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Mapping of ripening-related or -specific cDNA clones of tomato (*Lycopersicon esculentum*)

S. M. Kinzer, S. J. Schwager and M. A. Mutschler*

Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853, USA

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Summary. Nineteen ripening-related or -specific clones from *Lycopersicon esculentum* were mapped via RFLP analysis using an F_2 population from the cross *L. esculentum* × *L. pennellii* and cDNA or genomic clones of known map location. The map produced using cDNA and genomic clones of known map location corresponded well with previously published maps of tomato. The number of loci detected for each ripening-related or -specific clone varied from one to seven. These loci were located on all 12 chromosomes of the tomato genome. There was no significant clustering of ripening-related or -specific genes. Regions of very low recombination were observed. The clone for polygalacturonase (TOM6) mapped to a single region on chromosome 10, the same chromosome as the *nor* and *alc* ripening mutants. To fine map this chromosome, two backcross populations were produced from the cross of *L. esculentum* × *L. pimpenillifolium*, in which the *esculentum* parents used were homozygous for either the *alc* or the *nor*. The coding region for polygalacturonase is functionally unlinked to either of these two ripening mutants.

Key words: RFLPs – Clustering – *nor* – *alc*

Introduction

The transformation of unpalatable green fruit to edible, marketable tomatoes involves the coordinated physical and chemical changes known as ripening. In order to study the genetic regulation of tomato fruit ripening, Slater et al. (1985) isolated 19 classes of ripening-related or -specific clones (referred to as TOM clones) from a cDNA library prepared from mRNA from ripening tomato pericarp. Using similar techniques, Mansson et al. (1985) produced four clones (referred to as CGN clones). Most of the cloned genes are of unknown function. One of the clones (TOM6) is a gene coding for polygalacturonase (Grierson et al. 1986), another (TOM13) is wound inducible and is suspected to be a component of the ethylene biosynthesis pathway (Holdsworth et al. 1987).

Five of the TOM clones showed two patterns of mRNA accumulation during ripening. Levels of messenger RNAs corresponding to TOMs 5 and 6 rose to a maximum when lycopene content was 38 mg/g fresh weight (corresponding to a fully orange fruit), then declined slowly. The expression of mRNAs homologous to TOMs 13, 36, and 99 were similar, rising rapidly at first, declining, then rising again to peak expression also at a lycopene content of 38 mg/g (Maunders et al. 1987).

The 15 clones TOMs 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 96, 99, 111, and 114 were used to study the effects of the mutant *alc* on ripening processes (Mutschler et al. 1988). The level of messenger RNA homologous to TOM99 was not affected by *alc*. The other clones showed varying degrees of reduction of the levels of homologous mRNA. TOM5 exhibited the least reduction (to 66% of normal), and TOM6 the greatest to 10% of normal).

Since an excellent RFLP map of the tomato genome was available (Bernatzky and Tanksley 1986b), the ripening-related and ripening-specific clones could be readily mapped. Several articles provide thorough explanations of the underlying theory of mapping using restriction fragment length polymorphisms (RFLPs) (Beckman and Soller 1983, 1986). A map of the ripening-related or -specific clones would provide important information as to: (1) the number of loci homologous to each ripening clone; (2) whether there is any clustering of

* To whom correspondence should be addressed

ripening-related genes; (3) whether there is any clustering of loci exhibiting similar developmental expression patterns; (4) whether any of the clones map to a location near that of a known gene affecting fruit traits.

Few polymorphisms are found among domestic *Lycopersicon esculentum* lines, necessitating the use of interspecific crosses for mapping this species (Helentjaris et al. 1985; Miller 1989). Polymorphisms are easily detected between *L. esculentum* and *L. pennellii*, and a cross of these two parents has previously been used for isozyme and RFLP mapping (Tanksley and Rick 1980; Bernatzky and Tanksley 1986a, b).

Materials and methods

Plant materials

F_2 seeds were obtained by selfing F_1 plants from the cross *Lycopersicon esculentum* cv 'New Yorker' × *L. pennellii* (LA716). Leaf tissue of parents, F_1, and F_2 plants was harvested, washed in 0.05% Tween 20, frozen in liquid nitrogen, and stored at −80 °C until DNA extractions were made. Seventy-two plants were used for the F_2 mapping population.

For the backcross populations, chromosome marker stocks *L. esculentum hy-u-h-alc* and *u-nor* were used to make F_1 hybrids with *L. pimpinellifolium* (LA2189). The F_1 plants were backcrossed to the *esculentum* parent. Fresh leaf tissue harvested from parents, F_1, and BC_1 plants was used for DNA isolations. The *alc* and *nor* populations included 71 and 69 plants, respectively.

DNA preparation

For the F_2 population, DNA was extracted using the procedure of Vallejos et al. (1986), substituting 20 m*M* sodium bisulfite for the EDTA and β-mercaptoethanol in the extraction buffer, and eliminating the cesium purification steps. For the backcross populations, the method of Doyle and Dickson (1987) was used.

Genomic blots

Ten milligrams of DNA from each plant was digested with restriction enzymes (DraI, EcoRI, EcoRV, HindIII, SstI, XbaI, or XmnI), following the manufacturer's suggested conditions, with the addition of 2–4 m*M* spermidine to increase cutting efficiency. DNA fragments were loaded into 0.8% agarose gels and run overnight at 25 V (Maniatis et al. 1982). Gels were blotted onto Zetabind membrane (AMF CUNO, Inc.), using an alkaline transfer procedure.

Clones used

The mapping clones, total genomic (TG) and cDNA (CD) clones of known chromosomal location, were generously supplied by S. Tanksley (Bernatzky and Tanksley 1986a, b). Fifty-five mapping clones designating 62 chromosomal locations were used to characterize the F_2 population. Ripening-specific DNA clones were obtained from two sources, the TOM clones from D. Grierson (Slater et al. 1985) and the CGN clones from Calgene (Mansonn et al. 1985). Sixteen TOM clones (TOMs 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 94, 99, 111, 114, and 129) and 3 CGN clones (CGNs 9-24, 24-3, and 28-8) were used.

Six TG or CD clones and four mutants designating locations on chromosome 10 were used to characterize the *alc* BC population. Four TG or CD clones and two mutants designating locations on chromosome 10 were used to characterize the *nor* BC population.

Probe preparation

Insert DNA was isolated following digestion to release insert. Insert and vector DNA were separated in low-melting-point agarose and the insert fragments were random primed, using the Multiprime kit (Amersham). Probe was purified by chromatography over G-75 Sephadex (Maniatis et al. 1982).

Hybridizations

Genomic blots were prehybridized in 6× SSC, 0.5% SDS, 5× Denhardt's solution, and 100 mg/ml denatured salmon sperm DNA for 4–24 h at 65 °C. Probe was then added, and hybridizations were left for 24–28 h at 65 °C. Filters were washed through serial washings down to 0.5× SSC, 0.1% SDS at 65 °C (Maniatis et al. 1982). Filters were exposed to X-ray film, with an intensifying screen (DuPont Cronex Lightening Plus HC), and developed after 1–7 days.

Linkage and cluster analysis

The data were analyzed using the MAPMAKER program (Lander et al. 1987). The 'three point' command was used to determine the correct sequence for each linkage group. Where additional markers were mapped, the 'ripple' function was used to confirm orders. The Kosambi function of genetic distance was used.

Since no prior papers indicated a method for cluster analysis in RFLP maps, a method based on the observed number of closely linked loci was derived. The method is explained in the 'Results and discussion' section.

Homology testing

In cases where no recombinant plants were detected for two clones, tests were undertaken to determine whether these clones were homologous. Inserts of each clone were isolated from the plasmid vector (as described under 'Probe preparation'), and digested with a combination of restriction enzymes to obtain a restriction map of the insert, with each of the inserts being used as the probe on twin blots. To support the results of these homology tests, survey blot patterns of DNA of both parents and the F_1 digested with five enzymes were compared, using the clones in question as probes.

Results and discussion

The genetic linkage map

The complete map, created using both the mapping clones and the ripening-related clones, spans 1,158 centimorgans (cM) (Fig. 1). The 19 ripening-specific or -related clones, TOMs 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 94, 99, 111, 114, and 129, and CGNs 9-24, 24-3, and 28-8, mapped to 38 loci. None of the 3 CGN clones used was a duplicate of a TOM clone. The ripening-related and -specific loci are located on all 12 chromosomes. Because the mRNA homologous to most of these clones has been shown to be affected by the ripening mutant *alc*, the effects of *alc* on mRNA level must be transactive.

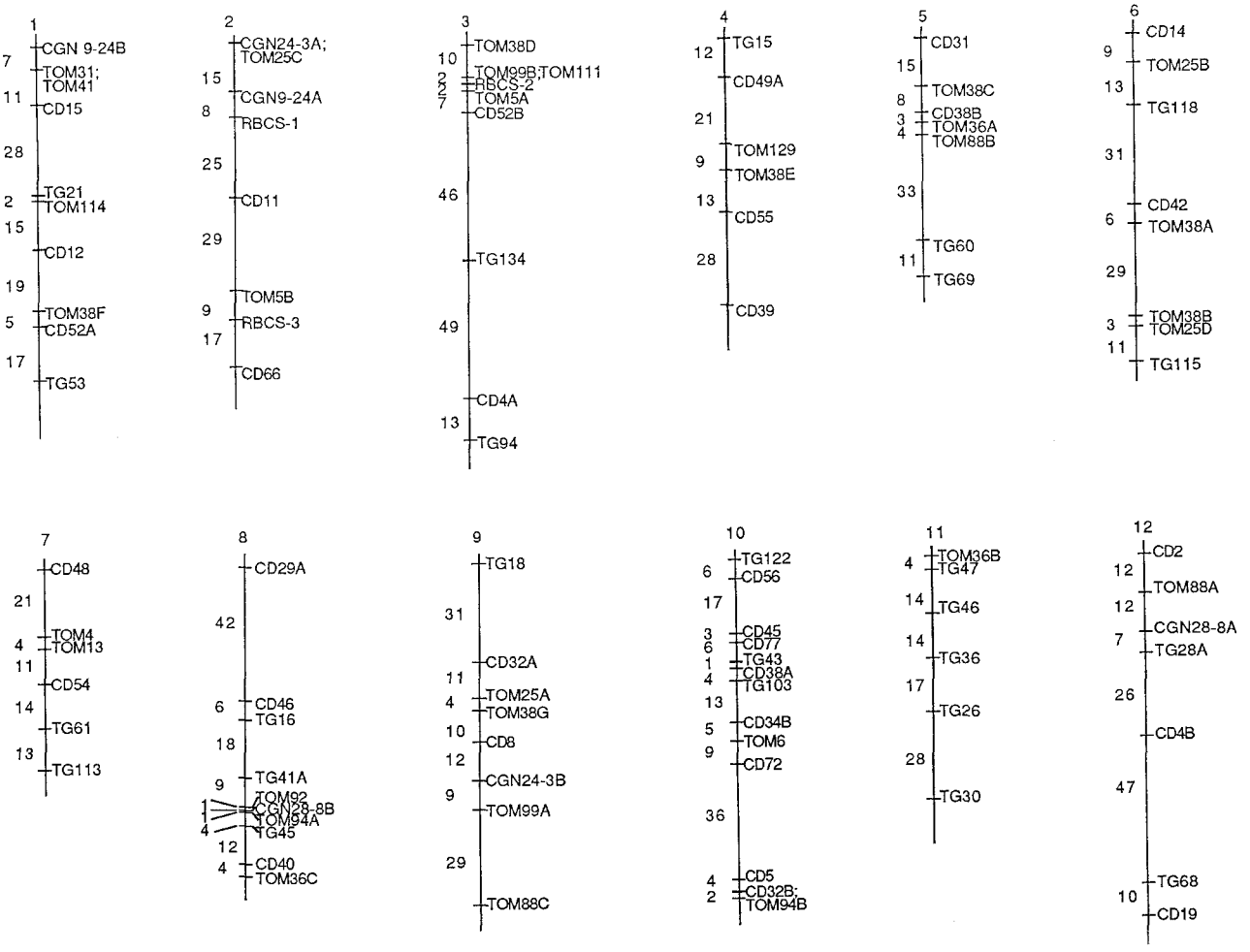

Fig. 1. RFLP map of tomato genome using clones of known map location (CD, RBCS, and TG) and ripening-specific or -related clones (CGN, or TOM). The *numbers* above the chromosomes indicate chromosome number

Nine of the ripening clones (47%) mapped to one locus each, 6 (32%) mapped to two loci each, 2 mapped to three loci, TOM25 mapped to four loci, and TOM38 mapped to seven loci (Fig. 1). There was agreement between the number of loci detected by RFLP analysis and the reported number of gene products detected by hybrid select translation as reported by Slater et al. (1985) for 3 TOM clones. The clones TOMs 6 and 13 corresponded to one translation product and TOM99 corresponded to two translation products. RFLP analysis detected one locus for TOMs 6 and 13, and two loci for TOM99. For 2 clones, the number of loci homologous to the clones was greater than published reports of their numbers of corresponding translation products. TOM5 and 36 corresponded to one and two translation products (Slater et al. 1985), but hybridized to two and three loci, respectively. Data are not available regarding the translation products corresponding to the other ripening-related clones. Exact agreement between the number of loci homologous to the clones and their numbers of corresponding translation products was not expected, since the numbers of regions homologous to a clone may over- or underestimate the number of loci actually coding for a message. Not all of the loci detected by a clone are necessarily active coding genes; some of the loci may be pseudogenes, incomplete or mutated copies of the active gene. Furthermore, each fragment with homology to a clone may contain more than one copy of the gene (Bernatzky and Tanksley 1986a).

The clones homologous to large numbers of loci, such as TOM25 and TOM38, may code for proteins needed in great abundance or at different stages during ripening, or they may indicate duplications. Unfortunately, the expression patterns of mRNAs homologous to TOM25 and 38 during ripening have not yet been characterized. The proportions of ripening clones mapping to one, two, or more loci agree well with the results of Bernatzky and Tanksley (1986a), who found that of 34 random cDNA clones from a leaf library, 53% corresponded to a single locus, and 32% corresponded to two loci in the tomato

genome. Since the spread of numbers of regions homologous to ripening clones is the same as that for random leaf cDNA clones, the spread of copy number does not provide evidence that the ripening clones with more regions have multiple copies for a reason related to ripening. However, loci homologous to TOM25 and TOM38 are tightly linked on both chromosomes 6 and 9 (3 and 4 cM, respectively), indicating the possibility of a duplication on these two chromosomes.

Suspected clustering of ripening cDNA clones

Clustering of loci is of interest because it might indicate an evolutionary, regulatory, or functional relationship among the clustered loci. The ripening-related and -specific loci are found on all 12 chromosomes of the tomato genome. Some regions homologous to ripening clones are found singly, others are in pairs or triplets (Fig. 1). No plants recombinant for the two loci were observed in a population of 72 plants for TOMs 31 and 41 on chromosome 1, TOM25C and CGN24-3A on chromosome 2, and TOMs 99B and 111 on chromosome 3. Homology tests and comparison of polymorphism patterns using five restriction enzymes showed that all three of these pairs of clones were nonhomologous.

There are other groups of closely linked loci – TOMs 36A and 88B are within approximately 4 cM on chromosome 5; TOMs 25D and 38B are within approximately 3 cM on chromosome 6; TOMs 4 and 13 are within approximately 4 cM on chromosome 7, and TOM25A and TOM38G are within approximately 4 cM on chromosome 9. The triplet TOM25A, 99B, and 111 are within a 4-cM range, and TOMs 92, 94A, and CGN28-8B are all within approximately 3 cM. With an F_2 population of 72 plants, measurement of precise linkage is not possible, and the values obtained under 5 cM may be under- or overestimates (Tanksley et al. 1988). Therefore, any of these tight linkages may or may not indicate a cluster. However, the first triplet of clones listed above is in a region of reduced recombination (Bernatzky and Tanksley 1986b), and the second triplet of clones may be in or near the region of reduced recombination near Aps-2 (Rick 1969).

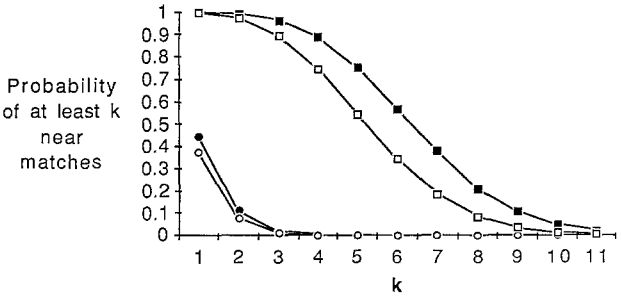

Fig. 2. The probability of at least k near-matches, at varying levels of m and r, with $n=38$. At $r=1200$, ● and ■ indicate $m=0$ and 5, respectively. At $r=1500$, ○ and □ indicate $m=0$ and 5, respectively

The question of whether the degree of clustering observed is statistically significant was examined. The analysis made several assumptions: (1) the ripening loci are unrelated by descent; (2) there is a fixed tomato genome size, which may be estimated by current information; (3) every region in the genome has an equal chance of containing a ripening locus; and (4) recombination is equally likely at any location throughout the genome. The first assumption is supported by the fact that the clones are nonhomologous. The size of the tomato genome has previously been estimated to be 1,200 cM (Bernatzky and Tanksley 1986b; Tanksley and Hewitt 1988). Since more recent data indicate a genome size of 1,500 cM (S. Tanksley, personal communication), both values are considered in this study.

The question of the randomness of locus position can be formulated as a problem of distributing balls into cells as follows: a set of n balls is distributed independently at random into a sequence of r cells, numbered 1 to r. A nonnegative integer is chosen for the threshold value m. The choice of m could be based upon the smallest resolvable distance of recombination, which is a function of the population size used (Tanksley et al. 1988). A *near-match* occurs when two balls are assigned to cells whose distance is m or less; the special case of $m=0$, when two balls are assigned to the same cell, is a *match*. Let k be the number of near-matches observed when n balls are distributed into r cells. The probability distribution of k is derived under the null hypothesis of independence and randomness. Under the alternative hypothesis that there is clustering among the locations of the balls, the number of near-matches tends to be greater than under the null hypothesis. The null hypothesis is therefore rejected if the observed value of k lies in the upper 5% tail of the null distribution of k.

In this experiment, $n=38$ (the number of loci homologous to the ripening clones) and $r=1{,}200$ or 1,500 (the size of the tomato genome). Two values for m are of interest, $m=0$, which corresponds to the occurrence of co-segregating pairs, and $m=5$, which corresponds to groups of loci linked within 5 cM, the approximate minimum resolvable distance for an F_2 population of 72 plants (Tanksley et al. 1988). The question is essentially: if 38 balls are distributed independently and at random into 1,200 (or 1,500) cells, what is the probability of at least k near-matches (or matches)? The answers are given in Fig. 2 and Table 1. A combinatorial derivation of the probability of exactly k near-matches for specified values of m, n, and r is reported by S. J. Schwager (in preparation). For $m=0$, this problem reduces to determining the distribution of the number of empty cells, a classical occupancy problem.

Three co-segregating pairs were observed, so $k=3$ for $m=0$. The probability of three or more matches in 0.0186 (Table 1). Thus, at a significance level of 5%, one would conclude that the loci are not distributed randomly. However, since one of the pairs (TOM99B and TOM111) is located within a region of reduced recombination, the assumption of equal likelihood of recombination throughout the tomato genome is violated. Omitting this questionable pair, the probability of two or more exact matches is 0.1125, leading one to accept the null hypothesis that the ripening loci are distributed randomly. When a genome size of 1,500 cM is considered, the same conclusions are drawn with $k=3$ and $k=2$ (Table 1).

Ten pairs of tightly linked loci were observed, so $k=10$ for $m=5$. Computational complexity makes exact calculations unmanageable at such high levels of n, r, m, and k, therefore, Table 1 contains empirical probabilities calculated by computer simulation for k greater than 4. The probability of ten or more near-matches within a genome of size 1,200 is estimated to be 0.0469 (Table 1), a borderline result when using a 5% significance level. However, four of these pairs are within suspected regions of reduced recombination, and the probability of six or more near-matches is 0.5675. With a genome size of 1,500, the probability of ten or more near-matches is estimated to be 0.0105, and the probability of six or more near-matches is 0.3462. Therefore, there is no substantial evidence for functional clustering of ripening-related loci.

A similar analysis could be performed for any number of clones (n), any size genome (r), and any near-match threshold (m). The number of near-match pairs (k) needed to constitute significant evidence of clustering increases with both increasing number of clones and increasing genome size

Comparison to existing maps

It is of interest to compare the framework map, which was generated using the mapping clones, to the previous maps produced by the originators of these clones (Bernatzky and Tanksley 1986b; Tanksley et al. 1988). Neither of the maps most recently published by Tanksley et al. was produced using the MAPMAKER program. For uniformity, more recent estimates of genetic distances, obtained from the MAPMAKER program, are used for these comparisons (S. Tanksley, personal communication).

The extreme similarity between our maps and the Tanksley maps is striking. The gene orders are identical for all clones located on both maps. Most of the linkage distances calculated between markers (the Kosambi function of the recombination fraction) also agree quite well. For example, on chromosome one, our estimates of linkage between loci CD15, TG21, CD12, and TG53 are 28, 17, and 41, compared with distances of 21, 15, and 45, respectively, on the Tanksley map. On the long arm of chromosome 10, we calculate as the distances between four of the markers CD34B, CD72, CD5, and CD32B, 14, 26, and 4, compared with 14, 31, and 5, respectively, on Tanksley's map.

Table 1. Probabilities of observing k or more near-matches with $n=38$[a]

k	$m=0$; $r=1200$	$m=0$; $r=1500$
0	1.0000	1.0000
1	0.4468	0.3767
2	0.1125	0.0772
3	0.0186	0.0103
4	0.0023	0.0011
5	0.0003	0.0002
k	$m=5$; $r=1200$	$m=5$; $r=1500$
0	1.0000	1.0000
1	0.9992	0.9961
2	0.9918	0.9708
3	0.9619	0.8936
4	0.8859	0.7460
5	0.7503	0.5471
6	0.5675	0.3462
7	0.3786	0.1864
8	0.2170	0.0853
9	0.1085	0.0327
10	0.0469	0.0105
11	0.0170	0.0030
12	0.0056	0.0008
13	0.0017	0.0002
14	0.0004	0.0000

[a] Obtained by exact calculation for $m=0$ (all k) and for $m=5$, $k\leq 5$; obtained by computer simulation for $m=5$, $k\geq 6$. 100,000 repetitions were performed to obtain the simulation probabilities for each combination of m and r

In a few cases the distances on the two maps do not seem to correspond well. For example, on chromosome 5, estimates of linkage between the four mapping clones CD31, CD38B, TG60, and TG69 are 23, 40, and 11 cM, while estimates from Tanksley's lab are 12, 25, and 24 cM, respectively. On chromosome 7, linkage estimates between the four clones CD48, CD54, TG61, and TG113 are 36, 14, and 13 cM, while those from Tanksley's lab are 61, 25, and 4 cM, respectively.

Considering the number of distances being estimated, the differences in map distances are most likely attributable to sampling error. Differences could also be a result of differing population sizes or of errors in scoring. Large variations among populations for recombination fractions have been observed in both tomato (A. Patterson, personal communication) and in rice (S. McCouch, personal communication). Differences in recombination could be due to a number of environmental or other factors affecting meiosis.

Table 2. Possible linkages between regions homologous to ripening clones and visible ripening mutants

Chromosome	One	Two	Three	Five	Six	Seven	Eight	Nine	Ten	Twelve
Ripening clones:	CGN9-24B TOM31 TOM41 TOM114	TOM5B	TOM5A TOM99B TOM111	TOM36A TOM38C TOM88A	TOM25B TOM25D TOM38A TOM38B TOM88B	TOM4 TOM13	CGN28-8B TOM36C TOM92 TOM94A	CGN24-3B TOM38G TOM88C TOM99A	TOM6 TOM94B	CGN28-8A 8A
Ripening mutants (position):	Never-ripe-2 (–) Unpigmented fruit epidermis (30)	Peach (67)	Yellow flesh (29)	Apricot (–) Ripening inhibitor (0)	Beta-carotene (106) Old gold crimson (106)	Green stripe (5)	Green flesh (44)	Never-ripe (–)	Alcobaca (5) Fruit-stripe (16) Non-ripening (15) Tangerine (95) Uniform (19)	High pigment (–)

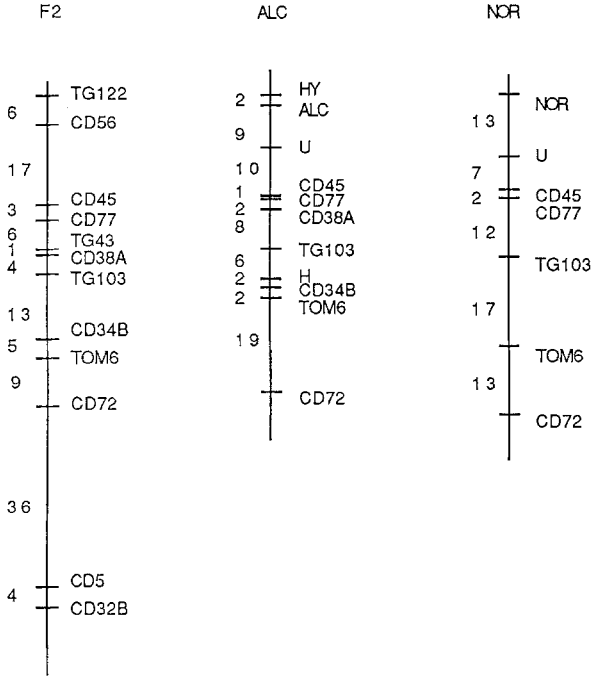

Fig. 3. Maps of chromosome 10 using an *L. esculentum* × *L. pennellii* F_2 population, and (*L. esculentum* × *L. pimpinellifolium*) × *L. esculentum* BC populations segregating for *alc* and *nor*

Locations of ripening-related loci versus classical ripening genes

The ripening-specific or -related loci homologous to the CGN and TOM clones may be related to previously identified and mapped visual ripening mutants. Table 2 provides a list of possible linkages between ripening cDNA loci and visible ripening mutants. It is extremely difficult to compare RFLP maps to the classical tomato linkage map, because of the scarcity of common markers. Therefore, any markers within the same general region are listed, but further testing is needed before drawing any firm conclusions. Some of the more interesting possibilities include the possible linkage of TOM36A and TOM38C to *rin* (ripening inhibitor) on chromosome 5, of TOM25A, TOM38G, TOM88C, TOM99A, or CGN 24-3B on chromosome 9 to *Nr* (Never-ripe), or of TOM6 (the PG clone) to *alc* (alcobaca), *u* (uniform shoulder), or *nor* (non-ripening) on chromosome 10.

Two backcross populations were analyzed to determine whether PG is closely linked to *alc*, *u*, or *nor*. The localization of TOM6 versus the ripening mutants could not be studied in an intraspecific cross of *L. esculentum* because of a lack of polymorphisms within the species. This agrees with previous studies (Helentjaris et al. 1985; Miller 1989). A cross of *L. esculentum* × *L. pennellii* also could not be used, because the latter parent produces green fruit, and many of the BC_1 fruit lack *esculentum*-type color development, making analysis of ripening mutants impossible. Therefore, BC_1 populations were obtained from the interspecific cross between *L. esculentum* and the red-fruited species, *L. pimpinellifolium*.

One backcross population segregated for the visible markers *u* and *nor*. The other segregated for *hy* (homozygous yellow), *u*, *h* (hairless), and *alc*. Restriction polymorphisms were not easy to locate; when surveyed with 16 enzymes, the *alc* population exhibited polymorphisms for 7 out of 11 chromosome 10 clones, and the *nor* population exhibited polymorphisms for only 5 out of 11 chromosome 10 clones. The inability to detect polymorphisms for two of the clones in the *nor* population, for which polymorphisms were detected in the *alc* population, is attributed to heterogeneity in the *L. pimpinellifolium* parent rather than within the *nor* line itself.

The RFLP maps of chromosome 10 from these two populations agreed well with the map generated from the F_2 population (Fig. 3), as well as with the published maps (Mutschler et al. 1987; Tanksley et al. 1988). When both the RFLP and visual traits are analyzed together, it is apparent that TOM6 is many map units away from *u*, *alc* and *nor*, and is in fact functionally unlinked.

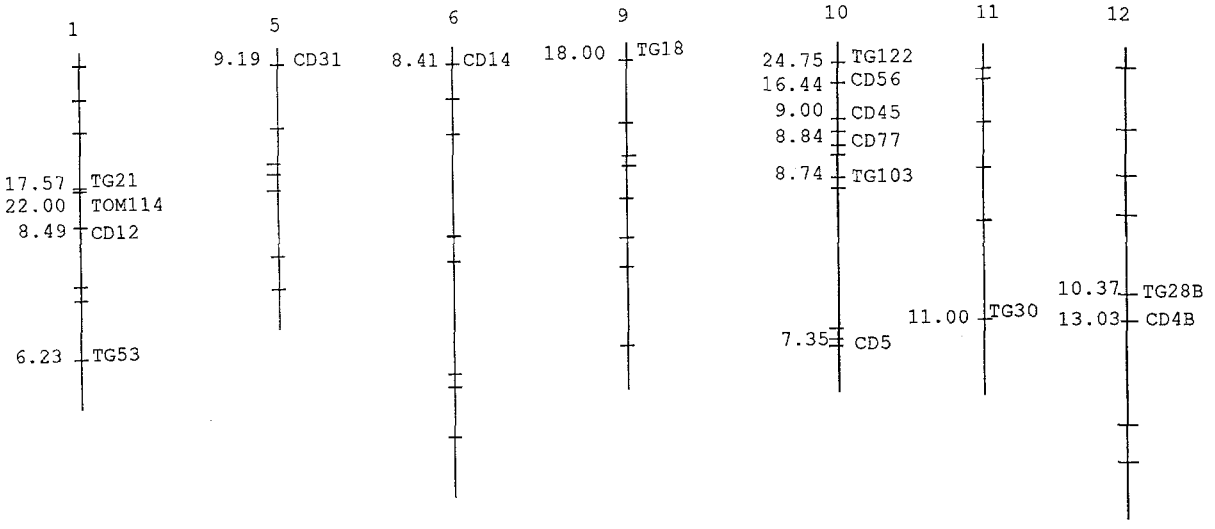

Fig. 4. Loci at which segregation does not fit Mendelian ratios for a single gene at 95% confidence level. Chi-square values are listed to left of each locus. *Unlabeled bars* indicate loci at which segregation fits Mendelian ratios for a single gene

Single gene ratios

Upon first examination of the F_2 RFLP data, it is obvious that some of the loci do not fit the 1:2:1 ratio expected of codominant genes, or the 3:1 ratio expected with a presence/absence marker. Significant deviations from an expected single-gene ratio will affect the recombination fraction, and thus the map distance calculated. Seven chromosomes have regions in which one or more clones do not fit the expected ratio (Fig. 4).

Three regions of ratio distortion are evident on chromosomes 1, 10, and 12. These segregation distortions agree with work by Bernatzky and Tanksley (1986b), Zamir and Tadmor (1986), Gadish and Zamir (1987), and Rick (1969). The cluster on chromosome 10 is of particular interest, because of the directional nature of the aberrancy; the distortion increases markedly for loci more distal on the short arm of the chromosome. Similar patterns of ratio distortion were observed during the mapping of the *Ge* (Gamete eliminator) and *X* (gametophytic factor) loci in *L. esculentum* (Rick 1966, 1970; Alexander 1972). The backcross population used to analyze the ripening clone and mutants on chromosome 10 more thoroughly did not exhibit aberrant segregation ratios. Data from previous papers (Rick 1969) do not indicate a lack of aberrant segregation in backcross populations of *L. esculentum* × *L. pennellii;* therefore, the lack of aberrancies on chromosome 10 detected in the BC population is probably due to the use of *L. pimpinellifolium* in the cross, rather than *L. pennellii*. The presence of aberrancies in the F_2 is likely to be an effect of the interaction of *pennellii* and *esculentum* genomes.

Segregation patterns not fitting Mendelian ratios are common in interspecific crosses (Grant 1975). This could be due to many factors affecting the plant life cycle between sporogenesis and seed germination. In the case of *L. esculentum* × *L. pennellii,* Gadish and Zamir (1987) suggested that the deviations are due to differential zygotic lethality.

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