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A Genetic Map of Tomato Based on BC_1 *Lycopersicon esculentum* × *Solanum lycopersicoides* Reveals Overall Synteny but Suppressed Recombination Between These Homeologous Genomes

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ABSTRACT

F_1 hybrids between the cultivated tomato (*Lycopersicon esculentum*) and the wild nightshade *Solanum lycopersicoides* are male sterile and unilaterally incompatible, breeding barriers that impede further crosses to tomato. Meiosis is disrupted in 2× hybrids, with reduced chiasma formation and frequent univalents, but is normal in allotetraploid hybrids, indicating the genomes are homeologous. In this study, a partially male-fertile F_1 was backcrossed to tomato, producing the first BC_1 population suitable for genetic mapping from this cross. BC_1 plants were genotyped at marker loci to study the transmission of wild alleles and to measure rates of homeologous recombination. The pattern of segregation distortion, in favor of homozygotes on chromosomes 2 and 5 and heterozygotes on chromosomes 6 and 9, suggested linkage to a small number of loci under selection on each chromosome. Genome ratios nonetheless fit Mendelian expectations. Resulting genetic maps were essentially colinear with existing tomato maps but showed an overall reduction in recombination of ~27%. Recombination suppression was observed for all chromosomes except 9 and 12, affected both proximal and distal regions, and was most severe on chromosome 10 (70% reduction). Recombination between markers on the long arm of this chromosome was completely eliminated, suggesting a lack of colinearity between *S. lycopersicoides* and *L. esculentum* homeologues in this region. Results are discussed with respect to phylogenetic relationships between the species and their potential use for studies of homeologous pairing and recombination in a diploid plant genome.

HIGH density molecular marker maps are now available for many crop plants and provide a useful framework for genome studies, gene cloning, quantitative trait loci (QTL) analysis, varietal development, and many other potential applications. Comparisons of genetic maps have been made for related species such as rice, maize, sorghum, and wheat (Ahn *et al.* 1993; Ahn and Tanksley 1993; Pereira *et al.* 1994; Paterson *et al.* 1995), tomato, potato, and pepper (Tanksley *et al.* 1992; Prince *et al.* 1993), and Arabidopsis and Brassica (Lagercrantz 1998) species. These studies revealed a surprisingly high level of marker synteny over large tracts of DNA and conservation of QTL for agronomic characters in species as diverged as maize and rice, which are separated by up to 65 million years of evolution and a 25-fold difference in DNA content; remarkably, even dicots and monocots have regions of conserved gene order (Paterson *et al.* 1996). These genomic similarities are expected to facilitate the identification and utilization of beneficial genes, alleles, and QTL across related plant taxa.

Due to the limited genetic variation inherent in many domesticated plant species, marker maps are sometimes based on recombination in interspecific hybrids. In tomato (*Lycopersicon esculentum* Mill.), the natural mating system and population bottlenecks during migrations of its direct ancestor (*L. esculentum* var. *cerasiforme*) and during domestication, as well as more recent breeding activities, depleted the crop of much of its natural diversity (Rick 1995). As a result, cultivated forms show little variation for molecular markers such as restriction fragment length polymorphisms (RFLPs), isozymes, or randomly amplified polymorphic DNA (Rick and Fobes 1975; Miller and Tanksley 1990; Williams and St. Clair 1993). Fortunately, the nine related wild species of Lycopersicon, each of which can be crossed to *L. esculentum*, provide a rich source of allelic diversity, not only for the construction of genetic maps, but for many economic traits as well. Maps based on crosses between *L. esculentum* and *L. cheesmanii*, *L. chmielewskii*, *L. hirsutum*, *L. pennellii*, *L. peruvianum*, or *L. pimpinellifolium* indicate a high degree of colinearity between these genomes (Paterson *et al.* 1990; Tanksley *et al.* 1992; Paran *et al.* 1995; Grandillo and Tanksley 1996; Bernacchi and Tanksley 1997; Fulton *et al.* 1997). Furthermore, corresponding F_1 hybrids exhibit normal chromosome pairing in meiosis and reasonably high fertility (Afify 1933; Lesley and Lesley 1943; McGuire

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and Rick 1954; Sawant 1958; Khush and Rick 1963). Therefore, the genomes of Lycopersicon species can be considered essentially colinear and homologous.

In contrast, F_1 hybrids of tomato with the wild nightshade *Solanum lycopersicoides*, its closest relative beyond the Lycopersicon clade, display incomplete chromosome pairing and high pollen sterility (Menzel 1962; Chetelat *et al.* 1997). Furthermore, synthetic allotetraploid hybrids display preferential pairing of homologous chromosomes and greatly increased fertility, indicating that the *L. esculentum* and *S. lycopersicoides* genomes are homeologous (Menzel 1964; Rick *et al.* 1986). Considering that hybrids are also unilaterally incompatible (*i.e.*, reject *L. esculentum* pollen), these combined breeding barriers probably explain why little progress was made in the utilization of *S. lycopersicoides* for many years after the first report of its hybridization with tomato (Rick 1951). More recently, a set of monosomic alien addition lines, each with a single *S. lycopersicoides* chromosome added to a 2× genome of *L. esculentum*, have been produced (Chetelat *et al.* 1998). In these aneuploids, homeologous chromosomes occasionally pair, resulting in recombinant diploid progeny with traits introgressed from *S. lycopersicoides* (Rick *et al.* 1988).

Gene transfer was also accomplished through rare, fertile F_1 hybrids that were directly backcrossed to tomato (Gradziel and Robinson 1989; Chetelat *et al.* 1997). Though the majority of BC_1 plants were sterile, a population of backcross-inbred lines could nonetheless be derived, which together represented nearly the entire donor genome through overlapping introgressed segments (Chetelat and Meglic 1999); severe segregation distortion and recombination suppression in many genomic regions were also revealed by this study. The objective of the present experiments was to study segregation and recombination in the BC_1 generation and to construct a corresponding linkage map, primarily of molecular markers. The BC_1 map was compared for synteny and recombination rates to existing maps of the tomato and potato genomes. The results are discussed in light of previous cytological studies of homeologous chromosome pairing observed during meiosis of the F_1 hybrid.

MATERIALS AND METHODS

Plant material and hybridizations: The parental genotypes used in this study were *L. esculentum* cv. VF36 and *S. lycopersicoides* accession LA2951. The wild parent was collected by Drs. Andrés Contreras and Rudolf Thomann at Quistagama/Cuisama, Camina, Tarapaca Province, Chile. An F_1 hybrid between them (90L4178) was obtained using *L. esculentum* as the pistillate parent as described previously (Chetelat *et al.* 1997). The first backcross to VF36 was made using the F_1 as staminate parent to bypass the unilateral incompatibility that prevents the reciprocal cross. Of the 281 BC_1 plants obtained by embryo culture (Neal and Topoleski 1983), 84 were used for construction of the BC_1 map; 65 additional plants were used to increase map resolution on chromosome 10.

Molecular marker analysis: DNA isolations, restriction enzyme (RE) digests, and Southern blots were performed as previously described (Chetelat and Meglic 1999). Genomic (TG) and cDNA (CT and CD) probes were provided by Dr. Steve Tanksley at Cornell University and were chosen based on their locations on the tomato RFLP map (Tanksley *et al.* 1992) to detect linkage on each chromosome arm. A polygalacturonase inhibitor protein (PGIP) cDNA probe was provided by Dr. Ann Powell at University of California, Davis. The map locations of previously unmapped RFLPs, isozyme loci, and morphological markers were determined in this study. Probes were amplified from plasmid DNA by PCR, purified on spin filters, and then labeled with [^{32}P]dCTP and/or [^{32}P]dATP using the random hexamer primer method as previously described (Chetelat and Meglic 1999). Blots were washed three times to a final stringency of 0.5× SSC at 65°.

Isozyme analysis was performed as described previously (Chetelat *et al.* 1997) using the following enzyme systems: aconitase (*Aco*), alcohol dehydrogenase (*Adh*), acid phosphatase (*Aps*), diaphorase (*Dia*), esterase (*Est*), formate dehydrogenase (*Fdh*), glutamate oxaloacetate transaminase (*Got*), malic enzyme (*Mae*), malate dehydrogenase (*Mdh*), 6-phosphogluconate dehydrogenase (*6Pgdh*), phosphoglucoisomerase (*Pgi*), phosphoglucomutase (*Pgm*), peroxidase (*Prx*), shikimate dehydrogenase (*Skdh*), and triosephosphate isomerase (*Tpi*).

Statistical analysis: Genome ratios (percentage recurrent parent genome) in the BC_1 population were calculated from marker data using QGENE version 2.3 (Nelson 1997). Simulated BC_1 populations were also generated and analyzed with this software. Genetic maps were constructed and drawn with MAPMAKER version 2.0 for Macintosh (Lander *et al.* 1987), using a threshold LOD of 3.0 and recombination fraction (RF) of 0.40 to determine linkage groups. All map units were calculated using the Kosambi mapping function (Kosambi 1944).

RESULTS

Survey of molecular marker polymorphisms: To identify informative molecular markers for map construction, three accessions of *S. lycopersicoides* (LA1964, LA2408, and LA2951) were compared to *L. esculentum* for polymorphisms using allozyme and RFLP (cDNA and genomic) markers. For the isozyme analysis, 50–100 plants per accession were genotyped at ∼36 loci, yielding an average polymorphism rate relative to *L. esculentum* of ∼75% in *S. lycopersicoides*, with no appreciable differences between accessions. This compares favorably to *L. pennellii* accession LA0716, which was used to generate the high density molecular marker map of tomato (Tanksley *et al.* 1992) and which had a polymorphism rate relative to *L. esculentum* of only 51% using isozymes. The high rate of allozyme polymorphism made it possible to determine map locations for a number of previously unmapped isozyme genes, including *Dia-2*, *-3*, and *-4* and *Mae-1* and *Mdh-1* (see below).

Even higher rates of polymorphism were detected between *S. lycopersicoides* and *L. esculentum* using RFLP markers. Eighty-one percent of 1151 tested probe × RE × accession combinations produced distinct banding patterns in the two species. The three *S. lycopersicoides*

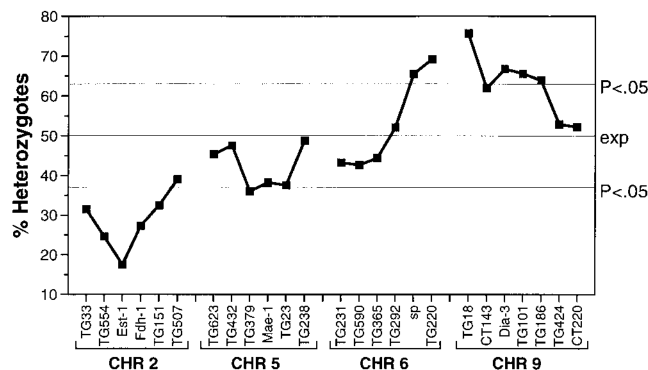

Figure 1.—Proportion of heterozygotes in BC_1 *L. esculentum* × *S. lycopersicoides* mapping population for markers on chromosomes showing significant segregation distortion. The middle line corresponds to the expected (exp) frequency of 50% heterozygous individuals; the outer two lines represent the threshold frequencies for significant segregation distortion, as indicated by χ^2 analysis (at $P \leq 0.05$).

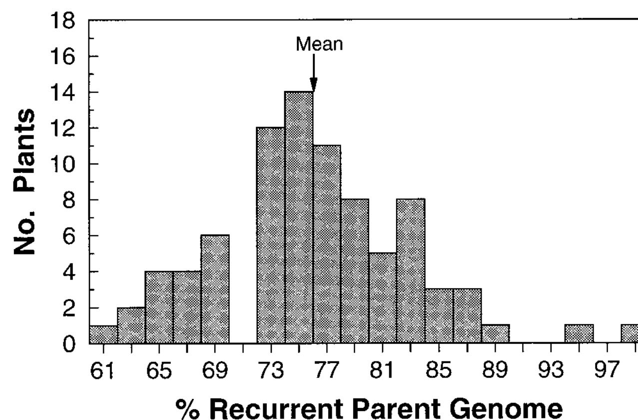

Figure 2.—Distribution of percentage recurrent parent genome values in BC_1 *L. esculentum* × *S. lycopersicoides* mapping population.

accessions had similar rates of polymorphism relative to *L. esculentum.* By comparison, *L. pennellii* was polymorphic for only 63% of 532 tested probe × RE combinations in the same study. Of the six restriction enzymes used (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I), *Dra*I and *Eco*RI were the most informative (85 and 84% polymorphism rate, respectively), while *Bam*HI was the least (40%).

Segregation distortion in BC_1: Based on these survey results, the BC_1 *L. esculentum* × *S. lycopersicoides* population (hereinafter designated BC-LS) was genotyped at 93 informative loci, consisting of 71 RFLPs, 20 isozymes, and 2 morphological markers. Single-locus segregations were consistent with the expected 1:1 Mendelian ratio at over 75% of loci. However, significant segregation distortions were indicated by chi-square analysis for loci on chromosomes 2, 5, 6, and 9 (Figure 1). On chromosomes 2 and 5, a deficiency of heterozygotes was observed. The most extreme distortion of this type was seen on chromosome 2, affecting most of the long arm, but most strongly around *Est-1.* The opposite trend, an excess of heterozygotes, was observed for distal markers on the short arm of chromosome 9 (most extreme toward TG18) and the end long arm of chromosome 6 (near TG220). For each chromosome, the degree of segregation distortion along the chromosome was consistent with selection at a single locus, positive or negative with respect to the *S. lycopersicoides* allele.

The distribution of genome ratios in the BC-LS population was approximately normal (Figure 2). The overall mean of 76.3% *L. esculentum* genome is quite close to the predicted value of 75% for a BC_1 population. This is consistent with the segregation trends outlined above: the number of loci showing an excess of homozygotes was roughly equal to the number showing an excess of heterozygotes, leading to minimal net effect on the average genomic constitution of the population. A wide range was seen in genome ratios, with two plants at or above 95% (Figure 2). By comparison, six simulated BC_1 populations generated with QGENE, consisting of population sizes, chromosome numbers, and marker density comparable to the BC-LS, yielded maximum genome ratios of 90% or less (data not shown).

Construction of BC_1 linkage map: Linkage maps based on marker data from the BC-LS population were generated with MAPMAKER and compared to the F_2 *L. esculentum* × *L. pennellii* (designated F2-LP) maps of Tanksley *et al.* (1992). The marker data assorted into 15 linkage groups, with 1 linkage group corresponding to each chromosome, except chromosomes 1, 4, and 12, which were represented by 2 linkage groups each (Figure 3). Adjacent markers on the two chromosome 1 linkage groups (TG83 and TG17) had a subthreshold LOD score of 2.0 in the BC-LS, but were separated by a recombination fraction of only 0.32 (or 37.4 cM), close to the 34 cM distance between these markers on the F2-LP map. For chromosome 4, adjacent markers on the two linkage groups had both a nonsignificant LOD and recombination fraction, for a distance that corresponds to 43.3 cM on the F2-LP map. The situation for chromosome 12 was similar to chromosome 1, with a subthreshold LOD of 2.1 for markers TG111 and *Dia-4*, but a significant recombination fraction of 0.32 (38 cM), suggesting linkage, and close to the approximate value of 36 cM from the F2-LP map.

Map synteny: For the purpose of determining map synteny, it was assumed that the RFLP, isozyme, and morphological markers would detect orthologous loci in *L. esculentum*, *L. pennellii*, and *S. lycopersicoides.* Indeed, with few exceptions, the order of markers in each of the linkage groups indicated by the BC-LS data was consistent with linkage relations predicted from the F2-LP maps (Figure 3). Therefore, previously mapped loci were used as "framework" markers as a starting point to compare different possible gene orders and to determine the placement of previously unmapped loci, such

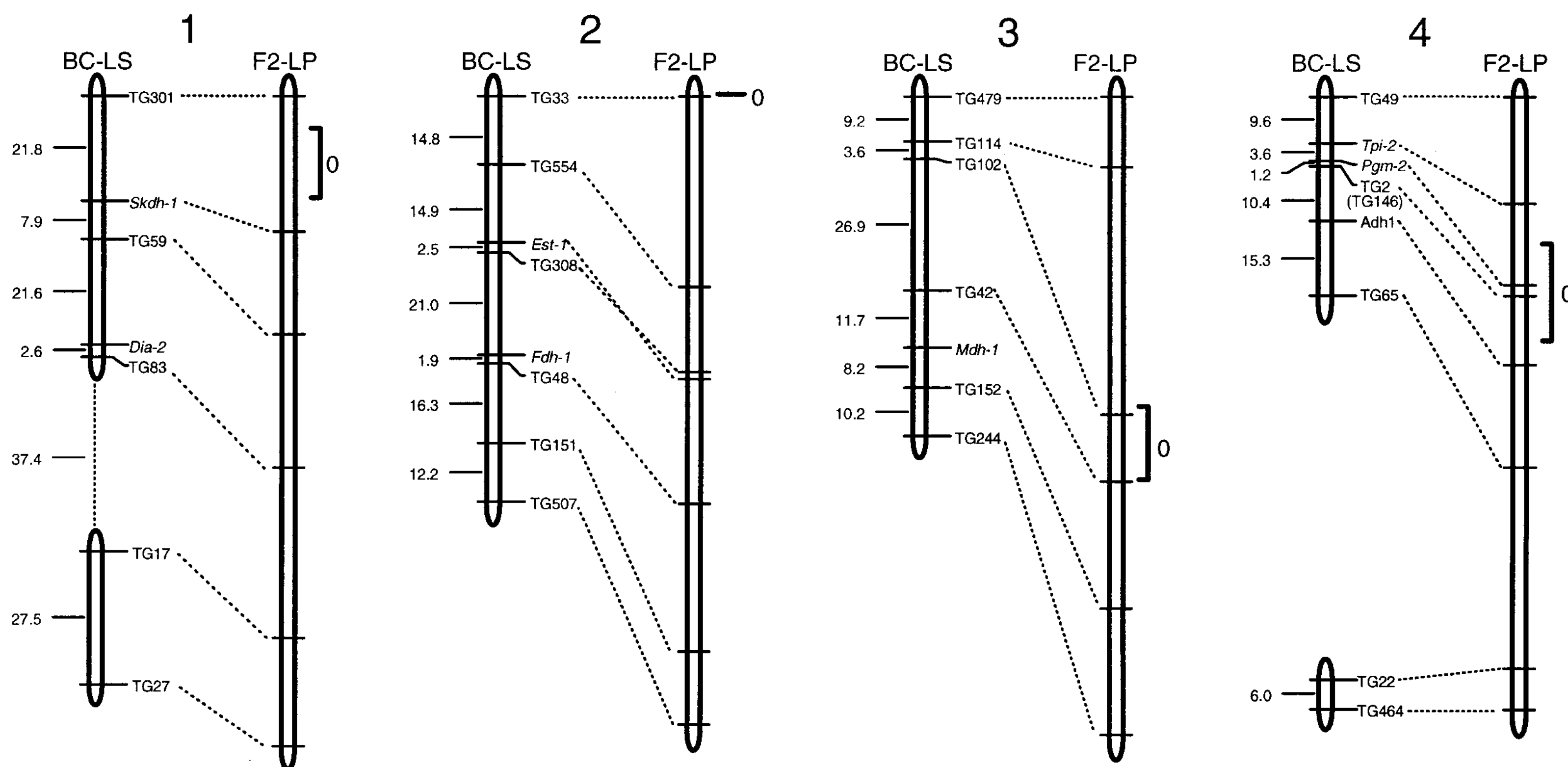

Figure 3.—Comparison of genetic maps based on recombination in BC_1 *L. esculentum* × *S. lycopersicoides* (BC-LS) and F_2 *L. esculentum* × *L. pennellii* (F2-LP; Tanksley *et al.* 1992). Dashed lines connecting BC-LS and F2-LP maps indicate shared markers. The indicated BC-LS linkage groups are statistically significant at LOD ≥ 3.0 and recombination fraction ≤0.4, except those connected by dashed lines wherein the LOD (but not RF) is subthreshold. Markers in parentheses are shown at their approximate locations. The position of centromeres on the F2-LP maps are from Fulton *et al.* (1997). All distances are Kosambi map units. Chromosomes are drawn to scale according to centimorgans.

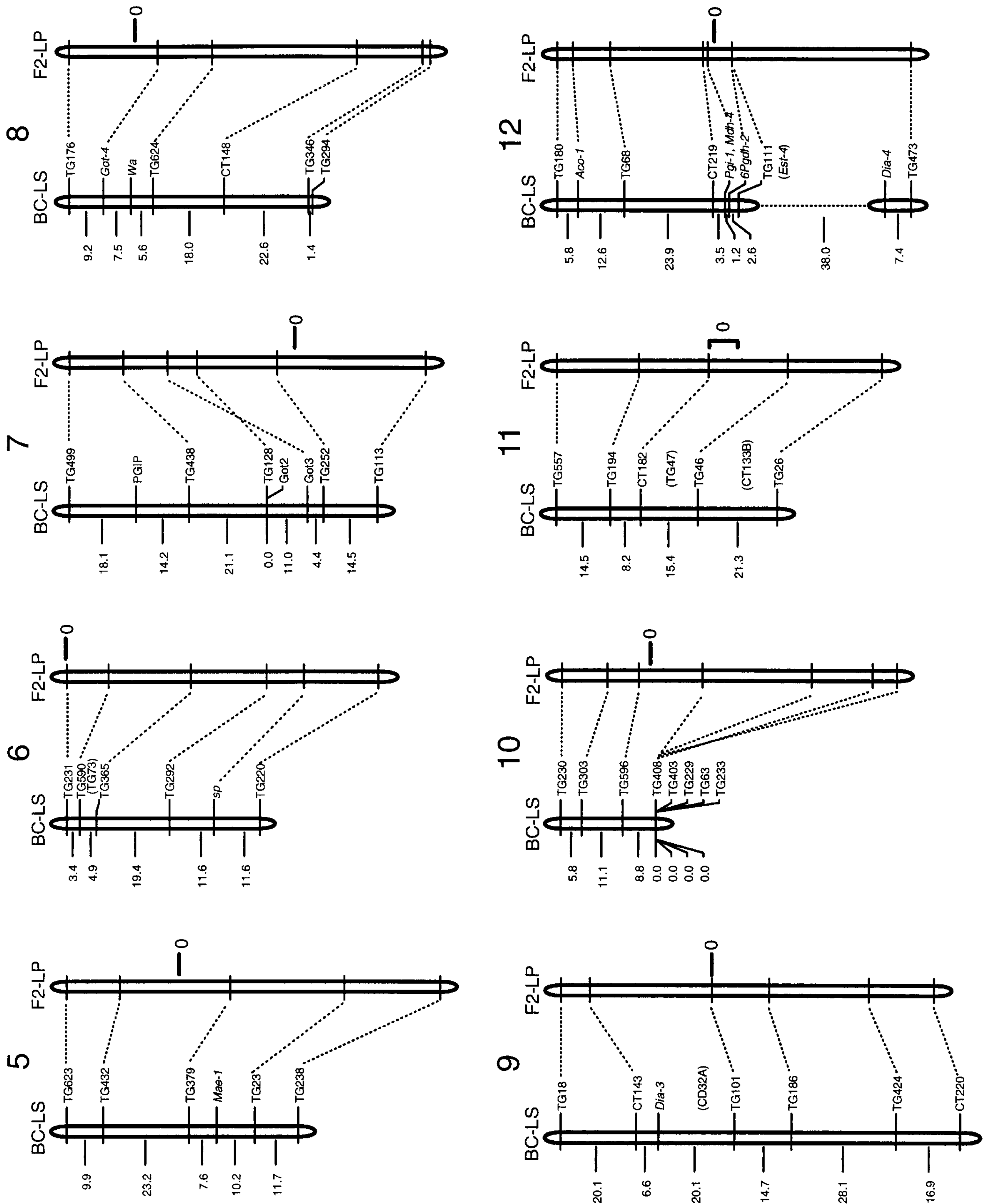

Figure 3.—*Continued.*

as isozyme coding genes, morphological markers, and secondary RFLP loci.

Apparent exceptions to overall map colinearity were found for markers on chromosomes 2, 4, and 7 (Figure 3). For chromosome 2, TG308 and *Est-1* were inverted relative to the F2-LP map. However, the position of *Est-1* on the F2-LP map was approximated from a separate population, indicating that its precise location is unknown and could be consistent with the BC-LS map. Furthermore, for all other markers on chromosome 2, the BC-LS and F2-LP maps were colinear. Analysis of backcross-inbred lines derived from the BC_1 (Chetelat and Meglic 1999) also supported colinearity on chromosome 2. For chromosome 4, the markers TG146 and CT133 showed unexpected linkage relations. TG146 mapped to the interval TG2–*Adh-1* rather than to the interval *Tpi-2–Pgm-2.* However, TG146 was a low confidence marker on both the BC-LS and F2-LP maps; hence minor differences in its locations are not likely to be significant. CT133, on the other hand, mapped to an entirely different linkage group: chromosome 11 instead of 4. Since the insert size following amplification of this probe was correct, the possibility of contamination with other sequences is unlikely. Therefore, the CT133 probe probably detected a secondary (duplicate) locus (*i.e.*, CT133B) on chromosome 11 for which the BC-LS population was polymorphic due to the greater genetic distance compared to F2-LP. No other chromosome 4 and 11 markers were exchanged in this fashion, indicating that a translocation between them is unlikely. Finally, the chromosome 7 marker *Got-3* mapped to a position proximal to TG128 and *Got-2* in the BC-LS population, instead of the opposite relationship as indicated by the F2-LP map (in which the position of *Got-3* is approximated from another population). The BC-LS map for this region is supported by analysis of the backcross-inbred lines, in that introgressed segments carrying the *S. lycopersicoides* alleles for TG252 and TG113 also carry *Got-3*, whereas introgressions spanning TG438 and TG128 are homozygous *L. esculentum* for *Got-3* (Chetelat and Meglic 1999).

Previously unmapped loci: Several isozyme loci were placed on the molecular map for the first time in the BC-LS population, including three diaphorase genes (*Dia-2*, *-3*, and *-4*), a malate dehydrogenase gene (*Mdh-1*), and one malic enzyme locus (*Mae-1*; Figure 3). *Dia-2* mapped to chromosome 1, at a position just proximal to TG83, a result consistent with previous data indicating a location distal to *Skdh-1* (Bournival *et al.* 1988). *Dia-3* mapped to the short arm of chromosome 9, between CT143 and TG101, a position consistent with earlier data indicating a locus on the short arm, at a distance of 23 cM from the *ah* (anthocyaninless of Hoffmann) gene (Chetelat *et al.* 1993). *Dia-4* mapped to chromosome 12L, proximal to TG473. *Mdh-1* mapped to chromosome 3, between the markers TG42 and TG142, a position consistent with previous data indicating a location on the long arm, between the genes *bls* (baby lea syndrome) and *sf* (solanifolia; Chetelat and DeVerna 1993). *Mae-1* mapped to chromosome 5L, between TG379 and TG23. A malic enzyme cDNA (LeME2) was independently mapped to this region of chromosome 5 in another F2-LP mapping population (Chetelat *et al.* 1999).

Of the two morphological loci on the BC-LS map, only the position of *sp* (self-pruning) relative to molecular markers was known (van Wordragen *et al.* 1996). The gene *Wa* (white anthers) was mapped previously to the interval between markers *dl* (dialytic) and *ae* (entirely anthocyaninless) and distal to *Got-4* on the long arm of cchromosome 8 (Rick *et al.* 1988). Its position on the BC-LS map, between *Got-4* and TG624, is consistent with these data. On the other hand, analysis of introgressed segments derived from the BC-LS population indicated *Wa* could be on either side of TG624, with a distal position slightly more likely (data not shown); however, fewer than 13 introgression lines were informative in this regard (*i.e.*, were heterozygous or homozygous for the *S. lycopersicoides* alleles at *Wa* and TG624), and therefore the position indicated by the BC-LS data is probably more accurate.

Comparison of recombination rates: For the majority of chromosomes, significant reductions in recombination rates relative to the F2-LP map were observed (Table 1; Figure 3). The total genetic length of all linkage groups in the BC-LS was only 73% of the distance between the same markers on the F2-LP map. The most extreme recombination reductions were seen on chromosomes 3, 6, and 10, which had only 55, 55, and 29%, respectively, of the total F2-LP map units for the same linkage groups. In contrast, recombination rates on chromosome 12 were normal and on chromosome 9 were somewhat higher than expected. Heterogeneity in recombination rates within individual chromosomes was also observed (Figure 3). The most extreme recombination reductions were generally in pericentric regions: this was the case for chromosomes 1, 2, 3, 7, and 8. The interval TG18–CT143 accounted for most of the increase in genetic length of chromosome 9 in BC-LS relative to F2-LP. Finally, recombination was virtually eliminated on the long arm of chromosome 10, from marker TG408 to TG233 (Figure 3), representing a distance of 53 cM on the F2-LP map. In contrast, recombination was reduced to a much lesser extent (69% of F2-LP) on the short arm of this chromosome.

Genome-wide reductions in recombination have previously been reported in male *vs.* female gametes (de Vicente and Tanksley 1991; Ganal and Tanksley 1996). In this study, the BC-LS maps are based on recombination in male gametes of the F_1 *L. esculentum* × *S. lycopersicoides* hybrid, whereas the F2-LP maps are an average of both male and female recombination rates. Therefore, to evaluate whether the recombination suppression noted above could be purely a product of the direction of the cross, the BC-LS maps were also compared to BC_1 *L. esculentum* × *L. pennellii* (BC-LP) maps

TABLE 1

Comparison of the genetic length of each chromosome in maps derived from BC_1 *L. esculentum* × *S. lycopersicoides* and F_2 and BC_1 *L. esculentum* × *L. pennellii* populations

	Total centimorgans			Map ratio	
Chromosome	BC-LS (♂) map	F2-LP map[a]	BC-LP (♂) map[a]	BC-LS/F2-LP	BC-LS/BC-LP
1	118	128	122	0.92	0.97
2	85	124	110	0.69	0.77
3	70	126	117	0.55	0.60
4[b]	46	68	69	0.68	0.67
5	63	97	107	0.64	0.59
6	51	92	103	0.55	0.50
7	83	92	98	0.90	0.85
8	64	92	83	0.70	0.77
9	107	97	118	1.10	0.91
10	26	90	85	0.29	0.31
11	59	83	93	0.72	0.64
12	95	91	93	1.04	1.02
All	867	1180	1198	0.73	0.72

For the BC-LS (this study) and BC-LP (de Vicente and Tanksley 1991) maps, the respective F_1 hybrids were used as male parents in crosses to cultivated tomato. The total lengths of F2-LP (Tanksley *et al.* 1992) and BC-LP maps were adjusted to include only marker intervals shared with the BC-LS map.

[a] F2- and BC-LP maps include only intervals tested in BC-LS; also, BC-LP map adjusted to include distal markers on F2-LP map by assuming a constant map ratio of BC-LP/F2-LP.

[b] Does not include map distances between two linkage groups for this chromosome on BC-LS map.

in which the F_1 hybrid was used as male parent (Table 1). This comparison produced essentially the same conclusions as the F2-LP: overall recombination in BC-LS was reduced ~25% relative to BC-LP, affecting all chromosomes (particularly 5, 6, and 10) except 12; no chromosomes had elevated recombination rates. These results reinforce the conclusion that the recombination suppression observed in this study was significant and widespread and not solely due to the direction of the cross.

DISCUSSION

The majority of marker loci scored in the BC-LS population segregated in the expected Mendelian fashion, with the exception of loci on chromosomes 2, 5, 6, and 9. Segregation distortion is common in wide crosses of tomato and other species (Zamir and Tadmor 1986), presumably reflecting the effects of competition among gametes or selection at postzygotic stages. We previously examined segregation patterns in a separate BC-LS population in which the F_1 hybrid was used as female parent (Chetelat *et al.* 1989). The chromosomal region around *Pgi-1* on chromosome 12 showed significant segregation distortion in favor of homozygotes in the earlier study, yet behaved normally in male-derived BC-LS. Conversely, the regions showing an excess of heterozygotes in the present study (chromosomes 6 and 9) behaved normally in the reciprocal cross. Although the two BC-LS populations are not strictly comparable (bridging lines derived from *L. pennellii* were used as male parent in the earlier study), the data do suggest that the direction of the cross plays an important role. Similarly, reciprocal backcross populations of *Aegilops tauschii* showed significant segregation distortion at loci on chromosome 5D, but only when the heterozygous F_1 was used as male parent (Faris *et al.* 1998). Such trends probably reflect the greater opportunity for competition among male than female gametes. This is supported by our observation of occasional aneuploids ($2n + 1$, $2n + 2$) in female-derived BC-LS (Chetelat *et al.* 1989), whereas male-derived BC-LS included only diploids (Chetelat *et al.* 1997), indicating a strong selection for balanced gametes in pollen.

Furthermore, by plotting the percentage of heterozygous individuals at each locus along the chromosome, the present study showed that the degree of segregation distortion peaked near one or two markers on each affected chromosome. This suggests the presence of a small number of loci under relatively strong selection pressure on each chromosome, rather than selection at multiple loci. Similarly, the *Ae. tauschii* study supported the presence of a single segregation distorter locus on each affected chromosome, except 5D, which had three distorter loci (Faris *et al.* 1998). The single-locus model implies that the indirect cause of skewed segregation at most (if not all) marker loci is linkage rather than direct selection. In this study, this trend may have been accentuated by the reduced recombination observed on most chromosomes, which would tend to mask the effects of weaker or opposite selection at secondary loci.

A large excess of heterozygotes for markers on chromosome 9S, particularly TG18, was observed in this study, the pattern of segregation distortion along the

chromosome suggesting selection at a distal locus. Similar results were obtained in a BC_1 *L. esculentum* × *L. peruvianum* population (Fulton *et al.* 1997), also created using the F_1 hybrid as male parent. In the *L. peruvianum* study, segregation distortion peaked at the most distal marker examined on 9S (GP39, ~14.4 cM distal to TG18), with a maximum value of 100% heterozygous individuals. The authors attributed these results to the action of the gamete promoter gene (*Gp*) in *L. peruvianum*, which is inherited preferentially over the *L. esculentum* allele in segregating populations (Pelham 1968; Fulton *et al.* 1997). The behavior and location of the segregation distorter gene on chromosome 9S in BC-LS are so similar to those reported for *Gp* that they suggest this gene may be conserved at a functional level in *L. peruvianum* and *S. lycopersicoides.*

The average percentage of recurrent parent genome in the BC-LS population was approximately normal. This contrasts with a series of backcross-inbred lines derived therefrom, which showed a strong tendency for accelerated recovery of the *L. esculentum* genome (Chetelat and Meglic 1999). Several factors account for this difference. First, only a subset of the BC-LS individuals was sufficiently fertile and compatible with *L. esculentum* to produce seed; these plants presumably had higher-than-average genome ratios, since the presence of multiple *S. lycopersicoides* chromosomal segments reduced fertility. Second, an excess of homozygotes on one chromosome tended to be balanced by an excess of heterozygotes on another; in later generations, individual families would have only a fraction of the genome represented; hence this compensatory effect would be absent. Third, the degree of segregation distortion was more severe in later backcrosses, affected more chromosomal regions than in BC-LS, and nearly always resulted in an excess of *L. esculentum* alleles. Last, homozygous *S. lycopersicoides* introgressions were strongly associated with sterility in backcross-inbred plants, thereby preventing fixation and increasing chances for the loss of wild alleles over successive generations.

The genetic maps based on BC-LS showed a high level of overall synteny with those based on F2-LP (Tanksley *et al.* 1992). Only minor differences in gene order were detected, all of which were either low confidence markers on the F2-LP map, or involved tightly linked gene pairs for which informative recombinants were rare. These few map differences certainly do not support the hypothesis that chromosomal rearrangements, such as inversions or translocations, distinguish the *S. lycopersicoides* genome from *L. esculentum.*

In particular, there was little evidence on most chromosomes for the existence of paracentric inversions, such as those reported in potato (*S. tuberosum*) relative to tomato (Tanksley *et al.* 1992). In a heterozygous F_1, paracentric inversions would normally be manifested as a recombination suppression within the inverted region, since crossovers would lead to deficiencies and duplications, neither of which is normally transmitted through male gametophytes of tomato. Recombination suppression was in fact observed for most chromosomes in this study (and virtually eliminated on chromosome 10L); however, with the exception of 10L, the regions with the most extreme recombination suppression in BC-LS did not correspond with the chromosome arms distinguished by inversions in potato *vs.* tomato, namely 5S, 9S, 10L, 11S, and 12S. For example, recombination was increased relative to the F2-LP map on chromosome 9S, normal on 12S, yet greatly reduced in other regions, such as chromosomes 3 and 6, over which the potato and tomato genomes are apparently colinear. In the case of chromosome 10, recombination was completely suppressed on the long arm, yet reduced by only 30% on the short arm (comparable to the genome-wide reduction). This result may indicate a chromosomal rearrangement such as a paracentric inversion on 10L in *S. lycopersicoides* relative to its *L. esculentum* homeologue.

Yet observations of chromosome pairing at pachytene in the F_1 hybrid provided little evidence of structural differentiation between the genomes (Menzel 1962). For example, chromosomes of the two species were visually similar and completely synapsed in all 23 euchromatic regions (chromosome 2S is heterochromatic), while only 4 out of the 20 heterochromatic regions showed differences in length; only sporadic inversion, deletion, or translocation configurations were observed. Since nearly all recombination nodules detected in tomato synaptonemal complexes are located in euchromatin (Sherman and Stack 1995), the normal synapsis and general absence of inversion loops in these regions strongly support the hypothesis of a high degree of colinearity between the *S. lycopersicoides* and *L. esculentum* genomes indicated by this study. This conclusion is also supported by molecular marker genotypes of backcross-inbred lines derived from BC-LS, which were generally concordant with the F2-LP map (Chetelat and Meglic 1999). However, introgressions representing *S. lycopersicoides* chromosome 10L (or segments thereof) were exceedingly rare and never homozygous in these lines, consistent with a severe recombination restriction and possible structural differentiation in this region.

On several chromosomes, the most pronounced map shrinkage was seen in pericentric marker intervals. This observation is consistent with the suppression of recombination inferred by marker distribution in the F2-LP map (Tanksley *et al.* 1992) and directly observed through recombination nodule frequencies at pachytene of *L. esculentum* (Sherman and Stack 1995). That recombination in pericentric regions would be reduced even further in BC-LS might suggest that differentiation between these homeologous genomes is greatest near centromeres. In meiosis of the F_1 *L. esculentum* × *S. lycopersicoides* hybrid, Menzel (1962) concluded that differences in pericentric heterochromatin inhibit chiasma formation in euchromatic regions on the same

arm, while enhancing it in euchromatic regions on the opposite arm. DNA sequence comparisons in Drosophila species found normal rates of interspecific divergence for genes in regions of reduced crossing over per physical distance, whereas the same regions showed very low rates of intraspecific variation (Begun and Aquadro 1992); available data in Lycopersicon are insufficient to estimate rates of interspecific differentiation along the chromosome, but do support the extremely low level of intraspecific sequence variability observed near centromeres (Stephan and Langley 1998). Therefore, there is little evidence that centromeric recombination suppression reflects a higher interspecific divergence in proximal regions.

Recombination suppression was not restricted to centromeric regions in BC-LS, however, as several chromosomes showed similar reductions along their entire length. This effect was even more pronounced in later generations, in which recombination in some intervals was reduced 200-fold or more (Chetelat and DeVerna 1993; Chetelat *et al.* 1993; Chetelat and Meglic 1999). In several lines, intact *S. lycopersicoides* chromosomes were transmitted through multiple generations apparently without recombination events. Studies are currently underway to ascertain whether these effects are associated with pairing suppression in the introgression lines. In the F_1 hybrid, chiasma frequency at metaphase I was reduced ~20% compared to *L. esculentum* (Menzel 1962), and an average of 2.8 univalents per cell was observed (Y. Ji and R. T. Chetelat, unpublished results). These values agree relatively well with the ~27% reduction in overall map length in BC-LS relative to F2-LP, suggesting that reduced chiasma frequency is the principal cause of decreased recombination rates. This effect is probably best explained by the high degree of DNA sequence divergence between *L. esculentum* and *S. lycopersicoides* homeologues predicted from their biosystematic relationships and confirmed by the high rate of marker polymorphism detected in this study. Additionally, the putative rearrangement on chromosome 10L may further reduce observed recombination frequencies.

Biosystematic studies indicate that *S. lycopersicoides* is the most distant wild relative of cultivated tomato (*L. esculentum*) with which it can be directly hybridized by conventional sexual crosses (Rick 1979). Taxonomically, *S. lycopersicoides* has been grouped with three other tomato-like nightshade species (*S. juglandifolium*, *S. ochranthum*, and *S. sitiens*) in series Juglandifolia (Correll 1962). A recent taxonomic revision (Spooner *et al.* 1993) places only *S. sitiens* in the same clade (subsection) as *S. lycopersicoides* to indicate their especially close relationship and subsumes the entire Lycopersicon genus under Solanum. The overall synteny between the *L. esculentum* and *S. lycopersicoides* genomes indicated by this study does suggest a closer relationship than either species exhibits to other Solanum taxa, such as potato (*S. tuberosum*), from which they are differentiated by several inversions. Also, despite the sterility, disrupted chromosome pairing, and recombination suppression exhibited by F_1 *L. esculentum* × *S. lycopersicoides* hybrids, it was possible to introgress a large portion of the nightshade genome into the cultigen (Chetelat and Meglic 1999). However, many introgressed regions resisted fixation due to recessive sterility factors and severe segregation distortion in later generations. These barriers to introgression could owe at least in part to deleterious recessives present in this allogamous (*i.e.*, heterozygous) wild species, but not expressed until artificial inbreeding in later generations. However, these trends also suggest a high degree of differentiation between the species at a sequence level, a hypothesis supported by the ~80% polymorphism rate observed in this study using single-copy RFLP probes. As a whole, our research reveals severe restrictions on recombination and introgression of *S. lycopersicoides* traits into *L. esculentum* and, hence, a wide phylogenetic hiatus between these taxa. The observed obstacles seem to exceed those for two species of the same genus and certainly impede attempts at experimental introgression.

Although these experiments were motivated by a desire to extend the limits of introgressive hybridization in tomato and have potential applications for tomato improvement, the results are also of more general significance to the study of homeologous recombination. In this field, polyploid wheat and its wild relatives represent the model system, with significant advantages in terms of ease of cytological studies and the availability of mutants for genes such as *Ph* that affect pairing and recombination. Among diploid species, tomato and its wild relatives, particularly *S. lycopersicoides*, provide excellent germplasm for studying homeologous pairing relationships. The maps of recombination presented herein complement earlier cytological work, and a variety of genetic stocks are now available, including alien addition, substitution, and introgression lines. We therefore suggest these species provide a practical system for studies of homeologous pairing and recombination in a diploid plant genome.

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