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Homoeologous pairing and recombination in *Solanum lycopersicoides* monosomic addition and substitution lines of tomato

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Abstract We compared meiotic pairing and recombination between tomato (*Lycopersicon esculentum*) and homoeologous *Solanum lycopersicoides* chromosomes in monosomic additions (MAs) and substitution lines (SLs), each representing a single chromosome of the nightshade in a tomato background. Three configurations of each alien chromosome and its two tomato homoeologues were detected by genomic in situ hybridization in MA-7, -8, and -10 at diakinesis/metaphase-I: 1 trivalent (III), 1 bivalent + 1 univalent (II+I), and 3 univalents (3I). The II+I category was by far the most common, and the univalent was from *S. lycopersicoides* 91–99.5% of the time, indicating a high degree of preferential (homologous) pairing. In the corresponding substitution lines, association of homoeologous chromosomes was much higher (up to 90% of the cells), presumably due to the absence of homologous partners. However, SL-10 showed a surprisingly high frequency of univalents (about 73%). Genome-wide analysis of chromosome pairing revealed a decrease in the average chiasma frequency for both monosomic additions and substitution lines. Recombination between tomato and the nightshade was restricted in all cases, the reduction being more severe in each monosomic addition than in the corresponding substitution line. Recombination rates in the substitutions were less than those observed for the same chromosomes in the first backcross generation. Chromosomes 8 and 10 showed the highest and the lowest rates of homoeologous recombination, respectively. No recombination was detected between markers on the long arm of chromosome 10, presumably due to the presence of a paracentric inversion differentiating the two genomes in this region. The frequency of homoeologous pairing at diakinesis/metaphase-I was significantly higher than the rate of homoeologous recombination detected in the progeny, suggesting a strong selection against recombinant products in meiotic or post-meiotic stages.

Keywords Tomato · Monosomic addition · Substitution line · Homoeologous pairing · Genomic in situ hybridization

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Introduction

The wild nightshade *Solanum lycopersicoides* Dun. possesses numerous economic traits of potential value for genetic improvement of the cultivated tomato, *Lycopersicon esculentum* Mill., including disease and insect resistance as well as low-temperature tolerance (Rick 1988). Although the two species are easily hybridized, the resulting diploid F_1 *L. esculentum* × *S. lycopersicoides* hybrids are both unilaterally incompatible with tomato (i.e., the style rejects the pollen of *L. esculentum*) and are essentially male sterile (Chetelat et al. 1997). These barriers were overcome by chromosome doubling to produce allotetraploids, from which sesquidiploid hybrids (two genomes of *L. esculentum* and one genome of *S. lycopersicoides*) were obtained. Monosomic additions (MAs), each containing one of the 12 *S. lycopersicoides* chromosomes added to the tomato genome (2n + 1), were identified from the progeny of the sesquidiploids (Chetelat et al. 1998). Although monosomic additions are potentially useful for gene mapping by chromosomal assignment and for construction of chromosome-specific libraries, they are not ideal genetic resources for breeding or introgression due to the inevitable segregation of alien chromosomes and potential loss of chromosome integrity by homoeologous recombination. To overcome these limitations, a set of introgression lines containing overlapping *S. lycopersicoides* chromosome segments in a diploid tomato background was developed (Chetelat and Meglic 2000). Heterozygous substitution lines (SLs), each containing single *S. lycop-*

ersicoides chromosomes, were also identified from the backcross progeny of *L. esculentum* × *S. lycopersicoides*; these included substitutions for chromosomes 6, 7, 8 and 10 (Chetelat and Meglic 2000).

Recombination suppression is frequently observed in interspecific crosses involving cultivated tomato and related wild species. Recombination is generally lower in later backcross generations than in the F_1 hybrid (Rick 1969, 1972), in progeny of male than of female meioses (De Vicente and Tanksley 1991) and in pericentromeric than in distal regions (Tanksley et al. 1992). In BC_1 *L. esculentum* × *S. lycopersicoides*, an average recombination suppression of about 27% was observed, affecting all chromosomes except 9 and 12, in distal as well as proximal intervals (Chetelat et al. 2000). Although the genomes were largely colinear, recombination between markers on the long arm of chromosome 10 was completely absent, suggesting a possible rearrangement in this region. In later backcross generations from the same intergeneric cross, recombination was suppressed to a much greater extent (up to 100-fold in some regions), and segregation ratios were severely distorted (Chetelat and Meglic 2000).

Meiosis in the diploid F_1 *L. esculentum* × *S. lycopersicoides* is noticeably disrupted, with reduced chiasma formation and frequent univalents at diakinesis and metaphase-I (Menzel 1962). Synthetic allotetraploids display preferential pairing of homologous chromosomes (i.e., mostly bivalents) and greatly increased fertility, suggesting that the chromosomes of *L. esculentum* and *S. lycopersicoides* are homoeologous (Menzel 1964; Rick et al. 1986). In contrast to diakinesis and metaphase-I, chromosome behavior in early prophase is relatively normal: at pachytene, for example, chromosomes of the 2× intergeneric hybrid are completely paired (Menzel 1962) and form normal synaptonemal complexes (Menzel and Price 1966). Therefore, the disrupted chromosome pairing in late prophase presumably results from reduced rates of crossing-over caused by a lack of sequence homology and/or minor structural changes.

Two methods were used in this study to further test this hypothesis. First, genomic in situ hybridization (GISH) was used to differentiate the *S. lycopersicoides* chromosomes from those of *L. esculentum*, and to estimate rates of homoeologous chromosome pairing in monosomic additions and substitution lines. Second, RFLP analysis of progeny from substitution lines and monosomic additions was used to measure rates of homoeologous recombination.

Materials and methods

Plant material

For the purpose of this study, we chose to focus on chromosomes 7, 8 and 10, because the corresponding monosomic additions and substitution lines are available and are relatively fertile. In addition, convenient dominant morphological markers exist for these three *S. lycopersicoides* homoeologues (Chetelat et al. 1998). Monosomic additions for *S. lycopersicoides* chromosomes 7, 8 and 10 were selected from the selfed progeny of known monosomic additions based on morphology and confirmed by chromosome counts. The corresponding heterozygous substitution lines were selected from the selfed progeny of known substitution lines (Chetelat and Meglic 2000) using RFLP markers. The confirmed monosomic additions and heterozygous substitution lines were selfed and/or backcrossed as female parents to tomato. Homoeologous recombination frequencies were estimated from the F_2 or BC progeny genotyped with selected RFLP markers along each chromosome. The sizes of the mapping populations ranged from 47 to 203 (average 117) BC or F_2 individuals, corresponding to 55–396 (average 188) meiotic products. Control genotypes were: (1) *L. esculentum* cv VF36 (a standard for genetic studies); (2) *S. lycopersicoides*, accessions LA2951 and LA1964, collected in Quistagama, Tarapaca, Chile and Chupapalca, Tacna, Peru, respectively; (3) F_1 *L. esculentum* (VF36) × *S. lycopersicoides* (LA2951), plant 90L4178; (4) a sesquidiploid hybrid (GH266) containing two genomes of *L. esculentum* (UC82B) and one genome of *S. lycopersicoides* (LA1964) (Rick et al. 1986); and (5) the *L. esculentum* primary trisomics for chromosomes 7, 8 and 10, all in the background of VF36. All stocks were obtained from the Tomato Genetics Resource Center at UC-Davis.

Chromosome preparation

Pollen mother cells (PMCs) were used to study meiotic chromosome pairing. Antheridia from young flower buds were fixed overnight in ethanol: acetic acid (3:1), then rinsed with distilled water and incubated in 0.01 M citrate buffer (0.01 M sodium citrate and 0.01 M citric acid, pH 4.5) for 5 min. To enhance chromosome spreading and morphology, the material was then incubated in an enzyme mixture consisting of 2% cellulase Onozuka RS (Yakult, Tokyo), 0.3% pectolyase (Sigma-aldrich) and 1.5% macerozyme R-200 (Yakult, Tokyo) in citrate buffer for 30 min at 37° (Escalante et al. 1998). The partially digested antheridia were rinsed with distilled water, and individual anthers were subsequently placed on a clean slide and macerated in a drop of fresh 3:1 ethanol: acetic acid with a pair of forceps. Slides were stored at –80° for up to a year before use.

Genomic in situ hybridization (GISH) and signal detection

For GISH, biotin-labeled *S. lycopersicoides* genomic DNA was used as a probe, and *L. esculentum* genomic DNA was used for blocking (or vice-versa). Probe labeling and GISH were performed essentially as described by Ji et al. (1997), but the signals from biotinylated probes were detected with one layer of Cy3-streptavidin (5 μg/ml; Jackson ImmunoResearch Lab).

Fluorescence microscopy

Slides were screened with a Zeiss Axioskop microscope equipped with DAPI, FITC (fluorescein isothiocyanate) and DAPI-FITC-rhodamine filter sets. Photographs were taken on Fujicolor 800 professional film. Prints were digitally scanned on a conventional flatbed scanner, and processed to build plates, which were printed on a color printer.

Analysis of genome-wide chromosome pairing

For genome-wide analysis of chromosome pairing, the chromosomes were stained with acetocarmine (1% in 45% acetic acid) for observation. Meiocytes at the diakinesis stage having well-spread chromosomes and good chromosome morphology were analyzed for the occurrence of ring bivalents, rod bivalents and univalents. Diakinesis cells instead of metaphase cells were used in this study

because ring and rod bivalents are easily distinguished at this stage. Chiasmate arm-frequency and pairing behavior follow the definitions from Ji et al. (1999). Pairing behavior is defined as the average chromosome composition of a cell at diakinesis, i.e., the average number of ring bivalents (total number of ring bivalents in N cells divided by N) + the average number of rod bivalents (total number of rod bivalents in N cells divided by N) + the average number of trivalents (total number of trivalents in N cells divided by N) + the average number of univalents (total number of univalents in N cells divided by N). Chiasmate arm-frequency is defined as the probability of a chromosome arm with chiasmata and is calculated as: the total number of chiasmate arms in N cells divided by the total number of arms in N cells. For diploid tomatoes including normal and substitution lines, one cell has a total of 48 arms; for monosomic additions and primary trisomics, one cell has a total of 50 arms.

Analysis of homoeologous recombination

RFLP analysis, including DNA isolation, digestion, Southern blotting, source of probes and hybridization were performed as described by Chetelat and Meglic (2000). Recombination frequencies in the backcross monosomic addition populations were estimated as the percentage of recombinant progeny. Recombination frequencies in the F_2 monosomic addition populations were estimated as one-half of the percentage of recombinant progeny. Linkage maps for heterozygous substitution populations (BC or F_2) were constructed using adjusted map distances as described previously in wheat (Dvorak and Appels 1986; Dubcovsky et al. 2000). Maximum-likelihood estimates of recombination fraction were obtained using MAPMAKER version 3.0 (Lander et al. 1987). The recombination frequencies were adjusted to reflect the effect of pairing failure and gametic selection according to the formulas described below. The adjusted recombination frequencies were then converted into map distances using Kosambi's mapping function.

Two classes of pairing configurations are formed during diakinesis/metaphase from the homoeologues of a heterozygous substitution in tomato: a bivalent with at least one chiasmate arm, or two univalents. The bivalents will divide and segregate normally during later stages of meiosis. In contrast, the univalents will segregate randomly to the poles or be lost, producing six possible gamete genotypes, including four types of balanced (n) gametes (recombinant and non-recombinant), a disomic (n + 1) and a nullisomic (n – 1) gamete. The n – 1 gametes will be eliminated because tomato does not tolerate deficiencies during gametophytic stages (pollen or egg); this is the reason that monosomics of tomato are not transmissible (Khush 1973). On the other hand, the n + 1 gametes will be selected against on the male side due to competition with normal n pollen; this selection is demonstrated by the tomato primary trisomics, which transmit their extra chromosomes at negligible rates through the male germ line (Khush 1973). In contrast, transmission through the female gamete does occur, although the rate varies greatly between individual chromosomes in primary trisomics (Khush 1973) or monosomic additions (Chetelat et al. 1998). For the purpose of estimating recombination frequencies, we therefore assume that n + 1 eggs can be normally fertilized.

These properties of tomato are incorporated into the following formulas adapted from Dubcovsky et al. (2000) for adjusting recombination frequencies. For a testcross using the substitution line as female parent, the adjusted recombination frequency (RF_{adj}) was calculated according to the following formula:

$$RF_{adj} = (R/N)[1 - pf(1 - i)^2],$$

where R is the number of recombinant chromosomes, N is the total number of chromosomes observed, i is the average univalent inclusion rate (i.e., the probability for an unpaired chromosome to be incorporated into the nucleus of a spore) and pf is the rate of pairing failure (i.e., the frequency with which the homoeologous pair form univalents).

For F_2 progeny of substitution lines, the adjusted recombination frequency can be similarly calculated according to the following formula:

$$RF_{adj} = (R/N) \cdot \cdot \frac{1 - pf[i^2 + (1 - i)^2] - pf(1 - i)^2[(1 - pf) + 2i(1 - i)pf]}{1 - pf(1 - pf)[i^2 + 2(1 - i)^2]}.$$

Excluding (R/N), the terms in each equation {e.g., $[1–pf(1–i)^2]$ in the first equation} represent a constant value for a given mapping population, and is hereinafter referred to as the "adjustment constant".

Although the inclusion rate (i) has not been measured in tomato, it was empirically determined to be approximately 1/4 in wheat (Sears 1953), a value that we used in the present calculations. We further assume that our pairing figures from the diakinesis stage provide a conservative estimate of pf at metaphase, since univalents detected during late prophase should remain unpaired in metaphase, whereas a small proportion of the bivalents might disassociate.

Results

Genome-wide chromosome pairing

Analysis of genome-wide chromosome pairing indicated that meiosis in F_1 *L. esculentum* × *S. lycopersicoides* is greatly disrupted. Univalents occurred at diakinesis in 83% of PMCs from the intergeneric F_1 (Fig. 1), with an average of 2.8 univalents per cell. In contrast, diakinesis PMCs of the control genotype (VF36) contained only bivalents (Table 1). The F_1 hybrid also formed fewer ring bivalents and more rod bivalents than VF36. The average number of chiasmata per cell in the F_1 was 13.7, about 25% lower than in VF36, which formed an average of 18.2 chiasmata per cell. The frequency of chiasmate arms in the F_1 was approximately 57%, about 25% lower than in VF36 (76%).

In general, all monosomic additions and substitution lines had slightly fewer chiasmata per cell than the control VF36, but a substantially greater number of chiasmata per cell than the diploid intergeneric hybrid (Table 1). Average chiasmate arm frequencies in monosomic additions and substitution lines were respectively 11% and 8% lower than in VF36. The number of chiasmata per cell in primary trisomics (i.e., 2n + 1 plants with an extra *L. esculentum* chromosome) was essentially the same as the diploid control (Table 1). Each monosomic addition formed slightly fewer chiasmata than the corresponding primary trisomic, with an average decrease of 0.8 chiasmata per cell, observed for the three chromosomes in this study (Table 1). The average chiasmate arm frequency in the three trisomics was 71.2%, about 6% lower than in VF36, but about 5% higher than in the corresponding monosomic additions.

In the genome-wide pairing study, the behavior of the 11 pairs of homologous *L. esculentum* chromosomes was not distinguished from that of the homoeologous set in each monosomic addition or substitution line. In order to estimate the effect on homologous pairing specifically, the average number of chiasmata per cell for the homoeologous set (from Table 2) was subtracted from the corresponding genome-wide values (from Table 1). Since the two sets of pairing data were taken from

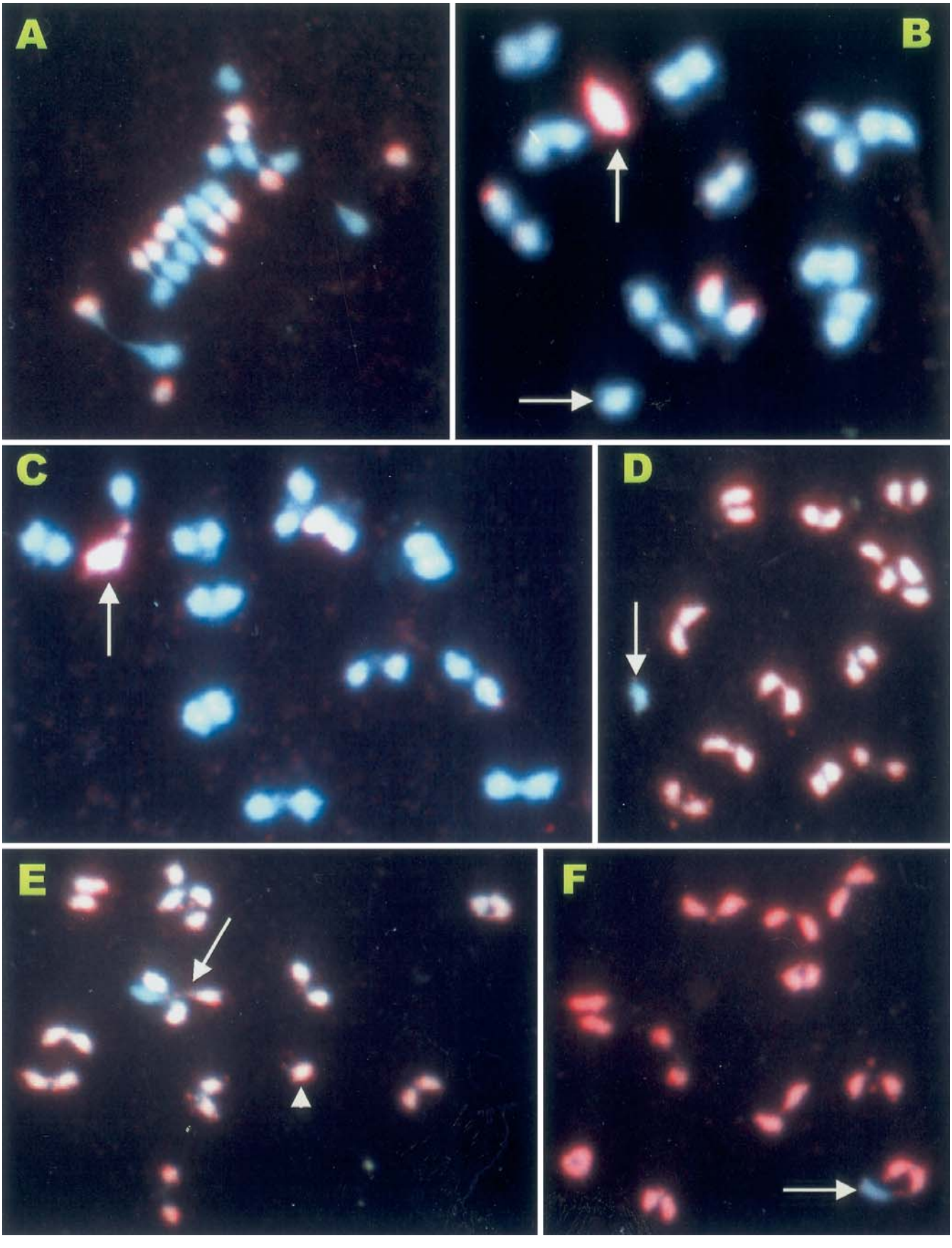
A
B
C
D
E
F

Table 1 Genome-wide chromosome pairing in *S. lycopersicoides* monosomic additions (MA), substitution lines (SL) and primary trisomics (Triplo) of tomato

Genotype[a]	Pairing behavior[b]	No. chiasmata/cell	Chiasmate arm frequency (%)	No. cells observed
VF36	6.2R + 5.8O	18.2	75.8	127
F_1 hybrid	3.13R + 7.45O + 2.84I	13.7	57.1	215
MA-7	5.09R + 6.80O + 0.09III + 0.95I	17.3	69.0	215
MA-8	4.68R + 7.11O + 0.17III + 0.91I	17.0	67.9	293
MA-10	4.55R + 7.33O + 0.06III + 1.06I	16.6	66.5	194
Average	4.77R + 7.08O + 0.11III + 0.97I	17.0	67.8	234
Triplo-7	5.76R + 5.86O + 0.34III + 0.74I	18.4	73.6	200
Triplo-8	5.18R + 6.47O + 0.23III + 1.01I	17.5	70.1	200
Triplo-10	5.13R + 6.48O + 0.25III + 1.03I	17.5	70.0	207
Average	5.36R + 6.27O + 0.27III + 0.93I	17.8	71.2	202
SL-7	4.69R + 7.14O + 0.33I	16.5	68.9	261
SL-8	5.18R + 6.72O + 0.20I	17.1	71.2	203
SL-10	5.24R + 6.02O + 1.48I	16.5	68.8	279
Average	5.04R + 6.63O + 0.67I	16.7	69.6	248

[a] VF36 = *L. esculentum* cv VF36, F_1 = F_1 *L. esculentum* × *S. lycopersicoides*
[b] See Materials and methods for formula. R = ring bivalent, O = rod bivalent, III = trivalent, I = univalent. Figures represent the average number of each chromosome configuration per cell

different populations and employed different cytological techniques, their integration can only provide an approximation of homologous chromosome associations. For the monosomic additions, the frequency of chiasmate arms among the 11 homologues (22 arms) estimated in this fashion was 68.1% (average for MA-7, -8 and -10), lower than the diploid VF36 (75.8%) or the primary trisomics (71.4%). The substitutions showed nearly normal frequencies of chiasmata among homologous chromosomes (average 72.3% for SL-7, -8 and -10) compared to VF36.

Homoeologous chromosome pairing

Genomic in situ hybridization (GISH) was used to differentiate *S. lycopersicoides* chromosomes from those of cultivated tomato in monosonic additions and substitution lines (Fig. 1). In the monosomic additions, three different configurations were formed from the single *S. lycopersicoides* chromosome and its two tomato homoeologues in PMCs at diakinesis/metaphase-I: 1 trivalent (III), 1 bivalent plus 1 univalent (II+I) and 3 univalents (3I). The II+I category was by far the most common (79–89% of cells) and the univalent was nearly always from *S. lycopersicoides* (91–99.5% of II+I configurations), indicating a strong preference for homologous pairing (Table 2). Nonetheless, homoeologous bivalents were detected for each monosomic addition, the frequency of which differed greatly between groups. For example, the frequency of homoeologous bivalents in MA-10 was about 16 × lower than that in MA-8. *S. lycopersicoides* chromosomes also paired with tomato homoeologues to form trivalents, albeit at very low rates, with MA-8 again showing the highest and MA-10 the lowest rate of homoeologous association (Table 2). Compared to the corresponding trisomics, monosomic additions showed a lower percentage of cells with one trivalent or three univalents, and a higher percentage of cells with a bivalent plus a univalent.

In the substitution lines, three different chromosome configurations of the two homoeologues were distinguishable using GISH (Fig. 1): a ring bivalent (chiasmata in both arms), a rod bivalent (chiasma in only one arm) and two univalents. Homoeologous chromosomes paired at much higher rates in substitution lines than in corresponding monosomic additions, presumably due to the absence of homologous partners (Table 2). As with the monosomic additions, SL-8 displayed the highest rate of homoeologous pairing, and SL-10 the lowest.

Fig. 1A–F Genomic in situ hybridization of F_1 *L. esculentum* × *S. lycopersicoides*, and chromosome-7 substitution (SL-7) and monosomic addition (MA-7) lines. (**A**) F_1 *L. esculentum* × *S. lycopersicoides* showing the occurrence of univalents at metaphase-I; (**B**) SL-7 at diakinesis showing unpaired homoeologous chromosomes (two univalents; *arrows*); (**C**) SL-7 showing paired homoeologous chromosomes (one bivalent; *arrow*); (**D**) MA-7 at diakinesis showing non-association of the alien chromosome with its tomato homoeologues (one univalent; *arrow*); (**E**) MA-7 showing association of the alien chromosome with one of its tomato homoeologues to form a bivalent (*arrow*) while the other forms a univalent (*arrowhead*); (**F**) MA-7 showing association of the alien chromosome with its two tomato homoeologues to form a trivalent (*arrow*). (**A**–**C**) Red = *S. lycopersicoides*, Blue = *L. esculentum*; (**D**–**F**) Blue = *S. lycopersicoides*, Red = *L. esculentum*

Homoeologous recombination

RFLP analysis of progeny from monosomic additions and substitution lines was used to estimate rates of homoeologous recombination. The map distances for progeny of heterozygous substitutions were adjusted (see Materials and methods for formulas) in order to reflect the observed rates of pairing failure (i.e., the univalent category in Table 2). Since univalent chromosomes result from a lack of chiasmata (i.e., crossovers) in either arm, their tendency to be excluded during meiosis would otherwise

Table 2 Frequency of chromosome configurations measured by genomic in situ hybridization

Genotype	% Of cells with each configuration[a]				No. chiasmata/ chromosome set[b]	Chiasmate arm frequency (%)[c]	No. cells observed
	III	II+I		3I			
		LL+S	LS+L				
MA-7	8.0	87.4	1.3	3.3	2.0	67.1	302
MA-8	16.7	71.7	7.2	4.4	2.1	69.3	293
MA-10	7.4	85.6	0.5	6.5	1.9	64.8	215
Average	10.7	81.6	3.0	4.7	2.0	67.2	270
		II+I (LL+L)					
Triplo-7	34	62		4	2.3	75.3	207
Triplo-8	23	65		12	2.0	66.3	200
Triplo-10	25	61		14	2.0	65.7	200
Average	27.3	62.7		10	2.1	69.1	202
	Ring II	Rod II		2 I			
SL-7	8.3	75.4		16.3	0.9	46.0	387
SL-8	27.6	62.6		9.8	1.2	58.9	203
SL-10	1.9	25.3		72.8	0.3	14.5	466
Average	12.6	54.4		33.0	0.8	39.8	352

[a] I = univalent, II = bivalent, III = trivalent, L = *L. esculentum* chromosome, S = *S. lycopersicoides* chromosome
[b] Chromosome set refers to the respective homoeologous chromosome pair for substitution lines, or the respective homologous chromosome pair along with its homoeologue chromosome for monosomic additions, or the respective trisomic chromosomes for the primary trisomics
[c] Chiasmate arm frequency for the respective chromosome set as defined in[b]

Table 3 Transmission rate of alien chromosomes in progeny of monosomic additions (MAs)

Genotype	Cross	Progeny[a]			Transmission rate[b] (% 2n + 1)
		2n	2n + 1	Total	
MA-7	BC (2n + 1) × 2n	154	35 (1)[c]	189	18.5
MA-8	BC (2n + 1) × 2n	142 (2)	61 (1)	203	30.0
	F_2 (2n + 1) × self	123 (2)	47 (3)	170	27.6
MA-10	BC (2n + 1) × 2n	115	52	167	31.1

[a] Classification of 2n or 2n + 1 was based on phenotype
[b] Transmission rate is defined as the percentage of trisomics in the backcross (BC) or F_2 progeny
[c] Figures in parentheses represent the number of recombinant progeny, determined by RFLP analysis

lead to an underestimate of the number of parental chromosomes; hence, an upward bias on recombination rates. Based on the frequency of univalents in each substitution, adjustment constants were used to calculate adjusted recombination frequencies as a fraction of the original estimates. These adjustment constants for backcrosses were 0.908, 0.945 and 0.591, and for the F_2s, 0.973, 0.991 and 0.421, for SL-7, -8 and -10, respectively.

Reduced recombination rates were observed in both F_2 and backcross populations of all monosomic additions and substitution lines compared to controls (Fig. 2). Much more-pronounced recombination suppression was observed in monosomic additions than in substitution lines, consistent with the lower rates of homoeologous chromosome pairing in the former. Chromosome 10 showed the lowest rate of homoeologous recombination in both substitution lines and monosomic additions. In fact, no recombination was detected between markers TG408-TG63 on the long arm of chromosome 10, in either monosomic additions or substitution lines, suggesting substantial structural differentiation between the genomes in this region. However, recombination in the adjacent region (TG596 – TG408) was reduced to a much lesser degree (Fig. 2). For all chromosomes, recombination was reduced to a greater extent in substitution lines than in the BC_1 *L. esculentum* × *S. lycopersicoides*; the total genetic length of chromosomes in the substitution lines was approximately one-half of that measured in the BC_1.

Marker analysis indicated that the extra chromosome in MA-8 was recombinant: the segment between TG330 and TG176 was from *S. lycopersicoides* and the segment between TG510 and CT68 was from *L. esculentum*. All other monosomic addition and substitution lines had intact *S. lycopersicoides* chromosomes.

Alien chromosome transmission rates

The transmission rates of the alien chromosomes were analyzed in backcross and F_2 progeny of monosomic additions and substitution lines; genotypes were determined using RFLP markers and, in the case of monosomic additions, by trisomic vs disomic phenotypes (Tables 3 and 4). Chromosome 7 showed the lowest transmission rates in both monosomic additions and substitution lines, while chromosome 10 showed the

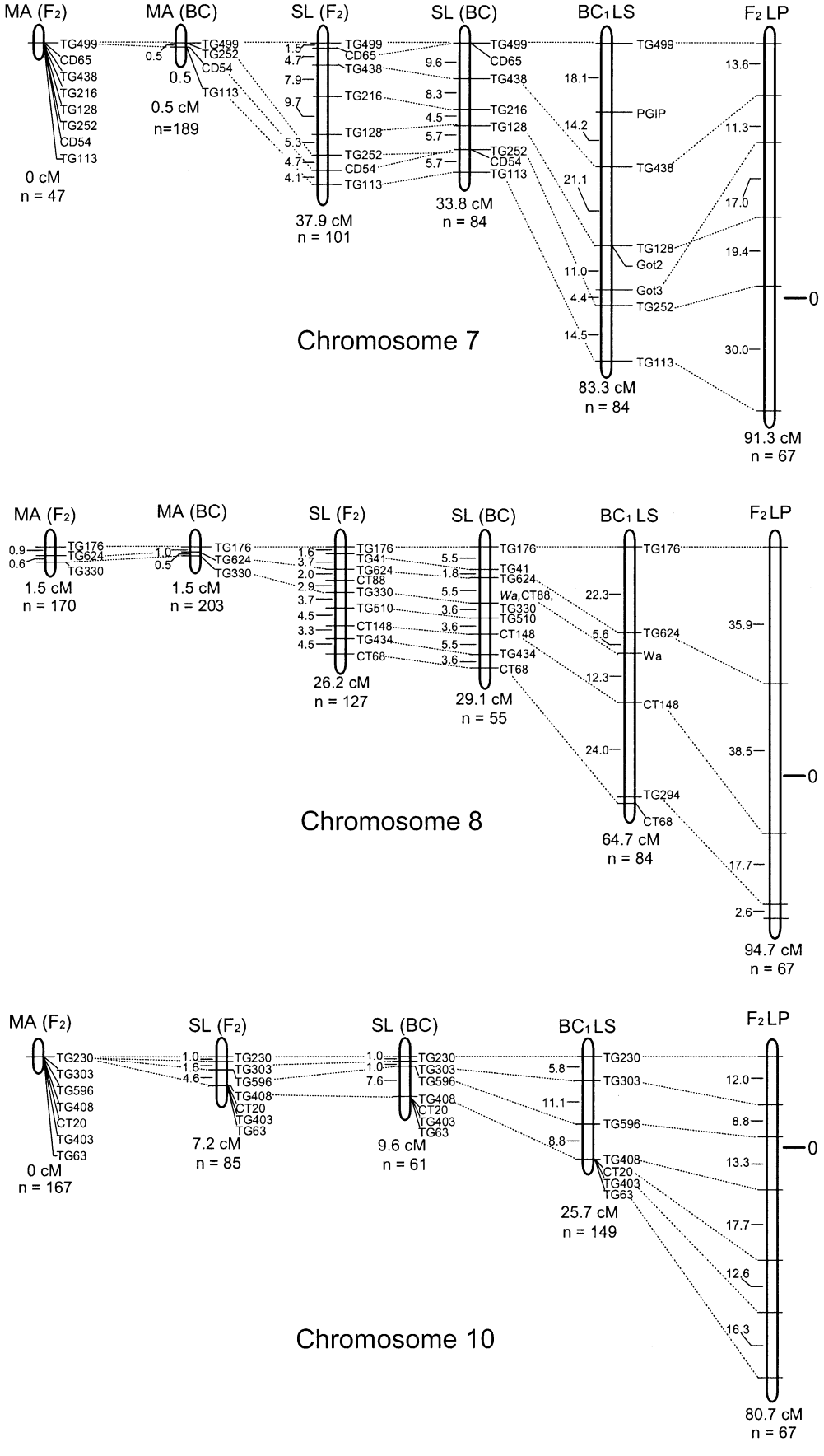

Fig. 2 Comparison of genetic maps of chromosomes 7, 8 and 10 based on recombination in F_2 or backcross (*BC*) progeny of *S. lycopersicoides* monosomic addition (*MA*) and substitution (*SL*) lines, BC_1 *L. esculentum* × *S. lycopersicoides* (BC_1 *LS*; from Chetelat et al. 2000) and F_2 *L. esculentum* × *L. pennellii* (F_2 *LP*; from Tanksley et al. 1992). Positions of centromeres on the F_2LP map are from Pillen et al. (1996). *Dashed lines* connecting maps indicate shared markers. All distances are Kosambi map units, with total genetic length (cM) and the number of individuals (n) in each mapping population indicated below each chromosome

Table 4 Transmission rate of alien chromosomes in backcross and F_2 progeny of heterozygous *S. lycopersicoides* substitution lines (SLs)

Genotype	Cross	Parental genotypes[a]				χ^2	Transmission rate[b] (%)
		+/+	+/S	S/S	Total		
SL-7	BC	54	3	–	57	45.6***	5.3
	F_2	24	16	0	40	30.4***	20.0
SL-8	BC	32	7	–	39	16.1***	17.9
	F_2	41	32	0	73	47.2***	21.9
SL-10	BC	35	17	–	52	6.3*	32.7
	F_2	23	32	4	59	12.7**	33.9

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

[a] +/+ = number of *L. esculentum* homozygotes, +/S = heterozygotes, S/S = *S. lycopersicoides* homozygotes. Recombinant chromosomes are not included. The progeny was genotyped using RFLP markers

[b] F_2 transmission rate = $\frac{(+/S + 2S/S)}{2(\text{total\#progeny})} \times 100\%$,

BC transmission rate = % heterozygotes

highest. The transmission rate of *S. lycopersicoides* chromosome 8 in the MA-8 F_2 population was 27.6%, close to that of the backcross, indicating that the extra chromosome was transmitted mainly through the female gametes. This interpretation is consistent with the absence of disomic additions (2n + 2) in the F_2 progeny of MA-8. In progeny of the substitution lines, segregation of the alien chromosome was significantly distorted from Mendelian expectations (i.e., 1:1 for backcross and 1:2:1 for F_2). In each case, a deficiency of genotypes heterozygous and/or homozygous for the *S. lycopersicoides* chromosome was observed (Table 4). For chromosomes 8 and 10, transmission rates in backcross and F_2 populations were similar. In contrast, the transmission rate of *S. lycopersicoides* chromosome 7 was much lower in the backcross than in the F_2 population, and was only about 10% of the expected Mendelian rate. This pronounced deficiency of heterozygotes observed in the progeny of female SL-7 suggests a strong gametic selection against ovules carrying the alien chromosome.

Discussion

The wild relatives of the cultivated tomato include nine species of *Lycopersicon*, native to Ecuador, Peru and Chile, all of which can be hybridized with the cultigen, albeit with varying degrees of difficulty. To-date, all genetic maps based on crosses between *L. esculentum* and related *Lycopersicon* species have indicated a strong conservation of gene repertoire and order along the chromosomes within this genus (Tanksley et al. 1992; Bernacchi and Tanksley 1997; Fulton et al. 1997). These observations are consistent with the normal chromosome pairing in meiosis and relatively high fertility of F_1 hybrids between *L. esculentum* and wild *Lycopersicon* species (Afify 1933; Khush and Rick 1963). Therefore, the chromosomes of the *Lycopersicon* species can be considered essentially colinear and homologous.

In contrast, our results with *S. lycopersicoides* monosomic additions and substitution lines in the background of cultivated tomato, including the observations of reduced pairing and recombination, indicate that the chromosomes of this nightshade species are homoeologous with those of *Lycopersicon*. The present experiments confirm earlier observations of disrupted pairing in the 2x and 3x intergeneric hybrids (Menzel 1962; Rick et al. 1986), and monosomic additions (Chetelat et al. 1998). In addition, we have extended results from conventional cytology by using GISH for a chromosome-specific analysis of homoeologous pairing in monosomic additions and substitution lines for chromosomes 7, 8 and 10. This study revealed a more-pronounced suppression of pairing between homoeologous chromosomes in monosomic additions than in corresponding substitution lines, presumably due to the opportunity for preferential homologous pairing in the former but not in the latter. Chromosomes of genomes with higher affinity have been shown to pair more frequently than those of more-distantly related genomes (Kimber and Yen 1990). Homologous chromosomes pair preferentially in monosomic additions, limiting the pairing between homoeologous chromosomes; in substitution lines, the alien chromosomes pair at higher rates with their tomato homoeologues due to the lack of competition. Furthermore, the fact that the *S. lycopersicoides* chromosomes pair at all with their tomato homoeologues in monosomic additions indicates that the two genomes must be closely related. Similar results were also observed in *Lolium* and *Festuca* hybrids (Cao et al. 2000).

The fact that *L. esculentum* chromosomes can be readily distinguished from their *S. lycopersicoides* homoeologues using standard GISH conditions suggests that the two genomes have diverged substantially at the DNA sequence level, at least in terms of dispersed repetitive sequences. Genomes sharing 85% or less sequence homology can be discriminated by the standard GISH protocol (Parokonny et al. 1997). This conclusion is consistent with RFLP analysis (Chetelat et al. 2000) using single- or low-copy probes that also indicated a high level of divergence between *L. esculentum* and *S. lycopersicoides* (average 75% polymorphism rate).

Our results with the substitution lines indicated more-severe recombination suppression than reported for the

same chromosomes in the *L. esculentum* × *S. lycopersicoides* BC_1 (Chetelat et al. 2000). This result could be due to the more-isogenic genetic background (i.e., pure *L. esculentum*) of the substitution lines. The same trend was observed for *L. pennellii* chromosomes during introgression into tomato (Rick 1969, 1972). Another factor contributing to the higher rate of recombination in BC_1 *L. esculentum* × *S. lycopersicoides* is the strong gametophytic selection among pollen of the relatively sterile F_1 hybrid; since selection would favor balanced gametes, for which normal chromosome assortment (i.e., crossing-over in each homoeologous pair) would be a prerequisite, the rate of recombination measured in the progeny could be higher than predicted from observations of pairing (Chetelat et al. 2000).

In the present study, estimates of recombination frequencies in the substitution lines were adjusted for the observed rates of pairing failure and the expected selection against aneuploid gametes demonstrated in previous studies. In contrast, the rate of pairing failure for individual chromosomes during meiosis of the intergeneric F_1 hybrid has not been determined. As a result, the unadjusted recombination frequencies from the F_1 are somewhat inflated relative to the adjusted values from the substitution lines. In addition, the frequencies of chiasmata for the homoeologous chromosome pairs in SL-7 (0.9) and SL-10 (0.3) are lower than the average number of chiasmata per chromosome for the *L. esculentum* × *S. lycopersicoides* F_1 (13.7/12 = 1.14, from Table 1). These trends suggest that the chiasmate arm frequencies are reduced at least for some of the chromosomes in the substitution line relative to the intergeneric hybrid. There is a 1:1 relationship between chiasmata and recombination nodules, which represent the sites of crossing-over (Sherman and Stack 1995). This provides evidence that the lower recombination rates observed in progeny of the substitution lines are at least partially due to crossover suppression. We also observed a higher recombination in substitution lines than in the corresponding monosomic additions; in this case, the presence of a pair of *L. esculentum* chromosomes for each *S. lycopersicoides* homoeologue in the monosomic additions resulted in largely homologous associations and a low rate of recombination.

Consistent with our pairing data, chromosome 10 showed the lowest recombination rate in the substitution lines and monosomic additions, and recombination between markers on the long arm was completely eliminated. This result could be explained by a structural rearrangement on *S. lycopersicoides* 10L relative to *L. esculentum* (Chetelat et al. 2000). Indeed, a genetic map of *S. lycopersicoides* chromosome 10 shows that the long arm is inverted relative to tomato, and has the same gene order as potato, *Solanum tuberosum* (Pertuze et al. 2002).

Reduced recombination in the backcross progeny of interspecific hybrids is a common phenomenon. In a study of recombination in *L. esculentum* × *L. pennellii* derivatives, Rick (1969, 1972) reported reduced recombination for chromosomes 3, 4, 8, 10 and 11, as each *L. pennellii* chromosome was substituted for its *L. esculentum* counterpart using morphological markers; consistent with our results, the most-severe reductions were observed in later backcross generations. The BC_1LS map revealed recombination suppression for both proximal and distal regions of most chromosomes (Chetelat et al. 2000). For some chromosomes, the most-extreme recombination reduction was observed in the pericentric regions, consistent with the higher marker density around centromeres reported in the *L. esculentum* × *L. pennellii* F_2 (Tanksley et al. 1992), and the much lower frequency of recombination nodules in centromeric heterochromatin (Sherman and Stack 1995). However, we also observed a noticeable exception for chromosome 10, in that recombination in the pericentric region between markers TG596 and TG408 (Pillen et al. 1996) was reduced to a much-lesser degree in this study. This was presumably due to a compensation effect, wherein the elimination of recombination on 10L due to the inversion was compensated for by higher recombination in the adjacent region of 10S. Compensation for low recombination in one region by elevated recombination elsewhere on the chromosome was also observed in *Gossypium* (Rhyne 1960), maize (Ji et al. 1999) and wheat (Dubcovsky et al. 1997).

Homoeologous trivalents or bivalents, formed from pairing of the alien *S. lycopersicoides* chromosome with its tomato homoeologues, were observed for each monosomic addition. The frequencies of homoeologous trivalents were relatively high, ranging from 7.4% for MA-10 to 16.7% for MA-8, while the frequencies of corresponding homoeologous bivalents were relatively low, ranging from 0.5% for MA-10 to 7.2% for MA-8. These observations demonstrate that *S. lycopersicoides* chromosomes can pair with their tomato homoeologues at appreciable rates. Homoeologous pairing in monosomic additions was also observed for other plant species, such as wheat, *Brassica* and *Oryza* (Dvorak 1978, 1987; This et al. 1990; Multani et al. 1994). Multani et al. (1994) studied homoeologous paring between *Oryza australiensis* and *Oryza sativa* chromosomes. The interspecific hybrids were obtained through embryo rescue and were highly sterile. In derived monosomic additions, the *O. australiensis* chromosomes paired with their homoeologues to form trivalents at a frequency of 7.5% to 24.0%. In these respects, the two rice genomes resemble the relationship between *L. esculentum* and *S. lycopersicoides*.

Our progeny tests of monosomic additions indicated a very low (in some cases zero) level of recombination. Therefore, the frequency of homoeologous pairing at diakinesis/metaphase-I did not correlate with the rate of recombination detected in the progeny. One possible reason for this discrepancy may be a strong selection against recombinant genotypes at gametophytic and/or post-syngamic stages. This is consistent with our observations of extreme segregation distortion in progeny of substitution lines (see below) and introgression lines (Chetelat and Meglic 2000), in which most regions of the genome showed a deficiency of *S. lycopersicoides* alleles.

In addition, some of the observed pairing between *L. esculentum* and *S. lycopersicoides* chromosomes could involve nonhomologous regions. If so, crossovers would lead to deficiencies, which are not tolerated in tomato, or duplications, which would be selected against during gametogenesis. Sequence divergence between *L. esculentum* and *S. lycopersicoides* chromosomes could lead to abortion of crossover events by the DNA mismatch repair system. In *Escherichia coli*, yeast and mammals, *Mut*S-type proteins are known to block recombination between imperfectly homologous sequences (Rayssiguier et al. 1989; De Wind et al. 1995; Selva et al. 1995). Lastly, distributive pairing, wherein homoeologous chromosomes pair for normal disjunction without a crossover, may occur in the monosomic additions. Distributive pairing was first reported in *Drosophila* (Grell 1962) and has also been observed in *Saccharomyces cerevisiae* (Loidl et al. 1994) and humans (Martin et al. 1986). However, our observation of chiasma formation between homoeologues does not support the distributive pairing model.

The alien *S. lycopersicoides* chromosomes were transmitted at frequencies ranging from 17% for MA-7 (BC) to 32% for MA-10 (BC). The transmission rates in backcross and F_2 progeny were very similar for MA-8, indicating that the extra *S. lycopersicoides* chromosome in MA-8 was transmitted mainly through female gametes. However, a low level of male transmission is possible since Rick et al. (1988) reported an appreciable rate (up to 3.5%) of trisomic progeny when MA-7, -8 or -9 were used as staminate parent in crosses to diploid tomato. Furthermore, in the case of the primary trisomics of tomato, only three (triplo-7, -8 and -10) transmit at all through male gametes, albeit at very low rates (Khush 1973). In monosomic additions of other species, such as rice, the alien chromosomes were also transmitted at relatively high rates through female gametes, but at very low or zero rates through male gametes (Multani et al. 1994).

Transmission of the alien *S. lycopersicoides* chromosomes was significantly distorted for all substitution lines, in both backcross and F_2 progeny. *S. lycopersicoides* chromosome 7 was transmitted at a much higher rate in F_2 than in BC progeny, suggesting inheritance of this chromosome through the female is restricted to a greater degree than via the male. This observation is consistent with the pattern of segregation distortion around *Got-3*, a marker for chromosome 7S, suggesting that a gene(s) in this region is under strong selection during development or fertilization of female gametes (Chetelat 1998).

S. lycopersicoides chromosomes do pair and recombine with *L. esculentum* chromosomes in monosomic additions and substitution lines, albeit at low rates. This should enable the selective introgression of genes from the nightshade that affect traits of economic importance in tomato. The fact that substitution lines displayed much higher rates of homoeologous pairing and recombination than corresponding monosomic additions indicates they are a better source of recombinants for introgression purposes. Similar results were obtained in wheat and have been used in the design of introgression of alien chromosome segments (Sears 1972). To-date, only four out of the 12 possible *S. lycopersicoides* substitution lines have been obtained. However, it should be possible to recover additional substitution lines in the progeny of the corresponding monosomic additions (of which ten are available), since each formed homoeologous bivalents, albeit at low frequencies. Furthermore, monosomic additions and substitution lines should be useful for chromosomal assignment of genes and DNA clones, as they have been in the *Triticeae* (Hart et al. 1980; Appels and Moran 1984); more recently, a series of maize/oat chromosome additions were used in this manner to map the maize genome (Ananiev et al. 1997). In conclusion, monosomic addition and substitution lines provide a means to access novel traits in *S. lycopersicoides*, as well as additional tools with which to analyze the tomato genome.

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