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Molecular mapping of chromosome segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*)

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Abstract The wild nightshade *Solanum lycopersicoides* (accession LA2951) was backcrossed to the cultivated tomato (*Lycopersicon esculentum* cv 'VF36'), then inbred through single-seed descent for several generations. Over 300 backcross-inbred families thereby derived were genotyped at 139 marker loci, consisting of RFLPs, allozymes, and monogenic morphological markers, to identify introgressed *S. lycopersicoides* chromosomes and segments thereof. The pattern of genotypes observed in the lines indicated a high degree of overall synteny between the *S. lycopersicoides* genome and that of tomato. Two putative single-copy RFLP probes revealed secondary loci in this wide cross. Recovery of the *L. esculentum* genome was more rapid than expected, with an average value in the BC_2 generation of 97.8%, versus the expected value of 87.5%. This was due to widespread segregation distortion that favored *L. esculentum* alleles as well as a tendency for plants homozygous for introgressed segments to be partially or completely male-sterile, thereby preventing the fixation of *S. lycopersicoides* markers in many lines. Despite these difficulties, nearly every *S. lycopersicoides* marker (or approximately 98% of the genome, measured in centi Morgans) was represented in at least 1 backcross-inbred line, with only a region on chromosome 4L missing from the population as a whole. Although the extent of transmission and fixation of introgressed segments varied according to chromosome, overall approximately 66% of the *S. lycopersicoides* genome was represented by homozygous introgressions with sufficient fertility to reproduce by self-pollination. An excess of terminal (vs. interstitial) segments was noted, and putative heterozygous substitutions for chromosomes 6, 7, 8, and 10 were found. Recombination within certain introgressed regions was reduced over 100-fold. These backcross-inbred lines are expected to facilitate the genetic analysis of traits identified in *S. lycopersicoides* and their transfer into horticultural tomatoes.

Key words Comparative mapping · Homoeologous recombination · Segregation distortion · Solanaceae · Tomato

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Introduction

The primary gene pools of many crop plants are so depleted in genetic variability that breeders have relied upon wild relatives for sources of disease resistance and other traits (Harlan 1976). Although crop germplasm collections contain thousands of potentially useful wild accessions, their utilization is sometimes hindered by hybridization barriers preventing interspecific crosses and/or by undesirable characteristics inherent in exotic germplasm. The advent of molecular marker maps for many crops permits a more systematic and efficient approach to introgression experiments (Tanksley and McCouch 1997). Marker-based estimates of genetic variability within and between accessions allow a more rational and efficient sampling of genebanks. Also, saturated marker maps make it is possible to introgress entire alien genomes by the construction of segmental substitution lines or other prebreds.

Recent applications of sexual and somatic hybridization techniques have enabled the introgression of genes from more distantly related wild species. In many cases, the use of molecular marker maps has facilitated a precise analysis of the progeny of such hybridization and introgression experiments. Examples include the addition of specific maize chromosomes to the oat genome (Riera-Lizarazu et al. 1996), tomato chromosomes added to potato (Garriga-Caldere et al. 1998), and various types of additions or segmental substitutions in rice (Brar and Khush 1997) and wheat (Friebe et al. 1996). The prebred

lines resulting from such wide hybridizations are useful as sources of economic traits (e.g., disease or insect resistance, stress tolerance, etc), as well as tools for genome mapping [e.g., QTL (quantitative trait locus) identification, map-based cloning, etc.].

These trends are well-illustrated in tomato (*Lycopersicon esculentum*), a crop possessing very little inherent diversity (Rick and Fobes 1975) but rich *ex situ* collections of over 1000 wild accessions (Chetelat and Rick 1998) representing nine *Lycopersicon* and four related *Solanum* taxa. Although crosses between tomato and all but two of these species are possible, surprisingly few prebred lines are available. This situation has been ameliorated by the development of a high-density restriction fragment length polymorphism (RFLP) map based on an F_2 *L. esculentum*×*L. pennellii* population (Tanksley et al. 1992). This map was used to characterize a set of 50 overlapping introgression lines representing an entire genome of *L. pennellii* in tomato (Eshed and Zamir 1995). These lines have subsequently proven useful for various genome mapping and introgression projects. Additionally, a recombinant inbred population resulting from the cross *L. esculentum*×*L. cheesmanii* is potentially useful for high-resolution mapping purposes (Paran et al. 1995). Other prebreds, such as backcross-inbred lines containing introgressed QTLs for specific traits [QTL-NILs (near-isogenic lines)], have been described for *L. hirsutum* and *L. pimpinellifolium* (Bernacchi et al. 1998). Collections of similar prebred lines representing the other tomato wild relatives would be valuable.

Advances in wide hybridization have extended the limits of introgression to include the nightshades *S. lycopersicoides* (Rick et al. 1986) and, to a lesser extent, *S. sitiens* (DeVerna et al. 1990). *S. lycopersicoides* possesses extreme cold tolerance, insect and disease resistances (Rick 1988) and possibly other horticultural traits expressed only in hybrids with tomato. Because F_1 *L. esculentum*×*S. lycopersicoides* hybrids are both unilaterally incompatible with tomato (i.e., style rejects pollen of *L. esculentum*) and essentially male-sterile (Chetelat et al. 1997), introgressing genes from this species has been difficult. A set of monosomic alien addition lines (MAALs) representing 10 out of 12 *S. lycopersicoides* chromosomes in a tomato background, have been assembled (Chetelat et al. 1998). Though potentially useful for the construction of chromosome-specific libraries (Ananiev et al. 1997) and for other mapping purposes, MAALs are not ideal as sources of prebred germplasm due to (1) the inevitable segregation of alien chromosomes in each generation (disomic additions would solve this limitation but are rare and infertile in tomato), and (2) the potential loss of chromosome integrity due to homoeologous recombination. Diploid recombinants identified in progenies of MAALs demonstrated the feasibility of transferring monogenic characters but did not provide an efficient method of genome-wide introgression (Rick et al. 1988).

The goal of the experiments described herein was to derive a set of overlapping, homozygous introgression lines representing the genome of *S. lycopersicoides* in a diploid tomato background. We previously described a single F_1 *L. esculentum*×*S. lycopersicoides* hybrid with moderate male fertility through which direct backcrosses to diploid *L. esculentum* were possible (Chetelat et al. 1997). We report herein the synthesis of a set of backcross-inbred lines derived from this hybrid and the identification of introgressed regions using molecular markers. Segregation of *S. lycopersicoides* chromosome segments and recombination with homoeologous *L. esculentum* regions was studied. The prospects for developing a complete library of homozygous introgressions, and their potential uses in breeding and genetics, are discussed.

Materials and Methods

Plant Material and Hybridizations

The parental genotypes used in this study, *L. esculentum* cv 'VF36' and *S. lycopersicoides* LA2951, the F_1 hybrid between them (90L4178), and the first backcross to 'VF36' are described in Chetelat et al. (1997). Of the 281 BC_1 plants obtained by embryo culture, 58 plants (21%) produced seed, the majority of which was obtained from pollinations with 'VF36'. BC_2s (and a limited number of BC_1s) were then inbred for a variable number of generations (up to 6) by self-pollination using single seed descent beyond the $BC_{(1–2)}F_2$ generation. Pollinations were carried out under field or greenhouse conditions; flowers were allowed to self-pollinate naturally, or, if they failed to set fruit (e.g., due to low fertility or stigma exsertion), were manually self-pollinated with a dissecting needle. Seed were treated with 2.5% sodium hypochlorite (half strength household bleach) for 30 min, rinsed in running water for several minutes, then germinated on moist filter paper in sandwich boxes at 25°C. After emergence and expansion of the cotyledons, seedlings were transplanted to soil in the greenhouse.

RFLP analysis

For the backcross-inbred families, a roughly equivalent amount of leaf material was collected from each of 6 plants per family and bulked into a single 50-ml centrifuge tube. DNA was isolated from the bulk leaf samples essentially as described by Chetelat et al. (1995), except that a polytron was used to grind samples, and volumes were scaled up approximately tenfold. DNA samples (approx. 10 µg DNA per reaction) were digested with the restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, and *Dra*I according to manufacturer's instructions. Restriction fragments were separated by electrophoresis at 30 V for 18 h through 0.8% agarose gels using a 1× Neutral Electrophoresis Buffer system. Gels were blotted onto Hybond N+ membrane (Amersham) in 10× SSC and blots dried for 2 h at 80°C in a vacuum oven.

Genomic (TG) and cDNA (CT and CD) probes were provided by Dr. Steve Tanksley at Cornell University and were chosen based on their map locations (Tanksley et al. 1992) to provide coverage of the entire genome at intervals of less than 30 cM. A polygalacturonase inhibitor protein (PGIP) cDNA probe was provided by Dr. Ann Powell at the University of California – Davis. The map locations of PGIP and other markers not on the Tanksley map were based on a BC_1 *L. esculentum*×*S. lycopersicoides* mapping population (Chetelat et al. 1999). Probes were amplified from plasmid DNA by the polymerase chain reaction (PCR) as described (Chetelat and DeVerna 1991), then labeled with [^{32}P]-dCTP and/or [^{32}P]-dATP using the random hexamer primer method (Feinberg and Vogelstein 1983). Blots were washed three times to a final stringency of 0.5× SSC at 65°C.

Statistical Analysis

For the calculation of segment lengths and genome ratios, the half-intervals flanking a marker locus were considered to be of the same genotype; in other words, introgressed segments were assumed to extend halfway between the outermost marker for which a given line carried the *S. lycopersicoides* allele and the first adjacent marker for which the same line was homozygous for the *L. esculentum* allele. Since adjacent segments in different lines may not overlap, estimates of *S. lycopersicoides* genome representation are maximal values. For missing marker data, plants were assumed to have the same genotype as that of flanking markers. If flanking markers were not concordant, as would be expected around recombination events, then introgressed segments were assumed to extend halfway between the nearest informative markers, as described above. The Tanksley map was used to estimate distances between markers, the length of chromosomes and introgressed segments, and the overall genome size for genome ratio calculations; although the BC_1 *L. esculentum*×*S. lycopersicoides* map was largely colinear, it exhibited reduced recombination in many regions and did not include all of the markers scored in the backcross-inbreds, hence was not used for the statistical analyses. Simulated backcross-inbred populations were generated and analyzed with QGENE version 2.3 (Nelson 1997).

Results and Discussion

Production of Introgression Lines

From the original cross *L. esculentum* cv 'VF36'×*S. lycopersicoides* LA2951, 58 BC_1s were produced that exhibited sufficient fertility for further backcrossing or selfing (Chetelat et al. 1997). Of these, 32 BC_1s eventually produced viable backcross-inbred lineages, while seed from the remaining 26 either failed to germinate or progeny were sterile. During inbreeding plants were selected on the basis of a number of morphological traits which distinguish *S. lycopersicoides* from *L. esculentum*. These included the following *S. lycopersicoides*-specific monogenic characters (see Fig. 1 for chromosomal locations): aubergine fruit (*Abg*), beta-carotene fruit (*B*), brilliant corolla (*Bco*), dialytic anthers (Dl^s), laciniate leaf (*Lac*), indeterminate habit (sp^+), clear leaf veins (obv^+), and white anthers (*Wa*) (Chetelat 1998; Rick et al. 1988, 1994, 1996). Additional phenotypic traits, including several novel variants not observed in either parent, were selected for during line development. Early selection was also based on 21 informative isozyme loci (Chetelat et al. 1997). For each allozyme or morphological marker, the objective was to identify fertile plants containing the *S. lycopersicoides* allele, preferably in the homozygous state. Given the small population sizes (generally 6–24 per family), selection in each generation was usually limited to single characters.

In this fashion, five out of the eight morphological characters (*B*, sp^+, *Lac*, Dl^s, and *Wa*), and 15 out of the 21 isozyme markers were each fixed in at least 1 backcross-inbred line through selection in early generations. Although each of these lines produced seed after artificial self-pollination, most exhibited reduced seed set. This fecundity problem was due to low pollen production and/or fertility in most cases, as evidenced by normal fruit set following pollination with 'VF36'. Sterility was more extreme in field plantings, where high temperatures (>37°C) regularly encountered at Davis during the summer can prevent fruit set even on normally fertile genotypes. Also, homozygous introgressions tended to be more sterile than heterozygotes; plants homozygous for large introgressions frequently produced no seed at all, despite intensive manual selfing. Such highly sterile lines required hand pollination under more moderate greenhouse conditions. Many other lines presented few, if any, discernible morphological or allozymic differences vis-à-vis the recurrent parent. These were inbred by selecting 1 plant at random within each inbred line from which to collect seed for the following generation.

RFLP Analysis and Map Synteny

In order to more precisely identify regions of the *S. lycopersicoides* genome that had been introgressed, a group of backcross-inbred families were analyzed with RFLP markers. The 311 families examined had been backcrossed and/or selfed for varying numbers of genera-

Table 1 Number of *L. esculentum*×*S. lycopersicoides* backcross-inbred families analyzed, average number of introgressed segments in each, average heterozygosity, and average genome ratio

Generation	Number of lines analyzed	Number of segments/line[a]		Percentage heterozygosity[b]		Genome ratio (%)[c]	
		+/S	S/S	Expected	Observed	Expected	Observed
BC_1F_5-F_6	12	1.2	1.2	1.6–3.1	2.2	75.0	96.8
BC_2F_2	9	2.1	0.22	12.5	5.0	87.5	96.5
BC_2F_3	73	1.7	0.30	6.25	4.2	87.5	97.1
BC_2F_4	146	1.1	0.58	3.13	2.0	87.5	98.0
BC_2F_5	63	0.68	0.63	1.56	1.0	87.5	98.5
BC_2F_6	4	0.50	0.75	0.78	1.1	87.5	98.3
BC_3	3	1.7	0	12.5	1.6	93.8	98.1
BC_3F_3	1	3	1	3.13	4.8	93.8	96.3
Total	311	1.2	0.54	–	2.4	–	97.8

[a] S/S=homozygous; +/S=heterozygous for *S. lycopersicoides* segments
[b] Calculated as percentage of marker loci heterozygous out of total scored for the group
[c] Pertentage recurrent parent (*L. esculentum*) genome

tions, with the majority falling in the range of BC_2F_3 – BC_2F_5 (Table 1). DNA samples from several plants in each family were pooled and genotyped at 110 previously mapped RFLP loci detected with 108 single- or low-copy genomic and cDNA probes. These markers covered over 98% of the tomato genome, measured in genetic distance obtained from the Tanksley map; the remaining 2% represented genetic intervals distal to the markers used on each arm (Fig. 1). The average distance between markers was 11.5 cM. Comparing genotypes for adjacent markers in each line indicated that the 108 probes detected *S. lycopersicoides*-specific alleles at the expected (i.e., homologous) genetic loci. The results establish a high degree of overall synteny between the *L. esculentum* and *S. lycopersicoides* genomes.

The inferred colinearity of the two genomes agrees with previously obtained comparative mapping data around specific *S. lycopersicoides* isozyme and morphological markers (Chetelat 1998; Chetelat and DeVerna 1993; Chetelat et al. 1989, 1993; Rick et al. 1988), as well as with cytological observations of nearly complete bivalent pairing of the F_1 pachytene chromosomes (Menzel 1962; Menzel and Price 1966). Furthermore, a linkage map based on BC_1 *L. esculentum*×*S. lycopersicoides* provided little evidence for large rearrangements, except on chromosome IDL (Chetelat et al. 1999). On the other hand, small structural differences could not be ruled out, and would be consistent with the recombination suppression observed in the BC_1 map as well as cytological evidence of reduced crossing-over in the F_1 hybrid: decreased chiasmata frequency and the presence of univalents at diakinesis and metaphase-I (Menzel 1962; Rick 1951). As a rule, however, there is a high level of synteny between various interspecific and intraspecific maps involving *Lycopersicon* genomes. For example, no differences in marker order were discovered in genetic maps based on *L. esculentum*×*L. pimpinellifolium* (Grandillo and Tanksley 1996), *L. esculentum*×*L. hirsutum* (Bernacchi and Tanksley 1997), *L. esculentum*×*L. pennellii* (Tanksley et al. 1992), and *L. peruvianum*×*L. peruvianum* (van Ooijen et al. 1994) hybrids.

The only exceptions to the overall synteny revealed by the present study were probes that detected secondary loci or those that produced banding patterns that were not concordant with flanking markers. In the former category, the putatively single-copy probe TG267 detected not only the expected locus on chromosome 1, but also a second locus (TG267B) on chromosome 3, between TG152 and TG244 (Fig. 1); since TG267 detected at least two restriction fragments in all tested DNA samples, including *L. esculentum*, it appears that the TG267B locus exists in both species and was revealed because of the wider cross employed in this population compared to the Tanksley map (F_2 *L. esculentum*×*L. pennellii*). This is consistent with the higher overall rate of isozyme and DNA polymorphism between *L. esculentum* and *S. lycopersicoides* than between the former and *L. pennellii*; for example, in a separate survey of over 1151 probe×RE combinations, accessions of *S. lycopersicoides* differed from *L. esculentum* on average approximately 80% of the time, versus 63% for *L. pennellii* LA716.

Results for 3 additional probes (CT20, CT79, and CT133) were not concordant with those of the expected flanking markers. Although each probe had an insert size consistent with the predicted values, CT20 and CT79 produced banding patterns in *L. esculentum* and *L. pennellii* that were inconsistent with published survey blots (obtained from the SolGenes database at http://probe.nalusda.gov), indicating problems with these 2 probes in the present study. Survey data were not available on SolGenes for CT133, a marker for chromosome 4. However, this probe hybridized to more than one restriction fragment in each parent (*L. esculentum* and *S. lycopersicoides*), suggesting the presence of a duplicate locus (i.e., CT133B) polymorphic in this wider cross. The distribution of marker genotypes in the 311 families analyzed indicated a close association between CT133B and the chromosome 11L marker TG105A. CT133B also showed linkage to chromosome 11 in the BC_1 *L. esculentum*×*S. lycopersicoides* mapping population. No evidence for a translocation involving chromosomes 4 and 11 was found in either the BC_1 or the backcross-inbreds.

Furthermore, there is little data in the literature to suggest the presence of other naturally occurring chromosomal rearrangements differentiating *Lycopersicon* species. Fulton et al. (1997) found evidence for a small translocation involving 1 marker on chromosome 9 (out of 113 markers tested) in the backcrosses of *L. esculentum*×*L. peruvianum*. Comparisons of more distantly related nightshade species, on the other hand, have detected genomic rearrangements. For example, the *S. etuberosum* genome shows numerous inversions and translocations compared to potato (*S. tuberosum*) (Perez et al. 1999). Several paracentric inversions distinguish the related potato (*S. tuberosum*) and tomato genomes (Tanksley et al. 1992), although no interchromosomal translocations were found. In light of the potato studies, as well as results presented herein, it is unlikely that the more closely related *L. esculentum* and *S. lycopersicoides* genomes are differentiated by translocations. However, a possible paracentric inversion on chromosome IDL was inferred from the BC_1 map (Chetelat et al. 1999).

Genome Ratio and Heterozygosity

Marker analysis of the 311 lines indicated a greater than expected recovery of the recurrent parent genome at all stages of backcrossing and selfing (Table 1). For example, plants in the BC_2F_3 – BC_2F_5 group had an average recurrent parent genome contribution of around 97.8%,

Fig. 1 Map of selected chromosome segments introgressed from *S. lycopersicoides* into *L. esculentum* backcross-inbreds. *Solid bars* represent segments that are homozygous in at least 1 line, *open bars* segments that have not been fixed (i.e. heterozygous)

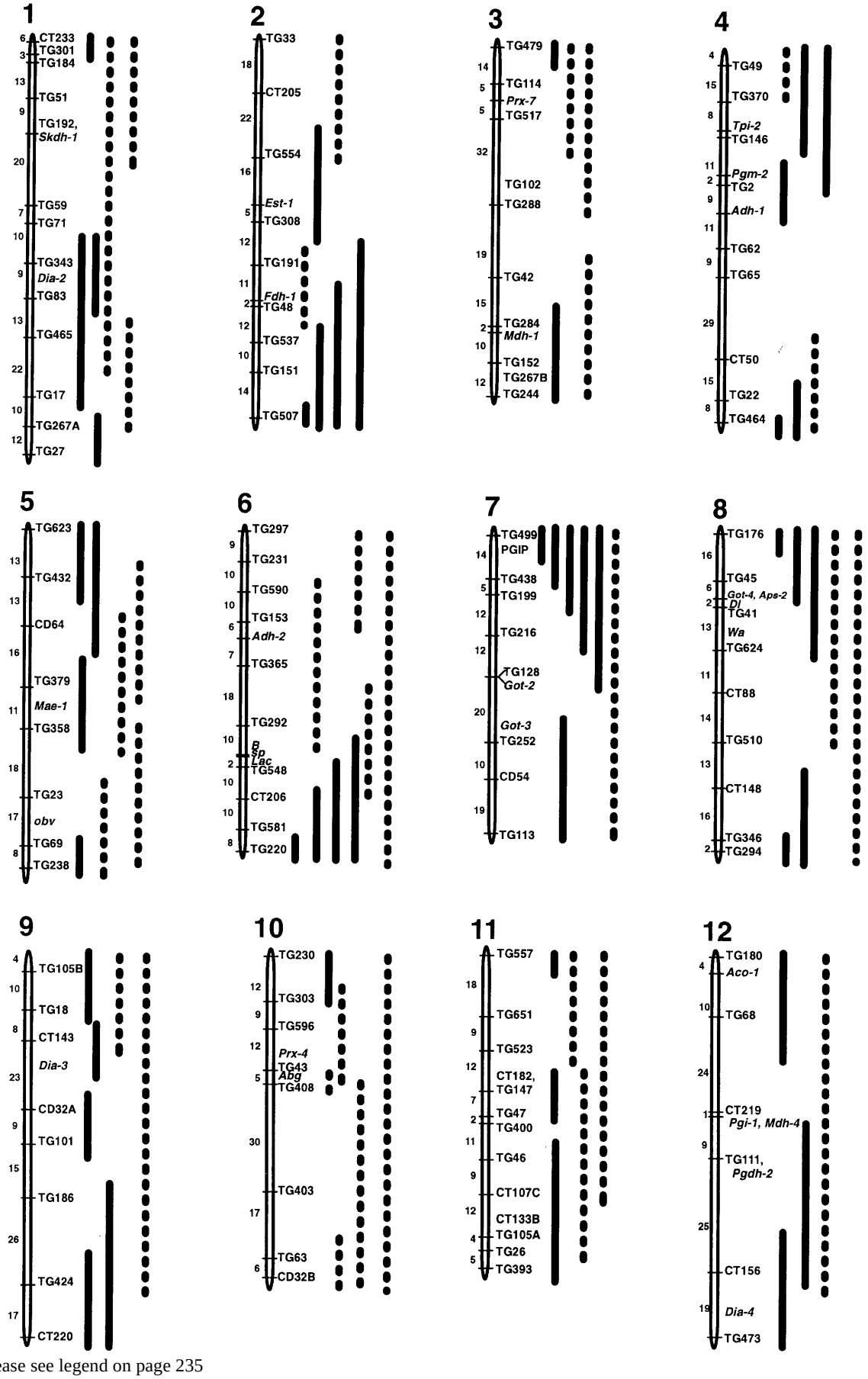

Fig. 1 Please see legend on page 235

compared to the expected 87.5%. Furthermore, with each generation of selfing from BC_2F_2 to BC_2F_6, the average genome ratio increased, indicating that *S. lycopersicoides* markers were being progressively eliminated. While the genome ratio of the population as a whole was skewed towards *L. esculentum*, a subset of 69 lines, chosen to maximize representation of the *S. lycopersicoides* genome, showed an average genome constitution closer to the expected value (Fig. 2). The average heterozygosity per locus was 2.4%, which was lower than expected for most generations (Table 1) due to an excess of plants homozygous for *L. esculentum* alleles and a deficiency of *S. lycopersicoides* homozygotes, at most loci.

Segregation distortion is a common phenomenon in wide crosses of many crop plants, including tomato. In backcross populations, alleles of the recurrent parent (normally *L. esculentum*) are often transmitted at a higher rate than those of the donor, thereby accelerating recovery of the recurrent parent genotype. For example, in the BC_1 *L. esculentum*×*S. lycopersicoides* population from which the present set of backcross-inbred lines was derived, segregation for 11 out of 24 loci deviated from the expected 1:1 ratio, the majority due to an excess of *L. esculentum* alleles (Chetelat et al. 1997). In addition, the process of inbreeding in the present study would have further altered transmission frequencies due to the deficiency of plants homozygous for *S. lycopersicoides* alleles at most loci. The few *S. lycopersicoides* homozygotes that were produced in progeny tests of specific segments were frequently sterile (see below). As a result, inbreeding created a greater opportunity for fixing *L. esculentum* alleles, and in this sense achieved the same objective as backcrossing. Comparable trends were observed following the inbreeding of F_1 *L. esculentum*×*L. cheesmanii* to produce a recombinant inbred population (Paran et al. 1995). After several generations of selfing, the average heterozygosity remained surprisingly high (15% vs. the expected 1.5% by F_7). This was due in part to a deficiency of *L. cheesmanii* homozygotes at many loci and the tendency towards reduced fertility of such genotypes.

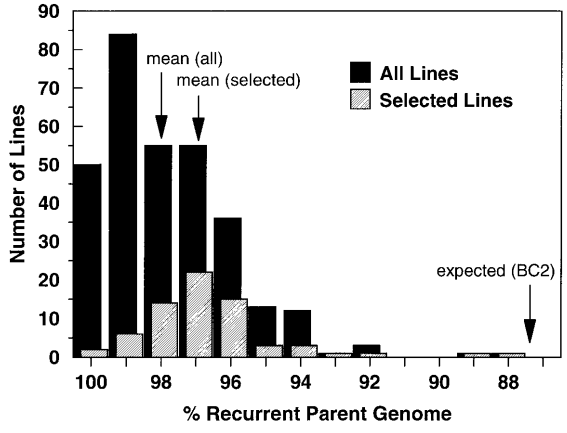

Fig. 2 Proportion of recurrent parent genome in backcross-inbred lines. *Solid bars* All families, *hatched bars* selected families only

Number, Size and Position of Introgressed Segments

Of the 311 lines analyzed in the present study, 39 (13%) had no detectable *S. lycopersicoides*-specific RFLP, isozyme, or morphological markers. A survey with randomly amplified polymorphic DNA (RAPD) markers found one or more putative *S. lycopersicoides* bands in about half of these lines, suggesting the possibility of introgressed DNA in regions not detected by the other types of markers; however, the reliability of the RAPD data is questionable, since many markers were ambiguous and did not integrate well with framework markers in the BC_1 mapping population. The remaining 272 lines (87%) had 0–7 heterozygous introgressed segments and 0–4 ho-

Table 2 Transmission of *S. lycopersicoides* segments detected in backcross-inbred derivatives and cumulative proportion of donor genome represented by homozygous and heterozygous segments

Chr.	Heterozygous segments		Homozygous segments		Maximum percentage genome coverage[a]	
	Number of segments	Average segment length (cM)	Number of segments	Average segment length (cM)	Homo only[b]	Het+Homo
1	28	31.3	6	10.2	58.8	100
2	49	25.9	17	26.5	92.8	100
3	32	19.9	7	6.9	25.0	100
4	16	27.1	9	15.6	57.7	74.9
5	38	26.4	20	25.7	81.6	100
6	33	42.3	18	21.7	34.4	100
7	27	20.5	21	20.8	100	100
8	40	37.1	11	14.5	70.6	100
9	38	32.9	14	25.7	100	100
10	16	23.4	2	11.3	18.3	100
11	18	28.0	4	18.3	74.4	100
12	25	34.9	31	21.2	85.4	100
All	359	29.6	160	20.7	66.0	97.7

[a] Based on the proportion of each chromosome's genetic length (in centiMorgan) represented by at least one introgression line family
[b] Includes some segments fixed in subsequent progeny testing

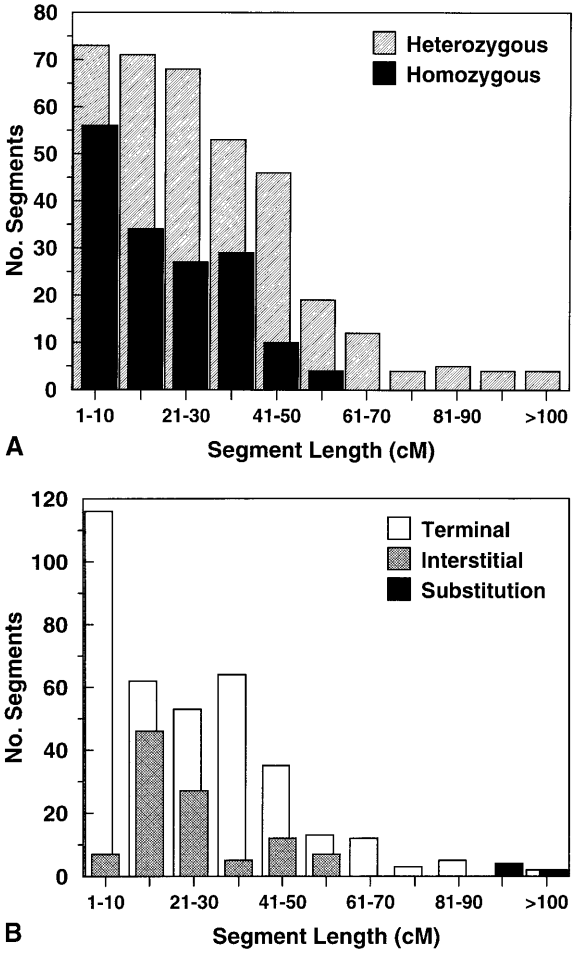

Fig. 3A, B Frequency distribution of recombinant chromosome segments according to genetic length (centiMorgans), detected in backcross-inbred *L. esculentum*×*S. lycopersicoides* derivatives. **A** genotype of introgressed segments (homozygous, heterozygous); **B** position of segment within chromosome (terminal, interstitial, or whole chromosome substitution)

mozygous ones. The average line had 1.2 heterozygous and 0.5 homozygous segments (Table 1). There were 71 lines with a single heterozygous segment and 39 with a single homozygous one.

S. lycopersicoides alleles for all but 2 markers (TG62 and TG65 on chromosome 4) were represented in at least 1 line (Fig. 1), indicating that introgression of up to 98% of the alien genome (measured in map units) was accomplished (Table 2). However, since the relationship between physical and genetic distance varies greatly within the genome (Sherman and Stack 1995; Tanksley et al. 1992), the proportion of *S. lycopersicoides* DNA transferred into tomato cannot be accurately reported. Furthermore, the transmission and fixation of *S. lycopersicoides* markers varied for individual chromosomes. For example, there was a greater number of introgressed segments representing *S. lycopersicoides* chromosome 12, the shortest of the set, than for chromosome 1, the longest (Table 2). Up to 100% of chromosomes 7 and 9 were covered by homozygous segments, while for chromosome 10 the coverage was only 18%. Heterozygous *S. lycopersicoides* segments tended to be longer (29.6 cM on average) than homozygous ones (20.7 cM) (Fig. 3A, Table 2). The largest heterozygous introgression was 100 cM, spanning the entire *S. lycopersicoides* chromosome 6. The largest homozygous segment, approximately 55 cM, was on chromosome 9 (Fig. 1). Overall, approximately 66% of the *S. lycopersicoides* genome was represented by homozygous introgressions (Table 2, Fig. 1).

The observed differences in segment length and genome coverage for homozygous versus heterozygous introgressions are best explained by the following trends revealed in this study: (1) plants homozygous for a given introgression were less fertile (particularly male-fertility) than heterozygotes, and (2) longer introgressions were associated with more extreme sterility than shorter ones, particularly when homozygous. Therefore, individual *S. lycopersicoides* genes associated with sterility in the background of *L. esculentum* appear to be largely recessive, and the effects of multiple genes at least partially additive.

The distribution of the introgressed segments along the chromosomes was not random. A majority of *S. lycopersicoides* introgressions were in terminal positions, defined herein as including the most distal marker for a given chromosome arm (Figs. 1, 3B). Out of 491 *S. lycopersicoides* segments identified, 385 (78%) were terminal and 106 (22%) were interstitial. The difference was more pronounced for homozygous introgressions: 91% of these were terminal. Similar trends were observed in a BC_1S_6 population derived from *L. esculentum*×*L. pennellii* (Eshed et al. 1992); RFLP mapping of *L. pennellii* segments indicated 76% were terminal and only 24% interstitial.

To test whether the apparent excess of terminal introgressions observed in the present study was abnormally high, we performed computer simulations with QGENE. The hypothetical genome consisted of 12 chromosomes, with an average of 12 markers and 100 cM per chromosome. For each of three simulations performed, 30 lines were chosen at random from a population of 100 BC_1F_4s and their segments categorized as to position on the chromosome. This analysis predicted an average of approximately 62% terminal, 38% interstitial introgressions, with little if any difference in the homozygous versus heterozygous classes. Further backcrossing and selfing would be expected to increase the proportion of interstitial introgressions. This analysis is consistent with the interpretation that the proportion of terminal introgressions in the *S. lycopersicoides* derivatives is abnormally high.

The maximum genetic length of terminal introgressions considerably exceeded that of interstitial segments (Fig. 3B). The longest segments observed were putative nonrecombinant *S. lycopersicoides* chromosomes (Fig. 3B); such ‘substitutions’, which were heterozygous in all instances, displayed the *S. lycopersicoides* alleles for all markers on a given homoeologue, and were identified for chromosomes 6, 7, 8, and 10 (Fig. 1). Since a terminal introgression would require a single crossover,

whereas an interstitial segment would require a minimum of two crossovers, the preponderance of the former type suggests recombination may be significantly reduced within introgressed regions. This is supported by the transmission of putatively nonrecombinant *S. lycopersicoides* chromosomes during several generations of backcrossing and selfing. The suspected recombination suppression was confirmed in progeny tests of specific introgressions (below).

Segregation and Recombination in Progeny Tests

For the purpose of identifying plants homozygous for specific introgressions or those carrying recombinant derivatives of larger segments, progeny tests were performed on specific backcross-inbred lines. In self progeny of heterozygotes, individual plants were analyzed for RFLP and/or isozyme loci within each introgressed segment. For nearly every *S. lycopersicoides* segment analyzed, segregation ratios showed significant distortion from the expected monogenic ratio of 1:2:1 (Table 3). A deficiency of plants homozygous for *S. lycopersicoides* segments was noted for all tested chromosomes, except the interval TG17 – TG27 on chromosome 1L, which showed the reverse tendency. In some instances, an excess of heterozygotes was observed, such as for the interval TG59 – TG83 on chromosome 1 (Table 3) and for *Got-2* on chromosome 7 (data not shown).

Despite the small populations analyzed, a clear trend for reduced recombination was observed in all intervals; recombination estimates were reduced over 100-fold in many regions, while in others no recombinants were observed (Table 3). Recombination suppression has been commonly observed in interspecific crosses involving tomato and wild *Lycopersicon* species. It is generally more pronounced in later backcross generations, in male versus female gametes and in centric rather than distal regions of chromosomes (deVicente and Tanksley 1991; Rick 1969, 1971; Tanksley et al. 1992). In backcross derivatives of *L. esculentum*×*L. pennellii*, recombination was suppressed up to tenfold, although most intervals were not affected to this extent (Rick 1971). In a set of *L. esculentum*×*L. cheesmanii* recombinant inbreds, recombination rates were, if anything, above normal, averaging twice that of the F_2 population from which they originated (Paran et al. 1995).

It appears, therefore, that recombination was suppressed to a greater extent in the backcross-inbred progeny of *L. esculentum*×*S. lycopersicoides* reported herein than in most crosses between *Lycopersicon* species. This difference is presumably caused by more substantial chromosome differentiation, at a sequence or structural level, vis-à-vis the more distantly related species. Meiosis in the diploid F_1 *L. esculentum*×*S. lycopersicoides* hybrid is noticeably disrupted, with frequent univalents and a an approximate 20% reduction in chiasmata formation (Menzel 1962; Rick 1951). In contrast, meiosis in F_1 hybrids between *L. esculentum* and other *Lycopersicon* spp. is relatively normal (Khush and Rick 1963; McGuire and Rick 1954). However, the degree to which meiosis is disrupted in F_1 *L. esculentum*×*S. lycopersicoides* is incommensurate with the dramatic reduction in recombination in certain regions of the genome (even to the zero level) observed in later backcrosses, hence cannot be solely responsible for it. On the other hand, our observations of radically modified segregation ratios suggest selective elimination of certain genotypes in gametogenesis, fertilization (e.g., pollen-tube growth), and/or post-syngamic processes. Thus, the results indicate that the observed reduction in recombination must owe not only to lowered crossing-over, but also to other factors, perhaps differential pre- and/or post-zygotic lethality.

Table 3 Segregation ratios observed for introgressed segments in progeny of self-pollinated heterozygotes, and recombination frequencies between distal markers on each segment

Chr.	Marker interval	Line numbers[a]	Parental genotypes				Recombinants[b]		Map units (cM)		Map ratio Observed/ expected
			+/+	+/S	S/S	X^2	SCO	DCO	Observed	Expected	
1	TG301-TG192	15–2, 5–8	4	18	1	9.10*	1	0	2.1	25.9	0.081
1	TG301-TG465	15–2	5	26	1	13.5**	4	0	5.9	26.1	0.23
1	TG83-TG17	20–9	9	8	2	5.63	0	0	0	34.1	0.00
1	TG17-TG27	21–4	0	25	4	16.3***	4	0	6.5	21.6	0.30
2	TG33-TG554	49–4	6	5	0	6.64*	0	0	0	38.0	0.00
3	TG284-TG152	14–8, 40–3	7	11	0	11.2**	0	0	0	18.0	0.00
4	TG49-TG146	24–14, 57–1	9	9	0	9.00*	0	0	0	25.2	0.00
4	*Tpi-2–Adh-1*	10–11, 10–9	57	52	1	57.4***	5	0	2.2	24.9	0.088
4	TG22-TG464	29–1	3	8	1	2.00	0	0	0	9.6	0.00
7	*Got-2–Got-3*	48–4	70	24	0	127***	3	0	1.56	17.4	0.090
11	TG557-TG523	24–11	22	28	0	20.1***	2	0	2.0	26.5	0.076
11	TG147-TG105	19–11	11	14	0	10.0**	9	1	18.2	40.6	0.45
12	TG180-TG111	45–7	17	29	0	15.7***	11	1	12.4	47.1	0.26

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

[a] Data pooled from more than one segregating family

[b] SCO=Single-crossover genotypes, DCO, double-crossover genotypes

In order to increase the proportion of the *S. lycopersicoides* genome represented by homozygous introgressions, it will be necessary to identify recombinants with smaller introgressions and therefore greater fecundity, particularly in the homozygous state. The pronounced recombination suppression and segregation distortion reported herein may significantly impede our efforts to develop a complete library of homozygous introgressions. Various strategies are being explored to increase recombination within introgressed regions or to improve the efficiency of detection, by, for example, enriching progeny of heterozygotes for recombinant gametes.

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