buffer system		Alleles 1	2	3	4
Esterase (E.C.3.1.1.2)	I	Est1	100	95	N	
		Est3	100	70	130	170*
Formate dehydrogenase (E.C.1.2.1.2)	I	Fdh	100			
Glutamate dehydrogenase (E.C.1.4.1.2.)	I	Gdh	100	85*	107*	
Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49)	I	G6pd	100	85	105	
Isocitrate dehydrogenase (E.C.1.1.1.42)	II	Idh	100	75*	105*	
Leucine aminopeptidase (E.C.3.4.1.1)	I	Lap1	100	97	103*	N
		Lap2	100	96*	104	104d*
Malate dehydrogenase (E.C.1.1.1.37)	II	Mdh1	100	125		
		Mdh2	100	110		
		Mdh3	100	40	130*	N
		Mdh4	100			
Menadione reductase	IIa	Mnr2	100			
		Mnr3	100	85*	120*	130
		Mnr4	100	110		
Shikimate dehydrogenase (E.C.1.1.1.25)	II	Shdh	100	70*	115	155
Sorbitol dehydrogenase (E.C.1.1.1.14)	I	Srdh	100	97*		
Superoxide dismutase (E.C.1.15.1.1.)	I	Sod1	100			
		Sod2	100	75*	140*	

* = rare alleles observed only in the bulked seed collections
d = double band
N = null allele

enzymes using recipes described by Mejnartowicz and Bergmann (1975) for leucine aminopeptidase, by Tsay and Taylor (1978) for formate dehydrogenase and by Cheliak and Pitel (1984) for the other enzymes.

The enzymes, buffer systems and number of loci and alleles are presented in *table 1.* The locus specifying the most anodally migrating isozymes was designated as 1, the next as 2, and so on. Within each locus, the most frequent allel was assigned the value of 100. Other alleles of the locus were described according to their mobility relative to the most frequent allele. Alleles lacking stain activity were designated as null (N).

The inheritance of allozyme polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the expected 1:1 ratio, using the Chi-square test. The Chi-square test was also used for the estimation of heterogeneity of results pooled over all trees. (Mather, 1963, chapter 2 :13 to 25 and chapter 7 :69 to 90).

Results and Discussion

Six enzymes had a single zone of activity (FDH, GDH, G6PD, IDH, SHDH, SRDH), while the rest had multiple zones, up to five for the MDH enzyme. The number of alleles observed at polymorphic loci varied from two to four.

A total of nineteen loci was identified in our material. Four of them (Fdh, Mdh4, Mnr2 and Sod1) were monomorphic, another four (Gdh, Idh, Srdh and Sod2) had rare alleles observed only in bulked collections (*Table 1 and Figure 1*). These eight loci were not take into further considerations. In Est1, Lap1 and Mdh3, three of 19 studied loci null allele were observed.

No significant deviation from the expected 1:1 segregation ratio was observed at any locus (*Table 2*). Also there was no significant deviation from this ratio in bulked samples for several heterozygous trees, where such an evaluation was possible with the heterogeneity test ("joint" in *Table 2*).

Esterase (EST)

Three zones of activity were found in our study (*Figure 1*). Est1 and Est 3 stained intensively and consistently, while Est2 stained faintly or was absent on many gels thus it was excluded from this study. Three alleles (one null) were detected at Est1, and four at Est3. Allele Est3-170 was observed only in the bulked collections.

In *Larix decidua* three loci were analysed by means of isoelectric focusing (Kosinski and Szmidt, 1984). Among other coniferous tree species, various number of easterase loci, from one in *Pinus attenuata* (Strauss and Conkle, 1986) to five in *P. torreyana* (Ledig and Conkle, 1983) were described.

It was not possible to determine the subunit structure in our larch embryo material because of blurred staining.

Glucose-6-phosphate dehydrogenase (G6PD)

One zone of activity was evident on gels stained for G6PD

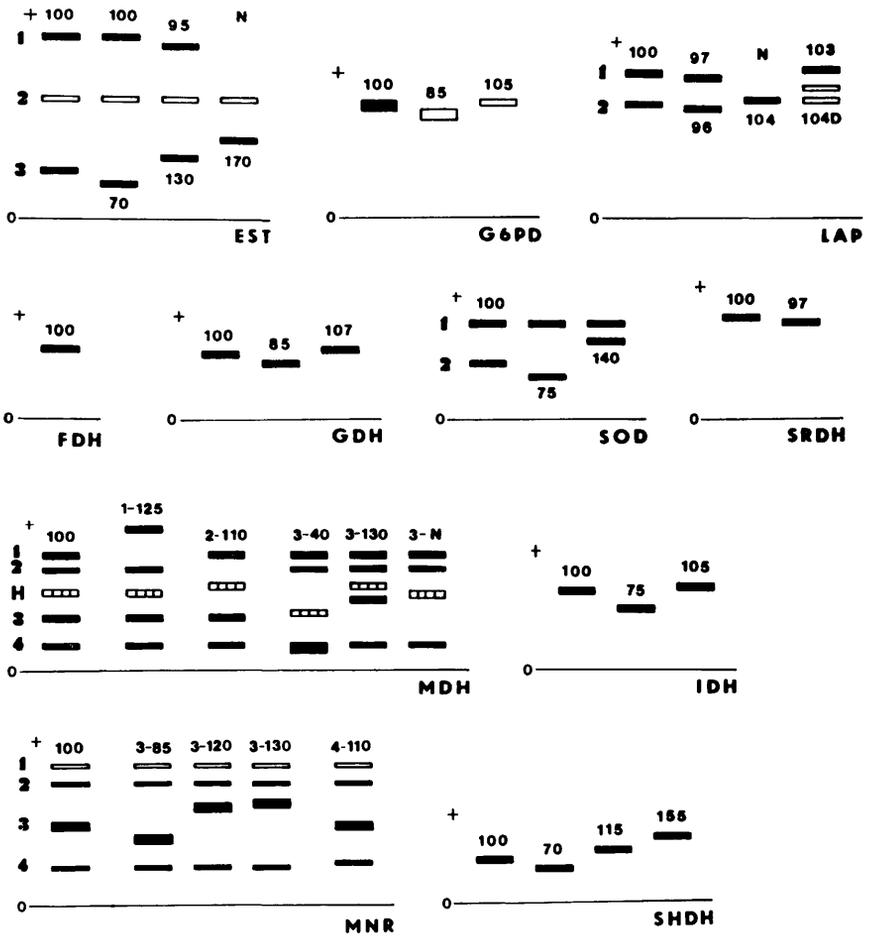

Figure 1. — Line diagrams of isozyme banding pattern of *Larix decidua* gamethophytes. The number above or under bands refer to the relative migration distance (see text for details) Bands black edged represent faint staining and hatched bands represent heterodimers (H). N — indicates null allele. Denotation of the type 3—130 indicates allel 130 at third locus (e. g. for MDH and MNR).

(*Figure 1*). In this zone, three alleles can be observed: the common, darkly staining allele 100 and faintly staining alleles 85 and 105.

One zone of activity for this enzyme was reported for *Larix laricina* (Cheliak and Pitel, 1985) and *Larix occidentalis* (Fins an Seeb, 1986). Some other conifer species had two zones of enzyme activity (Strauss and Conkle, 1986; Ledig and Conkle, 1983; O'Malley et al., 1979).

Because patterns 85 and 105 stained faintly, in both macrogametophyte and embryo tissues, it was not possible to determine the subunit structure of the G6PD enzyme in our material.

Leucine aminopeptidase (LAP)

According to an earlier observation on *Larix decidua* (Mejnartowicz and Bergmann, 1975) it was found that there exist two zones of activity with five patterns of this enzyme in the present study. These zones are encoded by two loci. Genetic control of LAP by two independent loci has been also reported for *Larix laricina* (Cheliak and Pitel, 1985) and for several other conifer species (Bergmann, 1973; Tigersted, 1973; Mejnartowicz, 1976; Rudin, 1977).

In bulked collections we observed additionally three rare alleles Lap1-97, Lap1-103, and Lap2-96 (*Figure 1*).

Heterozygous embryos of *Larix decidua* displayed two-banded monomeric patterns. Howewer the right analysis of heterozygous embryo is difficult because of small differences between bands mobility. LAP structure in *Larix decidua* agree with *Pinus sylvestris* (Rudin, 1977) and *P. muricata* (Millar, 1985).

Malate dehydrogenase (MDH)

Up to five sharply stained zones of MDH have been found. Four of them were designated as Mdh1, Mdh2, Mdh3, Mdh4, (Figure 1). The fifth zone located between Mdh2 and Mdh3 represents a heterodimer (H) between these two zones. Heterodimer MDH-bands were also observed in several other conifer species (O'Malley et al., 1979; El-Kassaby et al., 1982; Cheliak and Pitel, 1985).

Variation in Mdh2 and or Mdh3 results in a change of position of the heterodimer band so that it is always between the two loci. The heterodimer band existed even when a null allele appears at Mdh3. Similar heterodimers between active and null allele were observed in *Pseudotsuga menziesii* (El-Kassaby, 1981). Although the mechanism of catalysis is not know in details for any single enzyme, possible explantation could be that heterodimeric particles MDH2/MDH3 have one active site only on subparticle MDH2 or that they have two active sites on both subparticles. But for their catalytic process only one active site is necessery.

The Mdh4 locus was monomorphic. Two alleles were detected for Mdh1 and Mdh2 loci and four alleles for

Table 2. — Oberserved allozyme segregation in macrogametophytes of heterozygous trees and χ^2 tests for goodness of fit to 1:1 ratio and heterogeneity among employed trees.

Locus	Tree	Allelic combination	Observed segregation	Deviation $X^2(1)$	P	Heterogeneity $X^2(df)$	P
Est1	10-01	95/100	35;47	1,756	,10 - ,25	-	-
	MW 7	95/N	31;33	,063	,75 - ,90	-	-
	ST 1	100/N	30;39	1,174	,25 - ,50	-	-
	MW 15	100/N	28;31	,153	,50 - ,75	-	-
	ST 8	100/N	23;23	,000	>,99	-	-
	joint	100/N	81;93	,828	,25 - ,50	,499(2)	,75 - ,90
Est3	10-01	70/100	42;40	,049	,75 - ,90	-	-
	10-20	100/130	30;26	,286	,50 - ,75	-	-
G6pd	10-09	85/100	29;25	,296	,50 - ,75	-	-
	10-06	85/105	24;34	1,724	,10 - ,25	-	-
	SK 5	100/105	31;28	,153	,50 - ,75	-	-
Lap1	ST 6	97/100	22;21	,023	,75 - ,90	-	-
	SK 5	100/N	32;37	,362	,50 - ,75	-	-
	SK 7	100/N	33;27	,600	,25 - ,50	-	-
	joint	100/N	65;64	,008	,90 - ,95	,954(1)	,25 - ,50
Lap2	SK 7	100/104	31;29	,067	,75 - ,90	-	-
	ST 6	100/104	20;23	,209	,50 - ,75	-	-
	joint	100/104	51;52	,010	,90 - ,95	,266(1)	,50 - ,75
Mdh1	MW 3	100/125	29;28	,018	,90 - ,95	-	-
	MW 7	100/125	36;37	,014	,90 - ,95	-	-
	joint	100/125	65;65	,000	>,99	,032(1)	,75 - ,90
Mdh2	10-01	100/110	24;29	,472	,25 - ,50	-	-
Mdh3	MW 3	40/100	29;28	,018	,90 - ,95	-	-
	MW 7	40/100	38;35	,123	,50 - ,75	-	-
	BA425	40/100	15;15	,000	>,99	-	-
	joint	40/100	82;78	,100	,75	,041(2)	,97 - ,99
	15-63	100/N	26;30	,286	,50 - ,75	-	-
	15-88	100/N	10;14	,667	,25 - ,50	-	-
	joint	100/N	36;44	,800	,25 - ,50	,153(1)	,50 - ,75
Mnr3	MW 5	100/130	36;41	,325	,50 - ,75	-	-
	MW 8	100/130	46;35	1,494	,10 - ,25	-	-
	joint	100/130	82;76	,228	,50 - ,75	1,591(1)	,10 - ,25
Mnr4	MW 5	100/110	39;38	,013	,90 - ,95	-	-
	MW- 8	100/110	50;52	,039	,75 - ,90	-	-
	joint	100/110	89;90	,006	,90 - ,95	,046(1)	,75 - ,90
Shdh	10-20	100/115	28;26	,074	,75 - ,90	-	-
	10-25	100/155	37;41	,205	,50 - ,75	-	-

Mdh3, including a null allele. Allele Mdh3-40 was located below Mdh4 zone, although they have been observed as a one joined, thick band (see *Figure 1*).

Heterozygous embryos for the Mdh1 and Mdh3 loci displayed three-banded patterns, suggesting that their subunit structure are dimeric. Subunit structures for Mdh2 and Mdh4 were not investigated because the Mdh4 locus was monomorphic and the staining of Mdh2 were faint in the embryo.

Menadione reductase (MNR)

Larix decidua gels stained for this enzyme revealed four zones. Segregation was studied at two (Mnr3 and Mnr4) zones. Analysis of the genetic control of the upper two zones was not possible because of faintly stained first zone and monomorphic character of second zone.

Although we named these as menadione reductase zones, it must be mentioned, that the same patterns of mobility and activity of enzymes were received when the gels were stained with 2,6-dichlorophenolindophenol, a substrate for diaphorase (DIA). Similary, identical zymograms for some coniferous tree species (*Picea abies, Pinus contorta, P. jeffreyi, P. mugo, P. nigra, P. sylvestris* and *Pseudotsuga menziesii*) were observed by us when menadione or 2,6-dichlorophenolindophenol were used as substrat.

The equivalence of Dia3 and Mnr3 loci, was earlier noticed by Strauss and Conkle (1986) for *Pinus attenuata.*

It seems, that both substrates used gave a picture of the same enzyme or of some enzymes with low substrate specifity (to resolve this problem additional investigations are needed). For all four zones of this enzymatic activity observed in *L. decidua* we left arbitrarily the name menadione reductase until definite resolution of the question.

Mnr3 was coded with four alleles and Mnr4 with two only. Alleles Mnr3-85 and Mnr3-120 were observed only in bulked seed collections.

It was not possible to determine the subunit structure of MNR in our material because of blurred staining of the embryos.

Shikimate dehydrogenase (SHDH)

One zone of activity was evident on gels stained for SHDH. One monomorphic SHDH zone has been observed

in *Larix decidua* and *L. kaempferi* macrogametophytes by Bergmann and Ruetz (1987). In our study four alleles were detected at this locus (*Figure 1*). Heterozygous embryos displayed two-banded monomeric patterns. This agrees with the structure of SHDH in *Pinus ponderosa* (Linhart et al., 1981), *P. sylvestris* (Szmidt and Yazdani, 1984) and *P. muricata* (Millar, 1985).

Clear and intensive staining of SHDH variants in macrogametophyte and embryo samples as well as the considerable high polymorphism makes this enzyme especially useful as a genetic marker in *Larix decidua*.

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Genetics of Sweet Chestnut (Castanea sativa Mill.)

III. Genetic Analysis of Zymograms of Single Tree Offspring

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Summary

Genetic analysis of four enzyme systems (PGI, IDH, DIA, AP) in European sweet chestnut (*Castanea sativa* Mill.) is performed by a method utilizing single trees and their seed progenies, as offspring from controlled crosses were not available. A total of six variable gene loci with single-locus codominant modes of inheritance were intuitively postulated on the basis of adult zymograms alone. Subsequent genetic analysis supported the intuitive interpretation for only five of the six proposed loci. The results for the sixth, AP-A, demonstrate the necessity for genetic analysis of zymograms, the limitations of such analysis, and its usefulness for providing indications of distortive phenomena. These results are summarized as follows:

Observation of adult zymograms suggests complete codominance for AP-A, but qualitative analysis of the single tree progenies compels rejection of this hypothesis, instead suggesting the presence of a (recessive) null allele, A_0, in several maternal trees. The revised, null-allele-hypothesis is unequivocally supported by the qualitative tests in all maternal trees as well as by the quantitative tests in all maternal trees not possessing A_0. It must, however, be rejected on the grounds of the quantitative test for heterozygous maternal trees presumably possessing the null allele: A_0 was significantly overrepresented in two progeny sets, significantly underrepresented in a third, and showed no significant deviation from expectation for the remaining three. It is considered whether the null-allele-hypothesis must necessarily be false or whether other phenomena could have caused the seemingly erratic segregation distortion. Possible explanations are discussed in the light of the fact that, despite the apparently considerable frequency of A_0 in the population, no adult trees nor any seeds were found to be homozygous for it.

Key words: Electrophoresis, zymogram, genetic analysis, *Castanea*.

1. Introduction

Previous papers dealing with enzyme systems in sweet chestnut were limited to studies of the isoenzyme pheno-

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