USE OF NATURALLY-OCCURRING ENZYME VARIATION TO DETECT AND MAP GENES CONTROLLING QUANTITATIVE TRAITS IN AN INTERSPECIFIC BACKCROSS OF TOMATO

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SUMMARY

A backcross between the inbred parents, *Lycopersicon esculentum* (LA 490, recurrent parent) and *Solanum pennellii* (LA 716) constitutes ideal material for an investigation of linkage between enzyme marker genes and loci determining quantitative traits (QTL): the chromosomes of the parents are the same in number and are homosequential; the parents differ for well-defined alleles at 12 enzymatic loci, which represent at least 8 of the 12 chromosomes, and large differences exist between the parents for measurable metric traits.

The four metric traits investigated in this study were inherited in typical quantitative fashion; the marker segregations fit monogenic ratios for all except four loci, for which there were deviations of three favoured alleles of the recurrent parent, in agreement with frequent observations in interspecific hybrids. Association between QTL and mapped enzyme markers were detected statistically from calculations based on the normal distribution. In this manner a minimum of 21 QTL were mapped.

Two pairs of linked enzyme markers permitted application of a three-point mapping procedure by comparing the data with models expected for various positions of QTL. Data for three QTL fitted predictions specified for a locus closer to one marker than the other, and data for one QTL met all criteria save one for a locus between the two markers.

Certain QTL coding for stigma exsertion and leaf ratio have effects opposite to those expected from parental values; *i.e.*, for these traits both parents contribute alleles coding for positive and negative effects, resulting in the observed transgressive variation. When values observed for QTL linked with pairs of independent markers are compared with sums of separate values for each, significant epistatic interactions are detected. Although the extent of epistasis could not be determined precisely, it is probably not large. The paired interactions identified additional QTL whose individual effects were not otherwise detectable.

Enzymatic markers offer a number of advantages for the analysis of quantitative genetic variation which include: (a) lack of detectable effects on the morphology and physiology of the soma, (b) codominant expression, permitting exact identification of genotypes, and (c) lack of epistasis, allowing classification of any number of such markers segregating simultaneously. A tester stock with linked enzyme markers on each chromosome therefore could conceivably be utilized to analyze the total quantitative differences between two parents in a single segregating progeny. Such a tester stock might be especially useful for probing exotic germplasm for genes controlling quantitative characters of economic importance.

The methods of analysis outlined here could easily be adapted for analysis of F_2 populations or generations from other mating designs where a fuller array of genetic variation would be revealed.

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1. INTRODUCTION

SAX (1923) found an association of seed size (a quantitatively inherited character) with seed coat pigmentation (a discrete monogenic character). He attributed this correlation to linkage of seed size genetic factor(s) to the alleles for seed colour. A number of reports have since shown linkage of genetic factors controlling quantitative variation with discrete marker genes (for a review see Thompson and Thoday, 1979). The basic approach of using marker genes in segregations to detect genes controlling quantitative traits was put forth as a viable technique for systematically analyzing quantitative variation by Thoday (1961).

One problem inherent in this method of genetic analysis stems from the nature of the marker genes that one has classically been forced to use. Most of these markers are recessive, and when homozygous impart some morphological abnormality to the individual. The recessive-dominant allelic interaction also presents a problem. When using recessive marker alleles, the analysis is almost always confined to a backcross to the recessive homozygous tester stock. An F_2 generally would yield considerably less information and a backcross to the dominant homozygous parent would yield no information since the marker gene segregation would not be detected.

A second problem with classical markers is the possibility of confounding effects of the marker genes with effects of the quantitative genetic factors being investigated. In some cases this problem has been overcome, albeit at the expense of considerable effort and time (Breese and Mather, 1957, 1960; Jayakar et al., 1977).

To make this technique of analyzing quantitative genetic variation with marker genes more powerful and easily applicable, it would be desirable to have a large number of marker genes distributed throughout the genome with alleles interacting in a codominant fashion. The alleles of these marker genes should produce little or no effect on the morphology of the organism in question, and each marker should be unequivocally identifiable even if a large number are segregating in the same cross. Enzyme marker genes fit these qualifications and, as indicated by our experience in *Lycopersicon*, are ideal candidates for such study.

In applying a test for using enzyme markers for quantitative genetic analysis, we crossed individuals from two morphologically distinct species of tomato which were homozygous for allelic differences at 12 enzymic loci. By monitoring, in a backcross population, the segregation of these loci—most of which have been mapped (see Tanksley and Rick, 1980)—we have been able to map a number of the genetic factors controlling several quantitatively inherited characters. Although our experiments were limited to the first backcross, where recessive factors and some of the epistatic variation would not be expressed, the approach to be outlined could well be applied in analyzing the range of genetic variation found in an F_2 population. The potential of enzyme markers to accelerate selection in this backcross is treated elsewhere (Tanksley et al., 1981).

2. MATERIALS AND METHODS

Hybrids were made between *Lycopersicon esculentum* cv. VF36 (LA 490) and the related wild species, *Solanum pennellii* (LA 716 from Atico,

Peru). Both are highly inbred and homozygous for all tested loci. VF36 was used as the pistillate parent since unilateral compatibility precludes the reciprocal cross (Rick, 1960). Despite their allocation to different genera these two species are easy to hybridize, the F_1's experiencing nearly normal chromosome pairing and sufficiently high fertility to permit controlled introgression (Khush and Rick, 1963). Backcross seeds were obtained by using F_1 pollen in crosses to VF36.

(i) *Electrophoretic enzyme assays*

Five hundred BC_1 seeds sown in wooden flats in the greenhouse produced approximately 400 viable seedlings. Six weeks later when they were approximately 15 cm tall, these plants were transplanted into a 1 : 1 mixture of sand and soil supplemented with fertilizer—a treatment that stimulates new root growth and thus high levels of enzyme activity. One week later the plants were assayed via starch gel electrophoresis to determine their individual genotypes with respect to the following segregating enzyme loci: *Aps*-1, *Aps*-2, *Got*-2, *Prx*-1, *Prx*-2, *Prx*-4, *Prx*-7, *Est*-4, *Est*-7, *Pgm*-2, *Pgi*-1, and *Skdh*-1. Subsequently, these plants along with several replicates of the two parents and F_1 hybrid were transplanted in the field for measurement of quantitative traits. The mapped enzyme loci scored mark eight of the 12 chromosomes and two unmapped genes-*Est*-4 and *Pgi*-1—are known to be independent of the ten other enzymic genes—hence must mark significant additional parts of the genome, if not additional chromosomes. Methods of electrophoresis, enzyme extraction and map positions of the enzyme loci can be found in Tanksley and Rick (1980) and Tanksley (1981).

(ii) *Measurement of metric characters*

Measurements were taken on all individual plants of parents, F_1, and the backcross for the following quantitative traits: leaf ratio, stigma exsertion, fruit weight, and seed weight. Leaf ratio (width divided by length) differs between the orbicular segments of *S. pennellii* and the lanceolate ones of VF36. Stigma exsertion, measured from the mouth of the anther tube to the top of the stigma, varies from the exserted condition in *S. pennellii* to insertion in VF36. Means for these two characters were calculated from three independent measurements on each plant.

All plants were hand self-pollinated to assure adequate fruit set. Fruit weight was determined from a mean of six ripe fruits per plant, the minimum being two. A random sample of ten seeds from each plant was weighed to estimate the mean.

3. RESULTS AND DISCUSSION

(i) *Monogenic segregations*

The segregation at each locus was tested against the expected 1 : 1 ratio. In the total population, four of the twelve enzyme markers yielded ratios that deviated significantly from expectation (table 2). Segregations of *Aps*-2 (chromosome 8), *Skdh*-1 (chromosome 1), and *Prx*-7 (chromosome 3) showed a significant excess of *L. esculentum* homozygotes, whereas a significant excess of heterozygotes was observed for *Prx*-4 (chromosome

TABLE 1

Means, standard deviations, and ranges recorded for two parents, F_1 hybrid, and backcross population. ($P_1 = VF36$, $P_2 = $ S. pennellii, $LR = $ leaf ratio, St. Ex. = stigma exsertion, FW = fruit weight, SW = seed weight)

| | P_1 | | P_2 | | F_1 | | BC_1 | | BC_1 Range | |
	$\bar{X}$	s_x	$\bar{X}$	s_x	$\bar{X}$	s_x	$\bar{X}$	s_x	Max	Min
LR	1·90	0·23	0·96	0·05	1·39	0·14	1·55	0·16	2·07	1·18
St. Ex.	−1·78	0·58	1·61	0·33	1·77	0·51	0·14	1·29	3·67	−3·89
FW	205·10	25·60	2·73	0·63	8·95	2·51	30·64	18·39	99·90	5·60
SW	29·90	1·65	5·40	0·53	10·90	0·62	21·84	5·22	35·10	11·00

TABLE 2

Test for 1:1 segregation of enzymic marker genes in two subpopulations classified according to vigour, and test of heterogeneity for segregation ratios between these subpopulations. (E/E = homozygous for L. esculentum allele, E/P = heterozygous for S. pennellii allele)

| | More vigorous | | | | Weaker | | | | | |
	E/E	E/P	T	χ^2 (1:1)	E/E	E/P	T	χ^2 (1:1)	χ^2_T (1:1)	χ^2 (heterogeneity)
Aps-1	176	143	319	NS	68	63	131	NS	NS	NS
Aps-2	215	111	326	32·54‡	61	73	134	NS	18·00*	15·67‡
Skdh-1	126	97	223	NS	74	43	117	7·69†	10·24*	NS
Got-2	158	163	321	NS	75	59	134	NS	NS	NS
Prx-1	174	151	325	NS	70	64	134	NS	NS	NS
Prx-2	188	138	326	7·37†	61	73	134	NS	NS	5·16*
Prx-4	112	213	325	30·77‡	66	68	134	NS	22·67‡	8·13†
Prx-7	177	148	325	NS	82	52	134	6·28*	7·33*	NS
Est-4	163	159	322	NS	68	63	131	NS	NS	NS
Est-7	183	137	320	6·33*	62	72	134	NS	NS	4·10*
Pgi-1	154	162	316	NS	60	74	134	NS	NS	NS
Pgm-2	164	150	314	NS	59	75	134	NS	NS	NS

* Significant deviation at the 5 per cent level.
† Significant deviation at the 1 per cent level.
‡ Significant deviation at the 0·1 per cent level.

10). Our results are thus in general agreement with those of Rick (1969, 1972) for morphological markers on the three chromosomes, 3, 8, and 10. The latter investigations did not test chromosome 1.

In view of these distortions, each population was subdivided into stronger and weaker seedlings, the division being arbitrarily based on size of plant. The segregation data were compiled for each subgroup and Chi-square tests against $1:1$ and for heterogeneity between subgroups calculated (table 2).

Two of the loci with overall distorted segregations showed differential segregation between subpopulations. For *Aps*-2 the more vigorous subpopulation deviated $2:1$, *L. esculentum* homozygotes to heterozygotes, but segregation of the weaker group did not deviate significantly from $1:1$. For the *Prx*-4 locus, the distortion was of the opposite type: the more vigorous individuals segregated $2:1$ with an excess of heterozygotes, whereas the weaker group segregated approximately $1:1$. In the segregations of *Est*-7 and *Prx*-2, an interesting reversal occurred in the direction of skewing between subpopulations. The more vigorous deviated significantly toward an excess of the recurrent parent alleles, whereas the weaker showed a slight, non-significant excess of heterozygotes. Curiously, the massed data of these two subpopulations did not deviate significantly from $1:1$. Presumably the deviations of the weaker lot negated the significant distortions in the more vigorous.

Overall, seven significant deviations were encountered in the subpopulations and of these, six favoured the recurrent parent allele. In this respect the results are in general agreement with other tests of segregations in interspecific hybrids (Stephens, 1949; Rick, 1963, 1969, 1972). The fact that skewing at single loci differed significantly between the more vigorous and the weaker subpopulations for four of the twelve loci studied indicates that strong selection is acting at the seedling stage and can account for much of the overall skewness. Part of the single locus skewing may also be due to pollen competition during fertilization. The occurrence of this latter mechanism has been well documented (Zamir *et al.*, 1981).

(ii) *Segregation of quantitative traits*

Information concerning the behaviour of the four quantitative traits in parents, F_1, and backcross generations is summarized in table 1. Typical of quantitative characters in general, all exhibit continuous variation in the backcross. The genetic component of variation (broad sense heritability in comparisons between F_1 and BC) is relatively high for all except leaf ratio.

Leaf segments of *S. pennellii* are nearly circular in outline, having a mean leaf ratio (LR) of 0·96, whereas those of VF36 are lanceolate and have a mean LR of 1·90. Means of the F_1 and BC fall between the parental limits but demonstrate some degree of dominance of the wild parent.

In *S. pennellii* and the F_1, stigma exsertion is positive and nearly equal, revealing a high level of dominance of this parent, whereas the stigma is deeply inserted in the VF36 parent. The same degree of dominance is not revealed in BC, the mean value falling very close to the midpoint between P_1 and F_1. Segregation in BC is highly transgressive, greatly exceeding the limits of P_1 and F_1.

Fruit of VF36 are 76 times heavier than those of *S. pennellii*. The mean fruit weights of the F_1 and BC closely approximate the geometric means of the parental fruit weights in keeping with the observations in previous studies of the genetics of this character in tomatoes (MacArthur and Butler, 1938; Powers, 1942). The BC segregation is thus highly skewed toward the low parent. Dominance of small size is evident in the F_1 seed weight, but the BC mean very closely approximates the midpoint between P_1 and F_1.

(iii) *Detection of linkage between enzyme loci and quantitative-trait loci*

Following the convention of Geldermann (1975) we shall refer to the segregating loci affecting the quantitative traits under study as quantitative trait loci (QTL). In order to detect linkage of enzyme loci with QTL controlling genetic variation of the traits—leaf ratio, stigma exsertion, fruit weight, and seed weight—the backcross data were divided into two groups with respect to each enzyme locus; one, all heterozygotes, the other all homozygotes. Differences between the means were calculated and two-tailed significance levels computed for these differences based on the normal distribution:

$$z_{\bar{d}} = \frac{\bar{x}_1 - \bar{x}_2}{s_{\bar{d}}}$$

(table 3). A significant difference in the means between homozygotes and heterozygotes was interpreted as linkage of QTL to the enzyme locus.

(iv) *Estimating the minimum number of QTL*

The minimum number of QTL controlling a quantitative character was taken to be the total number of independent enzyme loci demonstrating a significant effect on that character. Since some of the enzyme loci are linked (*Skdh*-1 and *Prx*-1, *Prx*-2 and *Est*-7, *Est*-4 and *Pgi*-1), only one of the linked pair was counted if both showed significance. This conservative approach seemed appropriate since the same QTL could be responsible for significant effects associated with both linked enzyme loci.

No attempt was made to estimate the magnitude of the effect a QTL must have on a character in order to be detected, as this depends on a number of factors including heritability, degree of additivity, size of the backcross population sampled, and proximity to an enzyme marker.

(v) *Test for single vs. multiple QTL*

As already pointed out, more than one QTL can be linked to a given enzyme marker, the combined contributions causing the observed effect attributed to a marker. In certain instances there is a method of directly testing for single vs. multiple QTL associated with a given marker. This is restricted to QTL situated close to linked pairs of enzyme loci—in this case, *Skdh*-1–*Prx*-1, *Prx*-2–*Est*-7, and *Pgi*-1–*Est*-4. The logic behind the test is as follows: If a detected "effect" showing association with a given marker is caused by multiple QTL, equally distributed with similar effects, then the genetic cause of the "effect" cannot, with the type of data generated

TABLE 3

Test for difference between homozygotes and heterozygotes for each enzyme locus with respect to the quantitative characters: stigma exsertion, leaf ratio, fruit weight, and seed weight. (E/E = homozygous for L. esculentum allele, E/P = heterozygous for S. pennellii allele)

	Chromosome location	Stigma exsertion			Leaf ratio			Fruit weight (g/fruit)			Seed weight (mg/10 seeds)		
		E/E	E/P	Δ	E/E	E/P	Δ	E/E	E/P	Δ	E/E	E/P	Δ
Skdh-1	1	4·78/99*	5·66/81*	−0·88§	1·61/160	1·47/122	0·14§	32·60/96	26·50/69	5·10	24·38/93	19·12/68	5·26§
Prx-1	1	4·96/123	5·34/113	−0·38†	1·60/197	1·50/176	0·10§	32·90/130	28·18/115	4·72†	23·11/126	20·48/114	2·63§
Prx-2	2	4·74/128	5·61/110	−0·87§	1·56/206	1·54/169	0·02	37·48/132	22·72/114	14·76§	22·81/129	20·71/112	2·10‡
Est-7	2	4·71/124	5·65/109	−0·94§	1·56/205	1·55/169	0·01	35·51/127	25·70/115	9·81§	22·92/124	20·71/113	2·21§
Prx-7	3	5·31/128	4·95/110	0·36†	1·56/200	1·54/169	0·02	32·70/134	28·16/112	4·54†	21·77/132	21·91/109	−0·14
Pgm-2	4	5·17/115	5·16/115	0·01	1·59/185	1·51/179	0·08§	34·13/118	26·69/120	7·44‡	22·89/115	20·97/118	1·92‡
Aps-1	6	5·30/123	4·97/111	0·33†	1·56/200	1·54/166	0·02	30·99/129	30·47/110	0·52	21·60/124	22·25/110	−0·65
Got-2	7	4·76/113	5·50/123	−0·74§	1·59/186	1·51/185	0·08§	32·65/128	28·39/115	4·26	23·06/123	20·59/115	2·47§
Aps-2	8	5·02/144	5·33/93	−0·31	1·57/228	1·51/145	0·06§	33·20/144	27·17/100	6·03‡	22·76/142	20·60/97	2·16§
Prx-4	10	5·15/90	5·14/147	0·01	1·58/137	1·53/236	0·05‡	34·00/82	29·01/163	4·99	22·53/79	21·54/161	0·99
Est-4		5·29/125	4·97/107	0·32	1·53/184	1·57/184	−0·04†	34·23/132	29·23/108	3·00	21·24/130	22·50/105	−1·26
Pgi-1		5·31/116	5·03/114	0·28	1·54/169	1·56/196	−0·02	31·49/120	29·38/119	2·11	21·41/118	22·42/116	−1·01
minimum number of QTL (significant at 5 per cent level)		5			6			5			5		

* Number of plants in each class.
† Significant at the 5 per cent level.
‡ Significant at the 1 per cent level.
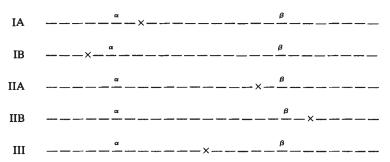§ Significant at the 0·1 per cent level.

here, be mapped to a specific point on the chromosome. If, on the other hand, a single QTL or a tightly linked cluster of QTL causes the observed effect, then it should in theory be possible to associate the effect more strongly with one marker than the other. In some cases it might be possible to map the effect to a precise locus on the chromosome relative to the two linked markers.

TABLE 4

Models and submodels giving all possible gene locations (α and β = isozymic loci, $\times$ = quantitative gene)

Model I	quantitative gene closer to α than β
Model II	quantitative gene closer to β than α
Model III	quantitative gene equidistant from α and β (between α and β)
Submodel A	quantitative gene between α and β
Submodel B	quantitative gene not between α and β

Combinations of models and submodels

All of the possible positions of a single QTL with respect to two linked markers are given in table 4. The possibilities are broken down into two considerations: (a) Is the QTL closer to one marker than the other (Models I–III)? (b) Is it between or to one side of the two linked markers (Submodels A, B)? If the backcross data are resolved into four classes representing all possible combinations of linked marker genotypes, the differences in the means between these classes can be used to answer these questions. Table

TABLE 5

Predicted differences between means of two linked loci combinations based on models I–III and submodels A, B (+ = positive value; − = negative value; 0 = no difference; NP = no prediction; A = E/E E/E, B = E/E E/P, C = E/P E/E, D = E/P E/P)

	Model						Submodel						
	I		II		III		A				B			
							I		II		I		II	
	Δ*	Δ†	Δ*	Δ†	Δ*	Δ†	Δ*	Δ†	Δ*	Δ†	Δ*	Δ†	Δ*	Δ†
B–C	+	−	−	+	0		+	−	−	+	+	−	−	+
A–B	NP	NP	+	−	0		+	−	+	−	0	0	+	−
C–D	NP	NP	+	−	0		+	−	+	−	0	0	+	−
A–C	+	−	NP	NP	0		+	−	−	+	+	−	0	0
B–D	+	−	NP	NP	0		+	−	−	+	+	−	0	0

* Recurrent parent has larger value for quantitative trait compared to F_1 (leaf ratio, fruit weight, seed weight).

† Recurrent parent has small value for quantitative trait compared to F_1 (stigma exsertion).

5 lists the predicted direction (+ or −) that these differences should take for different locations of a single QTL (different combinations of Models I–III and submodels A, B).

A breakdown of the data for linked enzyme markers showing significant effects on different quantitative characters is given in table 6. The statistical significance of the differences was tested using the normal distribution:

$$Z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{\text{MSE}}{n_1} + \dfrac{\text{MSE}}{n_2}}}.$$

The MSE (mean square error) was obtained from a series of 2-way analyses of variance using the linked loci as treatments. By matching the significant differences between the means with the predictions of the models in table 5, those "effects" that behave like single QTL can be mapped. Those cases for which no significant difference in the means was observed are candidates for multiple, recombining QTL.

(vi) *Detected QTL*

At least five QTL were detected for each of the traits studied, with the average being 5·2 (table 3). Six of the detected QTL were associated with linked pairs of enzyme markers, thereby permitting application of the test outlined above of single vs. multiple QTL. Three detected QTL fit models I or II from table 5, indicating that they are positioned closer to one marker than the other and suggesting that they might be single QTL. None of the detected QTL fits perfectly any submodel which would allow positioning between or to one side of the markers. However, one of the QTL for leaf ratio (*Skdh*-1–*Prx*-1) did fit the between-locus case (Model I, submodel A) for all but one of the predicted differences, indicating that it might reside between these two enzyme marker loci which themselves are 17 cm apart.

(vii) *Plus and minus effects of QTL*

By comparing values of the two parents and the F_1 hybrid one might predict whether the QTL contributed by each parent will have a positive or negative effect. For example, VF36 has fruit approximately 100 times heavier than *S. pennellii*. Thus the QTL contributed by VF36 should cause an increase in fruit weight. These predictions were met by all the QTL detected for fruit weight and seed weight. However, for the other two characters (stigma exsertion and leaf ratio), some QTL were detected which produced effects opposite to these predicted by the parental values. Thus one of the leaf ratio QTL contributed by *S. pennellii* caused a slight but significant increase in this character in BC_1 plants even though *S. pennellii* has a smaller leaf ratio value (table 3). For stigma exsertion the results were even more striking. Two of the five QTL detected, caused effects in the direction opposite to those predicted by the parents (table 3). Based on these observations, leaf ratio and stigma exsertion might be characters for which transgressive variation could be recovered in segregating generations. By combining a large number of positive or negative QTL contributed by both parents, some backcross individuals might exceed the

TABLE 6

Multiple comparisons of means for quantitative characters from genotypic combinations of linked enzyme loci
(E/E = homozygous for L. esculentum allele, E/P = heterozygous for S. pennellii allele)

Leaf Ratio

Genotypes α	β	$\bar{X}$	n	MSE	Genotypes	Δ	$s_{\bar{x}_1 - \bar{x}_2}$	Direction
Skdh-1	Prx-1							
A E/E	E/E	1·6074	124	0·019	B–C	0·0862	0·0368†	+
B E/E	E/P	1·6153	36		A–B	−0·0079	0·0261	
C E/P	E/E	1·5291	23		C–D	0·0749	0·0319†	+
D E/P	E/P	1·4542	98		A–C	0·0783	0·0313†	+
					B–D	0·1611	0·0269‡	+
Est-4	Pgi-1							
A E/E	E/E	1·5366	161	0·028	B–C	−0·0766	0·0711	
B E/E	E/P	1·5072	18		A–B	0·0294	0·0416	
C E/P	E/E	1·5838	8		C–D	0·0146	0·0605	
D E/P	E/P	1·5692	171		A–C	−0·0472	0·0606	
					B–D	−0·0620	0·0415	

Stigma Exsertion (mm)

Genotypes α	β	$\bar{X}$	n	MSE	Genotypes	Δ	$s_{\bar{x}_1 - \bar{x}_2}$	Direction
Skdh-1	Prx-1							
A E/E	E/E	4·7993	76	1·771	B–C	−1·1185	0·4511*	−
B E/E	E/P	4·7022	23		A–B	0·0971	0·3167	
C E/P	E/E	5·8207	14		C–D	0·1927	0·3916	
D E/P	E/P	5·6280	66		A–C	−1·0214	0·3870*	−
					B–D	−0·9258	0·3222*	−
Prx-2	Est-7							
A E/E	E/E	4·6640	117	1·495	B–C	0·3338	0·6162	
B E/E	E/P	5·6667	9		A–B	−1·0027	−0·4230*	−
C E/P	E/E	5·3329	7		C–D	−0·3173	0·4780	
D E/P	E/P	5·6502	100		A–C	−0·6689	0·4758	
					B–D	0·0165	0·4255	

Seed Weight (mg/10 Seeds)

Genotypes		$\bar{X}$	n	MSE		Δ		$s_{\bar{x}_1 - \bar{x}_2}$	Direction
α Skdh-1	β Prx-1								
A E/E	E/E	24.2959	74	20.956	B–C	6.4909	1.7344†	+
B E/E	E/P	24.7000	19		A–B	-0.4041	1.1773	
C E/P	E/E	18.2091	11		C–D	-1.0891	1.5076	
D E/P	E/P	19.2982	57		A–C	6.0868	1.4793‡	+
					B–D	5.4018	1.2127‡	+
Prx-2	Est-7							
A E/E	E/E	22.9538	117	24.940	B–C	-0.5076	2.4146	
B E/E	E/P	21.8638	11		A–B	1.0900	1.5749	
C E/P	E/E	22.3714	7		C–D	1.7861	1.9512	
D E/P	E/P	20.5853	102		A–C	0.5824	1.9432	
					B–D	1.2785	1.5849	

Fruit Weight (gm/ft)

Genotypes		$\bar{X}$	n	MSE		Δ		$s_{\bar{x}_1 - \bar{x}_2}$	Direction
α Skdh-1	β Prx-1								
A E/E	E/E	33.7013	76	300.568	B–C	-1.6150	6.3305	
B E/E	E/P	28.3100	20		A–B	5.3913	4.3570	
C E/P	E/E	29.9250	12		C–D	2.8969	5.5064	
D E/P	E/P	27.0281	57		A–C	3.7763	5.3854	
					B–D	1.2819	4.5057	
Prx-2	Est-7							
A E/E	E/E	36.2025	120	296.338	B–C	29.448	8.3231‡	+
B E/E	E/P	53.0909	11		A–B	16.8884	5.4230‡	–
C E/P	E/E	23.6429	7		C–D	0.8371	6.7219	
D E/P	E/P	22.8058	104		A–C	12.5596	6.6935*	+
					B–D	30.2851	5.4580‡	+

* Significant at the 5 per cent level.
† Significant at the 1 per cent level.
‡ Significant at the 0.1 per cent level.

parental extremes. This was clearly the case for stigma exsertion where some individuals had stigmas exserted or inserted to a degree greatly exceeding either parent (table 1).

(viii) *Test for epistatic interaction between QTL*

Interacting effects of detected QTL on the four metric characters were tested by 2-way analysis of variance in which pairs of marker loci served as treatments. Significant interaction terms from AOV were interpreted as indications of epistatic interaction between QTL and were observed for all four traits. Amongst the total of 274 possible comparisons for two-locus interactions, 18 showed significant interactions, 13 at a 5 per cent probability level, five at the 1 per cent level (table 7). In such a large number of traits, obviously not all comparisons with associated low probabilities can indicate real differences. Yet, in terms of the 1 per cent group alone, the total amount of interaction between loci is clearly more than expected by random deviation alone. Furthermore, of the seven interactions affecting seed weight, four are with QTL linked with *Pgm*-2, the remainder, with *Aps*-1. This delimitation, significant at $p = (21/66)^6 = 0.00104$, suggests that the activity of seed weight QTL linked with *Aps*-1 and *Pgm*-2 is contingent upon the presence or absence of other segregating QTL. In another situation, an unexpected type of interaction occurred for the effect

TABLE 7

Means for quantitative characters based on breakdown of genotypes for loci showing epistatic interaction. Pairs of numbers indicate genotypes (1 = homozygous for L. esculentum *allele; 2 = heterozygous for* S. pennellii *allele)*

Leaf ratio	1, 1	1, 2	2, 1	2, 2
Aps-1, *Aps*-2*‡	1·572	1·536	1·578	1·485
Aps-1, *Prx*-2‡	1·533	1·533	1·536	1·554
Aps-1, *Est*-7†	1·574	1·542	1·534	1·555
Skdh-1*, *Prx*-1*†	1·607	1·615	1·529	1·454
Skdh-1*, *Pgm*-2*†	1·660	1·556	1·494	1·438
Stigma exsertion (mm)				
Got-2*, *Pgm*-2†	4·723	4·922	5·717	5·346
Prx-2*, *Prx*-4†	4·911	4·623	5·455	5·707
Est-4, *Pgi*-1†	5·285	5·333	5·695	4·985
Fruit weight (g/fruit)				
Aps-2*, *Skdh*-1†	32·814	30·670	32·106	23·903
Aps-2*, *Pgi*-1†	33·273	32·581	29·304	24·565
Prx-2*, *Est*-7*†	36·203	53·091	23·643	22·806
Seed weight (mg/10 seeds)				
Aps-1, *Aps*-2*‡	21·947	21·106	23·797	19·950
Aps-1, *Prx*-2*‡	23·292	20·016	22·505	21·907
Aps-1, *Est*-7*‡	23·513	19·981	22·558	21·772
Pgm-2*, *Aps*-2*†	24·250	21·080	21·409	20·348
Pgm-2*, *Skdh*-1*†	25·656	19·534	23·137	19·009
Pgm-2*, *Prx*-1*†	24·793	21·280	21·934	19·714
Pgm-2*, *Est*-7†	24·650	23·141	21·662	20·340

* Single locus effect significant at 5 per cent level.
† Interaction significant at 5 per cent level.
‡ Interaction significant at 1 per cent level.

of the two linked markers *Prx*-2 and *Est*-7 with respect to fruit weight. The data in table 6 suggest that QTL linked to these two markers has a positive effect on fruit weight and that this QTL maps closer to *Prx*-2 than to *Est*-7. The breakdown of the two-locus groups in table 7 indicates a much larger effect in the presence of the *Est*-7 *S. pennellii* allele. In other words, the recombinant chromosome produces the greatest boost in fruit weight. Otherwise no clear patterns are discernible in the data.

The dilemma of determining which of the interactions with $P \leqq 0.05$ are real cannot be resolved with our data. At any rate, the highly significant interactions and the aforementioned indications of nonrandomness reveal the occurrence of some epistatic interaction.

Twelve of the 18 interactions included one or both markers which singly did not significantly affect the characters in question; all five of the highly significant interactions belong in this group. The relationship suggests that a number of the loci affecting the quantitative traits were not detected by single-locus analysis. It seems likely that these undetected QTL are highly epistatic, possibly producing both positive and negative effects on the character, depending on the allelic composition at other loci throughout the genome. If such were the case, they would probably not be detected by the test for differences between homozygous and heterozygous marker loci since they produce no consistent effect. The procedures for detecting QTL are therefore undoubtedly biased toward loci with a higher degree of additivity.

(ix) *Advantages of enzyme markers for investigating quantitative traits*

Many of the advantages of enzyme markers over morphological markers for genetic studies have been discussed previously (Brown and Allard, 1969; Nielsen and Scandalios, 1974; Tanksley and Rick, 1980). In contrast to the usual recessive/dominance interaction of morphological markers, most enzyme alleles interact in a codominant manner, in which F_2 segregations and backcrosses to either parent can be studied with comparable efficiency. Segregation of recessive morphological markers, on the other hand, can be detected in the backcross to only one parent, and in the F_2, far less information is yielded by a 3:1 than by the 1:2:1 ratios typical of allozymes.

Even if a large number of enzyme loci segregate simultaneously in the same progeny, the genotype for each locus can be determined unequivocally in all individuals. It is therefore theoretically possible to use several enzyme markers on every chromosome. If a backcross or F_2 were segregating for such an array of markers, a very clear picture of the genetic architecture of the investigated quantitative trait would be likely to emerge. Theoretically all of the QTL of major consequence affecting a trait could be mapped and characterized with data from a single segregating progeny. Since the contribution of the entire genome is observed simultaneously, tests for epistatic interactions between QTL would be straightforward. The results presented here as well as those of many other researchers (*e.g.*, Spickett and Thoday, 1966; Fasoulas and Allard, 1962) reveal that interactions between discrete QTL are common. In contrast, it is obviously difficult to establish the extent and nature of such interactions if only a few marker genes are segregating simultaneously—the usual situation with

morphological markers. Likewise, many epistatic interactions would not be detected by chromosome substitution techniques, in which the action of only a small portion of the genome is studied at a time.

For wide crosses, as in the present example, in which the desired enzyme variation is already present, no new markers need to be introduced. Thus, the time required in terms of number of generations and crosses is drastically reduced and the difficulties accompanying the introduction of extraneous genetic variation is avoided. Nevertheless, for the procedure to be more generally applicable, it might be possible to introduce into a single stock a series of rare enzyme alleles. This stock, which would differ at all relevant enzyme marker loci with respect to most other conspecific lines, could be used to test for any QTL-controlled trait. If enough allele differences were obtained, it should be possible in a single cross to test 90–100 per cent of the genome for QTL. In tomato, 20 enzymic genes have been mapped and another 10 should be located in the near future, so that a more complete coverage can be expected. For virtually all of these loci, rare alleles are available for incorporation into a single stock.

Up to the present time, the mapping of QTL has been mainly limited to *Drosophila* for which the generation time is relatively short and a good assortment of genetic markers is available. By decreasing the number of crosses and generations required to map the QTL of various quantitative traits, the use of enzyme markers in quantitative genetic studies might be extended to other organisms.

Finally, in breeding programmes in both animals and plants, the identification and mapping of major genes controlling important quantitative traits offer certain possibilities. Such genes found to be closely linked with one enzyme marker (better if situated between two enzyme markers) could be transferred with great efficiency and certainty. The codominant enzyme alleles could be selected in early development stages, deferring direct selection for the quantitative character in question until advanced generations. It might be especially useful for probing exotic germplasm for important genes and transferring them into desirable backgrounds. Some aspects of this method of quantitative gene transfer have been considered by Soller and Plotkin-Hazen (1977).

Tomato is an ideal model system for quantitative genetic studies using enzyme markers. In addition to the advanced status of enzyme genetic studies in this plant, there are a number of related, crossable species which possess characteristics of economic importance including drought tolerance, salt tolerance and low temperature tolerance (Rick, 1973, 1975, 1978; Vallejos, 1979; Epstein *et al.*, 1980). Presently there is great interest in transferring these traits into the cultivated tomato. Enzyme techniques as described in this paper might be used to study their inheritance and at the same time to facilitate their transfer into useful breeding lines.

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