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Inheritance and mapping of isoenzymes in pea (*Pisum sativum* L.)

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Summary. Three isoenzyme systems (amylase, esterase and glutamate oxaloacetate transaminase) were examined in seeds of pea (*Pisum sativum* L.) and shown to give clear variation in their band patterns on gel electrophoresis between different lines. The inheritance of these isoenzyme systems, and the location of their genes on the pea genome was investigated. Reciprocal crosses were made between lines, F2 seeds were analysed for segregation in the band patterns of the isoenzymes, and F2 plants were investigated to find linkage between the genes for these isoenzymes and genes for selected morphological markers. The results obtained showed that each of the investigated isoenzyme systems is genetically controlled by co-dominant alleles at a single locus. The gene for amylase was found to be on chromosome 2, linked to the loci *k* and *wb* (*wb* . . . 9 . . . *k* . . . 25 . . . *Amy*). The gene for esterase was found to be linked with the gene *Br* (chromosome 4) but the exact location is uncertain because of the lack of the morphological markers involved in the cross. The gene for glutamate oxaloacetate transaminase was found to be on chromosome 1 and linked with the loci *a* and *d* (*a* . . . 24 . . . *Got* . . . 41 . . . *d*).

Key words: Inheritance – Linkage – Gene mapping – Isoenzymes – *Pisum sativum* L.

Introduction

Electrophoretically variant enzymes have provided suitable material for studying genetic variation at the molecular level in higher plants. These variations have been detected with several enzyme systems in the higher plants (Frankel and Garber 1965; West and Garber 1967; Marshall and Allard 1969; Cherry and Ory 1973; Yamamoto 1975; Singh et al. 1977; Przybylska et al. 1982).

Amylase (E.C. 3.2.1.1.2) of higher plants exists as two distinctive forms, α and β amylase (Frydenberg and Nielsen 1965; Yamamoto 1975). The existence of genetic variation in amylase patterns in plants has been established in barley (Frydenberg and Nielsen 1965), maize (Scandalios 1966), *Vicia* (Yamamoto 1975), soybean (Gorman and Kiang 1977) and peas (Przybylska et al. 1982). Both the study of Frydenberg and Nielsen (1965) in barley and of Scandalios (1966) in maize strongly suggest simple Mendelian control of the amylases in the higher plants.

Esterase (E.C. 3.1.1.2) of higher plants has been used in genetic studies (Bassiri and Rouhani 1977; Gates and Boulter 1979; Hart et al. 1980). West and Garber (1967) determined the inheritance of two sites of esterase in the genus *Phaseolus* as a Mendelian monogenic inheritance. In *Pisum* Frankel and Garber (1965) extracted six detectable esterases from the germinating seeds of 12 varieties and found that the presence or absence of two of these esterases is determined by monogenic differences. Brown and Allard (1969) studied the inheritance of five enzyme systems including esterase in maize and found that each of these systems was governed by a single locus. In *Cucurbita* Wall and Whitaker (1971) stated that there are two forms of esterase controlled by co-dominant alleles.

Glutamate oxaloacetate transaminase or aspartate aminotransferase (GOT) (E.C. 2.6.1.1) in higher plants has been widely investigated, e.g. Rudin (1975) reported the monogenic inheritance of GOT of *Pinus sylvestris*, Guries and Ledig (1978) observed three zones of GOT activity in pitch pine. Przybylska et al. (1982) studied the electrophoretic patterns of several enzymes in *Pisum* and identified two independently varying zones of GOT activity, and in *Vicia faba* L. Suso and Moreno (1982) found that there were two zones of GOT banding pattern, "A" and "B", and that zone "B" variants were controlled by a locus with two alleles.

This paper presents the results of a study of the inheritance of electrophoretic variants with amylase, esterase and GOT systems in the seeds of peas (*Pisum sativum* L.). Linkages between the genes for these

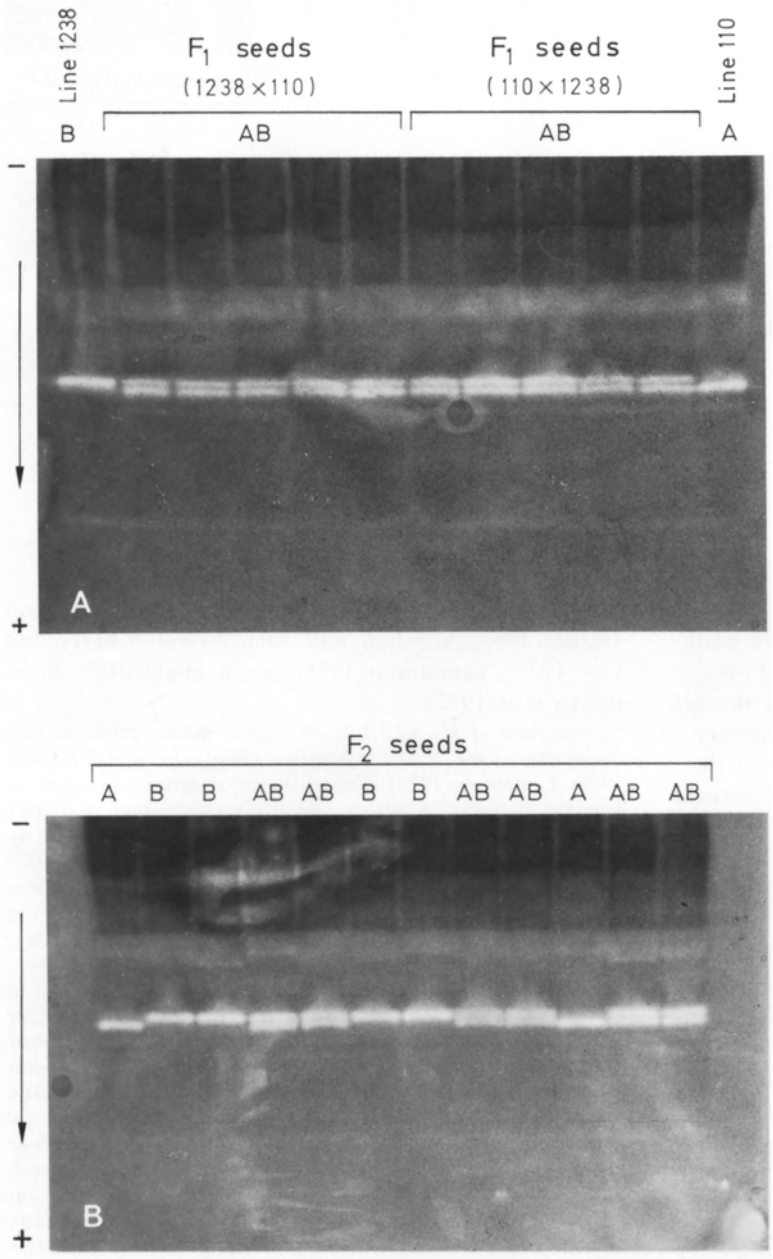

Fig. 1. A Polyacrylamide gel electrophoresis of amylase isoenzyme banding patterns of pea lines 110 (*A*) and 1238 (*B*) and their F_1 seeds in both straight and reciprocal crosses (*AB*). **B** Polyacrylamide gel electrophoresis of amylase isoenzyme banding patterns of the F_2 seeds (*A*, *B* and *AB*) of the cross I between pea lines 110 (*A*) and 1238 (*B*)

isoenzymes and selected morphological markers were investigated, to map locations of the enzyme structural genes onto the pea genome.

Materials and methods

Biological material

Seeds of pea lines under investigation were supplied by Dr. S. Blixt, Weibullsholm, Landskrona, Sweden.

Preparation of single seed extract

The testa was removed from a part of the seed and a quarter of the seed was cut off taking care that the radicle and the other parts of the embryo were not injured. The removed cotyledon tissue was then ground and 20 mg of the meal was suspended in 100 µl of 0.025 M sodium-phosphate pH 7.25 with 20% (w/v) sucrose. Isoenzymes were extracted at 4 °C for 16 h.

Polyacrylamide gel electrophoresis (PAGE)

PAGE under non-dissociating conditions was carried out by the method of Gabriel (1971). Seven and a half per cent acrylamide gels were used for the analysis of all the investigated isoenzymes.

Assay methods

Amylase bands were stained according to the method of Brewbaker et al. (1968). Gels were incubated for 5 min in

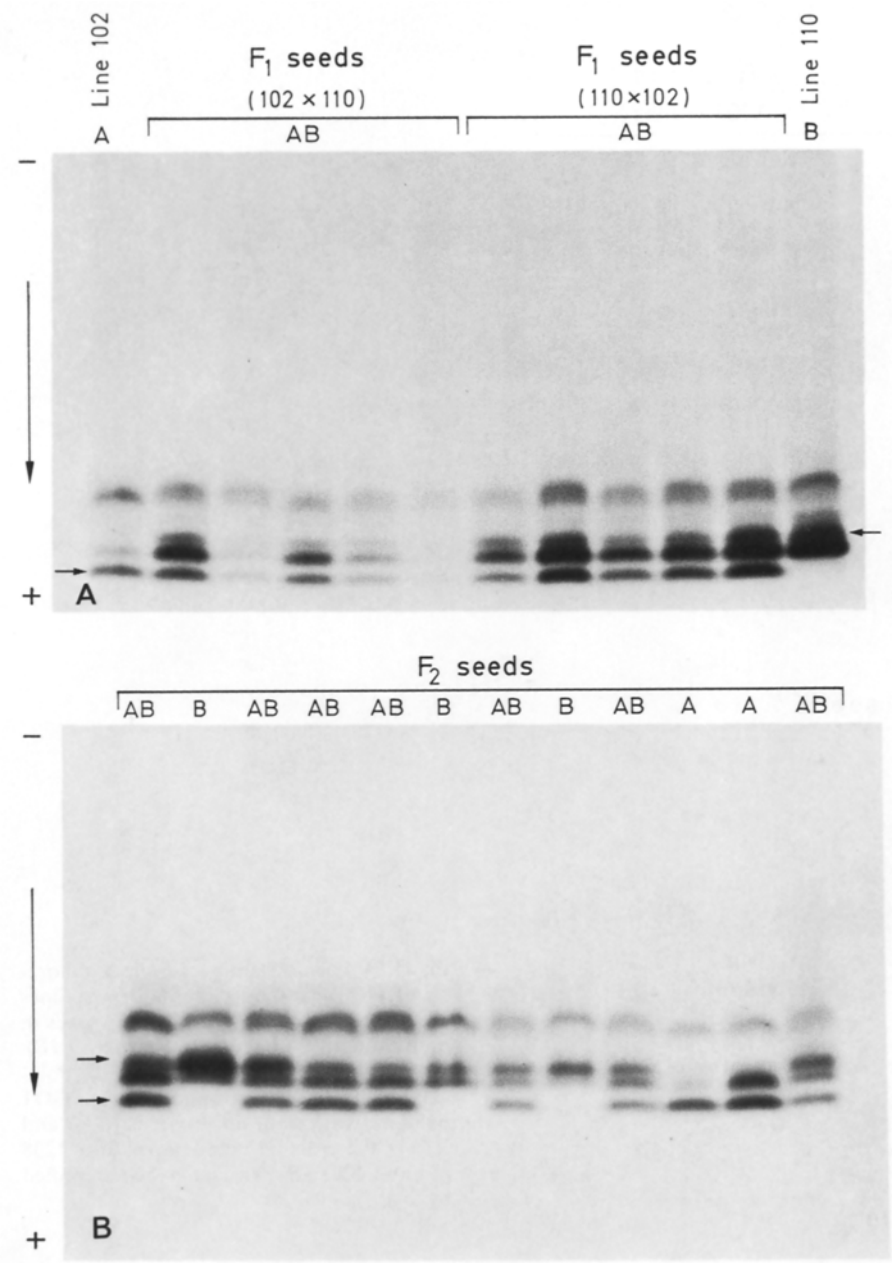

Fig. 2. A Polyacrylamide gel electrophoresis of esterase isoenzyme banding patterns of pea lines 102 (*A*) and 110 (*B*) and their F_1 seeds in both straight and reciprocal crosses (*AB*). Variant bands identified by >. **B** Polyacrylamide gel electrophoresis of esterase isoenzyme banding patterns of the F_2 seeds (*A*, *B* and *AB*) of the cross II between pea lines 102 (*A*) and 110 (*B*). Variant bands identified by >

0.2 M sodium acetate buffer pH 5.0, followed by incubation for 2 h in 1% (w/v) soluble starch in 0.2 M sodium acetate buffer pH 5.0. Gels were then washed for 5 min in 0.1 acetic acid followed by staining in potassium iodide/iodine solution (0.1 g potassium iodide, 20 mg iodine, 100 ml 0.1 M acetic acid). Esterase and GOT assays were as described by Shaw and Prasad (1970) with some modifications. Esterase bands were detected after pre-incubation of the gels for 5 min in 0.2 M sodium acetate buffer pH 5.0 containing α-naphthyl-acetate (1 mg/ml), and staining for 15 min at 37 °C in 0.2 M sodium acetate buffer pH 5.0 containing fast blue B salt (1 mg/ml). GOT bands were visualised by incubation for 10 min at 37 °C in 200 ml of a staining solution containing 0.2 M sodium acetate buffer pH 5.0, L-aspartic acid (500 mg), α-Ketoglutaric acid (70 mg), Pyridoxal-5-phosphate (10 mg) and fast blue B salt (200 mg). Developed gels were destained in methanol : acetic acid : water (50 : 7 : 43), photographed, and dried between layers of uncoated cellophane.

Statistical methods

Chi-square values were calculated following the formula given by Fisher (1925). The percentages of crossing-over and the probable error was calculated by the method of Immer (1930). One of the co-dominant factors in the allelic pair of the parental plants was considered as dominant, so that in a hybrid cross involving dominant and co-dominant factors, those factors were in coupling phase.

Nomenclamature and genotypes

Locus for amylase variant band patterns =	*Amy*
Locus for esterase variant band patterns =	*Est*
Locus for GOT variant band patterns =	*Got*

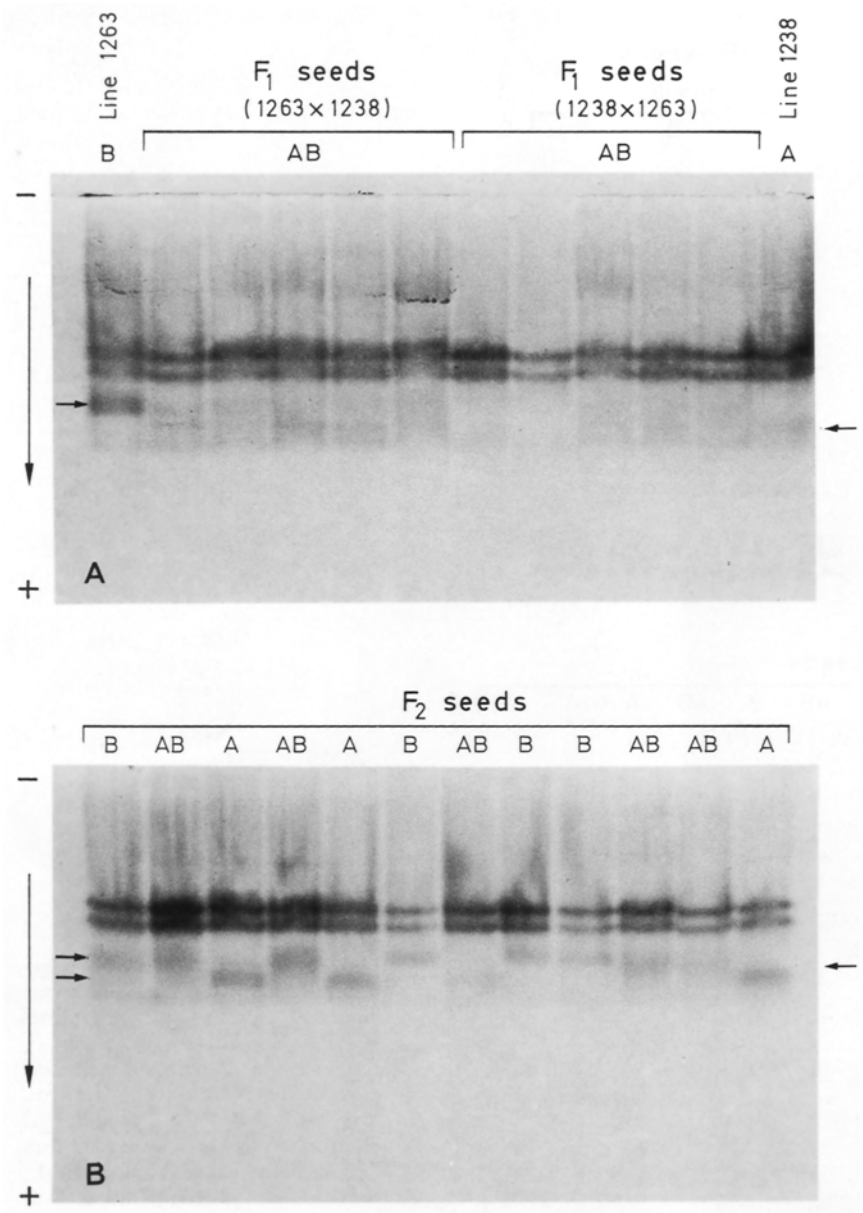

Fig. 3. A Polyacrylamide gel electrophoresis of GOT banding patterns of pea lines 1238 (*A*) and 1263 (*B*) and their F_1 seeds in both straight and reciprocal crosses (*AB*). Variant bands identified by >. **B** Polyacrylamide gel electrophoresis of GOT banding patterns of the F_2 seeds (*A*, *B* and *AB*) of the cross III between pea lines 1238 (*A*) and 1263 (*B*). Variant bands identified by >

Genotypes of pea lines were taken from Blixt (1981) and were as follows

102 *pa, a, Br, pre, tl, td, fl, le, mifo, Tra, r*
110 *a, td, D, n, v, fov, mifo, Tra, F, Fs*
1238 *b, k, Bra, dt, pr, wb, tl, d, fl, fr, fru, pro, coh, le, t, cp-1, te, gp, pur, sru, s, U, i, r*
1263 *a, Br, dt, Tra.*

Results

Preliminary investigations

In order to study the inheritance of amylase, esterase and GOT isoenzymes and locate the controlling genes onto pea genome, different lines of peas were analysed electrophoretically on polyacrylamide gels. The isoenzyme bands were detected in the gel according to the assay methods indicated in the materials and methods. Clear genetic variations in the band patterns of amylase, esterase and GOT were found between some of the tested lines which were subsequently used as parents in the following crosses.

Cross I. Reciprocal crosses were made between line 110 which has amylase (*Amy*) type "A" and line 1238 which has type "B" (Fig. 1 A). Type "A" has one band with faster migration, whereas type "B" has one band with slower migration (Rf values of 0.57 and 0.55, respectively, with respect to the dye front).

Cross II. Reciprocal crosses were made between line 102 which has esterase (*Est*) type "A" and line 110 which has type "B" (Fig. 2 A). Both types have two different zones of esterase bands, a nonvariant zone with one band of slow migration (Rf 0.55) and a variant zone of two bands in each type with Rf values of 0.69 and 0.65 in type "A" and 0.65 and 0.62 in type "B".

Cross III. Reciprocal crosses were made between line 1238 which has GOT (*Got*) type "A" and line 1263 which has type "B" (Fig. 3 A). Both types have two different zones of GOT bands, a nonvariant zone with two bands of Rf 0.31 and 0.28 for the fast and slow bands, respectively, and a variant zone which has one band in each type with faster migration in type "A" and slower migration in type "B" (Rf values 0.41 and 0.38, respectively).

Inheritance and mapping of amylase, esterase, and GOT genes

F1 plants of the straight and reciprocal crosses showed the expected dominant expression of the genes *D, Fl, Gp, I, K, Le, N, Pro, R, Te, Tl* and *Wb*. The band patterns of amylase and esterase in the Fl seeds were found to be the additive pattern 'AB' (i.e. contained bands from both the parental lines 'A' and 'B') whereas for GOT Fl seed the 'AB' patterns contained not only bands from both parental types 'A' and 'B' but also additional band of intermediate migration (Figs. 1 A, 2 A and 3 A). Statistical analysis of the F2 plants showed the known monogenic inheritance and dominance of the genes *A, Fl, Gp, I, K, Le, Pro, R, Te, N, Tl* and *Wb* (Tables 1 a, 2 a and 3 a), complementary genes, *A–D* (Tables 1 a and 3 a) and *Br–Bra* (Table 2 a). The segregation of amylase, esterase and GOT patterns (Figs. 1 B, 2 B and 3 B) of the F2 seeds with the parental and hybrid patterns are given in Tables 1 a, 2 a and 3 a. χ^2 values (Tables 1 a, 2 a and 3 a) for *Amy, Est* and *Got* support for the hypothesis that the controlling gene for each of the investigated isoenzyme systems occurs as a single pair of co-dominant alleles.

To investigate the linkage between the genes for amylase, esterase and GOT subunits and the genes for the selected morphological markers from the F2 data, Chi-square values of the segregation patterns for the isoenzyme subunits and the morphological markers were calculated in each cross (Tables 1 b, 2 b and 3 b). The calculated χ^2 values (Table 1 b) between *Amy* type 'A, AB and B' and the genes *a, d, fl, gp, i, k, le, pro, r, te, tl* and *wb*, show that the *Amy* locus segregates independently of the loci *a, d, fl, gp, i, le, pro, r, te* and *tl* and dependently with *K* and *wb*. These results indicate that the gene *Amy* for amylase subunits and the genes *k* for keel like wings and *wb* for waxless stipules are located on the same chromosome. On the other hand the genes *k* and *wb* showed the expected dependent segregation with a cross over value of 9.00 ± 1.76 between them (Table 1 b). Cross over values were calculated between the linked loci (Table 1 b). Values of 25.50 ± 3.02 and 33.50 ± 3.50 were found between the *Amy* gene and the genes *k* and *wb*, respectively.

Chi-square values (Table 2 b) between *Est* type 'A, AB and B' and the genes *Br-Bra, fl, le, n, r, te* and *tl*,

Table 1 (a). F_2 phenotypic classes, observed frequency and χ^2 values of the genes studied in cross I (line 110 × line 1238)

Gene	F_2 phenotypic classes and observed frequency			χ^2	Segregation ratio
	AA	*Aa*	*aa*		
Amy; A, AB, B	28	73	33	1.4473	1:2:1
A, a	98		36	0.2488	3:1
D, d	72		62	0.3454	9:7
Fl, fl	96		38	0.8060	3:1
Gp, gp	101		33	0.0100	3:1
I, i	100		34	0.0100	3:1
K, k	104		30	0.4876	3:1
Le, le	103		31	0.2488	3:1
Pro, pro	98		36	0.2488	3:1
R, r	98		36	0.2488	3:1
Te, te	100		34	0.0100	3:1
Tl, tl	96		38	0.8060	3:1
Wb, wb	105		29	0.8060	3:1

Table 1 (b). F_2 phenotypic classes, observed frequency, χ^2 and cross-over values of the pairs of genes studied in cross I (line 110 × line 1238). Segregation ratio is 9:3:3:1. Cp for coupling and Rp for repulsion

Gene pair	F_2 phenotypic classes and observed frequency				χ^2	Cross-over ± probable error
	AB	*Ab*	*aB*	*ab*		
Amy-a (Rp)	77	24	21	12	2.3316	
Amy[a]*-d* (Cp)	61	40	13	20	4.4720	
Amy-fl (Cp)	72	29	24	9	0.8456	
Amy-gp (Cp)	76	25	25	8	0.0232	
Amy-i (Cp)	77	24	23	10	0.5804	
Amy-k (Cp)	88	13	16	17	20.1626	25.50 ± 3.02
Amy-le (Cp)	77	24	26	7	0.3415	
Amy-pro (Cp)	72	29	26	7	1.0049	
Amy-r (Cp)	75	26	23	10	0.5274	
Amy-te (Cp)	75	26	25	8	0.0498	
Amy-tl (Cp)	81	20	23	10	1.9603	
Amy-wb (Cp)	85	16	20	13	8.1427	33.50 ± 3.50
k-wb (Cp)	99	5	6	24	67.2338	9.00 ± 1.76

[a] The segregation ratio is 27:21:9:7

Table 2 (a). F_2 phenotypic classes, observed frequency and χ^2 values of the genes studied in cross II (line 102 × line 110)

Gene	F_2 phenotypic classes and observed frequency			χ^2	Segregation ratio
	AA	*Aa*	*aa*		
Est; A, AB, B	32	69	33	0.1345	1:2:1
Br Bra, br bra	71		63	0.5803	9:7
Fl, fl	95		39	1.2040	3:1
Le, le	100		34	0.0100	3:1
N, n	102		32	0.0896	3:1
R, r	107		27	1.6816	3:1
Te, te	101		33	0.0100	3:1
Tl, tl	105		29	0.8060	3:1

Table 2 (b). Phenotypic classes, observed frequency, χ^2 and cross-over values of the pairs of genes studied in cross II (line 102 × line 110). Segregation ratio is 9:3:3:1. Cp for coupling and Rp for repulsion

Gene pair	F_2 phenotypic classes and observed frequency				χ^2	Cross-over ± probable error
	AB	*Ab*	*aB*	*ab*		
Est[a]*-Br* (Cp)	60	41	11	22	7.3579	28.43 ± 4.52
Est-fl (Rp)	69	32	26	7	2.6766	
Est-le (Rp)	76	25	24	9	0.1028	
Est-n (Cp)	75	26	27	6	0.8458	
Est-r (Rp)	78	23	29	4	3.1541	
Est-te (Rp)	73	28	28	5	2.0929	
Est-tl (Rp)	78	23	27	6	1.0845	

[a] The segregation ratio is 27:21:9:7

show independent segregation of *Est* locus with the loci *fl, le, n, r, te* and *tl* and linkage to the locus *Br* for bracteoles with cross over value of 28.43 ± 4.52 (Table 2 b) between them.

Chi-square values (Table 3 b) between Got type 'A, AB and B' and the genes *a, d, fl, gp, i, k, le, pro, r, te, tl* and *wb*, indicate that the gene for GOT subunits segregates independently of the loci *fl, gp, i, k, le, pro, r, te, tl* and *wb*, but indicate dependent segregation with the loci *a* and *d*. The linkages between the gene for GOT subunits and the loci *a* and *d* gave cross over values of 23.78 ± 3.00 and 40.28 ± 5.88 between *Got* locus and the loci *a* and *d*, respectively. These results indicate that the gene for GOT isoenzyme subunits and the genes *a* for absence of anthocyanin pigment and *d* for absence of maculum ring are located on the same chromosome.

Table 3 (a). F_2 phenotypic classes, observed frequency and χ^2 values of the genes studied in cross III (line 1238 × line 1263)

Gene	F_2 phenotypic classes and observed frequency			χ^2	Segregation ratio
	AA	*Aa*	*aa*		
Got: A, AB, B	24	66	36	2.5715	1:2:1
A, a	90		36	0.8572	3:1
D, d	71		55	0.0005	9:7
Fl, fl	102		24	2.3809	3:1
Gp, gp	92		34	0.2645	3:1
I, i	89		37	1.2804	3:1
K, k	97		29	0.2645	3:1
Le, le	98		28	0.5185	3:1
Pro, pro	93		33	0.0952	3:1
R, r	95		31	0.0105	3:1
Te, te	94		32	0.0105	3:1
Tl, tl	98		28	0.5185	3:1
Wb, wb	96		30	0.0952	3:1

Table 3 (b). F_2 phenotypic classes, observed frequency, χ^2 and cross-over values of the pairs of genes studied in cross III (line 1238 × line 1263). Segregation ratio 9:3:3:1. Cp for coupling and Rp for repulsion

Gene pair	F_2 phenotypic classes and observed frequency				χ^2	Cross-over ± probable error
	AB	*Ab*	*aB*	*ab*		
Got-a (Cp)	76	14	14	22	33.5485	23.78 ± 3.00
Got-d (Rp)	57	19	12	2	4.8148	40.28 ± 5.88
Got-fl (Rp)	75	15	27	9	4.0317	
Got-gp (Rp)	65	25	27	9	1.2098	
Got-i (Rp)	64	26	25	11	2.2258	
Got-k (Rp)	71	19	26	10	1.7178	
Got-le (Rp)	69	21	29	7	1.6614	
Got-pro (Rp)	67	23	26	10	1.0406	
Got-r (Rp)	69	21	26	10	1.1535	
Got-te (Rp)	70	20	24	12	2.7337	
Got-tl (Rp)	71	19	27	9	1.5484	
Got-wb (Rp)	67	23	29	7	1.5485	

Discussion

The results obtained from this study indicate that the genetic variation in the isoenzyme systems previously indicated in higher plants can be used in pea to map the controlling genes onto the genome. The three isoenzyme systems investigated, amylase, esterase and GOT were found to be controlled by co-dominant alleles at single loci. These results are in agreement with the results obtained by Frydenberg and Nielsen (1965) and Scandalios (1966) for amylase, Frankel and

Garber (1965); West and Garber (1967); Brown and Allard (1969) and Wall and Whitaker (1971) for esterase and MacDonald and Brewbaker (1972); Rudin (1975) and Suso and Moreno (1982) for glutamate oxaloacetate transaminase in other species. The observations that the Fl band pattern for GOT shows an additional band of intermediate mobility with respect to the variant parental bands suggests that this enzyme is a dimeric molecule occurring in three forms in the heterozygote; AA, AB and BB.

Appendix. Key to gene symbols and their chromosomal locations (from Blixt 1974)

Symbol	Location	Phenotype
a	1	Uncoloured flowers, seeds, axils
b	3	Flowers deep rose pink
Br	4	Flowers with bracts
Bra	3	As Br
coh	5	Reducing internode length
cp	5	Concavely curved pods
d	1	Absence of maculum ring
dt	–	Shortening distance from axil to first flower
F	3	Seed coat with violet spots
fl	6	Leaves and stipules without air pockets under epidermis
fov	3	Seed with deep impression over radicule
fr	3	Increasing number of stem branches
fru	4	As fr
Fs	5	As F
gp	5	Pods yellow
i	1	Green cotyledons
k	2	Wings reduced, keel like
le	4	Shortening internodes
mifo	2	Speeds with close standing ± regular small and shallow depressions
n	4	Thick and fleshy pod wall
pa	7	Dark green foliage color
pr	–	Shortens inflorescences
pre	–	As pr
pro	4	Stem branches growing out horizontally, then rising at 45° angle
pur	1	Pod color purple
r	7	Cotyledons wrinkled; starch phenotypically compound
s	2	Tragacanth excretion on seed coat outside; seeds stick together
sru	1	Pod along upper suture with anthocyanine stripe
t	1	Thicker stem
td	4	No dentation of foliage
te	5	Narrow pod
tl	7	Leaflets in the place of tendrils
Tra	4	Seed coat with "oily" spots from tragacanth excretion on inside
U	5	Seed coat violet colored
v	4	Pod-wall inside with patches of sclerenchyma
wb	2	Upper surface of stipules and lower of leaflets, stems and pods with very reduced wax layer

The F2 segregation results from cross I showed that the gene for amylase isoenzyme is on chromosome 2 and linked to the loci *k* and *wb* for keel like wings and waxless stipules, respectively. The deduced cross-over values (Table 1 b), predict a position of *Amy* in *Pisum;* wb . . . 9 . . . *k* . . . 25 . . . *Amy.*

Analysis of the data of cross II is more complex due to polymeric genes affecting the bracteole phenotype. The early studies of Lamprecht and Mrkos (1950) indicated that the gene *Br* causes (both in dominant and heterozygous states) the development of bracteoles, but later work by Lamprecht (1953) found that the development of bracteoles was dependent upon two polymeric genes in the dominant state. Apart from the earlier known gene *Br* dominance is necessary for the other gene, which is signified by the symbol *Bra.* From the gene map (Blixt, 1974; see Appendix), the genes *Br* and *Bra* are on chromosome 4 and 3, respectively. Esterase isoenzyme patterns were shown to be linked to the bracteole phenotype, and since the lines in this cross differ in their genotype with respect to *Br,* linkage must be between *Br* and *Est.* The lack of sufficient morphological markers in cross II makes the mapping of *Est* difficult, but the failure to detect linkage between *le* and *Est* suggests that *Est* is on the opposite side of *Br* to *le* i.e. the tentative map position is *Est* . . . 28 . . . *Br* . . . 60 . . . *le.* Further experiments will be necessary to confirm this location. It seems likely that this is only one of several esterase loci, since some esterase bands were found to be invariant in the present study.

The gene for glutamate oxaloacetate transaminase in *Pisum* was found to be on chromosome 1 by linkage with loci *a* and *d,* and the crossover values of 24 and 41 map units from the genes *a* and *d* respectively (Table 3 b) fix its position as lying between these loci; *a* . . . 24 . . . *Got* . . . 41 . . . *d.* The predicted value for the *a* . . . *d* distance, 65 map units, agrees well with that in the standard *Pisum* gene map (76 units; Blixt 1974). This *Got* locus does not correspond to the major isoenzyme bands, but to faster migrating minor bands; it appears to correspond to the GOT-1 locus of Przybylska et al. (1982).

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