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Inheritance and linkage of isozyme loci in pear (*Pyrus communis* L.)

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Abstract The polymorphism of 11 enzymes was analysed in 11 progenies from controlled crosses between pear varieties, using acrylamide and starch electrophoresis gels. Twenty-two loci were identified and segregation was scored for 20 of them. Three pairs of duplicated loci forming intergenic hybrid bands were detected, these correspond to equivalent duplicated genes in apple. A total of 49 active alleles and 1 null allele were identified. Joint segregation analysis revealed three linkage groups, which could all be related to existing groups on the apple map. The conservation of isozyme patterns, duplicated genes and linkage groups indicates a high degree of synteny between apple and pear.

Key words Isozymes · Inheritance · *Pyrus* · Pear

Introduction

Pear is the third most important temperate fruit species after grape and apple, with a world production of 9.7 million metric tons. Two main species of the genus *Pyrus* are cultivated: *Pyrus communis* L., the European pear, is grown in Europe and America; *Pyrus serotina* Redh. (syn. *P. pyrifolia* Nak.), the Asian pear or Nashi, is grown in Asia. The genus *Pyrus* belongs to the subfamily *Maloideae* of the *Rosaceae*, which also includes the genus *Malus* (apple). The basic chromosome number of the *Maloideae* (x = 17) is high compared to other *Rosaceae* subfamilies, suggesting a polyploid origin. Botanical data (Stebbins 1950), chemotaxonomical studies on phenolic compounds (Challice 1981) and isozyme inheritance studies (Chevreau and Laurens 1987; Weeden and Lamb 1987) support the hypothesis of an allopolyploid origin. The *Maloideae* probably arose as an amphidiploid of two ancestors from the *Spiraeoideae* (x = 9) and *Prunoideae* (x = 8) subfamilies. A recent molecular study of the chloroplast gene *rbc*L (Morgan et al. 1994) suggests spireaoids as the maternal ancestor of *Maloideae*.

Genetic studies in pear, as in many woody perennials, have been rare. The length of the juvenile period (average of 6 years), the space necessary to study large progenies and the high level of heterozygozity due to a strict gametophytic incompatibility have limited inheritance studies to a few morphological characters. The first study of pear isozymes was conducted by Santamour and Demuth (1980) to identify 6 ornamental cultivars of *P. calleryana* by peroxidase patterns. Peroxidase diversity has also been studied by Menendez and Daley (1986) on several species of the genus *Pyrus*, and by Jang et al. (1991, 1992) to classify 187 cultivars of Nashi. Isozyme variability of several enzyme systems was described by Cerezo and Socias i Company (1989) on pollen extracts of several pear varieties. More recently, DNA fingerprinting with a minisatellite probe from human myoglobin (Teramoto et al. 1993) and restriction fragment length polymorphism (RFLP) of mitochondrial DNA (Iketani et al. 1993) have also been applied to some *Pyrus* genotypes. No data on the inheritance of molecular markers has yet been reported for pear.

The present study investigates the genetic basis of 22 isozyme loci in 11 pear progenies of controlled crosses. The mode of inheritance and the observed linkages are discussed in comparison with those of apple.

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Materials and methods

The plant material was obtained from the pear breeding programme of the research station. Pollen and leaf samples were taken from

Table 1 Plant material – description of the progenies analysed

Code	Female parent	Male parent	Size of the progeny	Type of organ studied		
				Seedling leaves	Adult leaves	Pollen
A	Pierre Corneille	Comice	26	−	+	+
B	Conférence	Starking	36	+	−	−
C	Pierre Corneille	Delmoip	36	−	+	+
D	Harrow Sweet	P6 R30 A100	24	+	−	−
E	P6 R30 A100	Starking	36	+	−	−
F	P6 R30 A100	US 309	36	+	−	−
G	Comice	RR94	30	+	−	−
H	Comice	Passe Crassane	30	+	−	−
I	Williams	Passe Crassane	30	+	−	−
J	Comice	RR95	36	+	−	
K	Williams	RR94	60	+		−

unselected progenies from controlled crosses between pear varieties or hybrids (Table 1). Two progenies (A and C) consisted of adult unselected trees in the field, the others (B, D, E, F, G, H, I, J and K) consisted of young seedlings grown in greenhouse.

Pollen from flower buds prior to opening and young leaves from actively growing shoots were collected and kept at − 80°C. Pollen extracts were prepared by homogenization of 100 mg pollen in 0.5 ml 0.1 *M* TRIS KCl pH = 7 buffer. Leaf extracts were prepared by grinding 200 mg fresh leaves in 1.5 ml of the following extraction buffer: KH_2PO_4 (0.1 *M*), sucrose (7%), egg albumin (0.1%), ascorbic acid (0.4%), β-mercaptoethanol (0.15%), Triton X100 (0.02%), polyvinylpolypyrrolidone (8%), pH = 7.5. In both cases, centrifugation at 35,000 *g* for 1 h was applied.

Electrophoresis was performed in acrylamide gels for aspartate aminotransferase (AAT, EC 2.6.1.1), endopeptidase (ENP, EC 3.4.9.9), esterase (EST, EC 3.1.1.-), leucine aminopeptidase (LAP, EC 3.4.11.1), peroxidase (PRX, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1). Starch gels were used to separate alcohol dehydrogenase (ADH, EC 1.1.1.1), diaphorase (DIA, EC 1.6.99.-), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), phosphoglucoisomerase (PGI, EC 5.3.1.9) and phosphoglucomutase (PGM, EC 5.4.2.2). The composition of the gels were as described in Chevreau and Laurens 1987. Staining was performed according to Wendel and Weeden (1989).

Single-factor segregation at each locus and joint segregation of pairs of loci for possible linkage were examined using the LINKEM 1.0 software (Vowden and Ridout 1994). Single-factor segregation was tested by the chi-square statistic. Joint segregation of pairs of loci was tested by two methods: the standard chi-square independence test and the likelihood ratio test for linkage.

Results

Organ specificity

The 11 enzymes were investigated in leaf and pollen extracts. Most of them were active in both organs, but the highest activities were generally obtained from pollen. For example, LAP and PGD activities were detected in both organs, but scorable only from pollen extracts. In two cases, a clear organ specificity was observed: PRX activity could be detected only in leaves, and ADH only in pollen. This situation is very close to the isozyme organ specificity described in apple (Weeden 1986).

Organ specificity of individual isozyme loci was also observed in two cases. For EST and DIA, 2 loci were identified from leaf extracts, and a third one from pollen. The co-migration of pollen, leaf and mixtures of both extracts confirmed the organ specificity of these loci.

A developmental stage specificity was detected for PRX when zymograms from seedling leaves (progenies B, D, E, F, G, H, I, J and K) were compared with those from adult leaves (progenies A and C and grafted plants of the 12 parents). Leaves from young seedlings exhibited PRX activity only at 1 locus, whereas leaves from adult trees had complex PRX patterns with 5 scorable loci. Such specificity has already been described for several species (Bernal et al. 1994). A similar variation in the number of zones of activity was observed for SOD: four intense zones of activity were detected from adult leaf extracts, but only 1 zone was expressed in extracts from seedling leaves.

Aspartate aminotransferase

In pollen extracts, AAT zymograms consist of 3 zones of activity. The most anodal (AAT-1) was detected as very faint bands in pollen extracts and no polymorphism could be scored. In pollen as well as in leaf extracts AAT-2 consisted of well-separated bands (Fig. 1H). The correspondence between 3-band patterns in leaves and 2-band patterns in pollen indicates the dimeric structure of this enzyme. The total absence of activity in this zone in several samples suggests the existence of a null allele. A faint heterodimeric band between this null allele and an active one could be detected. The existence of 2 active alleles (*Aat-2a* and *Aat-2b*) and a null allele (*Aat-2n*) was confirmed by the segregations observed in 10 progenies (Table 2). A slower zone of activity (AAT-3) was scorable only from adult leaf extracts. Three-band patterns indicated a dimeric structure of the enzyme. The existence of 2 active alleles (*Aat-3a* and *Aat-3b*) was confirmed by the segregations observed in progenies A and C (Table 2).

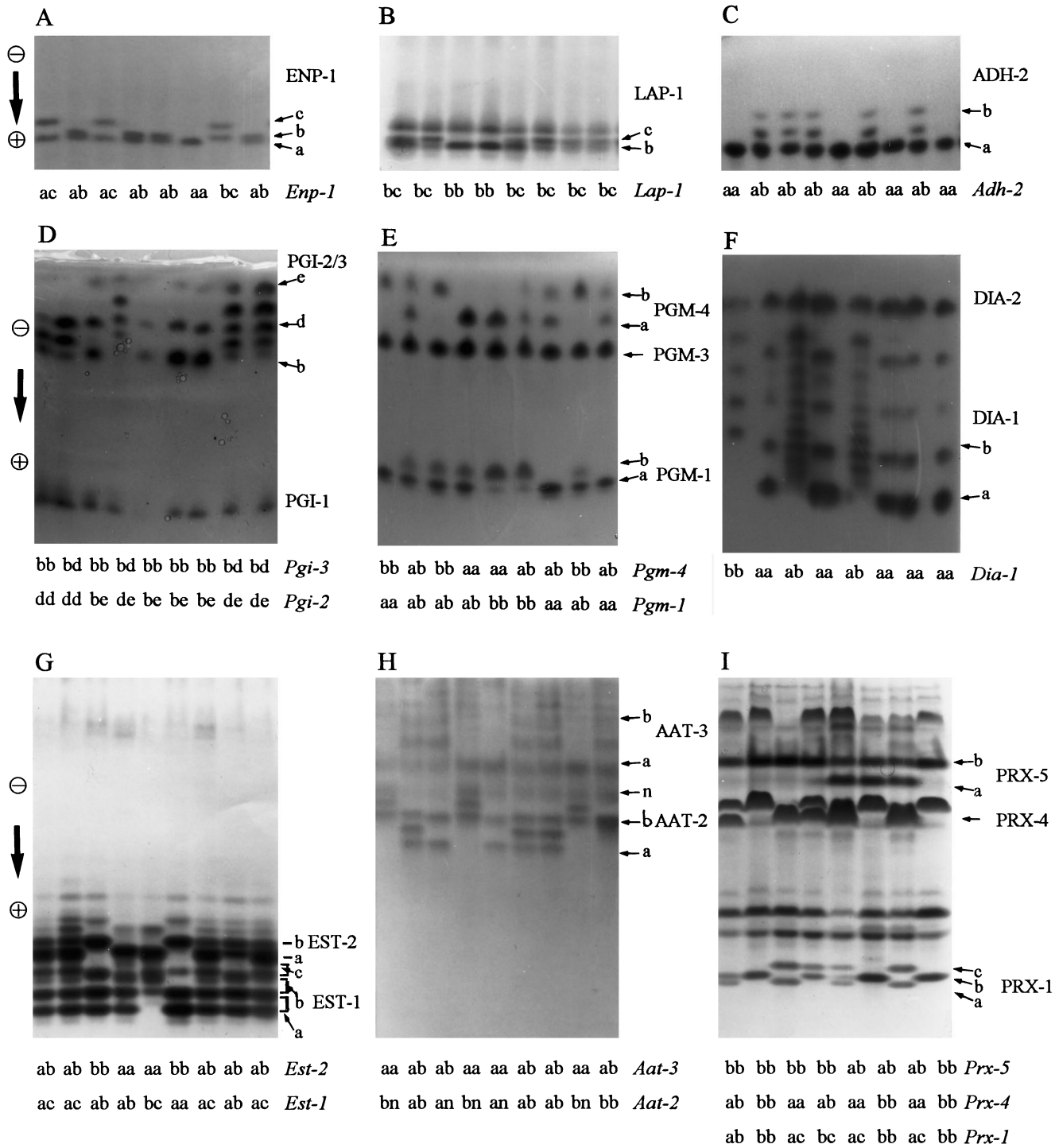

Fig. 1A–I Zymograms of nine enzyme systems from leaf and pollen pear extracts. **A** ENP from pollen extracts of progeny C and *Enp-1* genotypes; **B** LAP from pollen extracts of progeny C, and *Lap-1* genotypes; **C** ADH from pollen extracts of progeny C, and *Adh-2* genotypes; **D** PGI from leaf extracts of progeny K, and *Pgi-2*, *Pgi-3* genotypes **E** PGM from leaf extracts of progeny K, and *Pgm-1*, *Pgm-4* genotypes **F** DIA from leaf extracts of several parents, and *Dia-1* genotypes **G** EST from leaf extracts of progeny A, and *Est-1*, *Est-2* genotypes **H** AAT from leaf extracts of progeny A, and *Aat-2*, *Aat-3* genotypes; **I** PRX from leaf extracts of progeny A, and *Prx-1*, *Prx-4*, *Prx-5* genotypes

Table 2 Segregation observed for the different loci

Locus	Progeny	Genotypes ♀ × ♂	Observed segregation	Expected ratio	χ^2	*P*
Aat-2	A	ab × bn	10 ab : 5 an : 7 bb : 4 bn	1 : 1 : 1 : 1	3.23	0.36
	C	ab × ab	9 aa : 18 ab : 8 bb	1 : 2 : 1	0.09	0.96
	D	bn × ab	8 ab : 4 an : 5 bb : 7 bn	1 : 1 : 1 : 1	1.67	0.64
	E	ab × bb	21 ab : 15 bb	1 : 1	1.00	0.32
	F	ab × bb	16 ab : 20 bb	1 : 1	0.44	0.50
	G	bn × an	9 ab : 9 bn : 5 an : 5 nn	1 : 1 : 1 : 1	2.29	0.52
	H	bn × an	10 ab : 7 bn : 4 an : 6 nn	1 : 1 : 1 : 1	2.78	0.43
	I	bn × an	4 ab : 8 bn : 6 an : 8 nn	1 : 1 : 1 : 1	1.69	0.64
	J	bn × bn	7 bb : 22 bn : 7 nn	1 : 2 : 1	1.06	0.59
	K	bn × an	15 ab : 15 bn : 12 an : 17 nn	1 : 1 : 1 : 1	0.86	0.83
Aat-3	A	ab × aa	13 aa : 13 ab	1 : 1	0.00	1.00
	C	ab × aa	21 aa : 13 ab	1 : 1	1.88	0.17
Adh-2	A	ab × aa	12 aa : 14 ab	1 : 1	0.15	0.69
	C	ab × aa	17 aa : 18 ab	1 : 1	0.03	0.87
Dia-1	B	ab × aa	17 aa : 19 ab	1 : 1	0.11	0.74
	D	ab × aa	11 aa : 13 ab	1 : 1	0.17	0.68
	H	aa × ab	16 aa : 13 ab	1 : 1	0.31	0.58
	I	bb × ab	13 ab : 14 bb	1 : 1	0.04	0.85
	J	aa × ab	20 aa : 16 ab	1 : 1	0.44	0.50
Dia-3	A	ab × bc	8 ab : 10 ac : 5 bb : 3 bc	1 : 1 : 1 : 1	4.46	0.22
	C	ab × ab	6 aa : 22 ab : 7 bb	1 : 2 : 1	2.37	0.31
Enp-1	A	bd × bb	15 bb : 11 bd	1 : 1	0.62	0.43
	B	bc × bb	15 bc : 17 bb	1 : 1	0.13	0.72
	C	bd × bc	12 bb : 9 bc : 9 bd : 5 cd	1 : 1 : 1 : 1	2.83	0.42
	D	bb × ac	12 ab : 12 bc	1 : 1	0.00	1.00
	E	ac × bb	18 ab : 18 bc	1 : 1	0.00	1.00
	F	ac × bb	20 ab : 16 bc	1 : 1	0.44	0.50
	G	bb × bd	17 bb : 13 bd	1 : 1	0.53	0.47
	K	bb × bd	18 bb : 22 bd	1 : 1	0.40	0.53
Est-1	A	ac × ab	6 aa : 7 ab : 4 ac : 9 bc	1 : 1 : 1 : 1	2.00	0.57
	B	ab × ab	10 aa : 16 ab : 8 bb	1 : 2 : 1	0.35	0.84
	C	ac × bb	17 ab : 17 bc	1 : 1	0.00	1.00
	E	aa × ab	16 aa : 18 ab	1 : 1	0.12	0.73
	G	ab × aa	12 aa : 17 ab	1 : 1	0.86	0.35
	H	ab × ab	10 aa : 11 ab : 6 bb	1 : 2 : 1	2.11	0.35
	J	ab × bc	10 ab : 7 ac : 12 bb : 7 bc	1 : 1 : 1 : 1	2.00	0.57
Est-2	A	ab × ab	7 aa : 14 ab : 5 bb	1 : 2 : 1	0.46	0.79
	B	ab × ad	10 aa : 11 ab : 5 ad: 10 bd	1 : 1 : 1 : 1	2.44	0.49
	C	ab × bd	10 ab : 6 ad : 12 bb : 6 bd	1 : 1 : 1 : 1	3.18	0.37
	D	ad × bc	5 ab : 11 bd : 5 cd : 3 ac	1 : 1 : 1 : 1	6.00	0.11
	E	bc × ad	11 ab : 6 bd : 11 cd : 7 ac	1 : 1 : 1 : 1	2.37	0.50
	F	bc × ab	0 ab : 10 bb : 14 ac : 10 bc	1 : 1 : 1 : 1	12.6	< 0.01
	G	ab × bb	9 ab : 19 bb	1 : 1	3.57	0.06
	H	ab × ad	5 aa : 6 ab : 8 ad : 9 bd	1 : 1 : 1 : 1	1.43	0.70
	J	ab × bd	2 ab : 11 ad : 11 bb : 11 bd	1 : 1 : 1 : 1	6.94	0.07
	K	ab × ab	5 aa : 21 ab : 14 bb	1 : 2 : 1	4.15	0.13
Est-3	A	ab × ac	3 aa : 9 ac : 7 ab : 7 bc	1 : 1 : 1 : 1	2.92	0.40
	C	ab × ab	8 aa : 16 ab : 11 bb	1 : 2 : 1	0.77	0.68
Lap-1	A	bc × bc	6 bb : 16 bc : 4 cc	1 : 2 : 1	1.69	0.43
	C	bc × bc	10 bb : 16 bc : 8 cc	1 : 2 : 1	0.35	0.84
Pgd-1	A	bb × ab	14 ab : 12 bb	1 : 1	0.15	0.69
	C	bb × ab	18 ab : 17 bb	1 : 1	0.03	0.87
Pgi-2	A	dd × de	14 dd : 12 de	1 : 1	0.15	0.69
	B	dd × ad	20 dd : 16 ad	1 : 1	0.44	0.50
	C	dd × cd	15 cd : 20 dd	1 : 1	0.71	0.40
	D	bd × cd	5 dd : 4 bd : 9 cd : 3 bc	1 : 1 : 1 : 1	3.95	0.27
	E	cd × ad	9 dd : 12 ad : 8 cd : 6 ac	1 : 1 : 1 : 1	2.14	0.54
	F	cd × dd	16 cd : 16 dd	1 : 1	0.00	1.00
	G	de × de	6 dd : 23 (de + ee)[a]	1 : 3	0.29	0.80
	H	de × de	11 dd : 17 (de + ee)	1 : 3	2.33	0.15
	I	de × de	2 dd : 17 (de + ee)	1 : 3	2.12	0.15
	J	de × be	6 bd : 8 be : 22 (de + ee)	1 : 1 : 2	2.00	0.37

Table 2 Continued

Locus	Progeny	Genotypes ♀ × ♂	Observed segregation	Expected ratio	χ^2	*P*
Pgi-3	D	de × dd	10 de : 11 dd	1 : 1	0..05	0.83
	G	dd × bd	18 dd : 11 bd	1 : 1	1.69	0.19
	I	bd × dd	11 dd : 8 bd	1 : 1	0.47	0.50
	J	dd × ad	13 ad : 23 dd	1 : 1	2.78	0.10
Pgm-1	J	aa × ab	15 aa : 17 ab	1 : 1	0.13	0.72
	K	ab × ab	12 aa : 15 ab : 11 bb	1 : 2 : 1	1.74	0.42
Pgm-4	J	aa × ab	22 aa : 13 ab	1 : 1	2.31	0.13
	K	ab × ab	8 aa : 23 ab : 9 bb	1 : 2 : 1	0.95	0.62
Prx-1	A	ab × bc	4 ab : 4 ac : 8 bb : 10bc	1 : 1 : 1 : 1	3.00	0.25
	C	ab × ac	10 aa : 14 ac : 7 ab : 4 bc	1 : 1 : 1 : 1	3.00	0.10
Prx-3	H	bb × ab	11 bb : 19 ab	1 : 1	2.13	0.14
	I	bb × ab	20 bb : 16 ab	1 : 1	0.44	0.50
Prx-4	A	ab × ab	4 aa : 15 ab : 7 bb	1 : 2 : 1	1.31	0.52
	C	ab × aa	18 aa : 17 ab	1 : 1	0.03	0.87
	J	aa × ab	17 aa : 19 ab	1 : 1	0.11	0.74
Prx-5	A	bb × ab	12 ab : 14 bb	1 : 1	0.15	0.69
	J	ab × bb	16 ab : 18 bb	1 : 1	0.12	0.73
Sod-1	A	ab × aa	12 aa : 14 ab	1 : 1	0.15	0.69
	C	ab × ab	10 aa : 16 ab : 8 bb	1 : 2 : 1	0.35	0.84
	J	aa × ab	18 aa : 17 ab	1 : 1	0.03	0.87

[a] Genotypes within brackets are not distinguishable phenotypically because of the simultaneous presence of the d allele at a *Pgi-3* locus

Alcohol dehydrogenase

Strong activity of alcohol dehydrogenase was detected in pollen extracts (Fig. 1C). A 1 : 1 segregation between three-band and single-band patterns was observed in progenies A and C (Table 2), indicating a dimeric structure of this enzyme. The presence of a heterodimeric band in pollen extracts can be explained by the existence of a set of duplicated genes encoding ADH enzymes in pollen and forming an intergenic hybrid band. The difference in intensity constantly observed in the ADH patterns (a strong fast band and two fainter slower bands) further confirms this hypothesis. Thus, we postulated the existence of an *Adh-1* locus, homozygous for all of the samples tested in our work, and a polymorphic *Adh-2* locus with 2 active alleles, a and b.

Diaphorase

Very different patterns of DIA activity were observed in pollen and leaf samples. In leaf extracts (Fig. 1F), a 1 : 1 segregation was observed between 5-band and 12-band patterns. A slow band of intense activity was constantly present in these patterns. This suggests a tetrameric structure of this enzyme and the existence of two duplicated genes forming intergenic hybrid bands. The fast polymorphic locus *Dia-1* presented 2 active alleles (a and b), whereas the slow locus *Dia-2* was homozygous for all tested samples in our work. The segregations observed in 5 progenies (Table 2) were in agreement with this hypothesis.

In pollen extracts, DIA activity consisted of a single zone of intermediate mobility compared to DIA-1 and DIA-2 zones from leaf extracts. Segregation between 1- and 2-band patterns was observed, so no hypothesis could be inferred with respect to the structure of the enzyme. The hypothesis of a *Dia-3* locus with 3 active alleles (a, b and c) was in agreement with the segregations observed in progenies A and C (Table 2).

Endopeptidase

A single zone of ENP activity was constantly observed in pollen and leaf extracts (Fig. 1A). Four bands were observed and the hypothesis of a single locus (*Enp-1*) with 4 active alleles (a, b, c and d) was in very good agreement with the segregations observed in 8 progenies (Table 2). This enzyme seems to be monomeric in pear.

Esterase

Different patterns of EST activity were observed in pollen and leaf extracts, all suggesting a monomeric structure of this enzyme in pear. In the leaf extracts (Fig. 1G), a large fast zone displaying complex patterns was interpreted to be the product of two loci. The faster

one (*Est-1*) encoded doublets of bands of equal intensity. Three active alleles (a, b and c) with overlapping bands were identified. The segregations observed in 7 progenies confirmed this hypothesis (Table 2). A slightly slower locus (*Est-2*) adjacent to *Est-1* encoded single bands. Four active alleles (a, b, c and d) were identified. Among the segregations observed in 10 progenies, 7 were in good agreement with this hypothesis. However, segregation in progenies F, G and J was unexpected since one phenotypic class (ab) was totally missing or very low. This might be explained by a difficult scoring of the *Est-2a* band, which overlapped the *Est-1c* slower band.

In pollen extracts, a single zone of activity, slower than the EST-1 and EST-2 zones from leaf extracts, was observed. The patterns could be explained by the hypothesis of a *Est-3* locus encoding doublets of bands of equal intensity, without overlapping bands. Three active alleles were identified (a, b and c), and the segregations observed in progenies A and C (Table 2) confirmed this hypothesis.

Leucine aminopeptidase

The same zone of LAP activity was detected in pollen and leaves, but the resolution of the bands enabled scoring only from pollen extracts (Fig. 1B). Two- and 3-band patterns were observed, suggesting a monomeric struture of the enzyme and the presence of 2 loci. At the faster locus (*Lap-1*), 2 alleles (b and c) segregated in progenies A and C (Table 2), in good agreement with the expected ratio. The slower locus (*Lap-2*) was homozygous in these two progenies.

6-Phosphogluconate dehydrogenase

PGD activity was faint in leaf extracts, but very high in pollen extracts. Two zones of activity were observed but only the faster one exhibited polymorphism in progenies A and C. The segregation between 1-band and 2-band patterns could be explained by the hypothesis of 1 *Pgd-1* locus with 2 alleles, a and b (Table 2).

Phosphoglucoisomerase

PGI activity was high in leaf and pollen extracts and two zones could be distinguished (Fig. 1D). The faster one was very faint and apparently monomorphic; the slower zone consisted of 1- to 6-band patterns, in pollen as well as in leaf extracts. In these complex patterns 1 band of intermediate mobility was always present. Two to eight phenotypic classes were observed in the progenies, and these could be explained by the hypothesis of 2 duplicated genes, *Pgi-2* and *Pgi-3*, segregating independantly and encoding dimeric isozymes able to form intergenic hybrid bands. Five active alleles (a, b, c, d and e) shared by the 2 loci were identified. The 1-band pattern could be explained by the existence of the common allele (d) homozygous at the 2 loci. In 6 progenies, only 1 of the 2 loci was segregating, and we considered, that in this case *Pgi-2* was segregating and *Pgi-3* was homozygous dd. In 4 progenies both loci were segregating, and we proposed a genetic hypothesis that could best explain the observed phenotypic classes. As 7 of the 12 parents were involved in more than one cross, parental genotypes were assigned taking into account information accumulated from several progenies. In 4 progenies, the common allele (d) was present simultaneously at both loci. In these cases, some of the expected genotypic classes could not be distinguished phenotypically. However, overall, the segregations observed in 10 progenies were in good agreement with the ratios expected from this hypothesis (Table 2).

Phosphoglucomutase

PGM activity was very high in pollen extracts, but no polymorphism was detected in progenies A and C. Activity from leaf extracts could be consistently scored only on progenies J and K (Fig. 1E). As in most species, PGM zymograms in pear indicated a monomeric structure of this enzyme. A fast zone of activity presented 1- or 2-band patterns, which could be explained by the presence of a *Pgm-1* locus with 2 active alleles (a and b). A second zone of activity consisted of a band of high intensity, which was always present in all of the sample analysed. The third zone of activity also presented 1- or 2-band patterns, which could be explained by the presence of a *Pgm-4* locus with 2 active alleles (a and b). The segregations observed in progenies J and K (Table 2) were in agreement with these hypotheses.

Peroxidase

PRX activity was detected only in leaf extracts and was particularly intense in leaves from adult trees, where at least five zones of activity could be distinguished (Fig. 1I). One and 2-band patterns were observed in each zone of activity, suggesting a monomeric structure of the enzyme. Segregation in the faster zone of activity could be explained by the presence of 1 *Prx-1* locus with 3 active alleles (a, b and c). The second zone (PRX-2) appeared to be monomorphic in all of the progenies studied. Segregation in the third zone was only detected in samples from young seedling leaves. It could be explained by 1 *Prx-3* locus and 2 alleles (a and b). In the fourth and fifth zones of activity, segregations could be explained by 2 loci, *Prx-4* and *Prx-5*, and 2 alleles (a and b) at each locus. Due to a low level of polymorphism among the observed samples, only a few

segregations were observed (Table 2) . They were in good agreement with the proposed hypotheses.

Superoxide dismutase

Four intense zones of SOD activity were observed in pollen and adult leaf extracts, but only the faster zone was polymorphic in the progenies studied. Three different patterns were observed, each composed of a main intense band plus additional secondary bands. Because of this complexity, no hypothesis can be proposed regarding the structure of the enzyme. However, with the patterns being completely identical in pollen and leaf extracts, the existence of intragenic hybrid bands can be eliminated. The segregations observed in progenies A and C could be simply explained by the existence of a locus *Sod-1* and 2 active alleles (a and b) (Table 2).

Linkage relationships among isozyme loci

Among the 190 possible pairs of loci, only 132 could be tested, with at least 1 progeny providing informative results. Recombination fractions were considered to be significant when the LOD score exceeded 2.0. Three linkage groups were identified among the isozyme loci (Table 3). A linkage between *Aat-2* and *Pgi-2* was detected in all the progenies where it could be tested. Progenies G, H, I and J were excluded because of the impossibility to distinguish between *Pgi-2* de and ee phenotypes. The average estimate of the r value on progenies A, C, D, E and F is 0.086. For a more precise estimation of the pooled r value, an average weighted by the reciprocal of the standard errors is usually calculated. This formula cannot be used here because of the null value of the standard error in progeny C. Instead, an average weighted by the ration N/C (N = size of the progeny and C = number of phenotypic classes in segregation) can be proposed ; the corresponding r value is 0.061. A second linkage was detected in progenies A and C between *Aat-2* and *Lap-1*, with an average r value of 0.08. Finally, a very close linkage was detected between *Lap-1* and *Pgi-2* from progenies A and C, with a total absence of recombination in progeny A; the N/C-weighted average r value is 0.034. It can be concluded that these 3 loci belong to the same linkage group, *Aat-2* − (*Pgi-2*, *Lap-1*), the order of the last 2 loci being uncertain. Another

Table 3 Estimates of linkages between isozyme loci

Progeny	Parental genotypes	Progeny phenotypes	*n*	χ^2	(*df*)	*P*	LOD	r	(SE)
Aat-2/*Pgi-2*									
A	ab/dd × bn/de	7 ab/dd 3 ab/de 0 an/dd 5an/de 7 bb/dd 0 bb/de 0 bn/dd 4 bn/de	26	17.6	(3)	< 0.001	3.79	0.12	(0.06)
C	ab/dd × ab/cd	0 aa/cd 9 aa/dd 7 ab/cd 11 ab/dd 8 bb/cd 0 bb/dd	35	17.6	(2)	< 0.001	5.12	0.00	(0.00)
D	bn/bd × ab/cd	0 ab/bc 2 ab/bd 1 ab/cd 4 ab/dd 0 bb/bc 0 bb/bd 5 bb/cd 0 bb/dd 1 an/bc 2 an/bd 0 an/cd 1 an/dd 2 bn/bc 0 bn/bd 3 bn/cd 0 bn/dd	21	21.5	(9)	0.01	3.76	0.19	(0.06)
E	ab/cd × bb/ad	1 ab/ac 0 ab/cd 12 ab/ad 8 ab/dd 5 bb/ac 8 bb/cd 0 bb/ad 1 bb/dd	35	27.8	(3)	< 0.001	7.21	0.06	(0.04)
F	ab/cd × bb/dd	1 ab/cd 15 ab/dd 15 bb/cd 1 bb/dd	32	24.5	(1)	< 0.001	6.38	0.06	(0.04)
Aat-2/*Lap-1*									
A	ab/bc × bn/bc	0 ab/cc 0 ab/bb 10 ab/bc 3 an/cc 0 an/bb 2 an/bc 0 bb/cc 6 bb/bb 1 bb/bc 1 bn/cc 0 bn/bb 3 bn/bc	26	31.1	(6)	< 0.001	5.66	0.08	(0.04)
C	ab/bc × ab/bc	1 aa/bb 7 aa/bc 1 aa/cc 9 ab/bb 2 ab/bc 6 ab/cc 0 bb/bb 7 bb/bc 1 bb/cc	34	17.7	(4)	0.001	4.07	0.08	(0.04)
Lap-1/*Pgi-2*									
A	bc/dd × bc/de	6 bb/dd 0 bb/de 8 bc/dd 8 bc/de 0 cc/dd 4 cc/de	26	9.90	(2)	0.007	3.01	0.00	(0.00)
C	bc/dd × bc/cd	0 bb/cd 10 bb/dd 8 bc/cd 8 bc/dd 7 cc/cd 1 cc/dd	34	14.2	(2)	< 0.001	3.74	0.06	(0.06)
Adh-2/*Est-1*									
A	ab/ac × aa/ab	4 aa/aa 6 aa/ab 1 aa/ac 1 aa/bc 2 ab/aa 1 ab/ab 3 ab/ac 8 ab/bc	26	10.6	(3)	0.01	2.30	0.19	(0.08)
C	ab/ac × aa/bb	13 aa/ab 4 aa/bc 4 ab/ab 13 ab/bc	34	9.53	(1)	0.002	2.18	0.24	(0.07)
Prx-1/*Prx-4*									
A	ab/ab × bc/ab	0 ab/aa 4 ab/ab 0 ab/bb 4 ac/aa 0 ac/ab 0 ac/bb 0 bb/aa 1 bb/ab 7 bb/bb 0 bc/aa 10 bc/ab 0 bc/bb	26	47.3	(6)	< 0.001	9.30	0.02	(0.02)
C	ab/ab × ac/aa	5 aa/aa 0 aa/ab 13 ac/aa 0 ac/ab 0 ab/aa 7 ab/ab 0 bc/aa 4 bc/ab	29	29.0	(3)	< 0.001	8.73	0.00	(0.00)

n, Number of the progeny scored for both isozyme loci; χ^2, contengency table chi-square statistic, (*df*), degrees of freedom; *P*, corresponding probability; LOD, LOD-score value from the likelihood ratio test for linkage; only LOD-score values above 2.0 have been mentioned; r, recombination fraction estimated by the method of maximum likelihood; (SE) standard error of the estimate

very tight linkage group was detected between *Prx-1* and *Prx-4* in progenies A and C, with a total absence of recombination in progeny C; the N/C-weighted average r value is 0.007. Finally, a looser linkage was detected between *Adh-2* and *Est-1* in progenies A and C, with an N/C-weighted average r value of 0.226.

Discussion

Our results demonstrate that isozyme activity in pollen and leaf extracts of pear can be easily analysed by electrophoretic techniques very similar to those used for apple isozymes (Chevreau and Laurens 1987). The main modification concerned the composition of the extraction buffer for the leaf isozymes. A total number of 380 hybrids belonging to 11 progenies of controlled crosses were analysed. The level of polymorphism present in the 11 enzyme systems was explained by 20 segregating loci plus 2 monomorphic loci. A total number of 49 active alleles and a null allele were described. Most of the observed segregations were in agreement with the expected ratios. Only one case of skewed segregation ($P < 0.01$) was observed – for *Est-2* locus in the F population – and this was probably due to our difficulty in scoring the complex EST pattern.

Most of the enzyme systems analysed in this study show a great deal of similarity to apple isozymes. Patterns of AAT activity are very close to those described in apple (Manganaris and Alston 1987, 1988a). The presence of an *Aat-2* null allele forming hybrid bands with the active alleles has also been reported for apple. Similarly, patterns of activity observed in pear for ENP, LAP, PGD, PGM and SOD are identical to those described in apple by Manganaris and Alston (1988b, 1992a), Manganaris (1989) and Chevreau and Laurens (1987) but with slight differences in migration distances. Some differences between the two fruit species were observed with respect to EST and PRX activities. The *Est-1* locus in pear codes for doublets of bands, as in apple (Chevreau et al. 1985), but the organ specificity concerns different zones of the pattern. Apple PRX activity is coded by at least 8 genes (Manganaris and Alston 1992b), most of which encode a main band plus several secondary bands, contrary to our results on pear where only 5 genes seem to be involved, these coding for single bands.

The most striking similarities between apple and pear isozyme genetics concern the existence of duplicated loci and linkage relationships. As in pear, ADH activity from pollen extracts of apple has been reported to be under the control of a pair of duplicated loci showing fixed heterozygozity (Chevreau et al. 1985). PGI activity in apple is also under the control of a pair of duplicated genes forming intergenic hybrid bands (Weeden and Lamb 1987), but the level of polymorphism among apple varieties is much lower than that detected in pear in our study. Finally, a tetrameric structure of DIA was clearly observed in our study on pear, instead of the dimeric structure postulated to explain DIA patterns in apple (Weeden and Lamb 1987). The genetics of apple isozymes has now been extensively studied, and more than 50 loci have been described. On the basis of these published results plus our own unpublished results, the percentage of duplicated loci in apple can be estimated to be about 18%. Our study on pear isozymes detected the same proportion of duplicated loci (3/17), and the values are very similar to those reported for several ancient polyploid species with high basic chromosome numbers, such as *Magnoliaceae* or *Lauraceae* (13–29%) (Soltis and Soltis 1990). These isozyme data support the hypothesis that the high chromosome number of the *Maloideae* ($x = 17$) arose from ancient allopolyploidization.

The three linkage groups detected in this study on pear can be clearly related to linkage groups already decribed for apple. The *Aat-2* – (*Lap-1*, *Pgi-2*) linkage group in pear is very similar to the *Lap-2* – *Gpi-c* – *Aat-p* on apple linkage group 7 (Hemmat et al. 1994). The conservation of the linkage *Aat-p* – *Gpi-c* between apple and pear had already been indicated by a group working on development of linkage maps on several fruit crops (Weeden et al. 1994). The looser linkage between *Adh-2* and *Est-1* corresponds to the linkage of the same genes on the sixth linkage group of apple (Hemmat et al. 1994). Finally, the very close linkage between two PRX genes (*Prx-1* – *Prx-4*) is similar to the very closely linked *Prx-2* and *Prx-3* genes in apple (Manganaris and Alston 1992c).

The conservation of linkage groups between apple and pear and the evidence that the two species share duplications for the same enzymes indicate a high degree a synteny between these botanically related species. This situation can speed up considerably the progress of genetic knowledge in pear. Several groups are already working to establish genetic maps of apple in United States (Hemmat et al. 1994), Europe (King 1994) and New Zealand (Gardiner et al. 1994). The synteny between apple and pear should allow an efficient transfer of a number of apple markers to pear and a precise understanding of the degree of divergence between both fruit species.

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