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Structural Differences in Chromosomes Distinguish Species in the Tomato Clade

L.K. Anderson P.A. Covey L.R. Larsen P. Bedinger S.M. Stack

Department of Biology and Program in Plant Molecular Biology, Colorado State University, Fort Collins, Colo., USA

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Chromosome rearrangements · Evolution · F_1 hybrids · Synaptonemal complex · Tomato

Abstract
The tomato clade of Solanaceae is composed of 12 species that are all diploid with the same chromosome number and morphology. Species in the tomato clade are considered to have evolved primarily by genic changes rather than large-scale chromosomal rearrangements because pachytene chromosomes in F_1 hybrids synapse normally along their lengths and linkage maps of intra- and inter-specific hybrids are co-linear. However, small inversions have been reported between tomato and some of its wild relatives. Therefore, we reevaluated 5 F_1 hybrids using high-resolution, electron microscopic examination of pachytene chromosome (= synaptonemal complex) spreads to determine whether any minor structural changes had occurred among species in the tomato clade, which were not easily visible using light microscopic analysis of conventional chromosome squashes. Our study revealed a number of unexpected synaptic configurations such as mismatched kinetochores, inversion loops and reciprocal translocations. Most of these structural differences were in or close to heterochromatin that has comparatively few genes and little recombination, so they would be expected to have little effect on the evident colinearity of linkage maps, especially in euchromatin. However, these results demonstrate that substantial changes in chromosome structure have occurred among species within the tomato clade.

The tomato clade of *Solanum* (*Solanum* sect. Lycopersicon) [Peralta et al., 2008] includes 12 species and subspecies (fig. 1). All are diploid, except 2 natural tetraploid populations of *S. chilense* (2n = 4x = 48) [Chetelat and Ji, 2007], and share the same number (2n = 2x = 24) of acrocentric to metacentric chromosomes with large blocks of pericentric heterochromatin and distal euchromatic arms [Brown, 1949; Barton, 1950; Gottschalk, 1951]. The only exception to this generalization is chromosome 2 with a completely heterochromatic short arm including a distal nucleolus organizing region (NOR) [Barton, 1950; Gottschalk, 1951]. The similarity in chromosome morphology as well as colinearity of genetic markers among members of the tomato clade suggested that major chromosome rearrangements such as translocations and inversions are rare. Not all of the F_1 hybrids have similar high levels of fertility, but genic differences, not major chromosomal differences, appear to account for this variation in fertility because light microscopic examination of chromosome squashes from various tomato F_1 hybrids showed all 12 homeologous chromosomes synapsed completely along their lengths at pachytene and formed 12 bivalents that segregated appropriately at anaphase I [Rick, 1979; Quiros, 1991; Chetelat and Ji, 2007]. The most regularly observed chromosomal differences between tomato *(Solanum lycopersicum)* and certain wild species (particularly *S. pennellii*) are in the relative amounts of pericentric heterochromatin and the pattern of heterochromatic chromomeres, neither of which appear to have detrimental effects on meiotic synapsis or

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Lorinda Anderson
Department of Biology and Program in Plant Molecular Biology
Colorado State University
Fort Collins, CO 80523-1878 (USA)
Tel. +1 970 491 4856, Fax +1 970 491 0649, E-Mail lorinda.anderson@colostate.edu

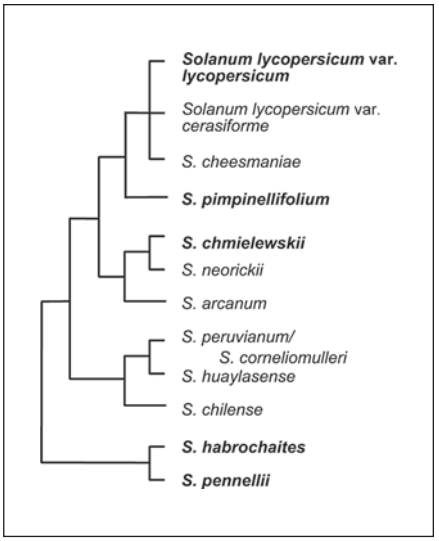

Fig. 1. Phylogenetic tree of the tomato clade, redrawn from [Moyle, 2008]. Tomato *(S. lycopersicum)* and the wild tomato species used in this study are indicated in bold.

recombination [Gottschalk, 1954; Khush and Rick, 1963]. Making fertile hybrids with wild species is particularly important for tomato breeding because cultivated tomato is autogamous (self-compatible) and has little genetic variability compared to its wild relatives, many of which are facultative or obligate out-crossers [Rick et al., 1978; Robertson and Labate, 2007]. The chromosomal stability of species in the tomato clade is 1 reason why they have been used as a model system for exploring the molecular and genetic basis of phenotypic variation in an ecological and evolutionary context [Moyle, 2008]. Such studies are more difficult in plant species that differ by numerous chromosomal rearrangements and/or ploidy level.

However, there are exceptions to the rule of chromosomal stability in the tomato clade. An inversion between tomato and *S. peruvianum* has been detected on the short arm of chromosome 6 around the nematode-resistance gene *Mi-1* [Seah et al., 2004] and may be related to an earlier cytological report of an inversion in this inter-specific hybrid [Lesley, 1950]. The inverted region is characterized by strong suppression of recombination in the F_1 hybrid. Similarly, high-resolution genetic mapping, molecular analysis, and FISH of DNA markers on chromosomes from cultivated tomato and *S. pennellii* demonstrated that these 2 species differ by a paracentric inversion in the short arm of chromosome 7 [van der Knaap et al., 2004]. This inversion seems to have occurred in the *S. pennellii* genome because genetic mapping of tomato × *S. pimpinellifolium* and tomato × *S. peruvianum* hybrids revealed higher levels of recombination among markers in this region compared to tomato × *S. pennellii* hybrids. Other chromosomal modifications may exist between tomato and S. *pennellii* based on differences in the location of markers on chromosome 1 in tomato using FISH compared to their expected positions using the tomato × *S. pennellii* EXPEN2000 genetic linkage map and the recombination nodule map for tomato [Sherman and Stack, 1995; Chang et al., 2007]. While the chromosomal rearrangements detected to date in *S. pennellii* relative to cultivated tomato appear to be small, they could have a significant negative impact on assembling the euchromatic genomic sequence for tomato (http://solgenomics.net/; Mueller, 2005 4473 /id) when markers are inverted or farther apart than expected based on their genetic map position in the EXPEN2000 map. On the other hand, chromosomal rearrangements could be used to help clarify evolutionary relationships among the wild relatives of tomato, which are still considered to be provisional (fig. 1) [Peralta et al., 2008].

Here, we report patterns of pachytene chromosome synapsis for 5 different F_1 hybrids from the tomato clade. Most of the hybrids were from cultivated tomato crossed with wild species ranging from the closely related *S. pimpinellifolium* to the more distantly related *S. pennellii* and *S. habrochaites* (fig. 1). One hybrid was a cross between 2 wild species, *S. pennellii* and *S. habrochaites*. For each hybrid, we examined spreads of complete sets of pachytene synaptonemal complexes (SCs) by electron microscopy. Each lateral element (LE) represents a chromosome from 1 of the species, and any significant differences in chromosome structure are reflected in the synaptic pattern of the SC. The higher resolution of electron microscopy can reveal irregularities in synapsis that are too small or too complex to be accurately interpreted by light microscopy of squashes of pachytene chromosomes [Gottschalk, 1951; Menzel, 1962]. Using this higher-resolution assay, we observed mismatched kinetochores, inversion loops, translocations, foldbacks and other synaptic irregularities, most of which had not been observed before in tomato inter-specific hybrids.

Materials and Methods

Plants

The 5 different interspecific tomato hybrids used in this study were produced by crossing the following parental species (with female parent indicated first): (1) *Solanum lycopersicum* (tomato – VF36) × *S. pimpinellifolium* (LA1589); (2) tomato (VF36) ×

Table 1. Type and frequencies of unusual synaptic configurations observed among F_1 hybrids in the tomato clade

F_1 hybrid	No. of observed SC sets	Average no. of SCs per set with mismatched kinetochores (min.–max.)	Maximum no. of SCs per set with inversion loops	No. of SC sets with translocation	Average no. of SCs per set with other synaptic irregularities[a] (min.–max.)			
					foldback	asynapsis	mismatched ends	combined irregularities
S. lycopersicum × *S. pimpinellifolium*	10	0.9 (0–2)	0	0	0.3 (0–2)	0.1 (0–1)	0.9 (0–4)	1.3 (0–5)
S. lycopersicum × *S. chmielewskii*	16	6.8 (4–9)	2	15	0	0.1 (0–1)	0.1 (0–1)	0.2 (0–1)
S. lycopersicum × *S. pennellii*	22	5.3 (3–7)	1	0	0.2 (0–2)	1.2 (0–3)	0.4 (0–2)	1.9 (0–4)
S. lycopersicum × *S. habrochaites*	22	4.0 (1–6)	1	0	0.3 (0–2)	0.6 (0–2)	0.8 (0–2)	1.6 (0–5)
S. pennellii × *S. habrochaites*	22	4.6 (2–7)	1	0	0.6 (0–1)	0.3 (0–1)	0.3 (0–1)	1.2 (0–2)

[a] Excluding mismatched kinetochores, inversion loops, and translocation configurations.

S. chmielewskii (LA1316); (3) tomato (VF36) × *S. habrochaites* (LA1777); (4) tomato (VF36) × *S. pennellii* (LA1340); (5) *S. habrochaites* (LA0407) × *S. pennellii* (LA0716). S. pennellii LA0716 is the accession used to develop the EXPEN2000 linkage map (http://solgenomics.net/). Otherwise, the accessions used to make the F_1 hybrids were chosen based on availability and overall plant health at the time of flowering. Hybrid plants were grown from seed to flowering in a controlled temperature greenhouse.

Chromosome Preparation

Spreads of pachytene synaptonemal complexes (SCs) were prepared as described in detail by Stack and Anderson [2009]. Briefly, primary microsporocytes in late pachytene were digested by cytohelicase in a hypertonic sugar-salt solution to remove their cell walls. The resulting protoplasts were burst in a hypotonic aqueous solution consisting of 0.05% IGEPAL® CA-630 (a nonionic detergent also known as Nonidet P40; Sigma), 1% bovine serum albumin, and 0.3% paraformaldehyde, and sets of SCs were allowed to settle onto a microscope slide coated with 0.6% Falcon plastic. The preparations were immediately fixed with a light spray of aqueous 4% paraformaldehyde totaling about 100 μl, allowed to dry for 2 h, dipped in aqueous 0.3% Photoflo 200, washed twice with water, then air-dried overnight. For electron microscopy, the slides were treated with DNase I for 15 min at 37°C, then washed and air-dried as described above. The slides were fixed for 10 min at room temperature using 2% glutaraldehyde and 2% paraformaldehyde in 0.3 M potassium phosphate buffer (final pH adjusted to 8.0), washed and air-dried as above. Slides were stained 10 min with 1% alcoholic phosphotungstic acid (PTA), washed in 95% alcohol, and air-dried. SC spreads at pachytene (as judged by synaptic extent and presence of kinetochores) in which the SCs were well-separated were located by phase light microscopy. These SC spreads on plastic were picked up onto grids and photographed using an AEI 801 electron microscope. Negatives were scanned at 600 dpi, and photographic montages were assembled using Adobe Photoshop CS2.

Fluorescence in situ Hybridization (FISH)

FISH was performed using SC spreads from the tomato × *S. pimpinellifolium* hybrid according to Chang et al. [2007]. Two BACs previously localized to tomato chromosomes 7 and 8 (http://sgn.cornell.edu/cview/map.pl?map_id=13) were used. BAC 216M19 contains the genetic marker T1123 and is located on the short arm of tomato chromosome 8, and BAC 215P04 contains the genetic marker T1401 and is located on the long arm of tomato chromosome 7.

Results

Pachytene SC spreads from a wild-type (non-F_1) tomato *S. lycopersicum* and each F_1 hybrid (fig. 2–5) revealed 12 bivalents by electron microscopy (EM), but synaptic irregularities were observed in every SC spread from all hybrid plants. These irregularities included mismatched kinetochores, inversion loops, foldbacks, mismatched ends, asynapsis (often with the axial elements having different lengths), and translocations. The type as well as the average number of unusual synaptic configurations observed per SC set varied between hybrids (table 1). Even within the same hybrid, there was substantial variation in the number and type of different synaptic configurations observed from set to set of SCs (table 1 and summarized below).

According to the most recent phylogenetic analysis, *S. pimpinellifolium* and tomato are closely related (fig. 1), and, as might be expected, the F_1 hybrid between these 2 species had the fewest synaptic irregularities among the

5 hybrids examined (table 1). On average, 1 SC per set had mismatched kinetochores (fig. 3), and 1 SC per set had mismatched ends. Among 10 SC sets from this hybrid, 2 sets had no SCs with mismatched kinetochores, and 1 set had 2 SCs with mismatched kinetochores. Similarly, some SC sets had neither foldbacks nor other synaptic irregularities (fig. 3A), while 1 SC set had 5 SCs with synaptic irregularities. We did not observe any inversion loops or partner trades due to translocations in this hybrid. Since usually only 1 SC per set had mismatched kinetochores, we were able to measure the relative length and arm ratio of all the SCs in these sets and narrow the range of possible SCs involved in the mismatch. We used FISH to determine that the SC that regularly exhibited mismatched kinetochores was chromosome 8 (fig. 3B).

S. chmielewskii is the next most closely related wild species to tomato (fig. 1). Unexpectedly, the tomato × *S. chmielewskii* F_1 hybrid had the most synaptic irregu-

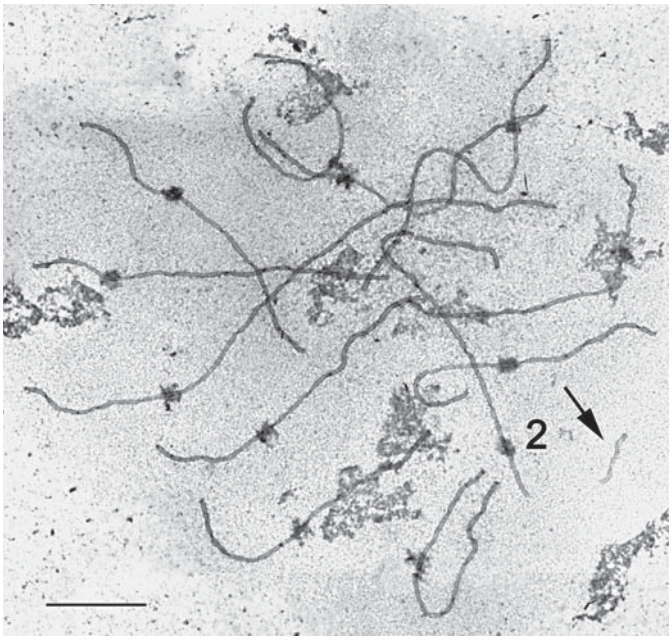

Fig. 2. Electron micrograph of an SC spread from tomato *(S. lycopersicum)* showing twelve bivalents with matched kinetochores. The short arm of chromosome 2 (marked by 2 at the chromosome 2 kinetochore in this and subsequent figures) is often broken (arrow points to SC fragment from short arm of chromosome 2). Scale bar = 10 μm.

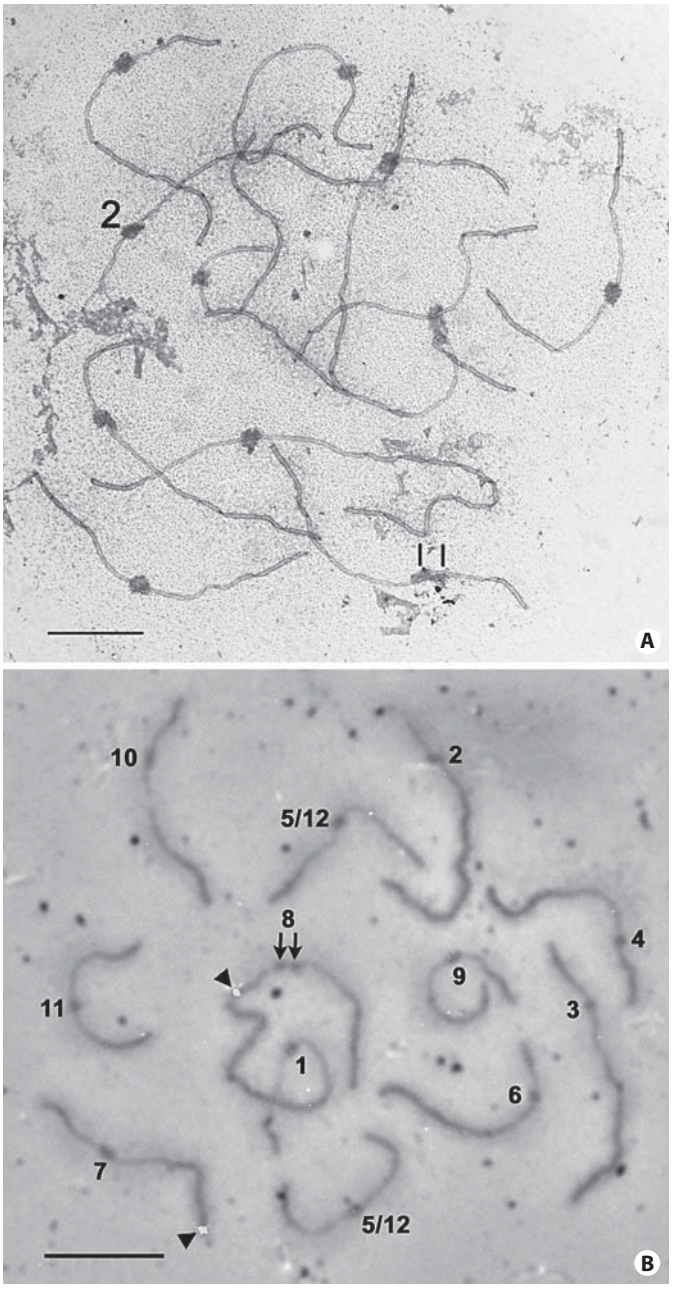

Fig. 3. SC spreads from *S. lycopersicum* × *S. pimpinellifolium* F_1 hybrid. **A** Electron micrograph showing 1 of the 12 bivalents with a mismatched kinetochore (bars). 2 = Kinetochore of SC 2. Scale bar = 5 μm. **B** Phase light micrograph with FISH signals (arrowheads) for BACs on chromosomes 7 and 8 (mismatched kinetochores – arrows) overlaid over the image. Each SC has been identified (see number next to each kinetochore) by relative length and arm ratio and/or BAC signal. Chromosomes 5 and 12 cannot be distinguished from one another by simple morphological criteria. SC in pericentric heterochromatin appears thinner than SC in distal euchromatin. Scale bar = 10 μm.

larities and rearrangements of any of the hybrids (table 1; fig. 4). We observed an average of almost 7 SCs per set with mismatched kinetochores. Two of the SCs with mismatched kinetochores also had inversion loops, and we could distinguish between the 2 SCs because the mismatched kinetochores were much closer together on 1 SC than on the other. Both inversions appear to be close to or within heterochromatin based on their proximity to the kinetochores and on the thin appearance of LEs in heterochromatic compared to euchromatic regions of chromosomes [Sherman and Stack, 1992]. In 2 of the SC sets, the SC with the closer mismatched kinetochores had 2 inversion loops immediately adjacent to one another (fig. 4C). This indicates that at least 1 of the chromosomes of tomato and *S. chmielewskii* differ by 2 closely adjacent inversions. We also observed a nearly whole arm translocation in this hybrid (fig. 4A, B). The breakpoints for the translocation apparently occurred in heterochromatin near the kinetochores, but the kinetochores themselves are not obviously mismatched (although they are asynapsed in some SC sets). This translocation configuration was observed in 15 of the 16 nuclei that we examined by EM. Presumably the other set had straight nonhomologous synapsis through the translocated segment as has been observed in other tomato translocation heterozygotes [Herickhoff et al., 1993]. Other irregular synaptic configurations such as foldbacks, asynapsis (that was not associated with inversion loops or with the translocation), and mismatched ends were rare (combined average of 0.2 per nucleus) compared to the other hybrids.

Within the tomato clade, *S. pennellii* and *S. habrochaites* are distant from cultivated tomato and close to each other (fig. 1). As expected from the phylogenetic tree, the modified synaptic configurations observed in the tomato × *S. pennellii* and tomato × S. *habrochaites* F_1 hybrids are similar in both type and frequency. The F_1 hybrids average 5.3 and 4.0 mismatched kinetochores per SC set, respectively, and a single inversion loop per set has been observed in both hybrids (table 1; fig. 5B, H). However, the inverted segment synapsed non-homologously

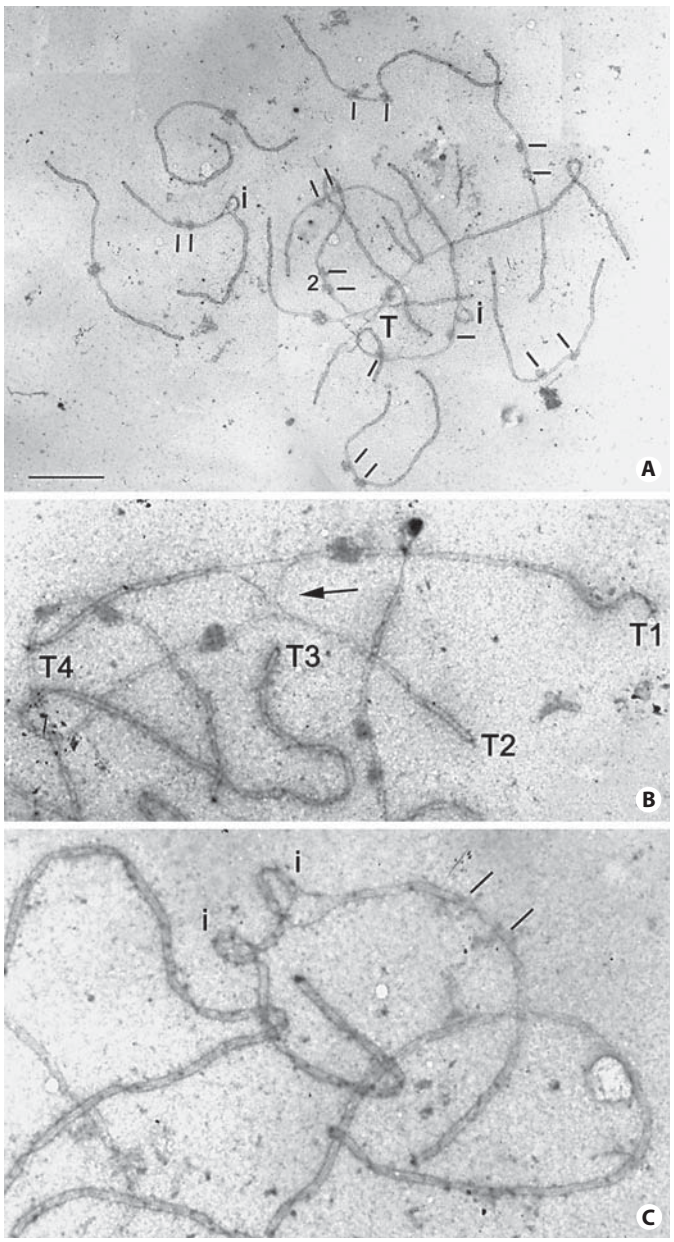

Fig. 4. Electron micrographs of SC spreads from *S. lycopersicum* × *S. chmielewskii* F_1 hybrid. **A** Montage of a complete SC set. Eight of 12 SCs have mismatched kinetochores (pairs of bars). Two SCs with mismatched kinetochores have inversion loops (i), but the inversion loop SC on the left of the spread has closely adjacent mismatched kinetochores while the inversion loop SC on the right of the spread has mismatched kinetochores separated by a long stretch of SC. In addition, 2 SCs are involved in a reciprocal translocation (indicated by T at the site of the breakpoint). 2 = Kinetochore of SC 2. **B** Higher magnification view of the translocation from a different SC spread. Each end of the SC involved in the translocation has been indicated (T1–T4) and the position of the translocation partner trade is indicated (arrow). The kinetochores are matched for both translocation chromosomes. **C** Higher magnification of an SC with two adjacent inversion loops (i) from a different SC spread. The kinetochores are mismatched and relatively close together. The same scale bar represents 5 μm for **A** and 2.5 μm for **B** and **C**.

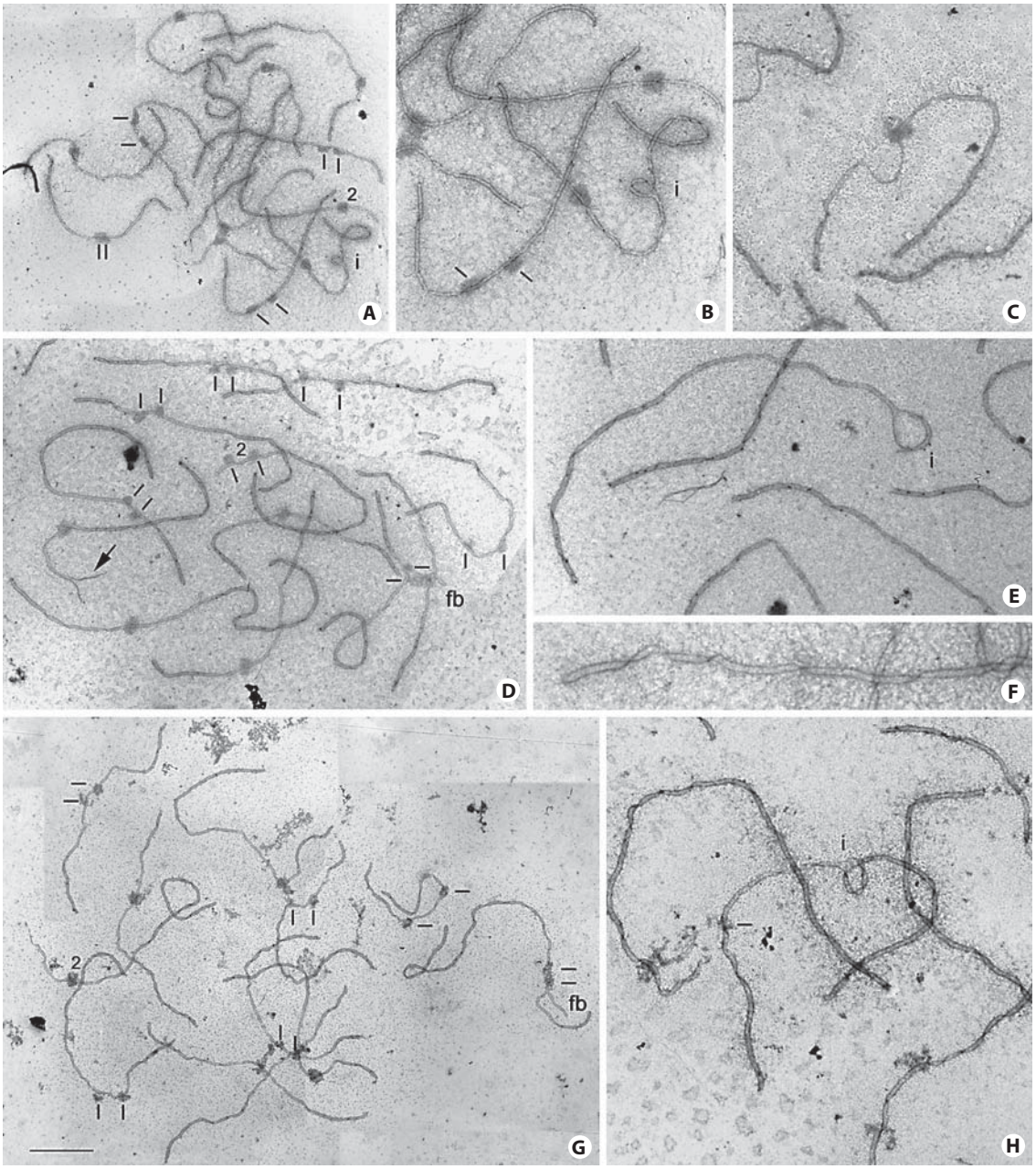

Fig. 5. Electron micrographs of SC spreads from F_1 hybrids. *S. lycopersicum* × S. *habrochaites* F_1 hybrid (**A–C**). **A** Montage of complete SC set. Four SCs have mismatched kinetochores (pairs of bars) and 1 SC has an inversion loop (i). 2 = Kinetochore of SC 2. **B** Higher magnification view of lower portion of B showing inversion loop (i) and mismatched kinetochores (bars). Each kinetochore is associated with one lateral element. **C** The two axial elements near the kinetochore differ in length. *S. habrochaites* × *S. pennellii* F_1 hybrid (**D–F**). **D** Montage of complete SC set. Seven SCs have mismatched kinetochores (pairs of bars), 1 SC has foldback (fb) loop near the kinetochore, and 1 end of 1 SC is asynapsed with axial elements of different lengths (arrow). **E** SC from a different set showing distal inversion loop. **F** Close-up of an SC from a different set showing one lateral element 'wrapped around' the other lateral element. This type of synapsis allows SC to form between lateral elements of different lengths. *S. lycopersicum* × *S. pennellii* F_1 hybrid (**G**, **H**). **G** Montage of complete SC set. Six SCs have mismatched kinetochores (pairs of bars), and 1 SC has a large foldback (fb) near the mismatched kinetochores. **H** SC from a different set with an inversion loop (i). The kinetochore for this SC is indicated (bar). Both inversion loops in **B** and **H** are located near the border of euchromatin (thicker LE) and heterochromatin (thinner LE). The same scale bar represents 5 μm for **A**, **D** and **G**, 2.5 μm for **B**, **C**, **E** and **H**, and 0.83 μm for **F**.

and did not form a loop in many nuclei (fig. 5G). In both hybrids, the inversion loop appeared to be located close to the euchromatin-heterochromatin border of the chromosome arm. The average number of foldbacks, asynapsis and mismatched ends were also similar at 1.6 or 1.9 per SC set for both hybrids.

Based on these observations and the phylogenetic tree, we expected the F_1 hybrid between *S. pennellii* × *S. habrochaites* to have few, if any, unusual synaptic configurations. Instead, this hybrid had nearly as many mismatched kinetochores and synaptic irregularities as the F_1 hybrids of the 2 wild species with cultivated tomato (table 1; fig. 5). The *S. pennellii* × *S. habrochaites* hybrid averaged 4.6 mismatched kinetochores per set and about 1.2 other synaptic irregularities per set. In addition, an inversion loop at the distal end of 1 SC was observed in SC spreads from this hybrid (fig. 5E). This inversion may correspond to distal asynapsis often observed in other SC sets from this hybrid (fig. 5D). Overall, these results indicate that the differences in synaptic configuration in the tomato × *S. pennellii* and tomato × *S. habrochaites* hybrids, while similar in overall frequency and type, are due to changes in different chromosomes with respect to the 2 wild species.

For all the hybrids, we noticed a tendency for SC spreads at early pachytene (as determined by the presence of early recombination nodules and/or small kinetochores [Anderson and Stack, 2005]) to have more mismatched kinetochores than late pachytene SC sets. Similarly, inversion loops and asynapsed regions of SCs were more likely to be present at early compared to late pachytene. Such observations indicate that the 2 LEs in an inter-specific hybrid are capable of at least some synaptic adjustment toward straight nonhomologous synapsis [Moses et al., 1982]. This is in contrast to previous observation of a lack of synaptic adjustment of inversion loops in maize [Anderson et al., 1988] and in *Allium cepa* × *Allium fistulosum* F_1 hybrids [Albini and Jones, 1990]. However, we did not do a comprehensive study in the tomato hybrids, so these results regarding synaptic adjustment are preliminary.

In the absence of FISH markers, the particular chromosomes involved in different synaptic configurations are difficult to determine. This is especially true given the difficulty of determining accurate arm ratios and relative chromosome lengths if the LEs are capable of synaptic adjustment. However, the heterochromatic short arm of chromosome 2 containing the NOR is often broken and can be identified in many SC spreads from the different hybrids. We found that each of the hybrids had a consistent pattern with regard to the kinetochore pattern for SC 2. All of the SC spreads from 2 hybrids, tomato × *S. chmielewskii* and *S. pennellii* × *S. habrochaites*, had mismatched kinetochores for SC 2, but none of the SC spreads from the other hybrids had mismatched kinetochores for SC 2. These results suggest that there is no adjustment of SC 2 that allows mismatched kinetochores to become matched at the same site. However, we also noted that neither tomato × *S. pennellii* (LA1340) nor tomato × *S. habrochaites* (LA1777) hybrids had mismatched kinetochores while the *S. pennellii* (LA0716) × *S. habrochaites* (LA0407) hybrid had mismatched kinetochores. This result was unexpected, but the accessions for *S. pennellii* and *S. habrochaites* used in these 3 crosses were not the same. Therefore, we examined SC spreads from a tomato × *S. habrochaites* (LA0407) hybrid. Although the data were limited, we observed 2 SC sets from this hybrid, and in both nuclei, the SC 2 kinetochores were mismatched. Therefore, it is likely that the 2 *S. habrochaites* accessions (LA0407 and LA1777) differ in the position of the kinetochore on SC 2.

We were able to count the number of late recombination nodules (RNs) that mark crossover sites in 34 late pachytene SC sets from the hybrids. The number of RNs ranged from 12 to 24 per SC set. Each SC (except one) had at least 1 RN as required for balanced segregation.

Discussion

Twelve bivalents were regularly observed in SC spreads for all F_1 hybrids except tomato × *S. chmielewskii* that had 10 bivalents plus a reciprocal translocation of 2 bivalents. Mismatched ends or asynapsis at the ends of SCs were only rarely observed, and synapsis at the telomeres and throughout the euchromatin was usually complete with no obvious irregularities in SC structure. These results are similar to observations made earlier using light microscopic observations of pachytene meiotic chromosomes from certain tomato F_1 hybrids [Khush and Rick, 1963; Rick and Yoder, 1988]. Since synapsis usually starts near telomeres in tomato [Stack and Anderson, 1986], the extensive, regular formation of SC in euchromatin is consistent with the high level of gene colinearity (homology) observed among members of the tomato clade [Rick, 1979; Tanksley et al., 1992; Chetelat and Ji, 2007]. The presence of RNs in numbers similar to those observed in cherry tomato also is consistent with balanced segregation of chromosomes at anaphase I and the generally high levels of fertility of the F_1 hybrids [Rick, 1979; Rick and

Yoder, 1988; Sherman and Stack, 1992]. Thus, at least with regard to euchromatin, the assertion that members of the tomato clade differ little in chromosome structure is supported by our results.

However, we also observed synaptic configurations in the F_1 hybrids that result from major structural rearrangements between the parental species. Paracentric inversion loops were observed in 4 of the 5 F_1 hybrids, and a reciprocal translocation was observed in the tomato × *S. chmielewskii* hybrid. In addition, mismatched kinetochores were observed in all of the hybrids (discussed in more detail below). In tomato × *S. pennellii* (LA1340) and tomato × *S. habrochaites* (LA1777) hybrids, the inversion loops were located in heterochromatin or close to the euchromatin-heterochromatin borders. Because of the low frequency of recombination in and close to pericentric heterochromatin [Sherman and Stack, 1995], inversions in these locations would be difficult to detect by genetic mapping, and neither inversion has been reported in genetic mapping studies [Tanksley et al., 1992; Bernacchi and Tanksley, 1997]. On the other hand, the inversion loop in the *S. pennellii* (LA0716) × *S. habrochaites* (LA0407) hybrid was located at the distal end of 1 SC in euchromatin and should be detectable using genetic as well as chromosomal approaches. The *S. habrochaites* LA0407 accession has not been used for genetic mapping, so it is possible that the inversion is present in this accession but not in the LA1777 accession that has been used for mapping [Bernacchi and Tanksley, 1997]. We noted a difference in kinetochore position for SC 2 between the two *S. habrochaites* accessions. However, we cannot exclude the possibility that this inversion arose spontaneously in the individual plant we assayed. Similarly, we cannot exclude the possibility that the reciprocal translocation difference in the tomato × *S. chmielewskii* hybrid is plant- or accession-specific because the genetic mapping study (that did not report a translocation) was done on a different *S. chmielewskii* accession (LA1028) than the one we used here (LA1316). It seems unlikely that the different inversions and 1 translocation we observed each arose in individual plants, but more work will be required to determine which structural changes are found consistently in each accession. Nevertheless, these results show that large changes in chromosome structure can and do occur among different members of the tomato clade.

The most common irregularity we observed in all of the hybrids was mismatched kinetochores. Mismatched kinetochores have not been reported in tomato interspecific hybrids before, although variations in heterochromatin amounts and patterns have been [Gottschalk, 1954; Khush and Rick, 1963]. Kinetochores are clearly visible in SC spreads, and mismatches are easy to recognize. In comparison, centromeres are more difficult to identify in chromosome squashes, and mismatches would have to be well-separated to be identified as such. There are at least 3 possible explanations for the mismatched kinetochores, all of which require some level of non-homologous synapsis and any one of which could apply to SCs between different hybrids as well as to different SCs in the same hybrid. (1) One explanation is that pericentric inversions involving breakpoints in the heterochromatin and/or heterochromatin-euchromatin border regions have occurred in some of the lineages, and non-homologous synapsis has occurred through the length of the inversion [Coyne et al., 1991 and references therein]. This is attractive because some inversions undoubtedly have occurred as indicated by the presence of small paracentric inversion loops in many of the hybrids we studied. Genetic mapping and FISH have also demonstrated the presence of paracentric inversions among species in the tomato clade [van der Knaap et al., 2004; Seah et al., 2004]. Although easily visible as mismatched kinetochores in SC spreads, pericentric inversions with breakpoints that occur in recombination-suppressed regions with relatively fewer genes would be expected to have little, if any, effect on genetic linkage maps and would be unlikely to show significant deviation from the marker colinearity usually observed in tomato × wild species hybrids [Frary et al., 2005; Chetelat and Ji, 2007]. (2) Observed differences in genome sizes (DNA amounts) between species in the tomato clade could provide a second explanation for mismatched kinetochores. Anderson et al. [1985] reported that the length of a complete set of SCs is strongly correlated with genome size in plants, i.e., differences in genome size would be expected to result in differences in SC length among tomato and its wild relatives. For species in the tomato clade, genome size varies from 0.85 pg/1 C to 1.23 pg/1 C, a 45% difference (table 2). Such differences in genome size could lead to mismatched kinetochores if the differences in DNA amount are not distributed equally throughout the chromosome arms, perhaps due to differential expansion or contraction of transposons [Wang et al., 2006; Chang et al., 2008]. Such changes in transposon frequency would be unlikely to alter gene order. (3) Finally, kinetochores could change locations epigenetically in homeologs from different species [Lamb et al., 2007].

Genetic maps have been made for all of the F_1 hybrids we examined [Rick, 1972; Paterson et al., 1988; Tanksley

Table 2. DNA genome size (C values in pg) for some species in the tomato clade

Species	1C (pg)	% difference in genome size compared to *S. lycopersicum*	Reference
S. lycopersicum (formerly *Lycopersicon esculentum*)	0.95	–	Bennett and Smith [1976]
S. habrochaites (formerly *L. hirsutum*)	0.93	–2	Bennett and Smith [1976]
S. pimpinellifolium (formerly *L. pimpinellifolium*)	0.85	–12	Bennett and Smith [1976]
S. pennellii (formerly *L. pennellii*)	1.23	+29	Arumuganathan and Earle [1991]
S. chmielewskii	No information	–	–

et al., 1992; Grandillo and Tanksley, 1996; Bernacchi and Tanksley, 1997, although not always the same accessions]. The population structure and the number of markers used to generate the maps varied in these different studies, but all of the maps were colinear and similar in size (around 1,200–1,350 cM except tomato × *S. chmielewskii* at 852 cM). All the maps also had marker segregation distortions, but the distortions were not necessarily related to phylogenetic distance. Bernacchi and Tanksley [1997] compared linkage maps of tomato × *S. pimpinellifolium* (E×PM), tomato × *S. habrochaites* (E×H), and tomato × *S. pennellii* (E×P) F_1 hybrids in detail [Bernacchi and Tanksley, 1997]. They observed that the overall map lengths among these hybrids did not differ much, but the genetic lengths of individual chromosomes could vary substantially. For example, they observed 50% more recombination on chromosome 1 but 40% less recombination on chromosome 11 for E×H compared to E×P. While crossing over is generally suppressed around centromeres (centromere effect) [see Tanksley et al., 1992 for references], genetic intervals around centromeres seemed to be particularly variable when comparing different maps. Five centromeric regions on chromosomes 1, 3, 4, 8 and 12 had more than a 2-fold increase of recombination in the E×H compared to E×P maps, but the situation was reversed for chromosomes 7, 2 and 9 (although to a lesser extent for the latter 2 chromosomes). Similarly, recombination around the centromere was less repressed for 6 of the chromosomes (1, 3, 4, 5, 11 and 12) in the E×PM hybrid compared to E×P [Grandillo and Tanksley, 1996]. Considering this mapping data together with our cytogenetic analysis of tomato interspecific hybrids, it is possible that chromosomes with mismatched kinetochores are more likely to have reductions in crossing over in proximal intervals compared to chromosomes without mismatched kinetochores. We plan to use FISH of mapped single-copy sequences to identify chromosomes with mismatched kinetochores in the F_1 hybrids, similar to that done for tomato × *S. pimpinellifolium* (fig. 3), to address this question in more detail in the future.

Based on the most current phylogenetic analysis using molecular, genetic, and morphological traits [Peralta et al., 2008], 2 of our results were unexpected. First, the tomato × *S. chmielewskii* F_1 hybrid exhibited the most differences in chromosome structure during synapsis even though tomato is closer on the phylogenetic tree to *S. chmielewskii* than it is to either *S. habrochaites* or *S. pennellii* (fig. 1). Second, the *S. pennellii* × *S. habrochaites* F_1 hybrid had a high frequency of structural differences even though the 2 species are close on the phylogenetic tree. This is in contrast to the F_1 hybrid between the closely-related tomato and *S. pimpinellifolium* species in which only 1 mismatched kinetochore was regularly observed. Thus, the number and type of structural rearrangements that have occurred between tomato species do not necessarily correlate with the current phylogenetic tree.

Members of the tomato clade have long been considered to have stable genomes in which speciation has occurred primarily by genic change and not large-scale chromosomal alterations [Rick, 1979; Chetelat and Ji, 2007; Moyle, 2008]. However, our results show that significant changes in chromosome structure have occurred among the different species. Many of these changes are in or close to heterochromatin where gene frequency is lower than in euchromatin [Wang et al., 2006]. Because

of this, structural changes in heterochromatin are difficult to detect using genetic mapping alone. With the exception of the translocation in the tomato × *S. chmielewskii*, none of the structural changes we observed would be expected in themselves to have major effects on F_1 hybrid fertility because complete haploid sets of genes from both species would be present. However, it is possible that the chromosomal changes we have observed could protect blocks of genes that are important in reducing fertility in the F_1 hybrids as well as in subsequent generations [Rieseberg et al., 1999; Noor et al., 2001; Lai et al., 2005; Rieseberg and Willis, 2007]. If so, this could be an important factor in restricting gene flow and defining species in the tomato clade.

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