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ORIGINAL PAPER

Comparative genetic linkage map of *Solanum* sect. *Juglandifolia*: evidence of chromosomal rearrangements and overall synteny with the tomatoes and related nightshades

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Abstract The two nightshades *Solanum ochranthum* and *S. juglandifolium* show genetic and morphological similarities to the tomatoes (*Solanum* sect. *Lycopersicon*), but are isolated from them by strong reproductive barriers. Their genetic relationships to tomato and other *Solanum* species were investigated using comparative genetic linkage maps obtained from an interspecific F_2 *S. ochranthum* × *S. juglandifolium* population. Sixty-six plants were screened using a total of 132 markers—CAPs, RFLPs and SSRs—previously mapped in tomato. Twelve linkage groups were identified, generally corresponding to the expected (syntenic) tomato chromosomes, with two exceptions. Chromosome 1 was composed of two linkage groups and chromosomes 8 and 12 were connected in one large linkage group, indicating a likely reciprocal translocation differentiating the two parental genomes. The total map length comprised 790 cM, representing a 42% reduction in recombination rate relative to the tomato reference map. Transmission ratio distortion affected one-third of the genome, with 13 putative TRD loci identified on 9 out of 12 chromosomes. Most regions were collinear with the tomato reference maps, including the long arm of chromosome 10, which is inverted relative to two other tomato-like nightshades, *S. lycopersicoides* and *S. sitiens*. The results support the status of *S. ochranthum* and *S. juglandifolium* as the nearest outgroup to the tomatoes and imply they are more closely related to cultivated tomato than predicted from crossing relationships, thus encouraging further attempts at hybridization and introgression between them.

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Introduction

The two tomato-like nightshades *S. ochranthum* and *S. juglandifolium* make up *Solanum* section *Juglandifolia* (Rydb.) Child. Both species are diploids ($2n = 24$), and grow as woody perennials with rampant, liana-like stems up to 30 m in length (Correll 1962; Rick 1988). Their fruit, especially those of *S. ochranthum*, are larger than any of the wild tomatoes, and after a long (8–9 month) period of maturation, emit an apple-like fragrance. Seeds are large and winged.

Molecular phylogenies have placed section *Juglandifolia* as the closest outgroup to *Solanum* sect. *Lycopersicon*, the group that includes cultivated tomato (*S. lycopersicum*, formerly *Lycopersicon esculentum*) and its 9–13 immediate wild relatives (Peralta and Spooner 2001). Basal to both groups is *Solanum* section *Lycopersicoides* (Child) Peralta, represented by the sister taxa *S. lycopersicoides* and *S. sitiens* (formerly *S. rickii*). Together with *S. lycopersicoides* and *S. sitiens* in section *Lycopersicoides*, *S. ochranthum* and *S. juglandifolium* share a number of morphological traits that place them in an intermediate position between tomato and potato. On the one hand they possess tomato-like characteristics that set them apart from most other *Solanum* species: their corollas are yellow

(flowers in subsect. *Petota* are mainly white, purple or blue), and their pedicels are articulated at the mid-position. On the other hand they lack certain tomato traits, such as the sterile anther appendages. Instead, pollen dehisces through terminal pores, as is typical for most species of *Solanum*. Their anthers are free (i.e., unattached), instead of fused, as in all tomatoes. Furthermore, strong reproductive barriers isolate these species from the tomato group (Rick 1988; Correll 1962; Child 1990; Stommel 2001; Smith and Peralta 2002). Using embryo culture, *S. lycopersicoides* and *S. sitiens* can be hybridized to tomato, but *S. ochranthum* and *S. juglandifolium* appear to be sexually incompatible with the tomato species in all combinations tested to date (Rick 1988). Although somatic hybrids have been obtained by protoplast fusion, they are highly sterile and have not, so far, provided a means for gene transfer (Stommel 2001).

Apart from the aforementioned traits, these two pairs of tomato-like nightshade species have little in common, either morphologically or with respect to autecology. *S. ochranthum* and *S. juglandifolium* are partially sympatric, native to the tropical rainforest regions at mid-elevations from Colombia to Southern Peru. These two species resemble each other closely: *S. juglandifolium* is a shorter, more sprawling version of *S. ochranthum*. In contrast *S. lycopersicoides* and *S. sitiens* are allopatric species that occupy dry and at times frost-prone areas at mid-to-high elevations in Southern Peru/Northern Chile (*S. lycopersicoides*) or the Atacama desert of Chile (*S. sitiens*; Correll 1958, 1962, Rick 1988). These two are perhaps more differentiated from one another, at least in terms of morphology and ecology, than are the *Juglandifolia* pair.

Cultivated tomato, like many other crop species, has been so depleted in genetic diversity—largely as a result of its domestication and early breeding history—that breeders must turn to related wild species for novel traits such as disease resistance (Rick and Fobes 1975; Bai and Lindhout 2007). Though not thoroughly tested, the sect. *Juglandifolia* species are expected to harbor novel traits, including tolerance to flooding stress, and resistance to pathogens prevalent in cool, humid conditions, such as late blight (Rick 1988). Understanding the genetic relationships between these two nightshades and cultivated tomato is an important element in evaluating their potential use in breeding programs, as well as their history of evolution and speciation.

One way to analyze relationships is through comparative genetic mapping. The advent of saturated maps of DNA based markers—initially RFLPs, now including SSRs, CAPs and others markers—made it possible to cross-map genomes using common sets of markers, thereby enabling comparisons of synteny relationships across related taxa. Comparative linkage maps have been generated for most agronomically important plant families, including the *Poaceae* (for review Devos and Gale 2000), *Brassicaceae* (Lagercrantz 1998), *Solanaceae* (Tanksley et al. 1992), *Fabaceae* (Boutin et al. 1995), *Compositae* (Burke et al. 2004), *Pinaceae* (Krutovsky et al. 2004) and *Rosaceae* (Dirlewanger et al. 2004). The solanaceous crop species—tomato, potato, eggplant and pepper—now possess one of the most detailed set of comparative maps available for any group of plants (Doganlar et al. 2002; Livingstone et al. 1999; Tanksley et al. 1992). In these and other examples, comparisons across genomes have uncovered a surprising amount of colinearity—even between monocots and dicots at a certain level—which enables the extrapolation of genetic data from well-characterized model species to less well-characterized relatives. Comparative maps also provide significant applications in plant breeding, and research on developmental and evolutionary genetics (Gale and Devos 1998).

Within the *Solanaceae,* comparative mapping revealed a high level of colinearity among species, with conserved gene order organized in reshuffled blocks of DNA. The genomes of tomato and potato (*S. tuberosum*) are differentiated by at least six large (whole arm) paracentric inversions involving 5S, 6S, 9S, 10L, 11S and 12S (Bonierbale et al. 1988; Bai et al. 2007). Twenty-three paracentric inversions and five translocations differentiate the genomes of tomato and eggplant (*S. melongena*), and even more rearrangements separate tomato from pepper (*Capsicum annuum*) (Doganlar et al. 2002; Livingstone et al. 1999). Direct comparisons of the Solanaceous genomes allowed reconstruction of the divergence of the *Solanaceae* lineages from a hypothetical common ancestor. Comparative analyses identified conserved segments even beyond the family level, for example between tomato/potato and *Arabidopsis* (Gebhardt et al. 2003; Ku et al. 2000), and between tomato and coffee (Lin et al. 2005).

In contrast to these numerous rearrangements at the family/genus level, gene order *within* the tomato group is highly conserved. Genetic maps based on interspecific crosses between *S. lycopersicum* and *S. chmielewskii* (Paterson et al. 1990), *S. pennellii* (Tanksley et al. 1992), *S. peruvianum* (van Ooijen et al. 1994, Fulton et al. 1997), *S. galapagense* (formerly *L. cheesmanii* f. *minor*; Paran et al. 1995), *S. pimpinellifolium* (Grandillo and Tanksley 1996), *S. habrochaites* (formerly *L. hirsutum*, Bernacchi and Tanksley 1997) or *S. neorickii* (formerly *L. parviflorum*; Fulton et al. 2000) are all highly collinear. One exception to this conserved gene order are segmental inversions (less than whole arm) between the genomes of tomato and *S. pennellii* on chromosome 7S of (Van der Knaap et al. 2004) and *S. peruvianum* on 6S (Seah et al. 2004). The genomes of *S. lycopersicoides* and *S. sitiens* lack the inversion of 10L that separates tomato from potato, which is thus a cyto-taxonomic marker unique to the tomato clade (Pertuzé et al. 2002).

The objective of the present study was to investigate the degree of macrosynteny in the *Solanum* sect. *Juglandifolia* species and the types of genome rearrangements relative to tomato and other Solanaceae. An applied goal was to assess their chromosomal affinities with the tomato genome, and thus the potential for eventual hybridization and introgression. A more basic objective was to obtain further insights into the history of genome evolution in *Solanum*.

Materials and methods

Plant materials

The parental species used in this study were *S. ochranthum* LA3650, from Choquemaray, Apurimac, Peru, and *S. juglandifolium* LA2788 from Quebrada La Buena, Antioquia, Colombia. Both accessions were collected by Charles Rick and Miguel Holle. Seeds were provided by the C. M. Rick Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, at Davis. Due to self-incompatibility of the parental species and their interspecific hybrid, two independent F_1 plants were crossed to obtain 66 pseudo-F_2 progeny. All plants were grown at the Vegetable Crops greenhouse facility at UC Davis.

Embryo culture

To insure germination, seeds of the parents were treated with half-strength household bleach (2.75% sodium hypochlorite) for 30 min, then rinsed extensively with water. F_1 and F_2 plants were obtained via embryo culture. Fruits were harvested ~45 days post-pollination, surface-sterilized for 10 min in 70% (v/v) ethanol and ~1.25% (w/v) sodium hypochlorite and rinsed. Ovules were extracted from fruit and embryos cultured on the HLH medium of Neal and Topoleski (1983), then transferred after 10–14 days to Gamborg's B-5 basal media with minimal organics (Sigma, St Louis, MO, USA) prepared according to Sacks et al. (1997). After 3–7 weeks of development, in vitro plantlets were transferred to soil, and acclimatized to greenhouse conditions.

Pollen stainability

Anthers of five flowers per F_1 plant (01L5288-1 and 01L5311-1) were squashed in acetocarmine (1% w/v in 50% v/v glacial acetic acid) on three different days. The number of viable grains—judged by the presence of stain in the cytoplasm and normal shape and size—was estimated out of a total of 100 grains using a light microscope at low magnification (200×).

Chromosome pairing

Chromosome associations during meiosis were evaluated in the F_1 hybrid 01L5288-1 using the acetocarmine squash method (Khush and Rick 1963). Developing floral buds were soaked in fixative (3:1 95% EtOH:glacial acetic acid with $FeCl_3$) for 24 h, washed 3× with 70% EtOH and stored at 4°C. The number of uni-, bi- and multivalents was recorded in eight individual cells using a Zeiss Axioskop compound microscope.

DNA isolation

Young leaves were harvested from mature plants of both parental accessions—for a better representation of the parental alleles, samples from five individuals per species were bulked—the two F_1 hybrids and the 66 F_2 plants. DNA was extracted by a modified CTAB method as previously described (Chetelat and Meglic 2000).

Choice of markers

Single copy markers—COS, COSII, RFLPs and SSRs—mapped previously in an interspecific F_2 *S. lycopersicum* × *S. pennellii* population (Tanksley et al. 1992; Fulton et al. 2000; Frary et al. 2005) were selected for this study. Markers were selected based on the confidence of their map position on reference maps (LOD scores ≥ 2) and their distribution in the genome so as to provide a whole genome coverage at an average spacing of 10 cM. Markers found to be informative in the *S. ochr.* × *S. jugl.* population were not always evenly spaced; a greater marker density was obtained around some centromeric regions, for instance (see "Results"). The tomato molecular marker maps EXPEN 2000, and to some extent EXPEN 1992, accessed via the SOL Genomics Network (SGN) database (http://www.sgn.cornell.edu), were used as reference maps. RFLPs were based on tomato genomic clones ('TG'; Zamir and Tanksley 1988; Miller and Tanksley 1990) and tomato leaf epidermal cDNA clones ('CT'; Yu and Blackburn 1991). COS and COSII (Conserved Orthologue Set) markers are based on single copy genes with corresponding orthologous loci in divergent taxa, and are thus well suited for comparative maps.

Conserved amplified polymorphic sequence (CAPS) technique

Primers for PCR-based CAPS markers were designed from sequence information available at SGN using the primer design program Primer3 (Rozen and Skaletsky 2000, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi), and assembled by Sigma Genosys and Operon Technologies.

The majority of the COSII loci were amplified using the *Universal Primers for Asterid Species* (UPA), available at SGN (Wu et al. 2006), for a few loci primers were designed as described above. PCR amplifications were conducted in a total volume of 20 µL in a thermal cycler (GeneAmp; Applied Biosystems). Each cycling reaction contained reaction buffer (1× final concentration, Applied Biosystems), $MgCl_2$ (1.5 mmol/l, Applied Biosystems) dNTPs (200 µmol/l, Applied Biosystems), bovine serum albumine (400 µg/µl, New England Biolabs), *Taq* polymerase 0.05 U/µl Applied Biosystems, 0.3 mmol/l primer (forward + reverse) and template DNA (100 ng). Amplification consisted of an initial denaturation for 5 min at 94°C, followed by 40 cycles of amplification with denaturation at 94°C for 30 s, annealing at 55–62°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The optimal annealing temperature was determined for each primer pair on parental genomic DNA in a gradient thermal cycler (Techne). Primer performance was evaluated by electrophoresis on 1.8–2.0% agarose (Amresco) followed by ethidium bromide staining. Primer pairs that resulted in single-band amplification were digested with a set of eight frequently cutting restriction enzymes (*Bso*BI, *Hinf*I, *Msp*I, *Ban*I, *Hae*III, *Sty*I, *Hae*II and *Hha*I; New England Biolabs or Promega). Digests were carried out according to the manufacturer's recommendations. Digested DNA fragments were separated on 1.8–2% agarose gels. If none of the restriction enzymes yielded polymorphic banding patterns among the parental genotypes a second set of digests was performed using eight additional restriction enzymes (*Ava*II, *Dra*I, *Nci*I, *Rsa*I, *Alu*I, *Dpn*I and *Dde*I).

Simple sequence repeats

Simple sequence repeats (SSRs) were derived from tomato ESTs (Frary et al. 2005). Primer sequence information for detecting SSRs was obtained from SGN. Primer assemblage and reaction mix preparation were as described above. PCR reaction conditions were based on those employed by Frary et al. (2005): after an initial denaturation for 5 min at 94°C, 40 cycles of amplification consisting of 30 s denaturation at 94°C for, 45 s annealing at 55°C and for 45 s extension at 72°C followed with a final extension of 72°C for 10 min. Depending on the size difference between parental fragments, samples were resolved on either 1.8–2% agarose and stained with ethidium bromide or on 5.2% polyacrylamide using a LiCor 4200 sequencing apparatus to detect fluorescently labeled fragments. For the latter, the tailed primer method was used to incorporate fluorescent dyes (IR-700 or IR-800) into PCR amplicons via a labeled M13 primer (TTTCCCAGTCACGACGTT; MWG-Biotech) that was added at 0.05 µg/µl to the PCR reaction mix.

RFLPs

Total genomic DNAs of both the parents, the interspecific hybrids and 66 F_2 individuals were digested separately with six restriction enzymes (*Eco*RI, *Eco*RV, *Hinf*I, *Xba*I, DraI, *Hae*III; New England Biolabs, Promega). Digestions were performed according to the manufacturer's instructions. Samples were electrophoresed on 0.8% agarose and blotted onto nylon membranes (Hybond-N+, Amersham). Probes were labeled with [^{32}P]-dCTP and [^{32}P]-dATP using the random hexamer primer method (Feinberg and Vogelstein 1983) and washed three times to a final stringency of 0.5× SSC before exposure to X-ray film (Kodak BioMax MS and Fuji Super RX) at −80°C. Surveys were conducted to identify probe/restriction enzyme combinations that produced polymorphisms between parental genotypes.

Linkage analysis

The χ^2 goodness-of-fit statistic was employed to test for deviations from expected Mendelian segregation ratios in the F_2 (1:2:1) at $P < 0.05$. Linkage analysis and map construction were performed with MapMaker version 2.0 for Macintosh (Lander et al. 1987). Linkage groups were assigned at LOD ≥ 4 and a recombination fraction ≤ 0.3. The linkage criterion was raised to LOD ≥ 6 in order to resolve a spurious association of two chromosomes (see "Results"). The most likely linear order of markers on each chromosome was determined by using the reference maps as a starting point, then testing consistency of the observed recombination frequencies with the expected gene orders by analyzing pairwise recombination fraction and LOD values. Alternate marker orders were compared with the 'Order', 'Try', and 'Ripple' functions. Recombination fraction units were converted into centimorgans using the mapping function of Kosambi (1944).

Results

Generation of the mapping population

Accessions of *S. ochranthum* and *S. juglandifolium* were cross pollinated using a bulk pollen sample from the latter applied to pistils of several plants of the former. Cross-incompatibility between the parental species was overcome by embryo rescue. The resulting F_1 hybrids (two plants) closely resembled each other and were intermediate between the parents for several distinguishing morphological traits: leaflet length/width ratio, leaflet number, and number of pseudostipules (*S. ochr.* > *S. jugl.*), and leaf surface texture and degree of pubescence (*S. ochr.* = smooth and hairy, *S. jugl.* = rugose, less hairy). A 'pseudo

F_2' population (66 plants) was generated by intercrossing the two F_1 plants—necessary for circumventing self-incompatibility, since both species are SI. The genotype of F_1 and F_2 plants was confirmed with DNA markers. Further evidence of hybridity and expected segregation was that F_2 plants varied for parental morphological characters described above (data not shown).

Pollen fertility and chromosome pairing

Pollen stainability averaged 38% in the two F_1 hybrids (39% in 01L5288-1 and 37% in 01L5311-1). The majority of the chromosomes paired normally at diakinesis/metaphase (5–8 bivalents/cell), but there were a substantial number of unpaired chromosomes (3–6 univalents/cell). Associations involving more than two chromosomes were observed in all 8 cells examined: 6 cells contained one trivalent, one cell contained two, and one cell contained a multivalent involving seven chromosomes.

Polymorphism rates

A genetic linkage map was constructed with 132 markers, consisting of 96 CAPS, 19 RFLPs and 17 SSRs. Slightly over half (51%) of loci were COSII markers, followed by 24% TG probes, 13% SSRs, 11% COS markers and 2% CT probes. The average spacing between adjacent markers on the reference map was 6 cM, ranging from 0 to 31.7 cM.

For the RFLP markers, 60/289 (21%) tested probe × restriction enzyme combinations were polymorphic. Polymorphism rates ranged from 0 to 80% for individual probes. Of the CAPS markers, 3.5% yielded single fragments that were polymorphic with respect to amplicon sizes (i.e., without RE digestion), all of which were COSII markers. A total of 2,052 CAPS × RE combinations were tested, resulting in an overall polymorphism rate of 12%. COS markers were the most polymorphic (17%), followed by 'TG' and COSII sequences which showed similar polymorphism rates (14 and 13%, respectively). Polymorphism rates were not corrected for fragment length, therefore those of 'TG' probes, which were generally shorter (data not shown), were likely underestimated. Polymorphism yield among SSRs that amplified as single fragments was 63% (17 out of 27).

Transmission ratio distortion

The chi-square goodness-of-fit statistic detected significant ($P < 0.05$) transmission ratio distortion (TRD) at one-third (32%) of all loci, six times more than the number of loci expected by chance alone (Fig. 1). Proximal and distal chromosome positions were equally affected. A minimum of 13 putative distorter (*trd*) loci (i.e., indicating location of the actual genes under selection) were inferred from these data, representing 9 of the 12 chromosomes (Table 1).

On chromosomes 2 and 9, deviation from Mendelian inheritance affected loci across nearly the entire length of the chromosome. TRD was most severe ($P < 0.0001$) on chromosomes 2, 5 and 9. One putative TRD locus was located at the upper end of chromosome 2 at a position predicted for the centromere (TG608; *trd2.1*), and a second towards the middle of the chromosome (C2_At4g20410 and C2_At4g30930; *trd2.2*) where the *juglandifolium* homozygote (J/J) reached >50% of the genotypic distribution at the expense of *ochranthum* homozygotes (O/O). All loci on this chromosome were distorted in the same fashion. The bias decreased towards the end of the long arm and was no longer significant at the most distal locus (C2_At4g37280).

The reverse situation was found on chromosome 5, where an excess of O/O genotypes was accompanied by a deficiency of J/J and a less pronounced decline of heterozygotes ($P < 0.0001$). The distortion peaked around T1584 (*trd5.1*). Markers on this chromosome showed either a highly significant deviation from Mendelian segregation, or no significant deviation. The sharp drop occurred between markers C2_At4g24830 and SSR115 and may therefore coincide with the centromeric position, which was predicted to lie in proximity of C2_At4g24830, albeit on its 'south' side.

On chromosome 9, an over-representation of O/O genotypes indicated the presence of another TRD locus of high significance ($P < 0.0001$), colocalizing with the centromere (TG291; *trd9.1*). While J/J homozygotes were suppressed throughout the length of chromosome 9, ratios for the heterozygous state were as expected on 9S but exceeded normal values on 9L. An increase of heterozygotes accompanied by a decline of O/O homozygotes was observed for all markers on 9L, and pointed to a second putative TRD locus around C2_At3g24050 (*trd9.2*).

Linkage groups 8 and 12 displayed similar patterns of marker segregation. Along the entire lengths of both linkage groups the number of heterozygotes was above, and the number of O/O homozygotes was below expected values. Segregation patterns were consistent with evidence (presented below) of a reciprocal translocation involving these two chromosomes.

Linkage groups

The 132 markers on the *S. ochranthum* × *S. juglandifolium* linkage map (hereafter *S. ochr.* × *S. jugl.*) were distributed over 12 linkage groups, corresponding to the 12 chromosomes of tomato, with the following exceptions (Fig. 2): tomato chromosome 1 was comprised of two loosely connected (LOD ≤ 1.98, RF > 0.28) linkage groups on the

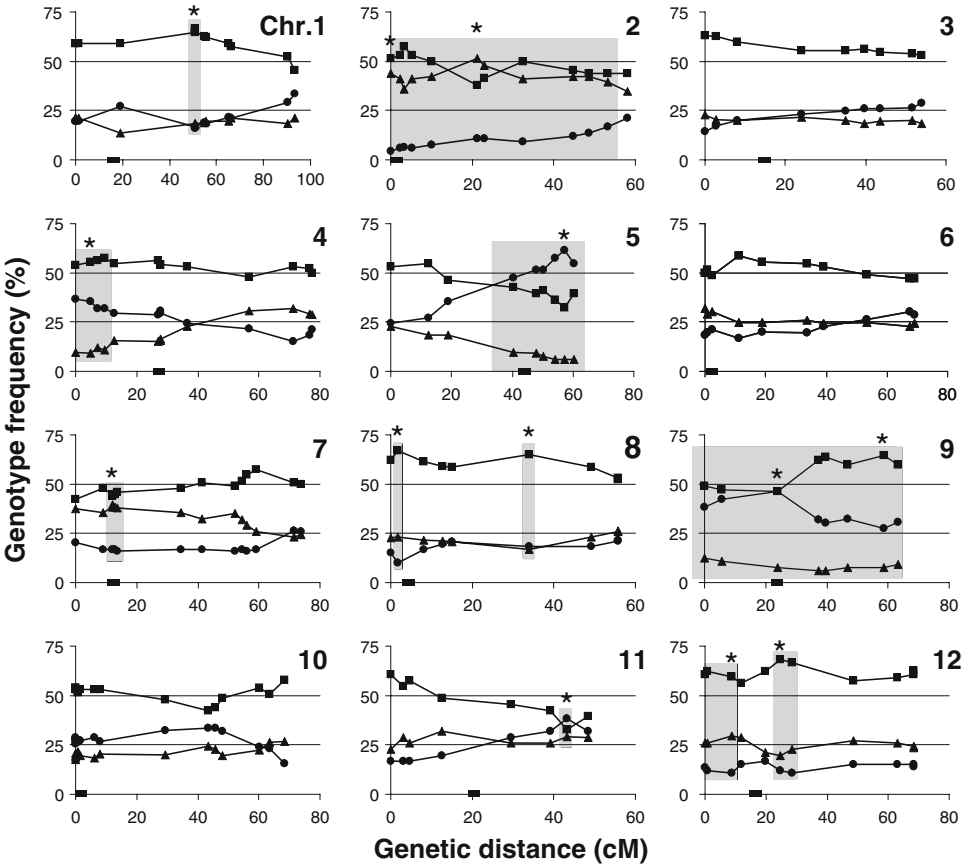

Fig. 1 Genotype frequencies observed for markers according to their position along the chromosome in F_2 *S. ochranthum* × *S. juglandifolium*. *Shaded areas* indicate markers showing a significant deviation from the expected Mendelian 1:2:1 segregation ($P < 0.05$). The locations of putative transmission ration distortion loci (*) are inferred from the strength and direction of the deviations. *Horizontal lines* indicate the expected frequencies of 50% for the heterozygotes and 25% for the homozygotes. The *X*-axis shows the genetic distance (cM) from the distal short arm to the distal long arm end of each chromosome, based on the tomato reference map (EXPEN 2000). *Black boxes* show putative centromere positions. *Filled circle* O/O, *filled square* O/J, *filled triangle* J/J, where *O ochranthum* and *J juglandifolium* alleles

S. ochr. × *S. jugl.* map, which were unassociated with markers on any other linkage group. Tomato chromosomes 5 and 9 were connected to a single large linkage group with both corresponding tomato chromosomes forming clearly delimited subgroups (i.e., loci order of the two subgroups was unaltered), suggesting a spurious association. The two linkage groups could be resolved by raising the threshold LOD to 6.

Tomato chromosomes 8 and 12 also emerged as a single linkage group which was almost twice as long as the average linkage group on the *S. ochr.* × *S. jugl.* map. Marker order was ambiguous along the merged linkage groups and higher stringencies did not result in a division into two balanced chromosomes. Within this subgroup linkage was strongest between markers SSR15 and C2_At42740 (LOD = 27, RF = 0), which map in proximity of the centromeres on tomato chromosomes 8 and 12, respectively. This result strongly suggests the existence of a reciprocal translocation involving these two chromosomes in one of the parental species. Thus, the F_1's would be heterozygous for the translocation, resulting in the appearance of linkage (pseudolinkage) between markers on the two chromosomes. Linkage groups were manually split at the putative interchange point (i.e., the point were cross-linkage was strongest) resulting in two putative, balanced chromosome pairs: (a) tomato chromosome 8 and 12 and (b) the translocated pair Ta and Tb (Fig. 3). Within each group computational analysis identified unambiguous marker positions that were also supported by LOD tables generated in MAPMAKER.

Map length

Under the scenario of either a translocated or the tomato chromosome 8/12 configuration, the total map length was the same, about 790 cM. This constitutes a 42% reduction relative to the distance covered by the same markers—1,363 cM—on the tomato reference map, EXPEN 2000 (http://www.sgn.cornell.edu). Linkage groups were heterogeneous with respect to map expansions or reductions, but all 12 displayed a net shrinkage compared to their tomato counterparts. Distal, proximal and intermediate regions were similarly affected. Clustering of markers around centromeric regions was observed on J2, J4, J6, J7 and J10. The same markers were also concentrated near the centromeres on the reference map, and the overall reduction in recombination in the *S. ochr.* × *S. jugl.* map accentuated this effect. Small regions of apparent map expansions were observed on all but one linkage group (J2). Average marker spacing was 6 cM.

Table 1 Marker loci showing significant deviations from Mendelian segregation ratios in F_2 *S. ochranthum* × *S. juglandifolium*, and inferred TRD loci under selection in each case

Chr	cM	Distorter Locus	Associated markers	Genotypic segregation				$\chi^2_{(df=2)}$	Allele frequencies		
				J/J	J/O	O/O	*P* value		J	O	$\chi^2_{(df=1)}$
1	50.8	*trd1.1*	C2_At2g45620	11	42	10	0.030	7.03	0.508	0.492	0.00
	50.8		TG460	12	43	11	0.048	6.09	0.508	0.492	0.00
2	0.0	*trd2.1*	TG608	29	34	3	0.000	20.55	0.697	0.303	9.47
	2.3		TG31	27	35	4	0.000	16.27	0.674	0.326	7.33
	3.3		TG33	22	35	4	0.003	11.95	0.648	0.352	4.74
	5.3		T1706	27	35	4	0.000	16.27	0.674	0.326	7.33
	10.0		C2_At4g20410	28	33	5	0.000	16.03	0.674	0.326	7.33
	21.3	*trd2.2*	C2_At4g30930	34	25	7	0.000	25.97	0.705	0.295	10.24
	22.9		C2_At4g21580	31	27	7	0.000	19.58	0.685	0.315	8.14
	32.4		T0759	27	33	6	0.001	13.36	0.659	0.341	6.06
	44.7		C2_At2g04700	28	30	8	0.002	12.67	0.652	0.348	5.47
	48.6		C2_At3g02300	28	29	9	0.003	11.91	0.644	0.356	4.91
	53.3		TG167	26	29	11	0.020	7.79	0.614	0.386	2.97
4	0.0		TG15	6	34	23	0.008	9.57	0.365	0.635	4.06
	4.9	*trd4.1*	SSR43	6	36	23	0.008	9.65	0.369	0.631	3.94
	7.2		TG581	8	37	21	0.048	6.09	0.402	0.598	2.18
	9.5		C2_At3g51010	7	38	21	0.024	7.45	0.394	0.606	2.56
5	40.5		C2_At4g24830	6	27	30	0.000	19.57	0.310	0.690	8.40
	47.8		C2_At1g67700	6	26	34	0.000	26.73	0.288	0.712	11.05
	50.1		C2_At1g10500	5	27	34	0.000	27.67	0.280	0.720	11.88
	54.0		TG351	4	24	38	0.000	NA	0.242	0.758	16.50
	57.1	*trd5.1*	T1584	4	21	40	0.000	NA	0.223	0.777	18.85
	60.2		TG185	4	26	36	0.000	NA	0.258	0.742	14.56
7	12.1		C2_At2g26590	25	29	11	0.034	6.78	0.608	0.392	2.60
	12.1	*trd7.1*	C2_At4g33250	26	29	11	0.020	7.79	0.614	0.386	2.97
	12.9		C2_At5g20180	25	29	11	0.034	6.78	0.608	0.392	2.60
	13.7		T1497	24	29	10	0.037	6.62	0.611	0.389	2.68
8	1.7	*trd8.1*	C2_At5g46630	14	41	6	0.009	9.33	0.566	0.434	0.80
	33.9	*trd8.2*	TG510	11	43	12	0.048	6.09	0.492	0.508	0.00
9	0.0		C2_At2g37240	8	32	25	0.012	8.91	0.369	0.631	3.94
	5.6		TG9	7	30	27	0.002	12.75	0.344	0.656	5.64
	23.9	*trd9.1*	TG291	5	30	30	0.000	19.62	0.308	0.692	8.86
	37.4		TG551	4	41	21	0.002	12.64	0.371	0.629	3.88
	39.7		TG144	4	42	20	0.002	12.67	0.379	0.621	3.41
	46.9		T0393	5	39	21	0.005	10.48	0.377	0.623	3.46
	58.6	*trd9.2*	C2_At3g24050	5	42	18	0.005	10.75	0.400	0.600	2.22
	63.4		SSR599	6	39	20	0.013	8.63	0.392	0.608	2.60
11	43.2	*trd11.1*	T0142	16	18	21	0.024	7.47	0.455	0.545	0.29
12	0.8		C2_At4g03280	17	41	8	0.042	6.33	0.568	0.432	0.97
	8.8	*trd12.1*	C2_At5g19690	19	38	7	0.034	6.75	0.594	0.406	1.89
	24.6	*trd12.2*	TG394	13	45	8	0.009	9.48	0.538	0.462	0.24
	28.5		SSR345	15	44	7	0.010	9.27	0.561	0.439	0.74

The list includes all markers with non-Mendelian genotypic ratios (χ^2 values with $P < 0.05$). Values represent the numbers of F_2 individuals in each genotypic class and computed allele frequencies. *J S. juglandifolium*, *O S. ochranthum*, χ^2 Chi-square goodness-of-fit statistic. Positions in cM are from the *ochranthum* × *juglandifolium* map

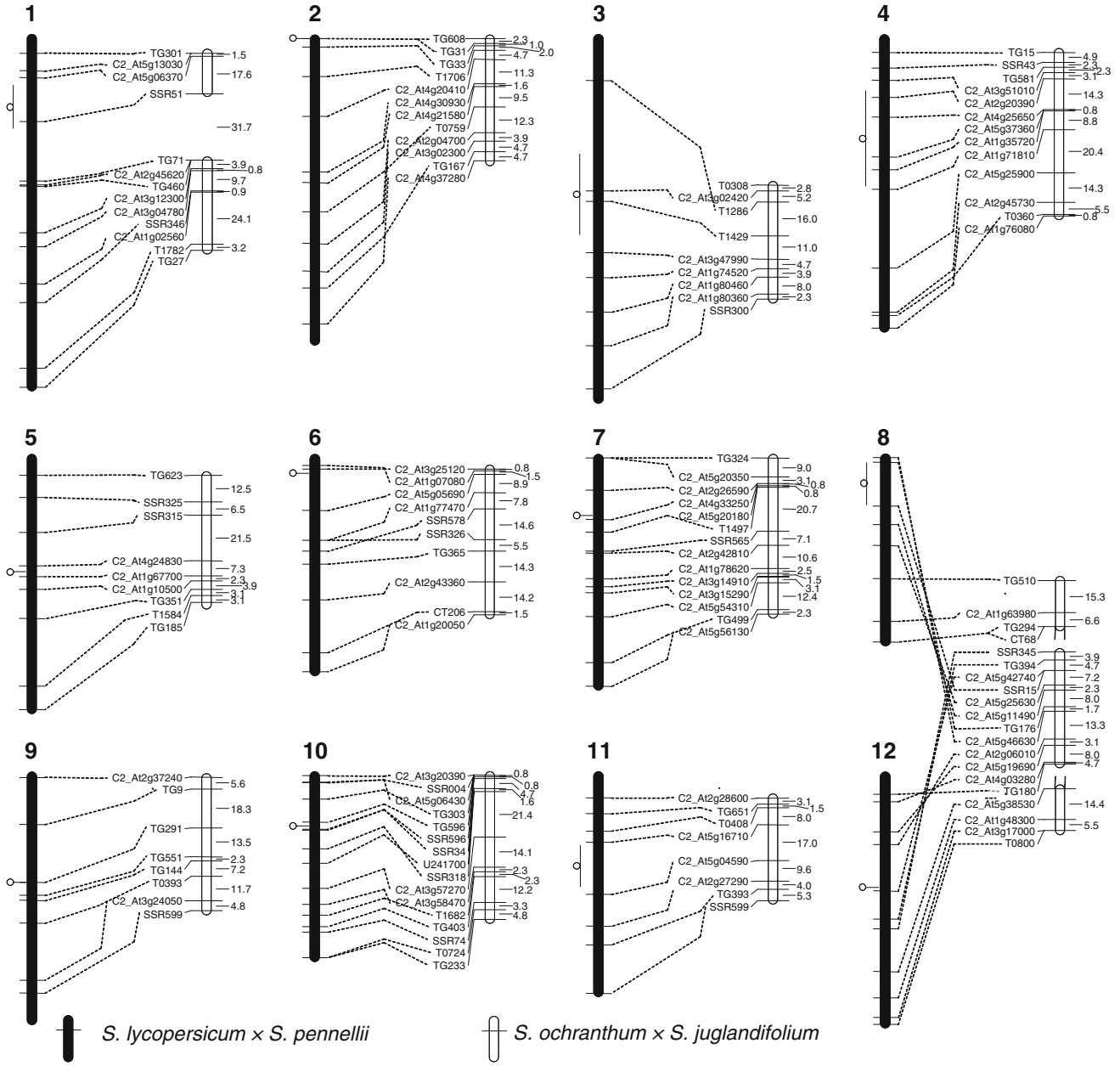

Fig. 2 Genetic linkage map of F_2 *S. ochranthum* × *S. juglandifolium* (*unshaded* chromosomes) and positions of corresponding markers on the reference map (*shaded*) of tomato (EXPEN 2000, http://www.sgn.cornell.edu). Common markers are joined by dotted lines. *Circles* indicate putative centromere positions (from Pillen et al. 1996). Map distances are in Kosambi centimorgans (cM). The fused linkage group of tomato chromosome 8 + 12 is shown as three linkage subunits

Map length reduction of individual linkage groups varied between 21% (J10) and 63% (J3). Strong overall length reductions was also observed on J2 (57%) and J11 (48%). The severe map shrinkage of linkage group 3 was mostly due to the nearly complete omission of the short arm (98% reduction) as a result of inverted positions of two markers, relative to the tomato-EXPEN 2000 map, accompanied by a tenfold size reduction: C2_At3g02420 and T1286 are separated by over 50 cM on the reference map but by only 5 cM on the F_2 *S. ochr.* × *S. jugl.* map. For the other linkage groups, recombination suppression was concentrated on just one of the two chromosomal arms: J10S (96%), J1S (87%) and J5L (76%).

Colinearity with the tomato genome

Overall marker order was highly conserved between F_2 *S. ochr.* × *S. jugl.* and the tomato reference map. No duplicated loci were detected—not surprising since only markers with simple banding patterns (RFLPs) or single

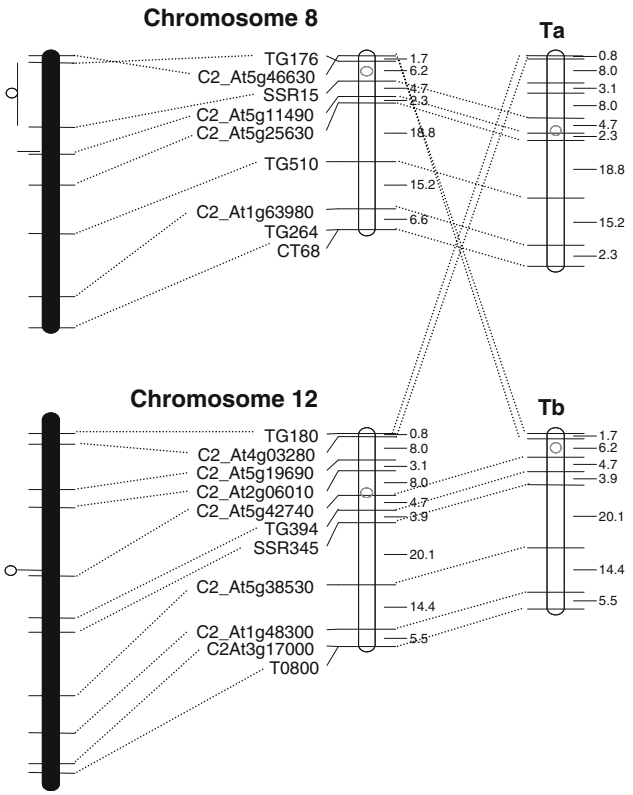

Fig. 3 Proposed reciprocal translocation involving the short arms of chromosome 8 and 12 (Ta and Tb) in either *S. ochranthum* or *S. juglandifolium*

amplicons (CAPS, SSRs) were included in the study. Inverted marker positions of adjacent loci were observed for pairs of markers on 7 out of 12 linkage groups: J1L, J2S, J3S, J6, J7L, J8S and J10L. Flipped positions could represent artifacts of the limited mapping power resulting from the small population size and/or reduced recombination in the F_2 *S. ochr.* × *S. jugl.* population. In four of these cases the flipped marker order was inferred at the 5% confidence level (i.e., where markers were spaced at >3 cM), specifically on J3S (C2_At3g02420 and T1286), in the centromeric region of J6 (SSR578 and SSR326), on J8S (TG176 and C2_At5g46630) and on J10L (U241700 and SSR318). Two markers mapped to different chromosomes: TG581, located on tomato 6L was placed on J4S, and T0308, on 10L in tomato, mapped to the distal end of J3S. T0308 demarcates the most distal position of the chromosome arm where the map was compressed, possibly as a result of an inversion. However, the apparent map shrinkage on J3S could also be an artifact of the limited number of markers—only two—for this region. Our efforts to increase that number were unsuccessful, as no markers with polymorphisms among the parental species were found in that region of the genome. A similar situation was found on J6S: the only two markers (C2_At3g25120 and C2_At1g07080) on this chromosome arm where inverted relative to tomato, thus implicating a whole-arm paracentric inversion scenario.

Discussion

Transmission ratio distortion

Significant departures from the expected 1:2:1 Mendelian ratio were observed in over one-third of the loci in F_2 *S. ochr.* × *S. jugl.*, affecting nine out of twelve chromosomes. Transmission ratio distortion is a phenomenon often observed in interspecific crosses between crop plants—including tomato—and their wild relatives (Zamir and Tadmor 1986, Wendel et al. 1987, Bonierbale et al. 1988). The extent of aberrant segregation generally increases with the level of divergence between species (Grant 1975; Zamir and Tadmor 1986). *Intra*specific crosses within *S. lycopersicum* (i.e., cultivars, landraces, and wild cherry tomatoes) generally produce 'normal' segregation ratios (Rick 1948). In a cross between *S. lycopersicum* and its close relative *S. pimpinellifolium*, only 8% of loci showed skewed segregation (Grandillo and Tanksley 1996). In wider crosses, a higher frequency of skewing is common, for example 51% of the loci deviated from expected Mendelian ratios in derivatives of *S. lycopersicum* × *S. cheesmaniae* (formerly *L. cheesmanii*), and 69% in *S. lycopersicum* × *S. neorickii* (formerly *L. parviflorum*) (Paterson et al. 1988, 1991). The frequency of non-Mendelian segregations reported herein (32%) is a little higher than observed in *S. sitiens* × *S. lycopersicoides* (24%) (Pertuzé et al. 2002).

A total of 13 putative TRD loci were identified—probably a conservative estimate because the effects of minor distorter loci could be hidden by nearby genes with large effects. Potential causes underlying TRD are complex, and could involve selection at several stages of development, including meiosis (meiotic drive, segregation distortion), gamete viability, pollen competitiveness, zygote viability and/or seedling vigor. A primary cause of TRD in interspecific hybrids is inviability selection, i.e., selection against particular allelic combinations that confer hybrid incompatibility (Moyle and Graham 2006). TRD loci may also go unnoticed because their effects are deleterious and prompt the selection for modifier genes at secondary loci that suppress them, a situation also known as 'genetic conflict' (Taylor and Ingvarsson 2003). Pollen killers that abort pollen carrying the non-driving allele have been reported in tobacco, wheat, rice, and tomato (Cameron and Moav 1957; Canady et al. 2005; Loegering and Sears 1963; Sano 1983). Gamete eliminators render only those gametes dysfunctional which contain the alternate allele in the

heterozygous sporophytic parent. Gamete promoters operate in the reverse fashion. Both have been observed in tomato (Pelham 1968; Rick 1966). The accumulation of sterility factors and modifier genes plays a central role in the establishment and reinforcement of reproductive barriers between species.

For several of the TRD loci detected in this study, *potentially* orthologous loci with similar map position and distortion effects have been found in other tomato interspecific crosses. For example, *trd2.2* mapped in close proximity to a distorter locus in BC_1 *S. lycopersicum* × *S. lycopersicoides* (Chetelat et al. 2000). Both studies also found markers subject to TRD on the long arm of chromosome 5 (Chetelat et al. 2000). Potentially orthologous TRD loci on chromosomes 4, 7, 8 and 9 were observed in the present study and in F_2 *S. sitiens* × *S. lycopersicoides* (Pertuzé et al. 2002). Interestingly, the locus on chromosome 4 (*trd4.1*) maps to the same region (the distal end of the short arm) in both populations, and is manifested by the nearly complete elimination of one homozygous class in each case. On chromosome 9, *trd9.1* in the present study mapped to the same general location—near the centromere—as a TRD locus in the *S. sitiens* × *S. lycopersicoides* population. The mode of action of the two factors was similar, though more severe in *S. sitiens* × *S. lycopersicoides*, with a nearly complete elimination on one homozygous class. Strong TRD along the entire chromosome 9 was also observed in *S. lycopersicum* × *S. lycopersicoides* derivatives (Chetelat et al. 2000). These similarities in map location and/or gene effects don't prove the existence of orthologous TRD loci in the different species, but they are suggestive.

Species relationships within sect. *Juglandifolia*

The sister taxa *S. ochranthum* and *S. juglandifolium* show a close morphological resemblance and have a similar ecology and geographic distribution (Rick 1988; Smith and Peralta 2002). Yet hybridization between the two is prevented by strong postzygotic reproductive barriers which we could overcome only with the aid of embryo rescue. Genetic control of crossing barriers is complex; chromosomal rearrangements are one underlying factor, and may reinforce other mechanisms (White 1978). In the present study, a reciprocal translocation in one parental genome was detected by linkage analysis and supported by our observations of reduced chromosome pairing and low pollen fertility (less than 50%) in the F_1 hybrid, phenotypes that are consistent with translocation heterozygosity (Burnham 1962). This conclusion is also consistent with our earlier studies of *S. sitiens* and *S. lycopersicoides*, two species that do *not* differ by chromosomal rearrangements: in this case, chromosome pairing in the F_1 hybrid was nearly normal, and fecundity high (Pertuzé et al. 2002; Rick 1979). However, since the effects of structural heterogeneity between *S. ochranthum* and *S. juglandifolium* would be expressed during meiosis of the F_1 hybrid, the strong reproductive barrier preventing their *initial* hybridization must be controlled by other factors.

Restricted fragment length polymorphisms are caused by base substitutions or insertion/deletions (Dvorak and Akhunov 2005) and therefore represent a measure of divergence at the DNA sequence level. The herein observed polymorphism rate of 21% is slightly lower than that reported for *S. lycopersicoides* and *S. sitiens* (27%; Pertuzé et al. 2002), consistent with sequence divergence estimates (based on the *waxy* gene) which indicated a slightly higher homology between *S. ochranthum* versus *S. juglandifolium* than *S. sitiens* vs. *S. lycopersicoides* (Peralta and Spooner 2001). The relatively greater difficulty in hybridizing *S. ochranthum* and *S. juglandifolium* may therefore seem surprising. However, crossability clearly does not vary in proportion to genetic relatedness or divergence. For example, cultivated tomato is more easily hybridized with *S. pennellii*, the basal taxon in the *Lycopersicon* clade, than with *S. peruvianum*, with which it shares a closer relationship based on molecular (Breto et al. 1993; Alvarez et al. 2001; Marshall et al. 2001; Spooner et al. 2005) and morphological phylogenies (Peralta et al. 2005). The existence of strong reproductive barriers between *S. juglandifolium* and *S. ochranthum* is consistent with the partial overlap of their geographic ranges (Smith and Peralta 2002), which would tend to reinforce species barriers. In contrast, *S. lycopersicoides* and *S. sitiens* are now entirely allopatric, and if they evolved under allopatry, then crossing barriers might not have been necessary.

Map length

The F_2 *S. ochr.* × *S. jugl.* map contained substantially fewer map units than other tomato maps: only 58% of the *S. lycopersicum* × *S. pennellii* map (EXPEN 2000, http://www.sgn.cornell.edu) and 67% of the *S. sitiens* × *S. lycopersicoides* map (Pertuzé et al. 2002). Map length compression could be caused by a variety of factors, of which some can be ruled out by our observations. The small population size used is an unlikely cause since the EXPEN 1992 map was based on a similar population size (67 vs. 66 herein) yet comprised over 1,300 map units (Tanksley et al. 1992). Similarly, the relatively low marker density in the present study (6 cM between markers vs. 0.6 cM for EXPEN 2000) is an unlikely cause of map compression since the number of markers used was similar to that of *S. sitiens* × *S. lycopersicoides*, in which genome-wide map reduction amounted to only a few percentage points. In addition, if undetected double crossover events

were responsible for the map size compression, larger intervals should be more severely affected, which we did not observe. Markers tend to cluster around centromeres (Tanksley et al. 1992). Enhanced clustering of markers at putative centromere positions was observed on two-thirds of all chromosomes (2, 4, 5, 6, 7, 8, 10 and 12). However, the effect on the over-all map length should be marginal because centromeric regions only represent a small portion of the total map units in the genome, and markers were chosen to cover all regions of the genome. It is well-established that sequence divergence among the parents leads to reduced recombination in wider crosses (Rick 1969; Bonierbale et al. 1988; Gebhardt et al. 1991; Burke et al. 2004). However, the phylogenetic distance within *Juglandifolia* seems to be smaller than that between the parental species of the longer tomato genetic linkage maps, as explained above.

Areas where recombination was eliminated almost completely may indicate inversion heterozygosity, which rarely produces viable recombinants (Livingstone and Rieseberg 2003). The short arm of chromosome J10 was reduced to just 0.8 cM. The residual recombination may represent a genotyping artifact or J10S may be rearranged among the parental species. Similar situations were found on J1S and J3S (87 and 98% reduction, respectively), but as chromosome 1 is subtelocentric, it is difficult to determine whether the observed shrinkage—inferred from just three markers—goes beyond the average genome-wide reduction. Likewise, the recombination suppression on J3S was based on only two markers.

Map length reduction might result from extreme transmission ratio distortion in some cases; for example if recombinant gametes are eliminated by selection, the actual recombination rates may be more normal (Rick 1969). In the present study, TRD affected most or all of chromosomes 2 and 9, always in direction of favoring one allele over the other, and thus might contribute to map length compression in these regions.

However, the genome-wide map reduction observed herein is more likely caused by factors that act genome-wide. For example, genes that control recombination frequency have been identified in several plants, most notably wheat (the *Ph* genes), but also in petunia (Maizonnier et al. 1984). The petunia genetic map is ca. ten-fold smaller than that of tomato (Strommer et al. 2002), while its haploid genome content is larger (1,200 vs. 950 Mb; Arumuganathan and Earle 1991). Like our F_2 *S. ochr.* × *S. jugl.* map, the genetic map of potato contains only about half the map units reported in tomato (Tanksley et al. 1992), suggesting variation in recombination rates are not unusual, at least within *Solanum.*

Alternatively, or in addition, recombination could be reduced or eliminated in one gamete. Male recombination is completely suppressed in *Drosophila* (Morgan 1912), and is lower than female recombination in wide crosses of tomato (de Vicente and Tanksley 1991; van Ooijen et al. 1994). A complete lack of male recombination would cause a 50% map shrinkage, similar to what we observed herein. Some combination of these factors—lower male recombination, structural and sequence divergence, pairing modifiers, etc.—seems the most likely explanation.

Other than the genome-wide map length reduction, the relative recombination rates across different marker intervals were similar to the tomato reference map, suggesting a high level of synteny between the genomes of sect. *Juglandifolia* and sect. *Lycopersicon*. A significant positive correlation of recombination frequencies across conserved linkage blocks was also observed among eggplant and tomato (Doganlar et al. 2002), a phylogenetically wider comparison than we report herein.

Putative speciation scenario within Sect. *Juglandifolia*

Our data suggest the genomes of *S. ochranthum* and *S. juglandifolium* are differentiated by a reciprocal whole-arm translocation. The possibility that this translocation is a feature of the individual accessions or plants used in this study, rather than the species as a whole, cannot be discounted. This rearrangement must be relatively recent, since these species are so closely related at the level of DNA sequence homology. If *S. ochranthum* and *S. juglandifolia* diverged under sympatry or parapatry, then reproductive barriers would have been required to prevent the diverging lineages from merging with their progenitor (Dobzhansky 1937). There are many cases where chromosomal rearrangements have been identified as causal factors in speciation processes of plants and animals (Livingstone and Rieseberg 2003). Likewise, rearrangements have been shown to be more common among sympatric versus allopatric species (Noor et al. 2001), suggesting that they may be advantageous under these conditions, possibly owing to their isolating effect. The situation, however, is paradoxical because the stronger the negative fitness effect of a chromosomal rearrangement, the greater is its potential to confer reproductive isolation, but at the same time the smaller its chance for fixation (i.e., the strongest barriers have the least chance to be fixed) (Livingstone and Rieseberg 2003; Burke et al. 2004).

Chromosomal rearrangements are generally assumed to be deleterious when heterozygous but not when homozygous (Levin 2002). Therefore, in self-incompatible species chromosomal rearrangements with underdominant effects may require genetic drift to be brought to fixation, making them only likely to occur in populations with small effective population sizes (Lande 1979; Lagercrantz 1998; Burke et al. 2004). Most known *S. ochranthum* and

S. juglandifolium populations are small in size and isolated (http://tgrc.ucdavis.edu; Smith and Peralta 2002), therefore it is likely that genetic drift and isolation by distance constitute important evolutionary forces. Alternatively, weakly underdominant rearrangements may be involved in speciation in sympatry or parapatry primarily through their effects on recombination (Rieseberg 2001; Noor et al. 2001). Sheltered from gene flow, isolating factors such as Dobzhansky–Muller incompatibilities have the chance to accumulate until eventually the reproductive barrier is complete (Navarro and Barton 2003). Empirical evidence in support for this theory was provided by a comparative analysis between potato (*S. tubersosum*) and tomato (Livingstone and Rieseberg 2003). In the case of sect. *Juglandifolia*, the difficulty of obtaining F_1 *S. ochranthum* × *S. juglandifolium* hybrids suggests other reproductive barriers play a more important role in maintaining their separation than structural changes of the chromosomes.

Colinearity with tomato

This study revealed a high level of synteny between the genomes of sect. *Juglandifolia* and tomato (*S. lycopersicum*). Highly conserved gene orders within rearranged blocks are a common finding, even across broad phylogenetic distances, such as those of tomato, potato, eggplant and pepper (Bonierbale et al. 1988; Tanksley et al. 1992; Livingstone et al. 1999; Doganlar et al. 2002). In addition to the reciprocal whole-arm translocation involving chromosomes 8 and 12, six other genomic regions showed deviations in marker order relative to tomato. Two loci mapped to different chromosomes, and one locus mapped to a different position within the same chromosome. Flipped positions of adjacent loci were suggestive of whole-arm inversions on J3S, J6S and J8S. However, these putative exceptions from colinearity are based on single-marker evidence only, thus may represent artifacts, e.g., the mapping of secondary or duplicated loci or genotyping errors. To substantiate these genetic changes, additional cytological and/or mapping data is needed. A recent FISH analysis showed a previously undetected paracentric inversion of 6S among the genomes of tomato and potato (Bai et al. 2007). Short inversions have also been detected in *S. peruvianum* chromosome 6S (Seah et al. 2004), and *S. pennellii* chromosome 7S (Van der Knaap et al. 2004). Therefore it is quite possible that the cases of inverted markers in the present study are real. Also noteworthy was the severe recombination suppression on J10S (96% map size reduction) which may be indicative of a whole-arm inversion, as mentioned above. An even stronger effect was seen on J3S, albeit solely based on the distance between two loci.

The number of the putative rearrangements reported herein is likely to be an underestimate of the chromosomal changes that differentiate the genomes of the two species. With an average marker density of 6 cM, mapping resolution is relatively coarse, and smaller rearrangements are unlikely to be detected. For example, the *peruvianum* and *pennellii* inversions cited above were detected only after fine-scale genetic and physical mapping, and cytological analysis. Still, in light of the relatively conserved genome structure seen amongst members of the tomato clade, the number of putative rearrangements in the *Juglandifolia* clade seems high.

The genomes of sect. *Lycopersicon* are essentially uniform, differentiated only by small rearrangements and gene substitutions, and thus considered homologous (Rick 1979; Tanksley et al. 1992; Paran et al. 1995; Grandillo and Tanksley 1996; Bernacchi and Tanksley 1997; Fulton et al. 1997; van der Knaap et al. 2004). Sect. *Lycopersicoides* is separated from tomato by a single paracentric whole-arm inversion on 10L. Since *Lycopersicoides* shares the potato configuration—assumed to be the ancestral state—the 10L inversion must have occurred relatively recently during evolution of the tomato lineage (Pertuzé et al. 2002), but before divergence of sect. *Juglandifolia,* since it shares the tomato (derived) arrangement. A total of just six paracentric whole-arm inversions separate the genomes of potato and tomato (Tanksley et al. 1992; Bai et al. 2007), four or five of these occurred in the tomato lineage: The inversions on tomato chromosome 6S, 9S, 10L and 11S are likely derived in tomato because the potato configuration is shared by pepper and eggplant (9S and 10L) or eggplant alone (11S, the state of 6S is unknown for pepper; Tanksley et al. 1992; Livingstone et al. 1999; Doganlar et al. 2002). Our evidence—albeit based on only two markers—suggests that the inversion of 6S occurred after the split of *Juglandifolia–Lycopersicon* clades. The configuration of chromosome 6S in sect. *Lycopersicoides* species is currently unknown (Pertuzé et al. 2002), however, the absence of recombinant introgressions involving this chromosome arm (Canady et al. 2005) is consistent with inversion heterozygosity.

The paracentric inversion of tomato 12S, on the other hand, is shared by tomato and eggplant, indicating that this represents the ancestral state and that a rearrangement occurred in the potato lineage. The paracentric inversion on tomato 5S, however, could have occurred in either the potato or the tomato ancestors because neither of the two states is shared by eggplant or pepper. Eggplant (*S. melongena*) is the most distantly related *Solanum* species, vis-à-vis tomato, for which a comparative genetic map exists; it differs from tomato by a total of 28 rearrangements (23 paracentric inversion and five translocations; Doganlar et al. 2002). The pepper lineage (*C. annuum*) in genus *Capsicum* has undergone extensive restructuring, presumably due to its high content of transposable elements. Pepper differs from tomato by a total of 22 breaks, composed of eight

paracentric, two pericentric inversions, five translocations as well as various forms of four dissociations or associations, some multiply nested (Livingstone et al. 1999).

Evolutionary rates may vary greatly even among lineages within the same family, and the number of changes cannot be equalized with evolutionary divergence time, as has been shown in the grasses (Gale and Devos 1998). Differences may reflect genome-specific abilities to fix rearrangements (Devos and Gale 2000) or external conditions during speciation: The tomato species are assumed to have evolved primarily through geographic isolation and adaptation (Peralta and Spooner 2005), consistent with the absence, in some species combinations, of strong crossing barriers, and the overall colinearity of species in the *Lycopersicon* clade; however, some species do show strong reproductive barriers, particularly in cases where geographic ranges overlap (e.g., *S. peruvianum* with *S. hirsutum*, *S. pennellii*, and *S. pimpinellifolium*). Present geographic distributions suggest *S. ochranthum* and *S. juglandifolium* originated via speciation in sym/parapatry, which is often associated with higher occurrences of chromosomal rearrangements than allopatric speciation (White 1978; Noor et al. 2001). Also noteworthy are the rearrangement classes observed in sect. *Juglandifolia*. A number of paracentric inversions and small translocations separate the tomato and potato clades (Bonierbale et al. 1988; Tanksley et al. 1992; Pertuzé et al. 2002; Bai et al. 2007), and *S. etuberosum* contains a number of rearrangements compared to potato (Perez et al. 1999). However, there are few large, whole arm reciprocal translocations of the type reported herein for the *Juglandifolia* species. Translocations do appear to have played an important role during the evolution of other *Solanaceae* species such as eggplant and pepper (Livingstone et al. 1999; Doganlar et al. 2002).

The relative frequencies of different rearrangement types are conserved across wide phylogenetic distances. The leading role of paracentric inversions appears to be a widespread phenomena in both plant and animal systems (Ranz et al. 2001; Doganlar et al. 2002), presumably because they confer the least selective disadvantage. Pericentric inversions appear to be extremely rare and are associated with a stronger selective disadvantage than other rearrangement types (Burnham 1962), although they do not produce a higher degree of semisterility than reciprocal translocations (Navarro and Ruiz 1997). In the heterozygous state translocations cause semisterility and are more detrimental than inversions (Burnham 1962), nonetheless they appear at a frequency intermediate to that of para- and pericentric inversions in the pepper/eggplant/potato/tomato divergence (Doganlar et al. 2002). The pepper species *C. annuum* and *C. chinense* are differentiated by a reciprocal translocation (Livingstone et al. 1999).

In the present study, several map features involved or were delimited by the positions of centromeres, including the chromosome 8/12 translocation, 3/4 of the single-marker translocations, and three stretches of near zero recombination (J1S, J3S and J10S). Centromeric and telomeric regions are prominent spots for chromosomal breakage and fusion in diverse plant systems (Lagercrantz 1998; Moore et al. 1997; Tanksley et al. 1992). Breakage often occurs in the heterochromatin surrounding centromeres (Khush and Rick 1963; Roberts 1965; Gill et al. 1980). Inversions may be triggered by homologous recombination between repetitive sequences within the heterochromatin of pericentromeric regions and of telomeres (Tanksley et al. 1992), and inverted chromosome arms are subsequently capped with new telomeric repeats to reestablish their stability (Yu and Blackburn 1991). Traces of the original telomeric repeats can be found at proximal positions in tomato in the form of interstitial telomeric repeats (Presting et al. 1996). If homologous recombination is functional in the creation of rearrangements, then regions that harbor repeats should serve as hotspots for structural changes. The increased flexibility seen in the transposable element-rich genomes of pepper and *Drosophila* are in support of this notion (Engels and Preston 1984; Robbins et al. 1989; Livingstone et al. 1999). In a comparison among *Brassicaceae* genomes, Lagercrantz (1998) observed that single transposed, "deviant" loci do not represent a random disturbance of colinearity but often collocate with junction points of conserved blocks. Findings herein seem to agree with that prediction as all putative rearrangements map to centromeric or telomeric regions, or other 'hotspots' for structural changes identified by cross-species comparisons.

Hotspots for rearrangements in sect. *Juglandifolia*

Chromosome 1

The *S. ochr.* × *S. jugl.* map is split into two linkage groups around the putative centromeric region, presumably due to a lack of marker saturation. In pepper, that same area marks the breakage point of a translocation with chromosome 8. Genome order is well preserved in all six genomes.

Chromosome 2

Marker content in J2 is well preserved compared to tomato, *Lycopersicoides* and potato.

Chromosome 3

The J3S map is much shorter than expected from the tomato maps, possibly due to a rearrangement. Further

evidence for chromosomal restructuring in this area is that a proximal marker on tomato chromosome 10L (T0308) mapped to distal J3S. Eggplant and pepper carry several small inversions and translocations in this region, suggesting it has been unstable during Solanaceae genome evolution (Doganlar et al. 2002).

Chromosome 4

A marker from tomato 6L (TG581) is translocated to J4S. Eggplant and sect. *Lycopersicoides* both show translocated areas between corresponding tomato chromosomes 4S and 10, while 4S in pepper is associated with tomato 5L, suggesting that the 4S region is prone to rearrangements in the Solanaceae.

Chromosome 5

This chromosome is collinear in *Juglandifolia*, *Lycopersicoides,* and tomato, but J5S is inverted relative to potato.

Chromosome 6

Eggplant and potato 6S are inverted relative to tomato, and *Juglandifolia* appears to share the former configuration. However, marker density is low on J6S—possibly because it is so short (Sherman and Stack 1995)—thus further evidence is needed to confirm its orientation.

Chromosome 7

Marker content and order are well conserved among the Solanaceae, including *Juglandifolia*.

Chromosome 8

Tomato chromosome 8 and 12 have undergone a reciprocal translocation involving the short arms in one of the *Juglandifolia* species. The translocation breakpoint is similar or identical to that of a translocation between chromosome 1 and 8 in pepper.

Chromosome 9

This chromosome is collinear among tomato, *Juglandifolia* and *Lycopersicoides*, but the short arm is inverted relative to potato.

Chromosome 10

Tomato and *Juglandifolia* share a paracentric inversion of 10L that is unique among the Solanaceae (see below). Interestingly, the spot on J10L that corresponds to the location of T0308 in tomato (translocated to J3S) shows some rearranged markers and marks the end of a stretch of severe map compression on J10. This area is located at/near the break point of the paracentric inversion in tomato 10L. Three loci from this region in tomato are scattered on chromosomes 4, 7 and 9 in *Lycopersicoides.* This region therefore appears to be an ancient hotspot for structural rearrangements in the Solanaceae.

Chromosome 11

Tomato, *Juglandifolia* and *Lycopersicoides* are collinear and inverted relative to potato, while other Solanaceae species show intra- and interchromosomal rearrangements.

Chromosome 12

Regions corresponding to tomato chromosome 12 have undergone multiple rearrangements in the potato, eggplant and the pepper lineages. Potato 12S is inverted relative to tomato and J8–J12. Eggplant chromosome 5 is a fusion of orthologous regions of tomato 5L and 12L, and eggplant 10S contains orthologous regions of tomato 12S. Pepper chromosomes 9 and 12 contain regions parts of tomato 12S. Finally, several loci from the distal end of tomato 12L are scattered across the genome in sect. *Lycopersicoides*.

Phylogenetic relationships

A major finding of this work is that the configuration of J10L is identical to that of tomato, unlike sect. *Lycopersicoides* which contains the ancestral arrangement shared by other Solanaceae, including potato, eggplant and pepper. This result supports sect. *Juglandifolia* as the closest outgroup to the tomatoes (sect. *Lycopersicon*), with sect. *Lycopersicoides* basal to both. Molecular phylogenies derived from GBSSI sequence and AFLP data also support these inferred relationships (Peralta and Spooner 2001; Spooner et al. 2005). The fact that reproductive barriers with sect. *Lycopersicon* are more pronounced vis-à-vis sect. *Juglandifolia* than sect. *Lycopersicoides* had supported the assumption that sect. *Lycopersicoides* was more closely related to the tomatoes (Rick 1979). The latter interpretation was also consistent with the more tomato-like morphology of this group, particularly *S. lycopersicoides*, as well as their similar ecology and distribution (Rick 1988). Thus our comparative mapping data help resolve a conflict between molecular and classical approaches to systematics.

One intriguing question remains unanswered: which of the species in sect. *Lycopersicon* is most closely related to sect. *Juglandifolia*. A single combined phylogenetic tree based on AFLP, GBSSI, cpDNA, ITS sequence and

morphological data supports *S. habrochaites* and *S. pennellii* as one clade that forms a basal polytomy with southern accessions of *S. peruvianum* and *S. chilense* (Spooner et al. 2005). Although several species of tomatoes overlap in their latitudinal distribution range with *S. ochranthum*, they mostly inhabit drier areas than the *Juglandifolia* species (http://tgrc.ucdavis.edu). Only one of the tomatoes, *S. habrochaites*, is sympatric with both *S. ochranthum* and *S. juglandifolium*. Interestingly, *S. habrochaites* also shows a superficial morphological resemblance with sect. *Juglandifolia*. However, these similarities could be caused by convergent evolution instead of shared ancestry. In southern Ecuador, *S. habrochaites* overlaps with *S. ochranthum* and *S. juglandifolium* in their likely center of diversity (at Leimebamba, Peru, *S. habrochaites* and *S. ochranthum* were collected from the same site). On the other hand, the flowers of *S. pennellii* suggest a closer affinity to the ancestral *Solanum* state: poricidal anthers lacking a sterile appendage are traits shared with the *Juglandifolia* and most other *Solanum* species.

Practical implications

The sect. *Juglandifolia* species have never been sexually hybridized with cultivated tomato (Rick 1979). Yet our results indicate tomato is more closely related to the *Juglandifolia* group than to either *S. lycopersicoides* or *S. sitiens,* both of which are experimentally cross-compatible with the cultigen. These strong breeding barriers, and the difficulty of growing *Juglandifolia* spp., probably explain why these wild nightshades have received relatively little attention to date. Our results suggest there might be more opportunity for germplasm introgression with cultivated tomato than previously assumed. The key may lie in identifying which species, *S. ochranthum* or *S. juglandifolium*, contains the putative translocated chromosome 8–12. This rearrangement would certainly complicate the transfer of any genes on those chromosomes, thus avoiding it would improve prospects for eventual introgression.

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