FOREST GENETICS 6(2):73–77, 1999

SHORT COMMUNICATION

INHERITANCE OF ISOZYME VARIANTS IN FIELD MAPLE (*ACER CAMPESTRE* L.)

L. Leinemann & Kathrin Bendixen
Institut für Forstgenetik und Forstpflanzenzüchtung der Universität Göttingen, Büsgenweg 2, D–37077 Göttingen, Germany

Received September 24, 1998; accepted February 20, 1999

ABSTRACT

Inheritance analysis of isozymes in field maple (*Acer campestre* L.) was conducted for the first time. 15 enzyme systems were investigated by horizontal starch gel electrophoresis. Six enzyme systems revealed satisfactory resolution and variation. In total, ten variable gene loci coding for the enzyme systems ADH, AP, IDH, AAT, PGM and PGI were identified.

Key words: *Acer campestre*, isozymes, inheritance.

INTRODUCTION

In competition with the main forest tree species, the occurrence of field maple is often limited to the woodland borders. Thus the distribution of field maple populations is different from those of the main forest tree species with a more continuous distribution, and investigations with respect to the differentiation of genetic structures in field maple are promising. In plant genetic studies, isozymes are widely used to describe the genetic structures of forest tree populations (HAMRICK & GODT 1989). A prerequisite for these investigations is the inheritance analysis of the observed isozyme phenotypes. Our objective in this study was the examination of the genetic control of isozymes in seed and bud tissue of field maple.

MATERIAL AND METHODS

Buds and single tree progenies from 30 open-pollinated trees and one bulked seed lot from field maple originating from the region of Miltenberg (Franken) were analysed by means of starchgel electrophoresis. The seeds were air-dried and stored at temperature of 5°C, the buds at temperature of –60°C.

Extraction of the enzymes

Seed and bud tissue samples were homogenised in 35 ml of 0,07 M Tris-HCl buffer pH 7.5. For stabilisation of the enzymes and protection against phenolics and heavy metals, 500 mg saccharose, 300 mg PVP, 15 mg DTT and 5 mg EDTA were dissolved in 10 ml extraction buffer.

Separation and staining of the enzymes

Initially, 300 seeds from the bulked seed lot were analysed to test various electrode and gel buffers and to get information about the variability of single enzyme systems.

The best results were obtained with the buffer system Ashton & Braden pH 8.1, and a Tris-citrate buffer system pH 7.4. The recipes are given in Table 1.

The staining of the enzymes was performed according to the recipes given by RICHARDSON *et al.* (1986) and CHELIAK and PITEL (1984). To avoid background staining that could influence the interpretation of the zymograms, the substrate specificity of the enzymes was tested by staining with and without substrate.

Genetic analysis

In order to study the ontogenetic stability of the enzyme expression under our running conditions, we compared the band patterns of seed and bud tissue.
The assumption that the observed phenotypes are completely under genetic control was analysed following the method of GILLET and HATTEMER (1989). This method can be applied to test a hypothesis of single locus codominant inheritance that assigns each isozyme phenotype to one genotype.

Three assumptions are necessary:
1.) regular meiotic segregation of the ovules
2.) random fertilisation of the ovules
3.) absence of viability selection in the offspring prior to the investigation.

Under these assumptions, the following quantitative and qualitative relations should be expected in the progenies of a open pollinated seed parent with a

Table 1. Recipes of the used buffer systems and running conditions.

Buffer system	Gel	Gel buffer (A)	Electrode buffer (B)	Running conditions
Ashton & Braden pH 8.1 PGI, PGM, GOT	29 starch 4 g saccharose	0.05 M Tris, 9mM citric acid 245 ml buffer A and 15 ml buffer B	0.19 M boric acid 0.042 M lithium hydroxide	constant 75mA 4 hrs
Tris Citro pH7.4 IDH, AP, ADH	29 starch 4 g saccharose	70 ml of buffer B and 130 ml H_2O	0.15 M Tris 0.043 M citric acid	180 mA for 5.5 hrs

putative heterozygous genotype A_iA_j.

(i) Each offspring must possess at least one of the alleles A_i, A_j.

(ii) The number of the heterozygous progenies with the phenotype A_iA_j (N_{ij}) is expected to be equal to the total number of homozygous progenies of phenotype A_iA_i (N_{ii}) and A_jA_j (N_{jj}).

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

(iii) The number of progenies with the phenotype A_iA_k (N_{ik}) is expected to be equal to those with the phenotype A_jA_k (N_{jk}).

$$N_{ik} = N_{jk}.$$

Deviations between observed and expected frequencies – (ii) and (iii) – were tested with the exact binomial goodness of fit test.

RESULTS

Isozyme phenotypes

No variation was observed for the enzyme systems 6-PGDH (E.C. 1.1.1.44), MDH (E.C.1.1.1.37), NDH (E.C.1.6.99.3) and MNR (E.C.1.6.99.2). No or only very faint staining were obtained for the enzyme systems FDH (E.C. 1.2.1.2) and GDH (E.C.1.4.1.3). No reliable resolution of the band patterns was observed for SKDH (E.C.1.1.1.25), ACO (E.C.4.2.1.3) and G-6-PDH (E.C.1.1.1.49).

Good resolution and variable band patterns were obtained for the enzyme systems LAP (E.C.3.4.11.1), IDH (E.C.1.1.1.42), PGI (E.C.5.3.1.9), PGM (E.C. 2.7.5.1), GOT/AAT (E.C.2.6.1.1) and ADH (E.C. 1.1.1.1). In the following, the band patterns of these enzyme systems are described.

Single enzyme zones are named according to their mobility in the gel. The fastest-migrating zone (most anodal) is named zone *A*, the second one zone *B* and so on. Corresponding to the relative mobility in the gel, the fastest-migrating band of a given zone is denoted as variant 1, the following as variant 2 etc.

When bud and seed tissue showed equal staining activity, the same band patterns were obtained under our electrophoretic conditions.

AP

Gels stained for aminopeptidase showed three clearly separated zones of activity. For LAP extracts of bud tissue only zones *A* and the *B* showed good staining activity. Zone *C* revealed higher activity when the substrate of AAP was added to the staining solution. In seed tissue, the variation of all three zones could be reliably scored.

One frequent and two rare variants were detected in zone *A*. The rare variants A_1 and A_3 occurred with frequencies of less than 2 %.

Zone *B* reveals much more variation, with two frequent variants (B_1 and B_2) and one rare variant (B_3). Two frequent variants (C_1 and C_2) were detected in zone *C*. The absence of hybrid bands in putative heterozygous genotypes suggests a monomeric structure of the AP enzyme system.

IDH

The IDH enzyme system showed one zone with two double-banded variants. Putative heterozygous individuals showed four bands of activity. One explanation for these uncommon patterns for an enzyme generally considered to be dimeric could be that hybrid bands occur between the faster bands and between the slower bands of each variant (for example see Fig. 1) but not between a fast and a slow-moving band. Variant A_2 is rare with frequencies below 2%.

PGI

The PGI system showed two clearly separated zones of activity. *Pgi–A* appears as a double banded nearly monomorphic zone. Another faster migrating variant was detected only twice. The lack of variation prohibited genetic analysis. Zone *B* showed four single-banded variants. Banding patterns of putative heterozy

Table 2. Inheritance analysis of isozymes in field maple. On the left, the proposed genotypes of maternal trees are grouped for each enzyme system. *N* is the size of the seed samples and P_1 and P_2 refer to the test equations (ii) and (iii). *P* represents the probability of equally or less likely samples. * significant deviation from the expectations on the 0.05 level. – hypothesis (iii) not testable.

Type	Tree #	*N*	Progenies						P_1	P_2
Adh–B			N_{11}	N_{22}	N_{33}	N_{12}	N_{13}	N_{23}		
B_2B_3	27	43	–	12	8	–	–	23	.760	–
B_1B_3	5	30	1	–	8	6	8	7	1.000	1.000
B_2B_3	19	33	–	13	6	–	–	14	.486	–
Got–B			N_{22}	N_{23}	N_{25}	N_{33}				
B_2B_3	25	29	17	10	1	1	–	–	.247	1.000
Got–C			N_{11}	N_{22}	N_{33}	N_{12}	N_{23}	N_{14}		
C_2C_3	27	43	–	22	2	–	19	–	.542	–
C_1C_2	4	35	2	13	–	18	–	2	.728	.500
C_1C_2	3	68	11	17	–	39	1	–	.222	1.000
C_1C_2	8	28	4	6	–	18	–	–	.185	–
C_2C_3	9	35	–	13	5	–	17	–	1.000	–
Lap–A			N_{11}	N_{12}	N_{22}					
A_1A_2	8	28	15	13	–				.851	–
Lap–B			N_{11}	N_{22}	N_{12}	N_{33}	N_{13}	N_{23}		
B_1B_2	27	43	20	6	17	–	–	–	.222	–
B_1B_2	3	68	14	17	37	–	–	–	.545	–
B_1B_2	5	30	10	5	15	–	–	–	1.000	–
B_1B_2	4	35	11	7	17	–	–	–	1.000	–
B_1B_3	25	20	4	–	3	1	6	6	1.000	0.508
Lap–C			N_{11}	N_{22}	N_{12}					
C_1C_2	9	35	15	1	19				.736	–
C_1C_2	27	23	14	–	9				.405	–
C_1C_2	25	20	5	3	12				.503	–
Pgm–B			N_{11}	N_{22}	N_{12}					
B_1B_2	27	43	14	9	20				.760	–
B_1B_2	3	69	18	21	30				.335	–
B_1B_2	4	61	14	14	33				.608	–
B_1B_2	5	30	3	10	17				.585	–
Pgm–C			N_{11}	N_{22}	N_{33}	N_{12}	N_{13}	N_{23}		
C_2C_3	27	43	–	2	9	11	8	13	.838	.847
C_1C_3	5	30	11	–	4	1	13	1	.850	1.000
C_1C_3	25	49	16	–	8	4	21	–	.765	.125
C_1C_2	4	62	20	10	–	26	3	3	.689	1.000
Idh–A			N_{11}	N_{22}	N_{12}					
A_1A_2	19	61	24	3	34				.443	–
Pgi–B			N_{11}	N_{22}	N_{33}	N_{12}	N_{23}			
B_2B_3	3	69	–	14	13	2	40		.142	.500
B_1B_2	5	30	2	19	–	8	1		*.024	1.000

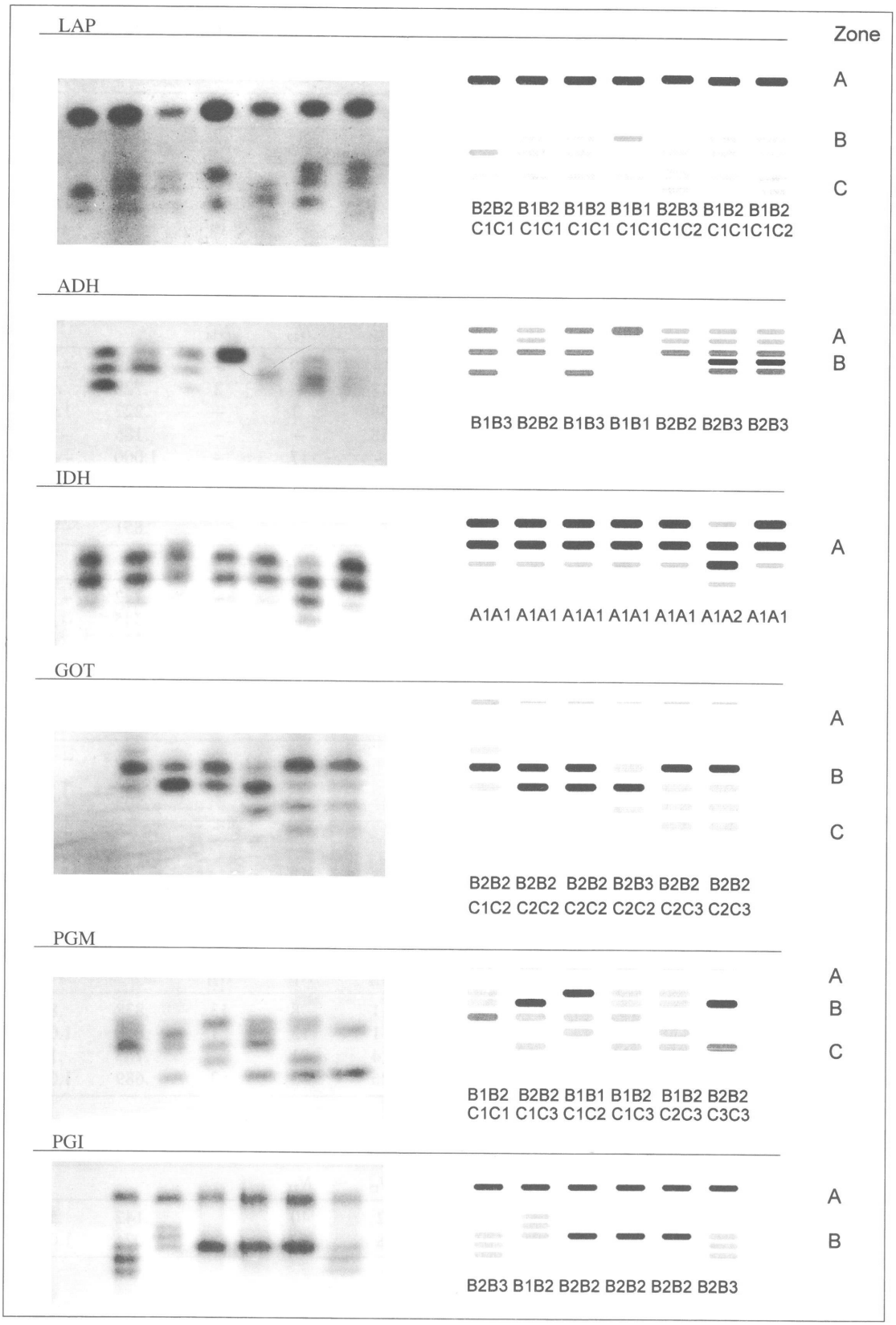

Figure 1. Examples of zymograms from bud tissue of field maple. Photos and schematic illustrations.

gous genotypes were always triple-banded, suggesting a dimeric subunit structure.

PGM

PGM showed three zones of activity. Variation was found in each zone. The zones *B* and *C* showed high activity in bud tissue as well as in seed tissue, whereas in seed tissue extracts the activity in zone *A* was often weak. Therefore, a reliable interpretation of the patterns in this zone was not possible. Zone B revealed two single-banded variants. Both variants occurred in frequencies of more than 40 %. Zone C reveals three variants with frequencies of more than 40 % for C_1 and C_3 and a frequency of about 20 % for C_2. The absence of hybrid bands of putative heterozygous individuals indicated a monomeric subunit structure.

GOT

GOT showed two clearly separated zones of enzyme activity. Zone *A* is variable, but staining especially in seeds was very faint. Therefore, inheritance analysis was not conducted for this zone. The band patterns in zone *B* suggested the hypothesis of two overlapping zones (see Fig. 1). The two zones were named according to the mobility of their most frequent variants. Six variants were observed in zone *B* with B_2 and B_3 being the most frequent ones. In zone *C* three variants occurred with the most frequent variant C_2. All variants appeared as single bands. Putative heterozygous individuals exhibit triple-banded patterns as expected for dimeric enzymes.

ADH

ADH showed one comparably broad zone of enzyme activity. According to our results, this zone presumably consists of two overlapping zones under control of two gene loci. Between the two loci, interlocus hybrid bands are visible. Zone *A* is characterised by the occurrence of one frequent single-banded variant. Zone B exhibits three variants with B_2 as the most frequent one. Putative heterozygous individuals showed triple-banded patterns indicating a dimeric subunit structure.

INHERITANCE ANALYSIS AND CONCLUSIONS

In this study inheritance analysis in field maple is conducted for the first time using a qualitative and quantitative approach. Each offspring possessed at least one maternal allele. The results of the exact binomial goodness-of-fit test support the hypothesis of a single-locus codominant mode of inheritance at ten gene loci.

The progeny of one single tree (# 5) showed significant deviation from equation (ii) at the gene locus *Pgi–B*. It is possible that one of the assumptions of the test hypotheses is violated. The data collected in this study are not adequate to verify the actual cause. A common explanation given in this context is viability selection in the offspring prior to the investigation.

LITERATURE

CHELIAK, W. M. & PITEL, J. A. 1984: Techniques for Starch Gel Electrophoresis of Enzymes from Forest Tree Species. Information Report PI–X–42. Chalk River Ontario. Petawawa National Forestry Institute, Canadian Forestry Service.

GILLET, E. & HATTEMER, H. H. 1989: Genetic analysis of isoenzyme phenotypes using single tree progenies. *Heredity* **63**:135–141.

HAMRICK, J. L. & GODT, M. J. W. 1989: Allozyme diversity in plant species. *In*: BROWN, A. H. D., CLEGG, M. T., KAHLER, A. L. & WEIR, B. S. Plant Population Genetics, Breeding, and Genetic Resources. pp. 43–63. Sinauer Associates, Sunderland, Mass.

RICHARDSON, B. J., BAVERSTOCK, P. R. & ADAMS, M. 1986: Allozyme Electrophoresis. Academic Press Inc., San Diego, California.