GENETIC VARIATION IN *ACER PSEUDOPLATANUS* L. I. INHERITANCE OF ISOZYME VARIANTS

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Received August 14, 2000; accepted December 12, 2000

ABSTRACT

Genetic analysis of 11 enzyme systems in *Acer pseudoplatanus* was performed utilizing single trees and their open pollinated progenies. A total of 25 gene loci were intuitively postulated on the basis of zymograms from mother trees. For 17 gene loci genetic analysis was conducted which supported the intuitive interpretation in all cases. For some systems, auch as AAT, PGI, PGM very complex band patterns were obtained. Difficulties in the interpretation of these patterns are discussed.

Key words: *Acer pseudoplatanus*, isozymes, inheritance, zymogram interpretation

INTRODUCTION

Mountain maple or sycamore maple (*Acer pseudoplatanus* L.) is an important tree species of the mixed montane forest zone in Central Europe. In the submontane zone it is found together with other noble hardwoods; in the montane zone primarily with European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*) and European silver fir (*Abies alba*). Its deep root system anchors the soil, making mountain maple an essential component in protection forests for erosion control and against snow slippage. The litter decays readily and under the shelter of the maple, beech and fir regenerate easily. The wood is of high quality (furniture, flooring etc.) and is always in demand. Last but not least its light green summer colors and the yellow fall coloring make it an attractive species in contrast to the dark green spruce and fir.

The importance of mountain maple has increased in recent years because of the conversion of pure spruce stands into multi-species mixed stands. As a result, the demand for plants from the nursery has increased and the demand for seed is considerable. Increasingly more attention is also paid to planting material of the proper provenance. This was not always possible since maple was a minor species with a very scattered occurrence.

In order to increase the stocking levels with maple, two seed orchards for the Bavarian Alps, one for the submontane/montane zone and one for the upper-montane/subalpine zone were established near our nursery in Laufen (Southeast Bavaria) in 1975. The intention of the seed orchards was to facilitate seed collection, obtain higher quality seed due to better pollination and greater genetic variation, and also to improve the stem/wood quality. Both seed orchards flower regularly and produce considerable amounts of seed. The seed is certified as "selected" reproductive material.

At the time the plus-trees were selected for inclusion in the seed orchards (early seventies) information on the genetic variation among forest tree populations was lacking. The selection was carried out solely on a phenotypic basis. Since the time of establishment of the orchards, the use of isoenzymes as gene markers has become a standard procedure for investigating the genetic composition of many forest tree species. Consequently it was our goal to look at the genetic composition of the two seed orchards. As a first step it was necessary to establish the isoenzyme analysis as a routine method for determining the genetic variation in mountain maple. A prerequisite is the inheritance analysis for the observed isozyme phenotypes, which we want to report on in this paper.

MATERIAL AND METHODS

Material

We investigated seed and bud tissue from the 83 mountain maple clones included in the two seed orchards near Laufen. The seed orchard I "Laufen Lebenau" contains 41 clones of the provenance region 810-10 "Alps, below 900 m", the seed orchard II "Laufen Brennmais" contains 42 clones of the provenance 810-11 "Alps, above 900 m".

In the fall of 1996 and 1998 we collected seeds

from each flowering clone as well as bud tissue during the winter. Seeds and buds were stored at -20 °C.

Electrophoresis methods

Extraction of enzymes

The samples were homogenized in 0.1M TRIS-HCl buffer with 3% PVP and 0.07 mmol.l^{-1} ß-mercapto-ethanol. The addition of further components (cystein, ascorbic acid, saccharose, NAD, NADP, EDTA, KCl, $MgCl_2$, glycerol, bovine albumin, DTT) did not result in further improvement of the enzyme activity nor the degree of separation. Because of the high enzyme activity of the seed and the buds, the amount of tissue used for the extraction should not be too large, otherwise the staining results in diffuse, unclear bands. With 70µl extraction buffer we extracted ⅓ of a seed or the green tissue from one bud, respectively.

Separation and staining of enzymes

The composition of the starch gels and the separation conditions are compiled in Table 1. The staining of enzymes was performed according to recipes given by CHELIAK & PITEL (1984) and KONNERT & MAURER (1995). To test the substrate specificity of the enzymes, the staining was carried out with and without substrate for each enzyme system on replicate slabs of the same gel.

Genetic analysis

No controlled crosses were available for the genetic analysis. Thus we carried out the analysis according to the method described by GILLET & HATTEMER (1989), which compares the mother tree with the open pollinated progeny. This method has been used successfully for the genetic analysis of zymograms by *Castanea sativa* (FINESCHI *et al.* 1989), *Cupressus sempervirens* L. (PAPAGEORGIOU 1993), *Alnus acuminata* (MURILLO & HATTEMER 1997), *Acer campestre* (LEINEMANN & BENDIXEN 1999), *Nothofagus nervosa* Phil. (MARCHELLI & GALLO).

The following conditions must be checked and met when using this method (GILLET & HATTEMER 1989):

- All progeny of a homozygous mother tree (e.g. A_iA_i) must possess the allele of the maternal tree (A_i)
- In case of a heterozygous mother tree (e.g. A_iA_j, $i \neq j$):

  a) each individual among the offspring must contain one of the maternal alleles A_i, A_j.

  b) the number of the heterozygous progenies A_iA_j (N_{ij}) is expected to be equal to the sum of homozygous progenies A_iA_i (N_{ii}) and A_jA_j (N_{jj})

$$N_{ij} = N_{ii} + N_{jj}$$

  c) the number of the heterozygous progenies A_iA_k (N_{ik}), is expected to be equal to the number of the heterozygous progenies A_jA_k

$$N_{ik} = N_{jk} \; (k \neq i, j)$$

The genetic analysis was carried out as follows: After analysing all 83 clones we registered all isoenzyme patterns and established hypotheses as to their genetic control. To test the respective hypothesis we investigated betwen 40 and 80 seeds from respective maternal trees. After comparing the observed phenotypes with the expected ones, our intuitive hypothesis was either accepted or discarded. The deviation between observed and expected values was tested with χ^2 was either accepted or discarded be goodness-of-fit tests.

Table 1. Separation systems for starch gel electrophoresis of enzymes in *Acer pseudoplatanus*.

Nr.	Compositions of gels	Electrode buffer / pH	Gel buffer / pH	Running conditions	
				I const. (mA)	Time (h)
1.	**TRIS-citrate system** 10.5 % starch + 4 % sucrose	0.135 TRIS-Citrate / 7.5	E-buffer add. 75 % dest. H_2O	170	5–5½
2.	**ASHTON-system** 10 % starch + 4 % sucrose	0.03 M LiOH- 0.2 M H_3BO_3 / 8.1	0.05 M TRIS-Citrate / 8.1 add. 10 % E.-buffer	80	4–5½
3.	**HISTIDIN-citrate system** 10.5 % starch +2 % sucrose; 1 % urea; 0.07 % EDTA	0.06 M HISTIDIN- Citrate / 6.2	E-buffer add. 75 % dest. H_2O	90	5–5½

Table 2. Enzyme systems investigated for inheritance.

Enzyme system (abbreviation) E.C. Number	Prospective controlling gene loci	Separation system (No. in Table 1)
Aspartate aminotransferase (AAT) E.C. 2.6.1.1	*Aat-A, -B, -C*	2
Alcohol dehydrogenase (ADH) E.C. 1.1.1.1	*Adh-A, -B*	1.3
Isocitrate dehydrogenase (IDH) E.C. 1.1.1.42	*Idh-A, -B*	1.3
Leucine aminopeptidase (LAP) E.C. 3.4.11.1	*Lap-A*	3
Malate dehydrogenase (MDH) E.C. 1.1.1.37	*Mdh-A, -B, -C, -D*	3
Menadion reductase (MNR) E.C. 1.6.99.1	*Mnr-A*	1
NADH-dehydrogenase (NDH) E.C. 1.6.99.2	*Ndh-A*	1
6-Phosphogluconate dehydrogenase (6-PGDH) E.C.1.1.1.44	*6-Pgdh-A, -B*	3
Phosphoglucose isomerase (PGI) E.C. 5.3.1.9	*Pgi-A, -B, -C*	2
Phosphoglucomutase (PGM) E.C. 2.7.5.1	*Pgm-A, -B, -C, -D*	2

RESULTS

The enzyme systems investigated are listed in Table 2. For GDH and G6PDH no or only very faint staining was obtained. For the systems MDH and LAP a complete genetic interpretation was not possible because of very complex banding patterns. For the other investigated systems a good resolution with variable band patterns was obtained. It is known that mountain maple is tetraploid and has 52 chromosomes (ROHMEDER & SCHÖNBACH 1959). However, in the zymograms we found patterns as would be expected for diploid organisms. Thus we assumed autopolyploidie and a disomal mode of inheritance in mountain maple. The autopolyploidie appears to be expressed more frequently for many enzyme systems by an increased number of gene loci. Similar results have been found in the hexaploid *Tilia* (FROMM, personal communication).

Figure 1 shows the banding patterns of allozymes for the different loci whereas in Figure 2 examples of zymograms for four highly variable systems are given together with their genetic interpretation. In Tables 3 to 9 N_{ij} denotes the observed number of offspring phenotypes, N the total sample size, χ^2_1 refers to tests of $N_{ij} = N_{ii} + N_{jj}$, χ^2_2 to tests of $N_{ik} = N_{jk}$ $(k \neq i, j)$.

Aspartate amino transferase (AAT)

AAT (also known as GOT) showed two zones of activity in the zymograms. In the faster migrating zone A, two single-banded and one triple-banded variant were observed in bud extracts. In seeds an additional triple-banded variant appeared. It was postulated that this zone is controlled by one locus, denoted *Aat-A*. The genetic analysis verified this assumption (Table 3). Each offspring possesses at least one maternal allele and there was no significant deviation from the expected 1:1 ratio of homozygous:heterozygous offspring. Three alleles were identified at this locus.

The interpretation of the phenotypes from zone B (Figure 2), which show one, three or five bands with differing intensity, is much more difficult. If one considers the dimeric structure of the enzyme AAT one can explain the patterns by a two loci control (*Aat-B* and *Aat-C*) and the appearance of inter- and intralocus hybrid-bands. One of the observed patterns shows only one intensively stained band. Other patterns differ only in the intensity of the bands, but not in their position. These observations indicate that enzymes controlled by the two loci partially overlap. It was assumed that the allele B_2 has the same position in the zymogram as the allele C_1. The same holds for the alleles B_3 and C_2. Therefore it is often difficult to match the respective pattern to a specific genotype since theoretically some patterns can correspond with different genotypes. For the present it appears that at the locus *Aat-B* three alleles were identified and four alleles at *Aat-C*.

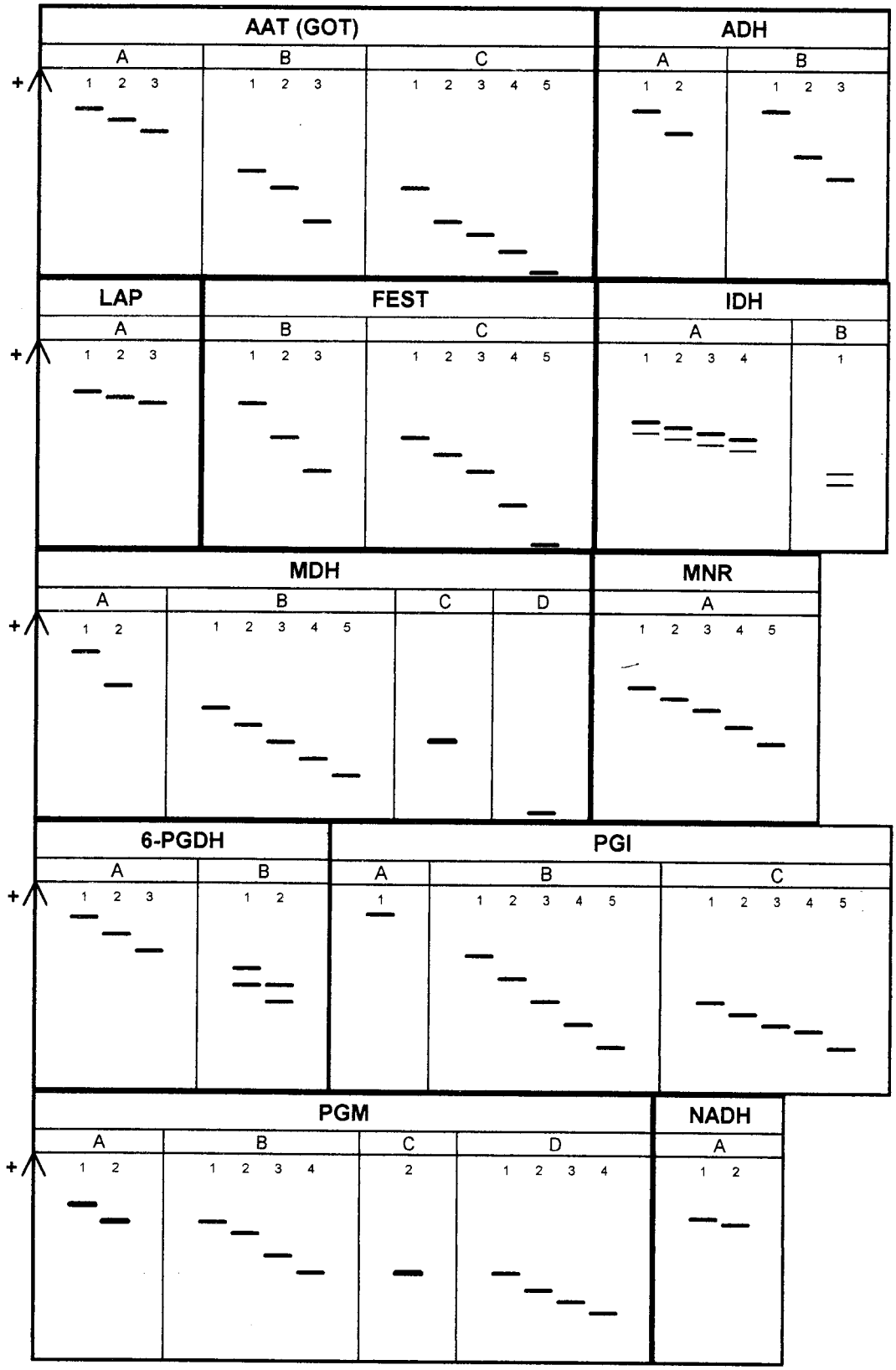

Figure 2. Zymograms from bud tissue of *Acer pseudoplatanus* for the enzyme systems AAT, ADH, PGI and PGM. Schematic illustration and designation of genotypes.

Table 3. Genetic analysis of *Aat-A, Aat-B & Aat-C* in *Acer pseudoplatanus*.

Maternal tree		Progeny											χ^2_1
Nr	Type	N_{11}	N_{12}	N_{13}	N_{14}	N_{22}	N_{23}	N_{24}	N_{33}	N_{34}	N_{44}	N	
39	A_1A_2	14	35			31						80	1.25 n.s.
47	A_1A_2	3	32			42	3					80	2.20 n.s.
2	A_1A_2	4	46			30						80	1.80 n.s.
65	A_2A_2		18			57						75	
49	A_2A_2		21			55						76	
8	B_1B_2		21			23		1			12	43	0.02 n.s.
	C_1C_4	10			20							43	0.09 n.s.
9	B_1B_2	2	15			24						41	2.95 n.s.
	C_1C_4	10			20						11	41	0.02 n.s.
65	B_2B_2		2			67	7					75	
	C_1C_1	23			52							75	
89*	B_2B_2		1			26	10					37	
	C_1C_2	3	10		10	4		10				37	1.53 n.s.
49	B_2B_2					65	11					76	
	C_1C_4	7			42						27	76	0.84 n.s.
47	B_2B_2					77	3					80	
	C_1C_4	9			41						30	80	0.05 n.s.
10**	B_2B_2					68	5					73	
	C_3C_4			5	10				13	27	18	73	0.28 n.s.
11***	B_2B_2					33	6					39	
	C_3C_4			3	6				7	13	10	39	0.53 n.s
35	B_2B_2					67	1					68	
	C_4C_4				7						61	68	
57	B_2B_2					42	8					50	
	C_4C_4				3						47	50	
2	B_2B_3					23	41		16			80	0.05 n.s.
	C_1C_1	15	10		55							80	
88	B_2B_3					30	22		12			64	0.25 n.s.
	C_1C_4	10			35						19	64	0.56 n.s.
39	B_2B_3					39	37			4		80	0.45 n.s.
	C_4C_4				8						72	80	

* $\chi^2_2 = 0.00$ n.s.; ** $\chi^2_2 = 1.67$ n.s.; *** $\chi^2_2 = 1.00$ n.s.

Alcohol dehydrogenase (ADH)

After staining ADH we found six different patterns in one zone with very clearly differentiated bands (Figure 2). The occurrence of up to eight bands within one pattern and their relative position toward each other could be explained by the assumption that this zone is controlled by two gene loci and the formation of inter- and intralocus hybrids (dimeric enzyme). In the sampled material from the seed orchards we found two alleles for both gene loci. From seed from other Bavarian maple stands we found a further allele (B_1 in Figure 1) at the gene locus *Adh-B*. For all clones the genetic analysis supports the intuitive hypotheses of a two-locus, codominant mode of inheritance (Table 4).

Isocitrate dehydrogenase (IDH)

Gels stained for IDH showed two zones of activity. The faster migrating zone (A) stained more rapidly and

Table 4. Genetic analysis of *Adh-A, Adh-B, Lap-A* and *Ndh-A* in *Acer pseudoplatanus*.

Maternal tree		Progeny							χ^2_1
Nr	Type	N_{11}	N_{12}	N_{13}	N_{22}	N_{23}	N_{33}	N	
				Adh-A, Adh-B					
2	A_1A_1	60						60	
	B_2B_2				54	6		60	
47	A_1A_1	63						63	
	B_2B_2				60	3		63	
18	A_1A_1	79	2					81	
	B_2B_3				38	40	3	81	0.01n.s.
8	A_1A_1	60						60	
	B_2B_3				23	24	13	60	2.40 n.s.
53	A_1A_1	75	5					80	
	B_3B_3					60	20	80	
6	A_1A_1	80	2					82	
	B_3B_3					15	67	82	
56	A_1A_2	42	38		60	20		80	0.20 n.s.
	B_2B_2							80	
1	A_1A_2	36	30		25	33	8	66	0.55 n.s.
	B_2B_3							66	0.00 n.s.
				Lap-A					
61	A_1A_1	48	2	5				55	
44	A_1A_1	52	7	1				60	
36	A_1A_2	24	30		12			66	0.54 n.s.
64	A_1A_2	18	32		20			70	0.51 n.s.
24	A_1A_3	15	32				21	68	0.24
25	A_1A_3	25	29				14	68	1.47
4	A_2A_2		8		52	1		61	
59	A_2A_2		8		42			50	
3	A_3A_3			4			61	65	
				Nadh-A					
25	A_1A_2	5	15		16			36	1.00 n.s.
12	A_2A_2				38			38	
13	A_2A_2				42			42	

more intensively than the slower migrating one (zone B). Zone A appears to be controlled by a single locus with four alleles encoding a dimeric structure. This hypotheses was confirmed by the results of the genetic analysis (Table 5). Isozymes of *Idh-A* stain as double bands of equal intensity. Presumable heterozygous trees and offsprings showed five-banded variants.

The slower migrating zone B stains weakly only after several hours. Despite high concentrations of the extract (which led to overstaining of the A-zone) bands were not clear enough to be evaluated. We assume that this zone is controlled by a further gene locus, *Idh-B*, but we were not able to test this hypothesis.

Leucineaminopeptidase (LAP)

Gels stained for LAP showed two variable zones of activity. The faster migrating one (zone A) appears only

Table 5. Genetic analysis of *Idh-A, Mdh-A* and *6-Pgdh-B* in *Acer pseudoplatanus*.

Maternal tree		Progeny											χ^2_1
Nr	Type	N_{11}	N_{12}	N_{13}	N_{14}	N_{22}	N_{23}	N_{24}	N_{33}	N_{34}	N_{44}	N	
						Idh-A							
39	A_1A_2		33			26						59	
10	A_2A_2					40						40	
8	A_2A_2					37	3					40	
18*	A_2A_3		1	1		39	42	2	4			89	
77	A_2A_3					31	28					59	
74	A_2A_3					17	19		4			40	
86	A_2A_4					22	2	16				40	
72	A_2A_4					40		18				58	
						Mdh-A							
12	A_1A_1	19	3									22	
54	A_1A_1	25	7									32	
27	A_1A_2	17	25			14						56	
24	A_1A_2	9	30			21						60	
17	A_2A_2		17			12						29	
21	A_2A_2		8			30						38	
						6-pgdh-B							
7	B_1B_1	13	27									40	
1	B_1B_2		18			22						40	
12	B_1B_2	2	21			17						40	
6	B_2B_2		17			57						74	

* $\chi^2_2 = 0.00$ n.s.

in gels stained for LAP. In this zone single-banded and double-banded variants occured. Therefore it was assumed that this zone is controlled by one gene locus, called *Lap-A*.

Three alleles were identified in the clone material. The enzyme has a monomeric subunit structure. The segregation analysis confirmed our hypothesis of genetic control (Table 4).

More difficult was the interpretation of the slower migrating zone (B). This zone appears in gels stained for LAP but also in gels stained for AAP, with only slight differences in the band patterns.

The comparison of the staining for LAP and AAP on two replicate slabs of the same gel shows that in this zone at least two gene loci overlap, so that a correct evaluation is not possible.

Malate dehydrogenase (MDH)

By staining for MDH very complicated patterns with numerous bands appear on the zymogram. Similar results have also been reported for this system for other species, e.g. *Abies alba* (HUSSENDÖRFER *et al.* 1995). The reason is that malate dehydrogenase (MDH), as found in higher plants, represents a complex system composed of various isozymes which function in different cell compartments (YANG & SCANDALIOS 1974). The different isozymes could have similar migration rates and overlap in the zymograms. In addition the system has a dimeric subunit structure and thus forms inter- and intralocus hybrids, wich complicates the analysis. By staining with the common tetrazolium method both the non-decarboxylating MDH and the oxalacetate-decarboxylating MDH can be stained,

Table 6. Genetic analysis of *Mnr*-A. For progenies only genotypes found in at least one seed sample are listed; (significance level: * α = 0.05).

Maternal tree		Progeny											
Nr	Type	N_{11}	N_{13}	N_{15}	N_{23}	N_{33}	N_{34}	N_{35}	N_{44}	N_{55}	N	χ^2_1	χ^2_2 [a]
15	A_1A_3	1	16	1		30	1	2			51	5.65*	0.33 n.s.
34	A_1A_3	1	18			28	2				49	2.57 n.s.	1.99 n.s.
51	A_1A_3		18			24					42	0.86 n.s.	
74	A_1A_3	4	19	1		21					45	0.82 n.s.	0.99 n.s.
47	A_2A_3				18	24					42	0.86 n.s.	
39	A_2A_3				5	13	2				20	1.20 n.s.	2.99 n.s.
43	A_3A_3		1			71					72		
13	A_3A_3		2		2	60	6				70		
69	A_3A_4					10	30		14		54	0.67 n.s.	
56	A_3A_4					15	28		12		55	0.02 n.s.	
78	A_3A_5		3	2		16		20		1	41	0.24 n.s.	0.20 n.s.
53	A_3A_5		1			18		24		2	45	0.36 n.s.	0.99 n.s.
86	A_3A_5					14		31		6	51	2.37 n.s.	
89	A_3A_5		2			15		18			35	0.27 n.s.	1.99 n.s.

[a] test statistics are inflated due to frequencies less than 5 and/or equal zero.

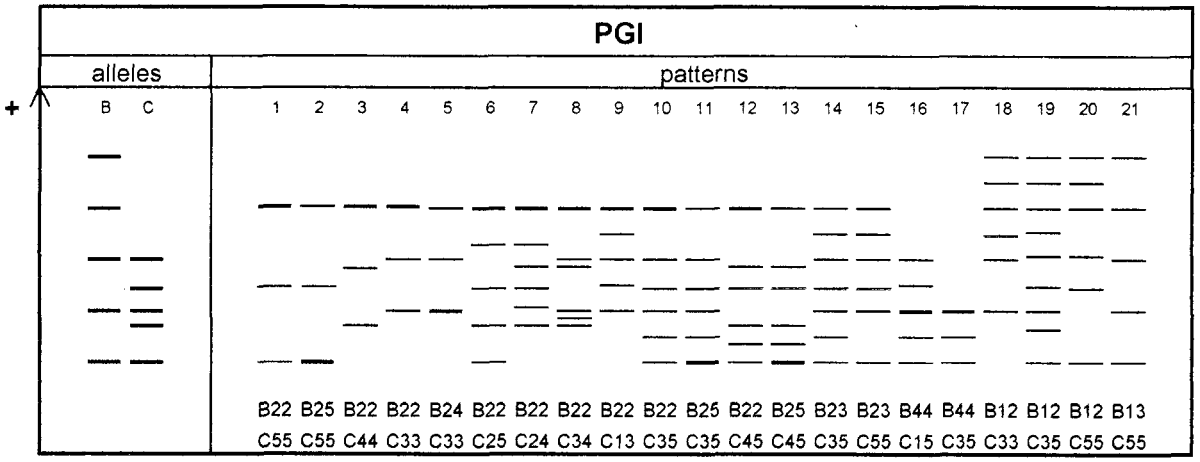

Figure 3. Survey of isozyme patterns at PGI in *Acer pseudoplatanus* and genetic interpretation based on the results of inheritance analysis.

by staining with Fast Blue BB only the oxalacetate-decarboxylating MDH (THORMANN & STEPHAN 1993, HUSSENDÖRFER *et al.* 1995). In the zymograms obtained by the tetrazolium staining method only the fastest migrating bands could be attributed to one gene locus, called *Mdh-A*, with two alleles in the studied material. Putative heterozygous trees for *Mdh-A* showed triple banded variants. For this gene locus the segregation of bands fit well with the expected ratio (Table 5). When staining with Fast Blue BB this gene locus does not appear in the zymogram. However the somewhat slower migrating zone seems to be controlled by two additional gene loci, *Mdh-B* and *Mdh-C*, whose enzyme forms inter- and intralocus hybrids which partially overlap. *Mdh-B* seems to have five alleles in the studied material, *Mdh-C* seems to be monomorphic. Staining with Fast Blue BB gives good, identifiable pattern only if the sample material is fresh. The longer it is stored the poorer the quality of the zymograms, especially for extracts of seed. This was also the reason

why we could only carry out a limited number of segregation analyses and that the interpretation must be regarded as provisional.

A further putative gene locus, *Mdh-D*, is located very close to the cathode and was made visible using both staining techniques. The gene locus was found to be monomorphic in the material we investigated.

Menadione reductase (MNR)

On gels stained for MNR only one zone was consistent enough to score and was included in our evaluation. This variable zone appears to be tetrameric; the patterns either had one band (supposedly homozygous trees) or had five bands (supposedly heterozygous trees). A single locus, codominant mode of inheritance was inferred. Five alleles were identified at this locus. The genetic analysis supports the intuitive hypothesis for nearly all tested clones (Table 6).

6-Phosphogluconate-dehydrogenase (6-PGDH)

Two zones of activity were found in gels stained for 6-PGDH. The faster migrating zone (A) stains weakly and shows single-banded and multi-banded variants. It was assumed that this zone is controlled by one locus, *6-Pgdh-A*, with three alleles and a dimeric structure of the enzyme (appearance of inter- and intralocus hybrid bands). The genetic analysis is not yet completed.

In the slower migrating zone (B) two double-banded variants and one triple banded variant appeared. Segregation data support the hypothesis of one diallelic locus, denoted *6-Pgdh-B* (Table 5). Homozygous genotypes were double-banded and heterozygous genotypes always triple-banded, suggesting a dimeric subunit structure.

Phosphoglucoseisomerase (PGI)

The system PGI is highly variable in mountain maple and the genetic evaluation is extremely difficult. When staining for PGI one obtains a faster migrating zone (A), which showed no variation in the material we investigated, and a slower migrating zone (B), with a high degree of variation. Zone A is probably controlled by one gene locus which we called *Pgi-A*. In the slower migrating zone eighteen different patterns were found for the bud tissue from the 83 investigated clones (nr. 1–18 in Figure 3). These patterns had three to nine clearly defined bands of different staining intensity. The resolution in this zone was high. In the seed extracts the number of patterns found was higher.

The observed patterns can only be explained under the assumption of a two locus control and the formation of intra- and interlocus hybrids (dimeric structure of the enzymes). The zones controlled by the two loci, called *Pgi-B* and *Pgi-C*, show substantial overlap; this means that bands expressed by these loci can be located in the zymograms in identical positions. For each of these two presumed loci five alleles are present. As a consequence, the genetic interpretation is difficult since many patterns can represent various genotypes. For each pattern found seeds from at least one clone were investigated. Based on the observations of the obtained band patterns we made the following assumptions:

- the enzymes controlled by *Pgi-B* are more active than those controlled by *Pgi-C* and thus stain more intensively;
- hybrid bands always stain more intensively;
- the corresponding band of an allele is always more intensive in homozygous than in heterozygous individuals.

Under this presumption, the genotypes of clones were intuitively postulated on the basis of observed patterns as indicated in Figure 3. The results of the genetic analysis support this hypothesis in most cases. In Table 7 are listed examples of genotypes as determined from the genetic analysis. Considerably more clones than those listed were investigated, whereby only two cases were found with deviations from the expected segregation ratio. For two clones the assumed genotype had to be corrected. For clone 62 on the basis of the zymogram pattern the genotype B_3B_5/C_3C_3 was postulated (Nr. 16 in Figure 3). However, none of the progenies (seeds) had the allele B_3, but all had the allele B_4, often as a homozygote. Similarly we found in seeds the genotypes C_1C_1, C_1C_5 and C_5C_5. Thus we had to correct our initial assumption: The maternal tree thus has the genotype B_4B_4/C_1C_5, which can also be explained by pattern 16 since the alleles B_3 and C_1, B_4 and C_3 as well as B_5 and C_5 lie at the same position (distance) on the zymogram.

Theoretically many more two locus combinations can occur than those listed in Figure 3. In further investigations on mountain maple in our laboratory we found many more patterns for PGI than those described here.

Phosphoglucomutase (PGM)

Two zones of activity were evident on gels stained for PGM. The faster migrating zone (A) was monomorphic and stained weaker than the slower migrating one (zone B). We presume that it is controlled by a single locus, denoted *Pgm-A*. In zone B various patterns with one to five bands were observed, whereby one of these bands is always more intensively stained than the others (see Figure 2). PGM is generally a monomeric system. Thus, a possible explanation for these band patterns is that

Table 7. Genetic analysis of *Pgi-B* and *Pgi-C* in *Acer pseudoplatanus*.

Maternal tree		Progeny																	χ^2_2
Nr	Type	N_{11}	N_{12}	N_{13}	N_{14}	N_{15}	N_{22}	N_{23}	N_{24}	N_{25}	N_{33}	N_{34}	N_{35}	N_{44}	N_{45}	N_{55}	N	χ^2_1	
90	B_1B_2	6	18				22										46	2.17 n.s.	
	C_3C_3			2							14		30				46		
98	B_1B_2		26				18										44	1.45 n.s.	
	C_5C_5												12		2	30	44		
69	B_1B_2	5	20				12			3							40		
	C_3C_5										1		15			24	40	2.0 n.s.	
91	B_1B_3	5	23	4				7			3						42	1.33 n.s.	8.53**
	C_5C_5												12		1	29	42		
54	B_2B_2						41			1							42		
	C_2C_5						1	3		13			7		3	15	42	0.31 n.s.	1.60 n.s.
13	B_2B_2						29	4	1								34		
	C_3C_3			2							11		21				34		
78	B_2B_2						19	4	1								24		-
	C_3C_4										3	7	4	7	3		24	0.89 n.s.	0.14 n.s.
18	B_2B_2		1				49	1									51		0.3(1.8)
	C_3C_5			1		2					4	1	23		4	16	51	0.21 n.s.	n.s.
39	B_2B_2		4				74										78		
	C_4C_5											9	9	7	18	26	78	0.60 n.s.	0.00 n.s.
21	B_2B_2						52	5									57		
	C_5C_5					1				1			8		5	42	57		
51	B_2B_2						39			3							42		
	C_1C_3	1		11		10					11		9				42	0.04 n.s.	0.05 n.s.
55	B_2B_3						16	22			4						42	0.09 n.s.	
	C_3C_5			1							13		18			10	42	0.61 n.s.	
81	B_2B_5						20	3		19							42	0.03 n.s.	2.99 n.s.
	C_5C_5					3							2		9	28	42		
61	B_2B_5						24			18							42	0.86 n.s.	
	C_3C_5												18			24	42	0.86 n.s.	
43	B_4B_4								23					12	5		40		
	C_3C_5										3		23		2	12	40	1.68 n.s.	1.99 n.s.
62	B_4B_4								22					17	3		42		
	C_1C_5	5		4		11							10			12	42	1.29 n.s.	2.57 n.s.

this zone is controlled by at least three gene loci. The genetic analysis demonstrated that this assumption was correct. The gene locus *Pgm-C* is always more intensively stained and is located between the gene loci *Pgm-B* and *Pgm-D* with four alleles respectively. Differentiating between the alleles B_4 and D_1 poses difficulty since they presumably lie at the same distance as C_1. In the material we investigated these two alleles were very rare and found only as heterozygotes. Data from segregation analysis are introduced in Table 9. In

Table 8. Genetic analysis of *Pgm-B* and *Pgm-D* in *Acer pseudoplatanus*.

Maternal tree		Progeny											χ^2_1
Nr	Type	N_{11}	N_{12}	N_{13}	N_{14}	N_{22}	N_{23}	N_{24}	N_{33}	N_{34}	N_{44}	N	
82	B_1B_1	24	5		7							36	
	D_2D_2		3			16		17				36	
50	B_1B_3	6		14					16			36	1.06 n.s.
	D_2D_2					25		11				36	
61	B_2B_2					38		4				42	
	D_2D_2					36		6				42	
47	B_2B_2					32	42					74	0.05 n.s.
	D_2D_4					24		38			12	74	
54	B_1B_3		1			7	18		16			42	0.61 n.s.
	D_2D_2		3			26		13				42	
71	B_2B_3					6	38	3	25			72	0.71 n.s.
	D_2D_4					16		35			21	72	0.06 n.s.
90	B_2B_3					13	22					42	0.09 n.s.
	D_2D_4					20		21	7		1	42	0.00 n.s.
62	B_2B_4				2	9		22			9	42	0.40 n.s.
	D_2D_2					39	3					42	
48	B_4B_4							2		5	42	50	
	D_1D_1	30	17		3							50	
51	B_4B_4							5		25	30	60	0.07 n.s.
	D_2D_4					13		31			16	60	
74	B_2B_2					25		5				30	
	D_4D_4							11		1	18	30	

the seed orchard material *Pgm-C* was monomorphic. In furtherinvestigations in Bavarian maple stands a second, faster migrating allele was identified, which lies in the same position as the allele B_3. In order to obtain a clear resolution of the patterns in this zone the staining has to be interrupted after 15–20 minutes to avoid overstaining.

Nicotinamide adenine dinucleotide dehydrogenase (NDH)

When staining for NADH two active zones appeared on the gels. Whereas the faster migrating zone (A) is stained intensively; the slower moving one (B) is very weak and diffuse, so that it was not evaluated. Zone A shows little variation. With the exception of one tree, all clones showed only one band. It is assumed that each zone is controlled by one gene locus. For *Ndh-A* two alleles were identified in the investigated clones.

DISCUSSION

The patterns found for mountain maple have much similarity with isoenzyme patterns found in other broadleaved and coniferous species, both in terms of the number of controlling gene loci as well as with respect to the primary structure of the enzymes. For the enzyme systems ADH, IDH, GOT and PGM the similarity with *Acer campestre* is high (LEINEMANN & BENDIXEN 1999), whereas the enzyme PGI, is much more variable in mountain maple. Comparable results for PGI have been reported only for *Acer rubrum* (TOBOLSKI & RICKY 1992). The paper however, refers only to clone identification and reports of high enzyme variability without giving a genetic interpretation. It is extremely difficult to evaluate this system in *Acer pseudoplatanus*. We want to demonstrate this for pattern 16 from Figure 3. If one only considers the position of the bands in the zymogram we can interpret pattern 16 as corresponding to the following two-locus genotypes:

B_3B_3/C_3C_5; B_4B_4/C_1C_5; B_5B_5/C_1C_3; B_4B_5/C_1C_1; B_3B_5/C_3C_3; B_3B_4/C_5C_5; B_3B_4/C_1C_5; B_3B_4/C_3C_5; B_3B_5/C_1C_3; B_3B_5/C_3C_3; B_3B_5/C_3C_5; B_4B_5/C_1C_3; B_4B_5/C_1C_5.

If one considers the different intensities of the bands, more interpretive options for interpretations remain possible. The intuitively accepted hypothesis that the clone with this pattern carries the genotype B_3B_5/C_3C_3 turned out to be incorrect; in fact the tree was of genotype B_4B_4/C_1C_5. However, this does not mean that this pattern also cannot represent other genotypes.

Overlapping bands also complicate the interpretation of the system AAT; However, here the number of patterns is much lower. The difference between some patterns, for example, results only in the intensity of staining of the slowest band. If the staining is very weak (for example, when the buds were collected too early in the fall), the differences are only slight and can lead to misinterpretation of the genotypes. Of all the patterns, it is almost impossible to differentiate between pattern B_2B_3/C_1C_4 (see Figure 2) and $B_2B_3/\ C_2C_4$. The correct determination of the genotype of a tree is only possible if one looks at both gene loci *Aat-B* and *Aat-C* in the progeny of a clone. If the genotype C_1C_1 occurs more frequently in the seed, but the genotype C_2C_2 is missing, the maternal tree is genotyped as B_2B_3/C_1C_4. In this way it was possible for us to correct misinterpretations in some cases. The situation is similar with genotypes B_1B_1/C_1C_4 and B_1B_2/C_1C_4. For clone 8 we assumed the genotype to be B_1B_1/C_1C_4 after we analyzed the buds. However, after the genetic analysis we had to revise our assumption. The clone was of genotype B_1B_2/C_1C_4. Overlapping also makes it difficult to interpret the system PGM.

Similar problems, as mentioned here, have been reported for the system 6-PGDH in beech (MÜLLER-STARCK & STARKE 1993) and spruce (KONNERT & MAURER 1995). As a consequence many investigators do not investigate these systems for these tree species (e.g. TUROK 1996). On the other hand, these systems are highly variable and if one does not evaluate them, much information on the genetic variation of individual trees and populations is lost. If one wants to avoid this loss of information, there is only the possibility of investigating the buds and seeds of the same tree. On the basis of patterns obtained by bud analysis one presents a hypothesis which then must be verified by analysing the seed of the respective mother tree.

ACKNOWLEDGMENT

The authors wish to thank Susanne Dawid, Susan Fuchs, Thomas Wimmer and Theresia Wimmer for technical assistance.

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