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Genetics of esterase isoenzymes in *Malus*

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Summary. Three main zones of esterase activity (EST-I, EST-III, EST-IV) identified in leaf extracts of cultivated apple and *Malus* species were determined by the genes *EST-1*, *EST-3* and *EST-4*, respectively. In addition to earlier reported alleles of *EST-1* (*a*, *b*) three further bands c, d and f were identified in the *EST-1* zone of which c was found to be determined by an allele, *c*. Two alleles, *a*, *b*, and a null allele were found for both the genes *EST-3* and *EST-4*. Differences in allelic frequency were observed between cultivars, rootstocks and *Malus* species. Allele *EST-1a* was rare amongst the rootstocks. The examination of *Malus* species and derivatives showed a geographical relationship. Allele *EST-1c* was confined to species of Asian origin, and *EST-1d* was confined to American species.

Key words: *Malus pumila* Mill – *Malus* species – Esterase – Genes *EST-1*, *EST-3*, *EST-4*

Introduction

Plant species contain large numbers of esterase (E.C.3.1.1.; EST) isoenzymes in many different tissues.

Maize (*Zea mays*) esterase isoenzymes, for which genetic analyses have been conducted, are encoded by ten genes (MacDonald and Brewbaker 1974). In barley (*Hordeum vulgare*) at least ten genes have been reported (Hvid and Nielsen 1977), three of them being tightly linked (Kahler and Allard 1970). In species of *Lycopersicon* seven esterase genes were detected (Tanksley and Rick 1980), and six genes have been identified in *Secale* sp. (Salinas and Benito 1985). In most cases esterases are monomers, with heterozygous individuals showing only the parental bands without 'hybrid' bands. However, in some crops both monomeric and dimeric esterases are present e.g. *Secale* sp. (Salinas and Benito 1985), *Lycopersicon* sp. (Tanksley and Rick 1980) and maize (Goodman and Stuber 1983). Complex esterase genes encoding multiple bands have been described in *Secale* sp. (Schmidt-Stohn and Wehling 1983) and maize (Mac Donald and Brewbaker 1974).

In apple Chevreau et al. (1985) described esterase polymorphism in pollen. Two zones were found to be controlled by two genes, which they designated '*Est-1*', with two alleles, *a*, *b*, and '*Est-2*', with three alleles, *a*, *b* and a null allele *n*. '*Est-2*' was detected only when β-naphthylacetate was used as the substrate in the staining solution. Using leaves Chevreau and Laurens (1987) detected only '*Est-1*' genotypes. Menendez et al. (1986) recorded 19 and 22 different patterns, respectively, for the two zones which they located – 'EST-a' (substrate α-naphthylacetate) and 'EST-b' (substrate β-naphthylacetate) – amongst 34 apple rootstocks.

The present study investigates further the genetic basis of the existing polymorphism using various tissues from a wider range of plant material.

Materials and methods

Plant material

Seedling progenies from controlled crosses were studied. In addition to young leaves, cotyledons, old leaves, bark and pollen were examined.

Scion varieties, rootstocks and *Malus* species were examined from the National Apple Collection, Brogdale, and the gene banks of Horticulture Research International, East Malling. Apart from a few occasions when flower buds or bark were sampled, actively growing leaves were usually used.

Sample preparation and electrophoretic procedure

The procedures used were those of Manganaris and Alston (1987) for glutamate oxaloacetate transaminase analysis, but electrophoresis was stopped after 3 h.

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In order to standardize the staining method two different buffers (0.2 *M* TRIS-HCl pH 6.0 or 0.1 *M* phosphate buffer pH 6.0) and three substrates (α- and β-naphthylacetate and α-naphthylbutyrate) were tried.

In a few sample preparations 1% w/v ascorbic acid was incorporated into the crushing solution. This resulted in a considerable drop of the pH, and after electrophoresis these gels showed activity only for the slow EST-IV zone.

Results

Description of zymograms

A large number of bands were distinguished on the basis of migrational distance. These bands were assigned to three zones: EST-I, EST-III and EST-IV (Fig. 1).

Zone EST-1. Using leaf and cotelydon extracts we detected two main groups of bands, designated a and b at the position of the leading intense band in each group. Two intense bands (homologous to those found from pollen), two lighter and more slowly migrating bands and two much fainter and faster migrating bands were observed for each group (Fig. 2). The minor bands were not always present, but when present they did not appear to be additional sites of variation since their positions appeared fixed in relation to the main bands in this zone. The two main bands in each group were equally intense, and the migrational distances of the main leading bands from the start of the 'running' gel were 80 mm and 78 mm for the a and b groups, respectively. When *Malus* species were examined, three more groups of bands were observed, c, d and f, either alone or in combinations (Fig. 2). The leading band of group c migrates the same distance (75 mm) as the trailing band of group b, and this overlapping causes difficulties in band identification. The leading band of group d migrates 67 mm from the origin, and the faint band at 76 mm, f, was observed only in a single case (*Malus fusca*). When two of these groups were present, a total of eight or seven bands were observed where leaves or cotyledon tissues were used. When bark or flower buds were used usually only the main bands of each group were observed; the leading band being most intense, while the trailing band was usually fainter or nearly absent (Fig. 3).

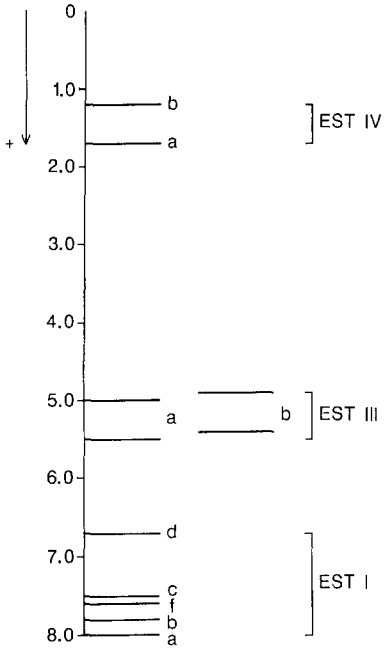

Fig. 1. Schematic representation showing the migration in cm of the main bands of EST-I, EST-III and EST-IV zones. Secondary bands are not shown

Zone EST-III. Activity in this zone sometimes appeared weak, and the clarity of the bands was usually poor making it difficult to score for this zone. In some cases, however, resolution was good (Fig. 4). This zone has two overlapping doublet positions, a and b (Fig. 1). When cotyledons, leaves and buds are used, the leading band of the doublet is more intense than the trailing band. However, when bark is used, the trailing band of each doublet is more intense. The fast doublet, a, migrates 50 mm and 55 mm from the start of the 'running' gel, while the overlapping trailing doublet, b, migrates 49 mm and 54 mm. Each doublet was accompanied by two faster faint bands (Fig. 4) when cotyledons were used.

Zone EST-IV. There are two major bands in this zone, a and b, which migrate 17 and 12 mm, respectively;

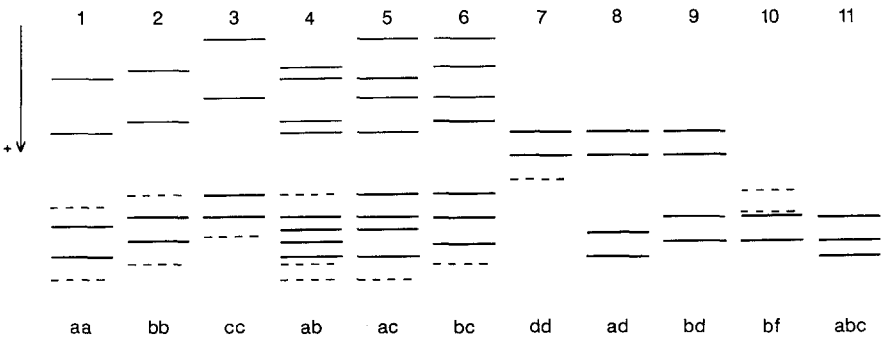

Fig. 2. Schematic representation of banding phenotypes in the EST-I zone and the corresponding *EST-1* genotypes. *Phenotypes 1–6* were observed in cotyledon samples and *phenotypes 7–11*, with either faint or no secondary bands, were observed in samples from flower buds

each is accompanied by one or two faster moving secondary bands. Bark samples produced the clearest zymograms, while leaves usually gave a dark background (Fig. 3).

In addition to the bands described above, some varieties showed a single band at different distances between zones EST-III and IV when leaves were used. In Fig. 3 cvs 'Reinette Clochard' and 'Winter Majetin' show a single band in different positions, while 'Jonathan', 'Starkrimson' and 'Granny Smith' do not show any activity in this area. It is possible that these bands are determined by another EST gene which in most cases does not show activity due to the presence of null alleles. Between Zone EST-IV and the origin of the 'running' gel also there were some bands present that showed polymorphism, but their resolution was poor making them difficult to score.

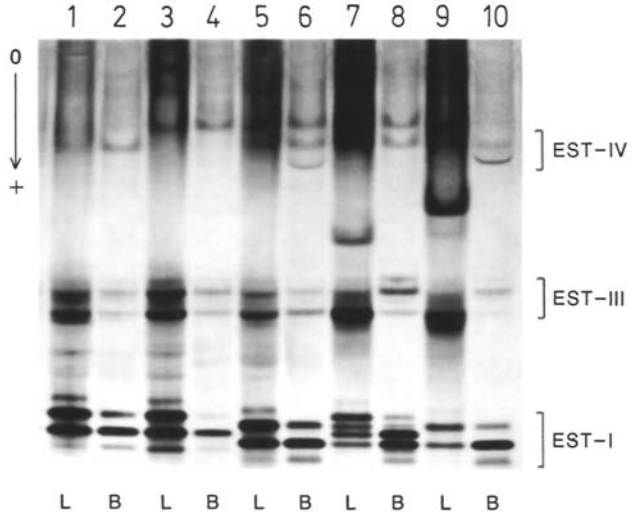

Fig. 3. Comparison of esterase zymograms from leaves (*L*) and bark (*B*) of the cys (left to right): *lanes 1, 2* 'Jonathan', *lanes 3, 4* 'Starkrimson', *lanes 5, 6* 'Granny Smith', *lanes 7, 8* 'Reinette Clochard', *lanes 9, 10* 'Winter Majetin'

In addition to the three anionic zones described, cathodal bands were observed when the polarity was reversed. However, these bands were not sharp and were difficult to delineate due to a dark background.

Plant part and developmental differences

EST-I activity was found in all of the tissues examined but there were differences in the intensity of the main bands and the number of the secondary bands (Fig. 3). Cotyledons and young leaves had higher activity than old or lyophilized leaves. The pattern from old leaf samples resembled that from bark.

EST-II was not detected in the bark and leaves. This isoenzyme was detected when pollen was examined with β-naphthylacetate incorporated into the staining solution (Chevreau 1984). Although in the present study pollen was used in a few cases and the gels stained using α-naphthylacetate, insufficient results were available to examine EST-II. This Isoenzyme appears to be tissue specific.

EST-IV showed greater activity from leaves than from bark, but both bark and lyophilized leaves gave clear bands for this zone, while fresh leaves and cotyledons gave hazy bands. Differences were observed in the activity of leaves between young and old trees. Samples from old trees (approximately 10 years) showed higher EST-I and EST-III activity, but young trees were more active for the EST-IV.

In a few cases where the roots were examined, activity was high, particularly in actively growing roots of young seedlings.

Genetic control

The results in Table 1 are in agreement with the hypothesis of a single gene *EST-1* with alleles *a*, *b*, and *c* determining polymorphism in the EST-I zone. Although segregating progenies were not available for bands d and f, it seems likely that they are coded by two alleles, tentatively named *d* and *f*. Eleven different phenotypes were

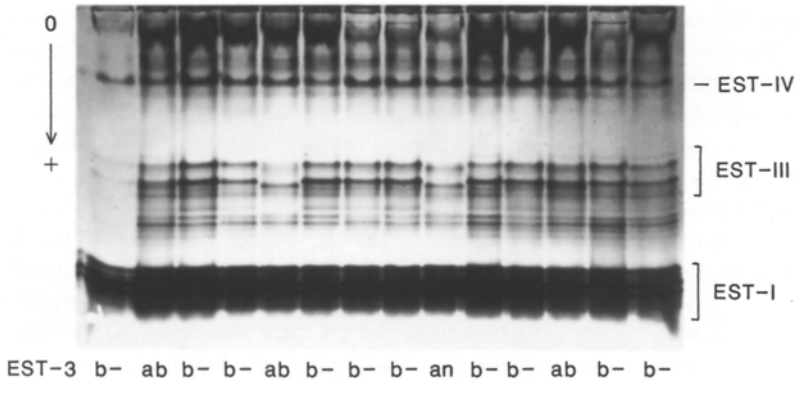

Fig. 4. Esterase zymograms using cotyledons from seedlings of the cross F49: 'Winter Majetin' (*bn*) × 'Northern Spy' (*ab*). *EST-3* genotypes are specified. *b-* includes both *bb* and *bn* genotypes

Table 1. Segregation for *EST-1*

Family		Parental genotypes	Progeny genotypes	Expected	χ^2	P
F27, 29[a]	('Delprim' × 'Katy')	ab × ab	6aa:12ab:10bb	1:2:1	1.71	0.42
F70	(A172-2 × A814-137)	ab × ab	4aa:15ab:9bb	1:2:1	1.93	0.38
F71	(A140-7 × A172-2)	ab × ab	13aa:11ab:8bb	1:2:1	4.69	0.10
F99	(A723-14 × 'Jester')	ab × ab	3aa:11ab:5bb	1:2:1	0.89	0.64
F100	(A723-5 × 'Jester')	ab × ab	12aa:18ab:4bb	1:2:1	3.88	0.14
F101	(A723-6 × 'Jester')	ab × ab	12aa:18ab:8bb	1:2:1	0.95	0.62
F132	('Katy × 'White Angel')	ab × ab	24aa:44ab:22bb	1:2:1	0.13	0.93
F140	('Glengyle Red' × 3762)	ab × ab	13aa:26ab:7bb	1:2:1	2.35	0.31
F5, 6[a]	('Idared' × T31-12)	bb × ab	22ab:34bb	1:1	2.57	0.11
F11, 21[a]	('Idared' × 'Fiesta')	bb × ab	31ab:29bb	1:1	0.07	0.79
F33	('Gala' × Elstar')	ab × bb	9ab:15bb	1:1	1.50	0.22
F32	('Fiesta' × 'Spartan')	ab × bb	6ab:7bb	1:1	0.08	0.78
F93	('Jonathan' × A849-7)	bb × ab	19ab:16bb	1:1	0.26	0.61
F102	('Northern Spy' × 'Worcester')	bb × ab	9ab:7bb	1:1	0.25	0.62
F7, 8	('Golden Delicious' × 'Cloden')	bb × ab	31ab:38bb	1:1	0.71	0.40
F12, 17[a]	(T30-24 × 'Cox')	ab × aa	13aa:15ab	1:1	0.14	0.70
F13, 14[a]	(T32-2 × 'Cox')	ab × aa	15aa:13ab	1:1	0.14	0.70
F16	('Cox' × T31-12)	aa × ab	10aa:4ab	1:1	2.57	0.11
F18	('Cox' × 'Fiesta')	aa × ab	12aa:4ab	1:1	4.00	0.05*
F23	('Vista Bella' × 'Katy')	aa × ab	10aa:11ab	1:1	0.05	0.83
F46	('Discovery' × 'Red Jade')	aa × ab	13aa:13ab	1:1	0.00	1.00
F104	(A721-19 × 'Cox')	ab × aa	7aa:9ab	1:1	0.25	0.62
F562	('Granny Smith' × SA219-21)	aa × ab	10aa:25ab	1:1	6.42	0.01**
F814	('Cox' × A467-74)	aa × ab	18aa:22ab	1:1	0.40	0.53
F115	('Kent' × 'Fiesta')	aa × ab	18aa:9ab	1:1	3.00	0.08
F41	('Cox × 'Baskatong')	aa × ac	25aa:38ac	1:1	2.68	0.10
F69	(3759 × 'Baskatong')	bb × ac	10ab:9bc	1:1	0.05	0.82
F244	('Wijcik' × 'Baskatong')	bb × ac	11ab:10bc:1abc[b]	1:1	0.05	0.83
F135	('Idared' × A679-12)	bb × ac	46ab:38bc	1:1	0.76	0.38
F567	(*M. florentina* × SA244-20)	cc × ab	5ac:5bc:7cc[c]	1:1	0.00	1.00

* $P \leq 0.05$; ** $P \leq 0.01$
[a] Pooled data from reciprocal crosses
[b] Triploid seedling deriving from unreduced pollen gamete
[c] Apomictic seedlings identical to the mother plant

found (Fig. 2), only 3 of which, *aa*, *ab* and *bb*, were present amongst the examined cultivars.

Triploid varieties heterozygous for *EST-1* appear with one allelic band stained more intensely than the other, while in diploids the bands were stained equally. In 'Blenheim Orange' (*EST1-abb*) band b was more intense than band a, while in 'Ribston Pippin' and 'Bramley's Seedling' (*EST-1aab*) band a was more intense. When β-naphthylacetate was used as a substrate for staining, the difference in the intensity of the red bands so produced was more obvious than the difference in the black bands produced by α-naphthylacetate. Amongst diploid varieties allelic bands in the homozygous state stained more intensely than individual allelic bands from the heterozygotes.

The results of segregating phenotypes in Table 2 are in agreement with the hypothesis that activity in zone EST-III is coded by a single gene, *EST-3*, with two active alleles α and *b* for the a and b doublets, respectively, and a null allele *n*. This gene was named *EST-3* since Chevreau et al. (1985) have already described an '*Est 2*' gene from pollen when staining with β-naphthylacetate as the substrate. Slight differences in band migration were observed, suggesting possibly more than two active alleles. Genotype *EST-3aa* was proposed for 'Prima', but the single band migrated between the normal a and b positions. Also, band a in 'Cox' and 'Miller's Seedling' is faster than the corresponding band in other cultivars.

The results in Table 3 suggest that a gene, *EST-4*, with two active alleles, *a* and *b*, coding for bands a and b, respectively, and a null allele *n* occurs. In one progeny (F589) and after combining *aa* × *ab* progenies a significant deviation from the 1:1 ratio was observed, producing a surplus of heterozygous (*ab*) seedlings.

Table 2. Segregation for *EST-3*

Family		Parental genotypes	Progeny genotypes	Expected	χ^2	P
F36	('Northern Spy' × 'Winter Majetin')	ab × bn	10an:25(bb+bn):11ab	1:2:1	0.38	0.82
F49	('Winter Majetin' × 'Northern Spy')	bn × ab	13an:22(bb+bn):8ab	1:2:1	1.18	0.55
F132	('Katy' × 'White Angel')	bn × ab	20an:49(bb+bn):17ab	1:2:1	1.88	0.41
F37	('Granny Smith' × 'Kent')	an × ab	4aa:5ab:4bb	1:2:1	0.66	0.72
F101	(A723-6 × 'Jester')	ab × ab	6ab:9(aa+an):4bn	1:2:1	0.48	0.79
F135	('Idared' × A679-12)	ab × an	23ab:39(aa+an):21bn	1:2:1	0.39	0.82
F2	(A463-70 × 'Granny Smith')	aa × ab	5aa:9ab	1:1	1.14	0.28
F11	('Idared' × 'Fiesta')	ab × aa	9aa:17ab	1:1	2.46	0.12
F21	('Fiesta' × 'Idared')	aa × ab	7aa:7ab	1:1	0.00	1.00
F41	('Cox' × 'Baskatong')	aa × ab	35aa:26ab	1:1	1.32	0.25
F69	(3759 × 'Baskatong')	aa × ab	10aa:9ab	1:1	0.05	0.82
F244	('Wijcik' × 'Baskatong')	aa × ab	12ab:10aa	1:1	0.18	0.89
F71	(A140-7 × A172-2)	ab × bb	14ab:12bb	1:1	0.15	0.69
F93	('Jonathan' × A849-7)	ab × bb	19ab:16bb	1:1	0.26	0.61
F27	('Delprim' × 'Katy')	aa × bn	5an:9ab	1:1	1.14	0.29
F29	('Katy- × 'Delprim')	bn × aa	3an:11ab	1:1	4.57	0.03*

* $P \leq 0.05$

Table 3. Segregation for *EST-4*

Family		Parental genotypes	Progeny genotypes	Expected	χ^2	P
F2	(A463-70 × 'Granny Smith')	ab × ab	7aa:14ab:6bb	1:2:1	0.11	0.94
F9, 10[a]	('Golden Delicious' × A463-70)	ab × ab	8aa:22ab:8bb	1:2:1	0.95	0.62
F99	(A723-14 × 'Jester')	ab × ab	7aa:7ab:5bb	1:2:1	1.74	0.42
F23	('Vista Bella' × 'Katy')	ab × ab	2aa:5ab:6bb	1:2:1	3.15	0.21
F93	('Jonathan' × A849-7)	an × ab	11ab:14(aa+an)[b]:10bn	1:2:1	1.46	0.48
F140	('Glengyle Red' × A3762)	an × ab	11ab:14(aa+an)[b]:12bn	1:2:1	2.24	0.33
F132	('Katy' × 'White Angel')	ab × bn	12an:22(bb+bn)[b]:8ab	1:2:1	0.85	0.65
F46	('Discovery' × 'Red Jade')	ab × bn	11an:11(bb+bn)[b]:4ab	1:2:1	4.38	0.11
F27	('Delprim' × 'Katy')	aa × ab	4aa:10ab	1:1	2.57	0.11
F29	('Katy' × 'Delprim')	ab × aa	4aa:10ab	1:1	2.57	0.11
F37	('Granny Smith' × 'Kent')	ab × aa	17aa:27ab:(1bb)	1:1	2.27	0.13
F41	('Cox' × 'Baskatong')	aa × ab	33aa:28ab	1:1	0.41	0.52
F1, 3[a]	(A463-70 × 'Cox')	ab × aa	16aa:20ab	1:1	0.44	0.51
F71	(A140-7 × A172-2)	ab × aa	14aa:18ab	1:1	0.50	0.48
F104	(A721-19 × 'Cox')	ab × aa	9aa:7ab	1:1	0.25	0.61
F135	('Idared' × A679-12)	aa × ab	37aa:48ab	1:1	1.42	0.24
F589	('Cox' × 'Greensleeves')	aa × ab	7aa:17ab	1:1	4.16	0.04*
F33	('Gala' × 'Elstar')	bb × ab	8bb:4ab	1:1	1.33	0.25
F101	(A723-6 × 'Jester')	bb × ab	20bb:18ab	1:1	0.11	0.74
F633	('Spencer's Seedless' × 'G. Carpenter')	bb × ab	38bb:57ab	1:1	3.80	0.05
F562	('Granny Smith' × SA219-21)	ab × bb	17bb:18ab	1:1	0.03	0.85
F244	('Wijcik' × 'Bskatong')	bb × ab	11b:11ab	1:1	0.00	1.00

* $P \leq 0.05$
[a] Pooled data from reciprocal crosses
[b] These genotypes could not be individually distinguished

Substrate specificity and choice of staining method

The reactions of the four esterase isoenzymes from leaf extracts to the three tested substrates are shown in Table 4. All variants coded by alleles at the same locus show the same reaction. The best substrate was α-naphthylacetate, while β-naphthylacetate gave good activity for EST-I only and α-naphthylbutyrate showed very little activity.

Two methods of staining were tested. The first method used 50 ml 0.2 *M* TRIS-HCl pH 6.0 and 150 mg α-

naphthylacetate as substrate (Chevreau 1984), and the second method used 50 ml 0.1 *M* phosphate buffer pH 6.0 and 10 mg of the same substrate (Devonshire 1975). In the second method the reaction was slow, and the staining could be stopped when all the expected bands had developed without overstaining. Activity of EST-I was greater than that of the other zones. Sometimes it was necessary to score *EST-1* after a few minutes of staining and the other zones after 1–2 h. The latter staining method was preferable for this reason.

Distribution of esterase classes

The distribution of the esterase alleles in cultivars, rootstocks and *Malus* species is presented in Table 5. The 6 rare phenotypes found for *EST-1* in *Malus* species (cc, dd, ad, bd, bf, abc) are not included in this table.

Among 83 cultivars (Table 6) two alleles (*a*, *b*) for *EST-1* and three alleles (*a*, *b*, *n*) for *EST-4* were found. The distribution of the three classes at the *EST-1* locus (*aa*, *ab*, *bb*) was close to that expected if the alleles were randomly distributed (1:2:1). However, with *EST-4* there was a surplus of genotype *aa* (42%) and a deficit of genotype *bb* (18%). Amongst the rootstocks (Table 7) there was a very low proportion of both *EST-1aa* (7%) and *EST-4bb* (11%) genotypes (Table 5). Finally, in *Malus* species (Table 8) the proportions of the three main classes for *EST-1* and *EST-4* indicated that alleles *a* and *b* were randomly distributed. Eight more phenotypic classes have occurred among these species.

Table 4. Substrate specificity of esterases from apple leaves

Isoenzyme	Substrate		
	α-Naphthyl-acetate	β-Naphthyl-acetate	α-Naphthyl-butyrate
EST-I	+	+	(+)
EST-II	–	–	–
EST-III	+	–	–
EST-IV	+	(+)	(+)

The sign + indicates that the esterase bands developed, (+) indicates a faint reaction and – indicates no band was observed

Discussion

The present study revealed two new esterase genes (*EST-3*, *EST-4*) each with two codominant alleles and a null allele. In addition, three new alleles were found for the gene *EST-1*. Chevreau (1984) described two *EST-1* alleles and three *EST-2* alleles, including a null allele. In all, four esterase genes *EST-1*, *EST-2*, *EST-3* and *EST-4* with five, three, three and three alleles, respectively, have now been found. *EST-2* was detected in pollen using β-naphthylacetate as the substrate in the staining solution (Chevreau 1984). In the present study no activity was detected for *EST-2* when the same substrate and other tissues (bark, leaves, buds) were used. The similar expression of isoenzymes only in the gametophytes has been reported in *Zea mays* (Sari Gorla et al. 1986) and *Lycopersicon* (Tanksley et al. 1981).

EST-3 has not been delineated sufficiently. Some methodological problems have to be overcome to ensure a faultless scoring and classification in the EST-III zone. The relative migration distance of the bands in this zone could be increased by changing the pH of the buffer. The classification in Tables 6, 7 and 8 was verified in many cases by progeny tests; in some others the *EST-3* genotype was not recorded.

Crosses of varieties showing activity in the area between the EST-III and EST-IV zones ('Winter Majetin', 'Reinette Clochard', 'Rome Beauty') could delineate the genetic basis of the existing polymorphism in that area.

When β-naphthylacetate was used as the substrate the dosage effects at the *EST-1* locus were more clearly visible than when α-naphthylacetate was used. This substrate could be useful to verify the genotype of triploid varieties.

Even where the genetic interpretation is straight-forward, the differences in banding patterns between differ-

Table 5. Distribution of *EST-1* and *EST-4* alleles among apple cultivars, selections, rootstocks and *Malus* species

Plant Material	Locus	Phenotypic class					Total no. of plants
		aa	ab	bb	bc	ac	
Cultivars	*EST-1*	17 (21%)	40 (48%)	26 (31%)	–	–	83
	EST-4	34 (42%)	33 (40%)	15 (18%)	–	–	82
Rootstocks	*EST-1*	3 (7%)	15 (34%)	25 (57%)	1 (2%)	–	44
	EST-4	26 (59%)	13 (30%)	5 (11%)	–	–	44
Malus species	*EST-1*	12 (28%)	11 (26%)	11 (26%)	7 (16%)	2 (5%)	43
	EST-4	13 (43%)	9 (30%)	8 (27%)	–	–	30

Table 6. Esterase genotypes of apple cultivars

Cultivar	*EST-1*	*EST-4*	*EST-3*
Akane	ab	ab	aa
Andre Briollay	bb	ab	–
Ashmead's Kernel	ab	ab	ab
Beauty of Bath	aa	aa	–
Belle de Boskoop	aa	bb	–
Blenheim Orange	abb	ab–	aaa
Bountiful	aa	ab	ab
Bramley's Seedling	ab	ab–	–
Charles Ross	aa	ab	–
Cloden	abb	aab	abb
Court Pendu Plat	ab	bb	bb
Cox	aa	aa	aa
Crawley Beauty	bb	ab	–
Delcorf	bb	bb	aa
Delprim	ab	aa	aa
Discovery	aa	ab	–
Early Victoria	ab	aa	ab
Edward VII	ab	bb	–
Elstar	bb	ab	aa
Egremont Russet	ab	–	–
Falstaff	ab	aa	–
Fiesta	ab	aa	aa
Florina	bb	aa	–
Gala	ab	bb	–
George Carpenter	aa	ab	ab
Gloster 69	bb	aa	aa
Golden Delicious	bb	ab	–
Golden Auvil	bb	ab	–
Starkspur Golden Delicious	bb	ab	–
Golden Noble	ab	ab	–
Granny Smith	aa	ab	ab
Gravenstein	bb	aa	–
Greensleeves	ab	ab	–
Grenadier	ab	aa	–
Holstein	aaa	aaa	ab–
Howgate Wonder	ab	ab	–
Idared	bb	aa	ab
Indo	bb	aa	–
Ingrid Marie	ab	aa	ab
James Grieve	aa	aa	ab
Jerseymac	ab	aa	–
Jester	ab	ab	ab
Jonagold	bbb	aab	–
Jonathan	bb	an	ab
Jupiter	ab–	aab	–
Katy	ab	ab	bn
Kent	aa	aa	ab
Kentish Codlin	aa	aa	–
Lane's Prince Albert	ab	bb	–
Laxton Superb 4n	aa	aa	ab
Leonie de Sonnaville	ab	aa	–
Liberty	ab	ab	bb
Lodi	ab	aa	bb
Lord Lambourne	ab	ab	–
Louiton	ab	aa	–
Marie Joseph D'Othee	ab	aa	–
Miller's Seedling	aa	ab	–
Monarch	ab	aa	–
Newton Wonder	bb	aa	–
Northern Spy	bb	aa	ab
Prima	bb	aa	aa
Reinette Clochard	ab	ab	ab
Reinette de France	abb	ab–	aab
Reverend W. Wilks	bb	ab	–
Rhode Island Greening	ab	ab	–
Ribston Pippin	ab	aab	–
Rome Beauty	ab	an	–
Double Red Rome Beauty	ab	an	–
Glengyle Red	ab	an	–
Scarlet Staymared	bb	bb	–
Spartan	bb	bb	ab
Spencer's Seedless	bb	bb	ab
Starkrimson	bb	bb	bb
Starkspur McIntosh	bb	bb	aa
Wijcik	bb	bb	aa
Suntan	aab	aab	–
Tydeman's Early	bb	bb	–
Vista Bella	aa	ab	ab
Wagener	aa	aa	–
Warden	bb	bb	–
White Transparent	ab	aa	–
Winter Majetin	aa	aa	bn
Worcester Pearmain	ab	bb	ab

ent tissues shows that care should be taken in comparing samples from different tissues. The results show that the age of the tissue also affects the pattern for esterases. For this reason this enzyme system was not used by Weeden and Lamb (1985) for identification purposes. However, since the genetic basis of this enzyme has now been established, esterase isoenzymes can be used reliably for identification purposes. This system could not distinguish between clones of the same variety, e.g. 'Golden Delicious Auvil' and 'Starkspur Golden Delicious', 'Double Red Rome Beauty' and 'Glengyle Red', 'Starkspur McIntosh' and 'Wijcik', nor between diploid M.13 and its tetraploid chimera. The differences in the esterase banding patterns between clones within the same rootstock which were observed by Menendez et al. (1986) were not reproduced; they might have been due to different physiological stages of the tissues and/or to the extraction technique used resulting in presence or absence of some bands.

When the pH of the crushing solution was reduced to approximately 2 and antioxidant factors omitted, only the *EST-4* gene showed activity, indicating that this gene codes for an esterase having properties different from those of EST-I and EST-III.

With the exception of *EST-1* all of the other esterase genes have null alleles. Null alleles also appear frequently amongst esterase systems in *Hordeum* sp. (Kahler and Allard 1970) and *Secale* sp. (Schmidt-Stohn and Wehling 1983).

Each allele at the *EST-1* locus codes at least two and at the most six bands. It is possible that this gene is compound and composed of tightly linked subunits, each subunit coding a single product. Alternatively, a single

Table 7. Esterase genotypes of apple rootstocks

Rootstock	*EST-1*	*EST-4*	*EST-3*
M.1	bb	aa	ab
M.2	ab	aa	bb
M.3	ab	bb	bb
M.4	ab	aa	bb
M.5	bb	aa	ab
M.6	bb	aa	aa
M.7	bb	ab	ab
M.8	aa	aa	bb
M.9	ab	ab	ab
M.10	bb	ab	ab
M.11	ab	ab	aa
M.12	bb	aa	ab
M.13	bb	ab	ab
M.14	bb	bb	ab
M.15	bb	bb	bb
M.16	bb	bb	ab
M.17	bb	aa	ab
M.18	bb	aa	aa
M.19	ab	ab	bb
M.20	aa	ab	bb
M.21	ab	aa	aa
M.23	bb	ab	ab
M.24	ab	aa	bb
M.25	bb	aa	bb
M.26	ab	ab	ab
M.27	bb	ab	bb
MM.101	bb	aa	aa
MM.102	bb	aa	aa
MM.104	ab	aa	ab
MM.105	ab	aa	ab
MM.106	bb	aa	ab
MM.109	ab	aa	ab
MM.110	bb	aa	bb
MM.111	bb	aa	ab
MM.112	ab	aa	ab
MM.113	bb	aa	ab
MM.115	ab	ab	ab
Merton 778	ab	aa	–
Metton 779	bb	aa	–
Ottawa 2	bb	ab	–
Ottawa 3	aa	aa	–
A2	bb	aa	–
Robusta 5	bc	bb	–
M.13 Chimera	bb	ab	–

Table 8. Esterase genotypes of *Malus* species and derivatives

	EST-1	*EST-4*
M. baccata flexilis	bb	–
M. baccata 'Gracilis'	ac	–
M. baccata jackii	bc	–
M. × *arnoldiana*	abc	–
M. × *hartwigii*	bc	–
M. × *robusta* 'Erecta'	aa	–
M. × *robusta* M.	bc	ab
M. × *robusta* o.p. (3760)	bc	aa
M. × *robusta* o.p. (3762)	ab	ab
M. × *zumi* o.p. (3752)	bc	ab
M. × *zumi* o.p. (3753)	bb	aa
M. 'Golden Hornet'	bb	aa
M. coronaria 'Charlottae'	dd	–
M. × *platycarpa*	ad	bb
M. × *floribunda*	ab	aa
M. floribunda brevipes	bc	–
M. × *scheideckeri* 'Exzellenz Thiel'	ab	–
M. × *scheideckeri* 'Hillieri'	ab	–
M. × 'Red Jade'	ab	bn
M. florentina	cc	–
M. fusca	bf	–
M. glaucescens	ab	–
M. hupehensis	bb	aa
M. hupehensis robusta o.p. (3759)	bb	–
M. hupehensis theifera	ab	–
M. prunifolia o.p.	dd	ab
M. pumila 'Niedzwetzkyana'	aa	bb
M. purpurea 'Aldenhamensis'	aa	–
M. × *moerlandsii*	ab	–
M. sargentii	bb	aa
M. sieboldii	bb	aa
M. toringo	ab	bb
M. 'White Angel'	ab	bn
M. × *atrosanguinea*	bb	–
M. × *sublobata*	bb	ab
M. sikkimensis	bb	–
M. silvestris o.p. (AR 537-1)	aa	bb
M. × *soulardii*	bd	bb
M. sieversii No. 3 (A781-1)	aa	aa
M. sieversii No. 3 (A781-2)	aa	aa
M. sieversii No. 4 (A782-1)	aa	aa
M. sieversii No. 4 (A782-2)	aa	aa
M. sieversii No. 6 (A784-2)	aa	bb
M. sieversii (AR 577-1)	aa	ab
M. sieversii (AR 577-4)	aa	aa
M. sieversii (AR 577-5)	ab	ab
M. spectabilis	bb	–
M. toringoides	bc	aa
M. toringoides o.p. (3755)	cc	–
M. transitoria	cc	ab
M. trilobata	cc	–
M. yunnanensis	bc	–
M. 'Baskatong'	ac	ab

product from the gene could be modified post-translationally to produce the different bands. So far it has been found that alteration of all bands always occurs simultaneously, and for this reason post-translational modification is the likely explanation for multiple allelic band. Complex esterase loci have also been described in *Secale* sp. (Schmidt-Stohn and Wehling 1983) and *Zea mays* (MacDonald and Brewbaker 1974).

The esterase isoenzymes could be useful research tools for studying the extent of allelic variability between cultivars of *Malus pumila* Mill. and between *Malus* species. Amongst the apple cultivars the *EST-1* alleles follow an even distribution. The predominance of the *EST-4a* allele is likely to be related to the fact that 16 (19%) of the cultivars studied are derivatives of 'Cox' (*EST-4aa*).

EST-1a predominates in cultivars that have been accepted in England as producing high quality apples ('Cox', 'Discovery', 'Fiesta', 'James Grieve'), although 'Elstar' and 'Worcester' have *EST-1bb*. This may be due

to the linkage of *EST-1a* with other genes carrying desirable agronomical characters, or it may be because most of them derive from 'Cox' (*EST-1aa*). Amongst the apple rootstocks there were deficiencies of alleles *EST-1a* and *EST-4b*. All six rootstocks of German origin (Hatton 1927) carry *EST-4b* either in the homozygous state (M.14, M.15, M.16) or in the heterozygous state (M.10, M.13, M.19), while *EST-4a* predominates in rootstocks of English origin (M.1, M.2, M.12). No correlation was found between any genotypic combination for EST and the dwarfing potential of the rootstocks.

Three additional alleles were detected amongst the *Malus* species. Both *Malus coronaria* 'Charlottae' and *Malus* × *platycarpa* (*M. coronaria* × *M. pulmila*) carry the rare allele *EST-1d*. According to Rehder (1954) these species belong to the section Chloromeles, a group of North American species. The same allele also appears in *M.* × *soulardii* (*M. ioensis* × *M. pumila*) and probably derives from *M. ioensis*, another member of the Chloromeles. Allele *EST-1c* appears in the Sorbomalus section (*M. yunnanensis*, *M. transitoria*, *M. toringoides* and *M. florentina*), in *M. trilobata*, the sole member of the Eriolobus Section, and in several *M. baccata* derivatives ('Baskatong', *M.* × zumi, *M.* × *robusta*, *M.* × *hartwigii*). All these originate from Asia or Europe. Two *Sorbus aria* seedlings were examined, and both showed the EST-Ic band only. Browicz (1970) suggested, on morphological grounds, that *M. florentina* is an intergeneric hybrid × *Malosorbus* (*Malus* × *Sorbus*). *M. florentina* is indigenous to Italy and Greece, and Browicz proposed its likely progenitors to be *M. sylvestris* (sensu lato) and included the cultivated apples and their escaped descendants, which many authors assign to *M. pumila*, and *S. torminalis*, that grow in the same region. However, the two *M. sylvestris* seedlings studied and the various *M. pumila* cultivars showed only a and b bands, whereas *M. florentina* and the two *Sorbus aria* seedlings showed only the c band. Therefore, *M. florentina* is unlikely to be derived from *M. sylvestris*. *M. transitoria* and others containing c bands may be ruled out as possible progenitors of *M. florentina* on account of their Asian origin. Finally, allele *EST-1f* was observed only in *M. fusca* (sorbomalus), a species of American origin. Further study of EST isoenzymes from a wider range of *Malus* species and derivatives of various origins is necessary in order to delineate the progenitors of the cultivated apple.

It is possible that other zones of activity exist that could become visible under certain conditions. Actively grown roots revealed six additional esterase genes in *Lycopersicon* sp. (Tanksley and Rick 1980). Apple roots showed good activity but no extra zones. However, actively growing roots were not examined.

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