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Genetics of Esterases in Species of *Lycopersicon*

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Summary. Improvements in plant culture and electrophoretic technique permit detection and genetic analysis of seven esterase loci in *Lycopersicon esculentum* and related species with homosequential chromosomes. At all of these loci except one, each allele codes for a single anodal band, and the electrophoretic variants are inherited in monogenic fashion. For the exceptional *Est-4*, allozymes are 1-3 banded in various combinations at four positions, and rare recombinants in one cross appeared at a frequency of 0.0005, suggesting the existence of several very tightly linked genes. *Est-2* segregated solely for intensity differences in dominant/recessive fashion; *Est-3* and *Est-4* behave as monomers; the remaining *Est-1, 5, 6*, and *7* – coding for contiguous bands in the region closest to the origin – are dimeric. The latter group are tightly linked inter se in the proximal portion of 2L (long arm of chromosome 2), the total map distance of the complex being approximately 1.5 cM; *Est-2* is situated on 9L between *ah* and *marm*; *Est-3* on 1L between *inv* and *dgt*; *Est-4* has not yet been located. Even in the interspecific hybrids, map distances are similar to the standard values for *L. esculentum*. Tandem duplication is hypothesized for the origin of the *Est-1, 5-7* complex, which adds another example to the growing list of linked mimic genes in the tomato genome. In respect to the position of their bands and tight inter se linkage, this series exactly parallels the EA, EB, EC esterase series in *Hordeum vulgare* – a fact which suggests great antiquity for this block of genes.

Key words: *Lycopersicon* – Gel electrophoresis – Esterases – Linkage – Angiosperm evolution

Introduction

Allozyme polymorphisms have proved to be highly useful for measuring the extent of variation within and between the tomato species. They have, in fact, provided virtually the only definitive data for assessing the nature of genetic variation in the wild species *Lycopersicon cheesmanii* (Rick and Fobes 1975a), *L. chmielewskii-parviflorum* complex (Rick et al. 1976), *L. pimpinellifolium* (Rick et al. 1977), and *L. hirsutum* (Rick et al. in press). Additionally, they have yielded clues to the evolution of the cultivated tomato *L. esculentum* (Rick and Fobes 1975b) and have greatly assisted in the formulation of systems for maintenance of accessions (Rick 1976). These studies were based on surveys of four enzyme systems – acid phosphatase, esterase, glutamate oxaloacetate transaminase, and perioxidase – embracing genes at 13 loci scattered throughout the genome. In such surveys the larger the number of loci assayed, the more strength and clarity lent to analysis and conclusions. Further, efficiency of the surveys increases in direct proportion to the number of loci analyzed in a given enzyme system. For these reasons we undertook the present study of the genetics and polymorphism of tomato esterases.

In the course of our investigations we have improved techniques to reveal a fuller scope of the phenotypic variation of the esterase complex. These improvements were formulated in the course of our recent survey of *L. hirsutum* (Rick et al. in press). Previously activity had been detected at only a single locus, *Est-1*, the bands of which were resolved by horizontal starch-gel electrophoresis and situated in the mid-anodal region. Although banding had been seen occasionally in other regions, it was never sufficiently consistent to permit accurate scoring. The modifications of technique that permit reading the other esterase phenotypes consist of changes in management of the plant material and in the staining procedure, details of which are presented in the next section.

After resolving the problems of technique, we attempted to delineate phenotypically the esterase loci by means of genetic segregation. The genetic variation essential at certain loci for this objective was found in *L. esculentum*

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and *L. pimpinellifolium*, which are so closely related that they behave genetically as if conspecific. For allozymes at other loci we had to extend the study to *L. parviflorum* and *Solanum pennellii*, which are congruous with the aforementioned species, and their hybrids have essentially normal meiosis. For several reasons the study was extended to determine linkage relations of the esterase genes. First, with appropriate crosses, data can be obtained simultaneously for both the pertinent monogenic segregations and for linkage tests. Second, linkage relations may elucidate phylogenetic relationships of genes coding for enzymes of similar catalytic properties. Further, the newly located genes themselves may serve as useful markers for future research. As the results reveal, the additional effort required for mapping is relatively modest when compared with the gains achieved.

Materials and Methods

Stocks

Information concerning the sources of various alleles studied in these experiments is provided in Table 1. In addition to these items, we utilized the standard series of tomato linkage testers and the set of primary trisomics in cv. VF36.

Plant Culture

Seeds were sown directly into nursery flats, and after approximately six weeks the seedlings were transplanted into new flats in a mixture of 1 part sand to 1 part soil. Such transplanting promotes new, rapid root growth, found to be necessary for expression of *Est-4*. After 5-10 days, plants were removed from the new mix and the roots washed in preparation for enzyme extraction in the laboratory.

Electrophoresis

The starch-gel electrophoresis equipment has already been described (Tanksley 1979) as well as the gel and reservoir buffer systems (Rick et al. 1974). Crude extracts were obtained by crushing the appropriate tissue with plexiglas rods and absorbing the liquid into paper wicks. The esterases were assayed from root tissue except *Est-3*, which was found in stems, leaves, and pollen. Wicks saturated with extract were placed at the origin of the starch gel for 20 minutes at 150 V, then removed, and the run was continued at 300-350 V for 4-5 hours.

The recipe for the esterase activity stain was developed during our recent survey of *L. hirsutum* (Rick et al. in press); the details are presented in that report. Ingredients for the stain are 5 ml 1% α-naphthyl butyrate (in 100% acetone), 100 mg Fast Blue RR Salt, 100 ml 0.1 M Na phosphate buffer pH 6.25.

Analysis of Linkage Data

The initial data for linkage of the esterase loci were analyzed on a Burroughs 6700 computer utilizing a contingency test program from SPSS (Statistical Package for the Social Sciences). We were able to analyze the linkage relationships among loci for BC's and F_2's segregating for up to 30 genes (isozymic and morphological) and in this fashion could test the possibility of linkage of the unmapped esterase loci with numerous loci already placed on the linkage map. The advantage of doing 20-30 point linkage tests

Table 1. Source and esterase genotypes of *Lycopersicon* accessions

Genotype	Accession number	Species	Site of collection	Province/ Department	Country
Est-1^1, *Est-4*^{3}	LA1338	*L. esculentum* var. *cerasiforme*	Puyo	Napo	Ecuador
Est-2^1, *Est-4*^{1} *Est-7*^{1}	247	*L. parviflorum*	Chavinillo	Huánuco	Perú
Est-2^1, *Est-4*^{1} *Est-7*^{1}	1319	*L. parviflorum*	Abancay	Apurimac	Perú
Est-1^5, *Est-2*^{1} *Est-4*^{1}, *Est-5*^{1} *Est-6*^{1}, *Est-7*^{2}	716	*S. pennellii*	Atico	Arequipa	Perú
Est-3^1	1602	*L. pimpinellifolium*	Punchauca	Lima	Perú
Est-4^2	1645	*L. pimpinellifolium*	Quebrada de Armendariz	Lima	Perú
Est-4^3	410	*L. esculentum*	Guayaquil	Guayas	Ecuador
Est-4^4	1521	*L. pimpinelliforium*	Asia-El Piñon	Lima	Perú
Est-4^5	1606	*L. pimpinellifolium*	Tambo de Mora	Ica	Perú
Est-4^6	1679	*L. pimpinellifolium*	Tambo de Mora	Ica	Perú
Est-4^7	1386	*L. esculentum* var. *cerasiforme*	Balsas	Amazonas	Perú
Normal alleles at all *Est* loci	490	*L. esculentum*	cv. VF36	bred at Univ. Cal.	
same	1108	*L. esculentum*	stock of *inv* – *dgt*	bred at Univ. Cal.	
same	1164	*L. esculentum*	stock of *ah* – *marm*	bred at Univ. Cal.	

versus the classical 2 or 3 point test is obvious. Map distances between esterase loci and other linked genes could be computed directly from backcross data, but for F_2 data estimates were obtained by applying maximum likelihood equations given by Allard (1956). The calculations were simplified by programing the equations into an HP-65 hand calculator.

Results

Phenotypes and Inheritance

The array of isozymes in the standard *esculentum* phenotype and the extent of variation encountered in the material studied here are illustrated in Figs. 1-11. These bands were assigned to their respective gene loci by studying the nature of segregation in crosses between the variant types. According to the simple Mendelian criterion, segregation into three phenotypic classes – the two parental homozygotes and their heterozygote – constitutes the maximum genetic variation expected for a single locus in a given cross; the corollary is that segregation into a larger number of phenotypic classes represents activity at more than one locus. Application of this criterion to our data reveals the existence of at least seven loci, designated by the symbols *Est-1* to *Est-7*.

The previous assignment of *Est-1* to the single gene detected in the early studies poses a dilemma in the assignment of additional symbols because the *Est-1* bands appear midway in the gel in respect to bands for the other loci in the complex. Throughout our studies we have attempted to assign numbers to the loci in order, starting at the anodal end and moving toward the origin; however, it was a foregone conclusion that, as techniques improved and additional material was studied, additional loci would be detected, and the system must accommodate symbols for the respective new genes. Since we have described considerable polymorphy for *Est-1* in several tomato species, it is important to preserve this notation for the intended locus. We therefore choose to follow the original system with the exception of retaining the *Est-1* symbol for its intended locus. The sequence thus starts with *Est-2* at the anodal end of the array, proceeding to *Est-7* in the most proximal position. In this fashion the bands for *Est-1* fall between those of *Est-4* and *Est-5* as indicated in Figures 1 and 2.

Est-2. A single band is situated in the borate front; to date we have not been able to effect a separation between them. The only variation that we have observed is segregation of a strong band, characteristic of *L. parviflorum* and *S. pennellii* vs. a weak band in *L. esculentum*. Doubts concerning the validity of this gene were dispelled by (1) the normal Mendelian ratios of 3 strong : 1 weak observed in F_2 progenies (Table 2) and (2) the linkage relations presented below. *Est-2* can be assayed reliably from root tissue and with moderate success from leaves, stems, and pollen. Intensive research on adjustment of the electrophoretic parameters might resolve the banding of this locus and separate it from the borate front. In its present status, this locus has limited usefulness.

Est-3. In the normal type a single band migrates to a position 4.5 mm behind that of *Est-2*. The gene expresses activity in actively growing leaves, stems, and pollen with relatively little activity detected in roots. Two variants have been encountered: one advanced 2 mm, the other retarded 1 mm behind the + band. Inheritance is of the typical monomeric type (having only parental bands, not hybrid heterodimeric bands, (Table 2, Fig. 3).

Est-4. The *Est-4* situation is particularly interesting be-

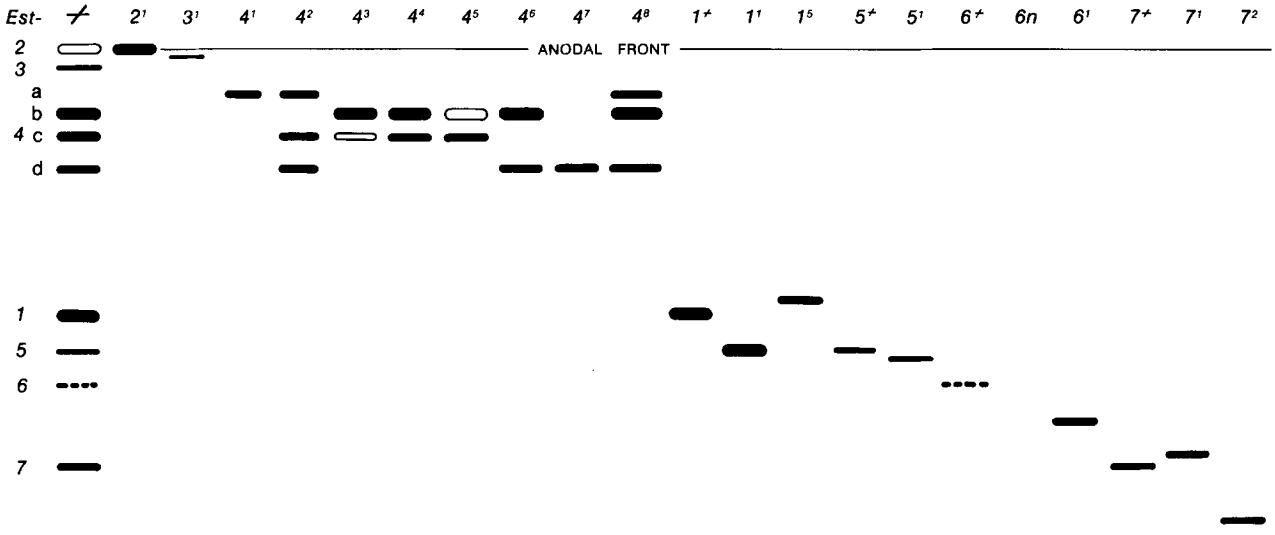


Fig. 1. Banding phenotypes of esterase alleles

cause it is more complex in several respects than the other esterase loci. In the material surveyed in this report, four different band positions can be recognized, which are designated a-d, proceeding from the anodal end toward the origin (Fig. 1). Although we have discovered single-banded stocks for each of these positions, the majority are multiple-banded in many different combinations of a-d. Certain single-banded lines may exhibit additional faint bands; for example, LA410 has a characteristically strong b band and a faint c band (Fig. 4); LA1606, strong c and faint b. The three-banded phenotype bcd is characteristic of nearly all of the cultivated and wild extra-Andean lines of *L. esculentum*. Typical of the other tested enzyme systems, *Est-4* has a great diversity of alleles in *L. pimpinellifolium*. A single band in the upper (a) position is fixed in all tested *L. parviflorum* accessions and in the LA716 collection of *S. pennellii* used in these studies. These species were therefore used as parents in several crosses because they are the unique sources of *Est-4*^{1}. The complete array of different phenotypes surveyed in this investigation is illustrated in Figure 1. Banding of *Est-4* like that of most of the genes in the esterase complex was detected only in root tissue; however, we found it absolutely necessary for the roots to be growing actively – an end that was achieved by the method of plant culture described above.

Table 2. Summary of esterase segregations omitting *Est-4*

Parent x		Parent y		Generation	Progeny			χ^2	P
Acc. No.	Allele	Acc. No.	Allele		x/x	x/y	y/y		
LA490	*Est-1*$^+$	LA716	*Est-1*^{5}	BC(y)		420	417	0.011	0.9-0.95
1338	*Est-1*^{1}	1319	*Est-1*$^+$	F_2	33	84	38	1.413	0.3-0.5
1164	*Est-2*$^+$	247	*Est-2*^{1}	BC(x)	54	64		0.847	0.3-0.5
1164	*Est-2*$^+$	247	*Est-2*^{1}	F_2	115	344		0.0065	0.9-0.95
1108	*Est-3*$^+$	1602	*Est-3*^{1}	F_2	106	248	123	1.968	0.3-0.5
490	*Est-5*$^+$	716	*Est-5*^{1}	BC(x)	248	240		0.131	0.7-0.8
490	*Est-5*$^+$	716	*Est-5*^{1}	BC(y)		423	414	0.097	0.7-0.8
490	*Est-6*$^+$	716	*Est-6*^{1}	BC(x)	250	238		0.295	0.5-0.7
490	*Est-7*$^+$	716	*Est-7*^{1}	BC(x)	251	237		0.402	0.5-0.7
490	*Est-7*$^+$	716	*Est-7*^{1}	BC(y)		418	419	0.0012	0.95-0.98
1338	*Est-7*$^+$	1319	*Est-7*^{1}	F_2	40	78	37	0.122	0.9-0.95

Table 3. Summary of *Est-4* segregations

Parent x		Parent y		Generation	Progeny			Recombinants	χ^2	P
Acc. No.	Allele (Bands)	Acc. No.	Allele (Bands)		x/x	x/y	y/y			
LA247	*1* (a)	Various *esculentum*	+ (b–d)	F_2	167	322	166	1 (a/b,c)	0.114	0.7-0.8
				BC		399	440	2 (a/c)	2.551	0.1-0.2
716	*1* (a)	LA490	+ (b–d)	F_2	44	95	70		8.20	0.01-0.02
				BC	218	257			3.20	0.05-0.1
				BC		873	794		3.74	0.05-0.1
1319	*1* (a)	1388	*3* (b)	F_2	43	75	41		0.170	0.9-0.95
410	*3* (b)	1386	*7* (d)	F_2	15	36	25		2.26	0.5-0.7
1521	*4* (b,c)	1679	*6* (b,d)	F_2	21	47	16		1.19	0.7-0.8
1521	*4* (b,c)	1386	*7* (d)	F_2	69	131	81		2.31	0.3-0.5
1606	*5* (c)	1679	*6* (b,d)	F_2	27	74	28		1.81	0.3-0.5
1606	*5* (c)	1645	*2* (a,b,d)	F_2	136	242	134		1.54	0.5-0.7
1606	*5* (c)	409	+ (b–d)	F_2	6	42			4.00	0.02-0.05
								Total individuals		5,134
								Total tested gametes		7,287
								Total resolvable gametes		6,543

To ascertain the genetic interrelationships between these pure-breeding band types, we intercrossed seven of them in various combinations and grew the respective F_1 hybrids. From the F_1's we derived F_2 and BC generations, which were assayed for *Est-4* segregations. The results of this program are summarized in Table 3 and representative segregations illustrated in Fig. 5 and 6. Segregations of all these lines were of monomeric type. It is also clear in these results that in all except one combination of parents, the progeny segregated into solely the parental and hybrid phenotypes. In other words, virtually all of the progenies indicate segregation at a single locus and control of each of the multiple banding patterns by single alleles.

The sole exceptions to monogenic segregation were observed in large segregating families of crosses between *L. esculentum* (b-d) × *L. parviflorum* (a): two plants of an identical new combination with bands at the a and c positions were encountered in the first backcross to *L. parviflorum*. An additional exception with bands at the a, b, and c positions (Fig. 7) appeared in a large F_2 familiy of the same cross. These three exceptions were found in a total of 5,134 plants of the segregating progenies, or, expressed in terms of the number of tested gametes, a total of 7,287. These figures do not permit a direct calculation of recombination frequency because the combination of bands in the parental stocks would not permit detection of recombinants in the a × b and b × d crosses and only a limited number of recombinants in the crosses between b,c and b,d and between c and b-d. Subtracting the number of tested gametes for these three crosses from the total, we obtain a difference of 6,545 gametes unequivocally tested for recombination. The frequency of recombinants amongst the latter group is 0.0005, which for inherent reasons is a maximum estimate.

Thus, the generation of new phenotypes via recombination between the parental types takes place at an exceedingly low rate. Although the appearance of the three purported recombinants suggests activity of two or more tightly linked genes, our data are too limited to permit a subdivision of this complex and to establish a linear order of its components. We therefore choose, as a temporary expedient, to assign all variants to one locus, *Est-4*. Determination of the fine-structural details must await future research.

In a parallel example, Nielson (1977) ascertained that multiple-banded phenotypes are encoded by closely linked genes in the salivary amylases of the bank vole. Here too, single, double, and triple-banded phenotypes were observed, and cosegregation of multiple bands was encountered in certain crosses, but the criterion for establishing the duplicate nature of the locus was quantitative variation in enzyme activity.

It is of interest to note that in many respects the *Est-4* complex resembles that of *Prx-4* (Rick and Fobes 1976). In both situations the majority of alleles are multiple banded with bands in various positions agreeing in morphology and position with those found a single-banded alleles. In both series genetic tests between alleles yielded results which are adequately explained by a single-locus model. The only exception to the latter generalization is our detection of a very low rate of recombination in one cross between $Est\text{-}4^+$ and $Est\text{-}4^1$. It is noteworthy that both series are also concordant in respect to their monomeric nature.

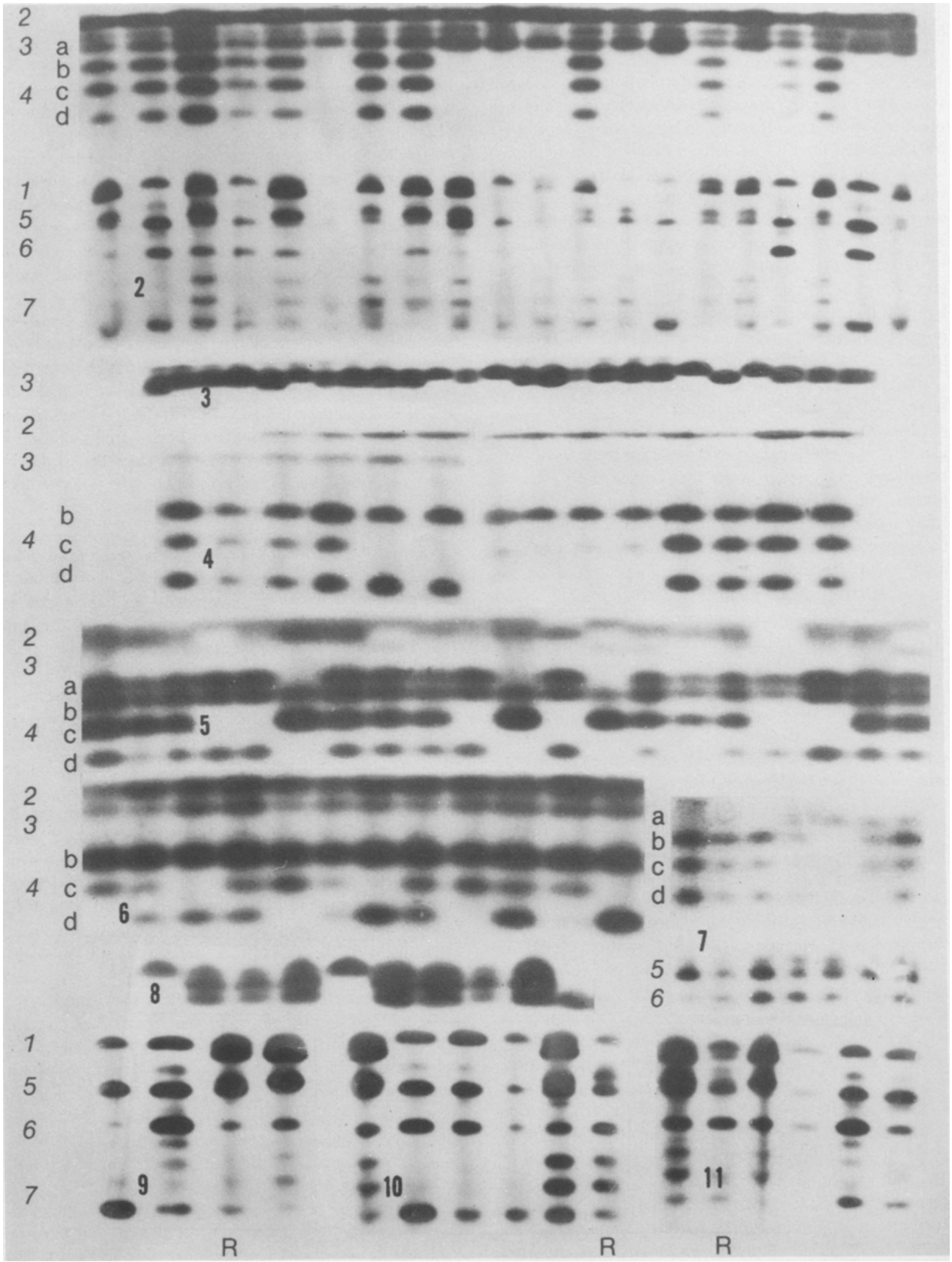
Est-1. Variation at this locus will not be described in detail because activity in this region has been described previously in our surveys of genetic variation in several tomato species (Rick and Fobes 1975a, b; Rick et al. 1976, 1978, in press). Figure 1 illustrates the banding patterns of a new allele encountered in this study. Progenies of all tested combinations of alleles segregate in monogenic fashion (Table 2). Detectable activity of *Est-1*, like that of *Est-5*, *Est-6*, and *Est-7*, is confined almost exclusively to root tissue.

Est-5. A single band appears 3.5 mm retarded from the + band of *Est-1*. A variant position retarded 1 mm is found in the Atico accession of *S. pennellii*. In backcrosses of the F_1 to both parents from the cross between this line and standard *L. esculentum*, the segregations behave according to a monogenic scheme (Table 2).

Lack of a heterodimer in this cross suggests a monomeric structure for the enzyme; however, other variant alleles of this locus have recently been found in accessions of *L. hirsutum*, which in crosses with *L. esculentum* display a heterodimer indicating the enzyme is dimeric. It is not known why the heterodimer was not observed in crosses with LA716, but the same features were observed for *Est-1* (Figs. 2, 9-11), which from segregation with other alleles is also known to be dimeric (Fig. 8). Differences in the structure of the two allozymes might prevent random recombination of subunits. Possibly such divergence might reflect the presumed ancient derivation of the two species.

Est-6. In standard *L. esculentum* the *Est-6* band is very weak and appears 6 mm cathodal to *Est-1*. Accession LA716 (*S. pennellii*) possesses an allele that codes for a strong band retarded 4 mm from the + position. Inheritance is again monogenic (Table 2); however, the + allele is so weak and unreliably expressed that heterozygotes cannot be distinguished from homozygotes in the backcross to *S. pennellii*. All segregations were therefore studied in the backcross to *L. esculentum*.

Est-7. The most cathodal position of the tomato esterases is occupied by the single band of *Est-7*, the band of $Est\text{-}7^+$ being retarded 15 mm with respect to $Est\text{-}1^+$. The *esculentum* band is relatively weak, but the allele prevailing in *L. parviflorum* codes for a fairly strong band accelerated 2 mm from the + position and that of *S. pennel-*



Figs. 2-11. Phenotypes of esterase genes in various progenies. Anodal direction above. **2.** Full array of esterase banding for BC LA490 × (F_1 LA490 × LA716). Note segregation of *Est-4* $^{+}$/*Est-4*^{1}, *Est-1*$^{+}$/*Est-1*^{5}, *Est-5*$^{+}$*Est-5*^{1}, *Est-7*$^{+}$/*Est-7*^{2}. **3.** F_2 segregation for *Est-3*$^{+}$/*Est-3*^{1} (LA490 × LA1602). **4.** Zymograms of stocks with various alleles of *Est-4*. Reading from left to right, first four plants: *Est-4*$^{+}$; next two: *Est-4*^{6}; next four: *Est-4*^{5}; last four: *Est-4*$^{+}$. **5.** F_2 segregation for *Est-2*$^{+}$/*Est-2*^{1}, *Est-4*^{4}/*Est-4*^{8}. **6.** F_2 segregation for *Est-4*^{4}/*Est-4*^{6}. **7.** F_2 segregation for *Est-4*$^{+}$/*Est-4*^{1}. Note possible recombinant (bands a, b, c) in second position from right. **8.** F_2 segregation for the *Est-1*^{1}/*Est-1*^{1} dimer. **9-11.** BCLA716 × (F_1 LA490 × LA716) showing various recombinants (denoted by R). Segregation for *Est-1*$^{+}$/*Est-1*^{5}, *Est-5*$^{+}$/*Est-5*^{1}, *Est-7*$^{+}$/*Est-7*^{2}. **9.** *Est-1*$^{+}$/*Est-1*^{5}, *Est-5*$^{+}$/*Est-5*^{1}, *Est-7*^{2}/*Est-7*^{2} recombinant. **10.** *Est-1*^{5}/*Est-1*^{5}, *Est-5*$^{+}$/*Est-5*^{1}, *Est-7*$^{+}$/*Est-7*^{2} recombinant. **11.** *Est-1*$^{+}$/*Est-1*^{5}, *Est-5*^{1}/*Est-5*^{1}, *Est-7*^{2}/*Est-7*^{2} recombinant

lii LA716, a band retarded 8 mm from +. In crosses between plants differing for these alleles, the progenies segregate in monogenic fashion (Table 2). A null allele has also been found.

To conclude regarding this series of genes, the inheritance of each is of codominant, monogenic type except for *Est-2*, for which the only detected variants are strong and weak alleles with banding in one position, thus conforming to a recessive-dominant scheme. Of the remaining genes, *Est-3* and *Est-4* behave as monomers, while the *Est-1*, *Est-5*, and *Est-7* behave as dimers. The structure of *Est-6* allozymes is unknown. Figure 2 illustrates simultaneous segregation at most of the esterase loci.

Several of the esterase alleles are characterized by differences in banding intensity in addition to differences in position. Whereas the former are generally less dependable than the latter for classifying phenotypes, the consistent association of the differences in intensity with their respective alleles in segregating progenies confirms that the differences are real. As stated above, we could detect consistent band intensity differences, even with the complex *Est-4* phenotypes.

Linkage

The results of the linkage survey are presented for each member of the esterase series in numerical order as follows.

Est-1. The locus of *Est-1* was previously situated at position 29 on the proximal portion of the long arm of chromosome 2 (Rick and Fobes 1977). Accordingly, no crosses were made with variants of *Est-1* except as they might bear on the linkage relationships between that gene and other members of the esterase series. The merits of pursuing such tactics are revealed below.

Est-2. Tests between *L. parviflorum* (*Est-2*^{1}) and various standard linkage tester stocks proved negative except for those against markers of chromosome 9. The first detection was sensed in the F_2 of a cross with an *esculentum* tester having the markers *ah* and *marm* on the long arm of 9. The data (Table 4) show a highly significant dissociation between *Est-2*^{1} and *ah* and a dissociation of borderline significance between *Est-2*^{1} and *marm*. The estimated map distances place *Est-2* between the two markers – an order supported by the absence of any homozygous *ah* – *Est-2* – *marm* double crossovers. On the basis of this discovery, we proceeded with a backcross to the *esculentum* marker stock. The results of this test (Table 4) confirm the F_2 results and yield more precise information concerning map locations. In the BC test both paired comparisons differ with great significance from random association, and the three-point test consistently points to a locus of *Est-2* between *ah* and *marm* at about position 38, some 14 cM from *ah* and 30 from *marm*. The overall map distance estimated for *ah* – *marm*, 44 cM, compares favorably with the standard value of 38 cM (Rick 1975). Our study therefore places *Est-2* in a specific region of a chromosome not previously marked with electrophoretic loci.

Est-3. As in the case of *Est-2*, we systematically tested a variant of *Est-3* (*Est-3*^{1}, LA1602, *L. pimpinellifolium*) with standard linkage testers. The only test to yield significant indices of linkage was that with a stock of *inv* – *dgt*, markers in the distal portion of the long arm of chromosome 1 (Table 5). In this instance, a large F_2 provided data that locate *Est-3*, thanks again to the versatility of the three-point test. All paired comparisons between the three genes have highly significant values. The estimated map distances position *Est-3* at 145, between *inv* and *dgt*, somewhat closer to the former. The sum of the two contiguous sections equals that of the total distance (8 cM), which, in turn, agrees reasonably well with the standard map distance of 12 cM. The latter is probably the more accurate estimate. The recovery of one double recombinant is compatible with the aforementioned gene order.

Est-4. To date we have not succeeded in situating *Est-4*, despite an ardent chase around the linkage map. Tests

Table 4. Linkage analysis of *Est-2*

Pedigree	Phenotypic classes							Linkages			
		+	+	+	*ah*	*marm*	+ *ah marm*		*ah–Est-2*	*Est-2–marm*	*ah–marm*
F_2 LA1164 x LA247	*Est-2*$^+$	18		22	7	5	χ^2	21.48***	4.47*	0.60
	Est-2^1	95		25	14	0	P	28 cM	38 cM	50 cM
BC to LA1164	*Est-2*$^+$	2		16	7	29	χ^2	56.57***	16.01***	4.63*
	Est-2^1	39		7	17	1	P	14 cM	30 cM	40 cM

* Significant at the 0.05 level
*** Significant at the 0.001 level

Table 5. Linkage analysis of *Est-3* from F_2 of LA1108 × LA1602

Est-3 genotypes	Phenotypes of morphological markers			
	+ +	*+ inv*	*dgt +*	*dgt inv*
+/+	1	7	6	92
+/1	230	7	11	0
1/1	122	1	0	0
Calculation	Linkages			
	inv–Est-3	*Est-3–dgt*	*inv–dgt*	
χ^2	394.63***	375.42***	269.65***	
Map distance	3.5 cM	4.5 cM	8.0 cM	

*** Significant at the 0.001 level

have been completed against most of the standard linkage testers and the primary trisomics for chromosomes 1, 3, 4, 5, and 6.

Est-5, 6, and 7. In approaching the problems of locating these remaining genes we made the fortunate choice of *L. parviflorum* and *S. pennellii* as test parents because both possess variant alleles at most of the *Est* loci. Thus, crosses with *esculentum* linkage testers also provided tests for independence between the *Est* loci themselves. In the final analysis, our large-scale backcrosses between F_1 (*L. esculentum* × *S. pennellii*) and both parents provided all the data necessary for placing these genes. For the backcross to *L. esculentum* it was possible to score segregation at *Est-5*, *6*, and *7*. The second family, a backcross to *S. pennellii*, could be scored satisfactorily for *Est-1*, *5*, and *7* (Fig. 9-11) and yielded similar results. Of the possible 20 recombinant classes among these progenies, only nine were observed (Table 6) and from the gene order proposed (see below) these can all be interpreted as results of single crossover events within a tightly linked gene complex. All other possible gene orders require that two or more of these observed classes be products of multiple crossovers, the repeated occurrence of which is so improbable that those gene orders must be rejected. The estimated linkage values from the above two backcrosses are:

(1) *Est-1* – 0.84 cM – *Est-5* – 0.84 cM – *Est-7*,

(2) *Est-6* – 0.82 cM – *Est-5* – 0.62 cM – *Est-7* – 9.8 cM – *Prx-2*.

Table 6. Linkage analysis amongst several *Est* loci and *Prx-2*

Pedigree	Genotype					N	Linkage
	Est-1	*Est-5*	*Est-6*	*Est-7*	*Prx-2*		
	5/5	*1/1*		*1/1*		411	*Est-1*
	+/5	*+/1*		*+/1*		412	0.84 cM
BC LA716	*+/5*	*+/1*		*1/1*		6	
×							*Est-5*
(LA490 × LA716)	*5/5*	*1/1*		*+/1*		1	0.84 cM
	+/5	*1/1*		*1/1*		2	
	5/5	*+/1*		*+/1*		5	*Est-7*
		+/+	*+/+*	*+/+*	*+/+*	229	*Est-6*
		+/1	*+/1*	*+/1*	*+/+*	30	0.82 cM
BC LA490		*+/+*	*+/1*	*+/+*	*+/+*	1	*Est-5*
×		*+/1*	*+/1*	*+/+*	*+/+*	3	0.62 cM
(LA490 ×		*+/1*	*+/1*	*+/1*	*+/1*	204	*Est-7*
LA716)		*+/+*	*+/+*	*+/+*	*+/1*	18	9.8 cM
		+/1	*+/+*	*+/1*	*+/1*	3	*Prx-2*
	+/+			*1/1*		33	
	+/1			*+/+*		2	*Est-1*
F_2 LA1338							2 cM
×	*+/1*			*+/1*		78	
LA1319	*+/1*			*1/1*		4	*Est-7*
	1/1			*+/+*		38	

The combined data yield this map for the region:

$$\frac{Est\text{-}1}{Est\text{-}6} - 0.8 - Est\text{-}5 - 0.6 - Est\text{-}7 - 10 - Prx\text{-}2.$$

A third family, an F_2 of *L. esculentum* × *L. parviflorum*, yielded an estimate of 2 cM for the *Est-1* – *Est-7* interval. The previously established locus of *Est-1* at position 29 and of *Prx-2* at 41 on chromosome 2 provide the framework necessary for establishing the location of the remaining genes; in fact, the *Est-1* – *Prx-2* distance estimated from these data conforms remarkably well with the previously determined distance (Rick and Fobes 1977). Future tests will be needed to establish the *Est-1* – *Est-6* order and to determine the map distances with greater precision. Unquestionably they constitute a tight cluster of genes, yet one that is separable by recombination.

Discussion

Monogenic Segregation

The segregations summarized in Tables 2 and 3 largely speak for themselves. Only two of the 24 populations deviate significantly from Mendelian expectations, and, in both instances, the deviations are not significant at the 0.01 level. Such discrepancies are, in fact, not unusual in crosses with *S. pennellii* (Rick 1969, 1972). The evidence for bona fide monogenic segregation at all loci is therefore compelling. The real problem with these data lies not in deviations, but in extreme conformity with expected values. The results of goodness-of-fit tests presented in these tables reveal that, of the 24 tested progenies, five fit Mendelian expectations with probability values of 0.9 or higher. For one cross P exceeds 0.95, and, for another, 0.98. The probability of such events happening by random assortment of gametes is remote. We have no explanation for this curious situation, but can state that most of the gels were scored by us together or by each of us separately. In all cases, our scores were in complete agreement. Furthermore, considering the number of different isozymes simultaneously scored on each gel, it is not conceivable how any subjective bias could have affected the classification to provide a close fit.

Linkage

The information presented in the preceding section affiliates all except one of the *Est* loci to their respective chromosomes and provides good approximations of their positions. The significance of these data is underscored by the fact that the linkage estimates for distances between the standard markers *Est-1* – *Prx-2*, *inv* – *dgt*, *ah* – *marm* agree well with values previously ascertained within *L. esculentum*. These agreements might not be expected because the latter two comparisons were made in crosses with *L. parviflorum*. Such results are noteworthy because both deal with regions fairly close to centromeres – regions that exhibited depressed recombination in *esculentum-pennellii* hybrids (Rick 1969, 1972). A closer phylogenetic relationship between *L. esculentum* and *L. parviflorum* than between the former and *S. pennellii* is thereby suggested – a relationship also indicated by comparisons of F_1 hybrid fertilities.

A point of primary interest in our results is the remarkable series of tightly linked loci – *Est-1*, *Est-5*, *Est-6*, and *Est-7* – comprising a maximum total distance of 2 cM on the linkage map of chromosome 2. These genes not only are closely approximated on the chromosome, but also code for products that are so similar that they migrate to contiguous positions in electrophoresis. Comparable situations in tomato isozymes have been encountered in *Got-2* – *Got-3* on chromosome 7 and *Prx-6* – *Prx-7* on chromosome 3 (Rick and Fobes 1977) and *Prx-2* – *Prx-3* on chromosome 2 (Rick et al. 1979). These constitute additional examples of the general phenomenon of linked mimics in the tomato genome, the first described cases being morphological mutants of the dwarf (*d*-chromosome 2), jointless (*j*-chromosome 11), hairless (*hl, ini*-chromosome 11), wiry (*w*-chromosome 4), and overwilting (*flc, not*-chromosome 7) series (Rick 1971).

This phenomenon is by means limited to *Lycopersicon esculentum*. Amongst other examples in flowering plants, the following cases can be cited. Two situations are known in barley – the esterases EA, EB, and EC (Kahler and Allard 1970), which form a remarkable parallel to our example, and the dramatic cluster of 5 (amongst 11 known) genes for powdery mildew resistance on chromosome 5 (Hiura 1960). Another example concerned with 'physiological' traits is a series of mutants affecting photosynthesis in maize. No less than seven of fourteen genes mapped by A-B translocations are situated on chromosome 1 (Leto and Miles 1979, personal communication). It should be observed in this example, however, that each of the seven linked genes affect different compounds in the photosynthetic process. Amongst animals, a spectacular case of linked genes with similar functions is reported by Goldsmith and Basehoar (1978) for genes coding for structural proteins of the chorion in *Bombyx mori*. Fifteen of the 16 tested genes cosegregated in a group linked with testers of chromosome 2. Since females of this species, in which crossing over is not detected, were used as the heterozygous parents of testcrosses, linkage intensities were not measured and the validity of the linked loci could not be resolved.

A reasonable hypothesis for the origin of this linked

esterase series is gene duplication. The argument is based on the following points. 1) Similarity in gene function. The enzyme products of all these genes catalyze the same reaction, at least in vitro, since they all react with the same substrate. The aforementioned 11 genes in barley control resistance to *Erysiphe graminis hordei*, but are distinguishable by their differential reactions with biotypes of the pathogen. 2) Similarity in molecular structure. The products encoded by all genes of this tightly linked complex are dimers except *Est-6*, whose determination is unknown; whereas those of the remaining *Est* genes are monomers. 3) Electrophoretic contiguity (as observed above). 4) Similarity in expression. All of the esterase loci have characteristic patterns of expression. For example, *Est-3* is found in leaves, stems and pollen; whereas *Est-2* is far more ubiquitous. The only loci which have expression limited to roots are *Est-4* and the linked series, *Est-1,5,6,7*. We can further distinguish *Est-4* from the series by its sensitivity to the growth rate of the roots. The expression of the series is much less sensitive to this environmental factor. If we group the loci according to similarity of expression, the linked cluster again shows the closest relationship. 5) Linkage relations (as observed above). For those isozyme loci that are so tightly linked – *Prx-2,3* and *Est-1, 5, 6, 7* – it is tempting to propose that the duplications are of a tandem nature, resulting from errors in pairing and crossing over. In the absence of any other well documented mechanisms capable of producing such tightly linked duplications, this is presently the only tenable hypothesis.

A topic related to gene duplication is concerned with the astonishing similarity between the distribution of esterases in *Lycopersicon* reported here and those in *Hordeum* (Kahler and Allard 1970). The genetics of maize esterases has also been studied in respect to monogenic segregation and delineation of loci (MacDonald and Brewbaker 1974), but not, as far as we are aware, in respect to linkage. In the case of barley, it is intriguing that seven loci can also be distinguished and that the three genes that code for the least mobile proteins are all tightly linked inter se. Independence was found between two tested of the four remaining loci and also between them and the three aforementioned tightly linked series. Recombination values for all tests between the three linked genes were less than 1 cM. Thus, in respect to migration rates and linkage relations, the tomato example closely parallels that of barley. The cases differ in the number of linked loci and in the multiple banding and monomeric structure of the linked barley genes vs. the single banding and dimeric structure of those in the tomato.

The evolutionary implications of this situation are intriguing. It would be remarkable indeed if the nearly identical linkage patterns in barley and tomato evolved independently. Although various investigations intimate that tandem duplications are fairly common in the evolution of isozyme systems, it is asking a great deal of chance variation to account for the very tight linkage of all of the slow migrating esterase loci in both species. In contrast, fewer assumptions are required by the proposal that both systems are ancient and originated only once in a common ancestor of the two genomes. The very tightness of the linkage per se would have tended to preserve this gene block, yet it would not be surprising if structural rearrangements did not disperse it in certain angiosperm groups. The argument for antiquity is bolstered by the absence of any detectable epistatic interactions (the formation of inter-locus heterodimers expected in tandem duplications) – also a feature of the morphological mimic mutant series (Rick 1971). If this supposition were correct, a great antiquity must be assumed for the system because it would have to have antedated the divergence of monocots from dicots and would therefore trace back to the early stages of angiosperm evolution. Until more critical data can be obtained, further discussion of our highly speculative suggestion would not be warranted. The formulation of any hypothesis will clearly depend on the investigation of esterase segregations in other plant groups, particularly in related genera of the Solanaceae – a project that we have initiated.

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