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ORIGINAL PAPER

A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*

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Abstract We report herein the development of a pepper genetic linkage map which comprises 299 orthologous markers between the pepper and tomato genomes (including 263 conserved ortholog set II or COSII markers). The expected position of additional 288 COSII markers was inferred in the pepper map via pepper–tomato synteny, bringing the total orthologous markers in the pepper genome to 587. While pepper maps have been previously reported, this is the first complete map in the sense that all markers could be placed in 12 linkage groups corresponding to the 12 chromosomes. The map presented herein is relevant to the genomes of cultivated *C. annuum* and wild *C. annuum* (as well as related *Capsicum* species) which differ by a reciprocal chromosome translocation. This map is also unique in that it is largely based on COSII markers, which permits the inference of a detailed syntenic relationship between the pepper and tomato genomes—shedding new light on chromosome evolution in the Solanaceae. Since divergence from their last common ancestor is approximately 20 million years ago, the two genomes have become differentiated by a minimum number of 19 inversions and 6 chromosome translocations, as well as numerous putative single gene transpositions. Nevertheless, the two genomes share 35 conserved syntenic segments (CSSs) within which gene/marker order is well preserved. The high resolution COSII synteny map described herein provides a platform for cross-reference of genetic and genomic information (including the tomato genome sequence) between pepper and tomato and therefore will facilitate both applied and basic research in pepper.

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Introduction

Pepper (*Capsicum*) was one of the first plant species subjected to comparative genetic mapping using DNA-based markers (Prince et al. 1993; Tanksley et al. 1988). Since then, numerous genetic maps (including integrated maps) have followed based on tomato and pepper restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers—each improving genome coverage, marker density or insights into synteny (Ben-Chaim et al. 2006; Caranta et al. 1997a, b; Kang et al. 2001; Lee et al. 2004; Lefebvre and Palloix 1996; Lefebvre et al. 1995, 2002; Livingstone et al. 1999; Minamiyama et al. 2006; Paran

et al. 2004). However, none of these previous studies have resulted in a complete genetic map of the pepper genome in which 12 linkage groups correspond to the 12 pepper chromosomes. RFLP markers were the first syntenous markers used to align maps of genera of the Solanaceae. However, RFLP markers have been largely replaced by a new generation of molecular markers (e.g. SSRs, AFLPs) offering a tremendous advance in cost, efficiency, throughput and sensitivity for plant genomics. The weakness of these new markers is their limited ability to be used in comparative genomics (Ben-Chaim et al. 2006).

The goal of the current study was to construct a complete map of the pepper genome based on orthologous markers that are already mapped in the tomato genome. In pursuit of this goal, we have relied heavily on a large set of publicly accessible PCR-based orthologous gene markers—termed conserved ortholog set or COSII markers (Wu et al. 2006). These COSII markers represent conserved, single copy genes in the families Solanaceae and Rubiaceae, and are anchored directly to the *Arabidopsis* genome (Wu et al. 2006). They are currently being mapped in a wide sample of species throughout these two families. Moreover, they are PCR-based and can be readily assayed on standard agarose gels—making them accessible to most breeders/geneticists in a broad range of research environments.

Using an F_2 population from the cross *C. frutescens* × *C. annuum* (Ben-Chaim et al. 2006) we have mapped 299 orthologous markers between pepper and tomato including 263 COSII markers and 36 tomato-derived markers. The result is the first complete pepper genetic map that is comprises 12 linkage groups (named P1-12 based on synteny with tomato chromosomes T1-12) corresponding to the 12 chromosomes in the pepper genome (named chromosome I–XII by Pochard 1970). Based on the COSII markers, the likely cytogenetic events have been constructed, which account for the differentiation of the pepper genome relative to the tomato genome. Conserved pepper–tomato synteny has allowed us to predict the position of 288 tomato COSII markers in the pepper map—bringing the total number of mapped and predicted markers on the pepper map to 661 (including 74 non-orthologous markers) for an average density of one marker every 2.4 cM throughout the genome. Since this complete pepper genetic map ties directly to tomato whose genome is currently being sequenced (http://www.sgn.cornell.edu/about/tomato_sequencing.pl) and other solanaceous species for which large sequence databases are available, it should facilitate the sharing of genomic resources among these genera.

Based on this genetic map, we have also developed a model to explain the chromosome translocation event that differentiates the cultivated *C. annuum* from wild *C. annuum* and related species (e.g. *C. frutescens*, *C. chinense*). The cultivated *C. annuum* possesses a unique karyotype relative to the wild forms. Cultivated *C. annuum* comprises ten meta- or submetacentric chromosomes and two acrocentric chromosomes (Koompai 1976; Lanteri and Pickersgill 1993; Pickersgill 1971; Pochard 1970; Tanksley 1984), which were named as chromosome I–XII and each of which was assigned a color via trisomic analyses by Pochard (1970). In contrast, most wild forms of *C. annuum*, as well as the closely related species of *C. frutescens* and *C. chinense*, all comprise 11 meta- or submetacentric chromosomes and only one acrocentric chromosome (Koompai 1976; Lanteri and Pickersgill 1993; Pickersgill 1971; Tanksley 1984). It is generally accepted that this change in karyotype was due to a single reciprocal translocation that occurred in the lineage leading to cultivated *C. annuum* (Koompai 1976; Lanteri and Pickersgill 1993; Pickersgill 1971; Tanksley 1984). The translocation was presumably between two metacentric chromosomes in the ancestral genome, which resulted in a metacentric and an acrocentric chromosome now visible in the karyotype of cultivated *C. annuum* (Koompai 1976; Lanteri and Pickersgill 1993; Pickersgill 1971). A combination of cytogenetic and genetic mapping has led to the conclusion that the two chromosomes resulting from this translocation correspond to the largest (Chromosome I) and the smallest (Chromosome XII) chromosomes in the karyotype of cultivated *C. annuum* (Pochard 1970; Tanksley 1984).

An important component of our model comes from the earlier studies of ribosomal genes in the *Capsicum* genomes. The largest cluster of R45S genes (R45S-A) is located at short arm of the second acrocentric chromosome (Chromosome XI) (Pochard 1970; Tanksley et al. 1988). This chromosome is apparently conserved in the genomes of *C. annuum*, *C. chinense* and *C. frutescens* (corresponding to tomato chromosome 2 which also bears the same R45S cluster) and was apparently not involved in the reciprocal translocation. However, karyotype analysis has also revealed that Chromosome I (in cultivated *C. annuum*) contains a satellite constriction (presumably containing 45S ribosomal genes) (Pochard 1970). This result was confirmed by genetic mapping with an R45S probe, which placed a second R45S gene cluster (R45S-B) near the putative centromere of Chromosome I (Tanksley et al. 1988). However, the R45S-B cluster is apparently relatively small since it is not readily detected by in situ hybridization (Tanksley et al. 1988). Finally, the wild species *C. chinense* (and possibly *C. frutescens*) contain another larger R45S gene cluster (R45S-C) absent in cultivated *C. annuum*. This cluster comprises the majority of the entire arm of a small metacentric chromosome and did not show significant linkage to any previously mapped markers (Tanksley et al. 1988). It is these two last chromosomes (the ones bearing R45S-B and R45S-C) that we hypothesize were involved in

the translocation that gave rise to the modern, cultivated *C. annuum* karyotype—an event that may have triggered by illegitimate pairing between the R45S-B and R45S-C clusters.

Materials and methods

Genetic mapping on pepper

The mapping population was an F_2 population of 94 individuals from the interspecific cross *C. frutescens* var. BG 2814-6 × *C. annuum* cv. NuMex RNaky (Ben-Chaim et al. 2006). Universal primers for the COSII markers (Wu et al. 2006; primers listed in supplementary Table S1), based on sequence alignments of orthologs from multiple solanaceous species, were used to amplify orthologous fragments from the above two parents. If the COSII primers used for tomato did not provide suitable polymorphic fragments for mapping in pepper, a second primer pair was designed in a different region of the same sequence alignment using the method described in Wu et al. (2006). Amplicon size difference between the two parents was used to genotype the mapping population directly; otherwise the single band amplicons were purified and sequenced. The sequences of the two parents were then aligned and examined for polymorphism using the program CAPSdesigner (http://www.sgn.cornell.edu/tools/caps_designer/caps_input.pl). Thereafter, the mapping population was genotyped via cleaved amplified polymorphic sequence (CAPS) assays (Konieczny and Ausubel 1993). In the cases where CAPS assays were not feasible, other single nucleotide polymorphisms (SNPs) were exploited for mapping using derived cleaved amplified polymorphic sequence (dCAPS) assays designed using the program dCAPS (Neff et al. 1998; Neff et al. 2002).

In order to cover the genome regions where COSII markers were not available, we mapped an additional set of 36 tomato-derived single copy markers in the pepper population. As a result, the 299 orthologous markers cover the entire tomato map with an average density of one marker per $5 \sim 10$ cM. Tomato-derived markers were either mapped in pepper by RFLP (Ben-Chaim et al. 2006) or by CAPS/dCAPS (this work). For CAPS markers, primers were designed in the tomato cDNA or BAC end sequences and used to amplify orthologous fragments from the two pepper parents. Then the same method as that described above for COSII markers were used to genotype these markers in the pepper mapping population. In addition, this population had been previously genotyped for a number of non-orthologous markers such as SSR markers (Ben-Chaim et al. 2006). These non-orthologous markers were used only in cases where they could assist in ordering orthologous markers and are mentioned only in that context.

A translocation event between the two pepper parents—*C. annuum* and *C. frutscens* was investigated in this work. Three related genes, *Idh-1* (NADP-dependent isocitrate dehydrogenase), *Pgm-2* (cytosolic phosphoglucomutase) and *Skdh-1* (dehydroquinate dehydratase/shikimate dehydrogenase) were mapped as CAPS markers on pepper. Primers were designed in the coding sequences of the three genes, respectively, (GenBank accession no. AY572426 for *Idh-1*, AJ240054 for *Pgm-2*, and L32794 for *Skdh-1*) and used to amplify the orthologous fragments from the two pepper parents. Then the same method as that described above for COSII markers were used to genotype these markers in the pepper mapping population. Information regarding all mapped markers can be found in supplementary Table S1 and S2. Marker sequences are available at ftp://ftp.sgn.cornell.edu/COSII/pepper_mapping/.

The above combined marker set was used to construct a genetic linkage map using Mapmaker software (Lander et al. 1987). We first estimated linkage groups using command "group 3, 0.2", and then established a framework (i.e. markers ordered at LOD ≥ 3) for each group using the "ripple" command. Subsequently, we calculated map distance using framework markers only and by the Kosambi mapping function (Kosambi 1944). Finally, we positioned additional markers in the intervals between framework markers using commands "try" and "ripple".

Genetic mapping in tomato

The tomato map, used in comparison to the pepper map, had been previously generated using an interspecific cross *S. lycopersicum* × *S. pennellii* (Frary et al. 2005; Fulton et al. 2002). Currently, more than 2,500 markers have been mapped in this population of which 877 are COSII markers (Wu et al. 2006). For the purpose of comparison with pepper, we prepared a modified tomato genetic map of which the framework is based predominantly on the COSII markers (supplementary Fig. S1). The complete tomato map is available at Solanaceae Genomics Network (http://www.sgn.cornell.edu/cview/map.pl?map_id=9&show_offsets=1&show_ruler=1) and bulk download of all COSII marker information is available at SGN FTP site (ftp://ftp.sgn.cornell.edu/COSII/).

Simulation analyses of the single gene transpositions

A set of 12 chromosomes with map distances equal to those found here for the pepper genome was the starting point for identifying single gene transpositions. For a scenario with n rearrangements between the two genomes (including k translocations and $n–k$ inversions), we first decided the steps on which the translocations would occur by choosing k steps without replacement from $\{1, 2, \ldots, n\}$. On an

inversion step we chose a chromosome at random with probability proportional to its length in cM, then chose two points at random on the chromosome and inverted the segment in between. On a translocation step, we chose two chromosomes with probability proportional to their length in cM, and then chose a point at random on each one. With probability ½, we flipped the orientation of the second chromosome chosen and then performed the translocation. The algorithm was implemented in a C++ program (gene_transposition_simulation.cpp in supplementary materials) using the random number generator "dr250()", which has been thoroughly tested and known to have good properties. We ran the above process for 100,000 times. For each replicate we counted the number of segments in bins of 10 cM. Then we averaged the values from all replicates to get the expected number of segments in each bin.

Results

COSII marker polymorphism

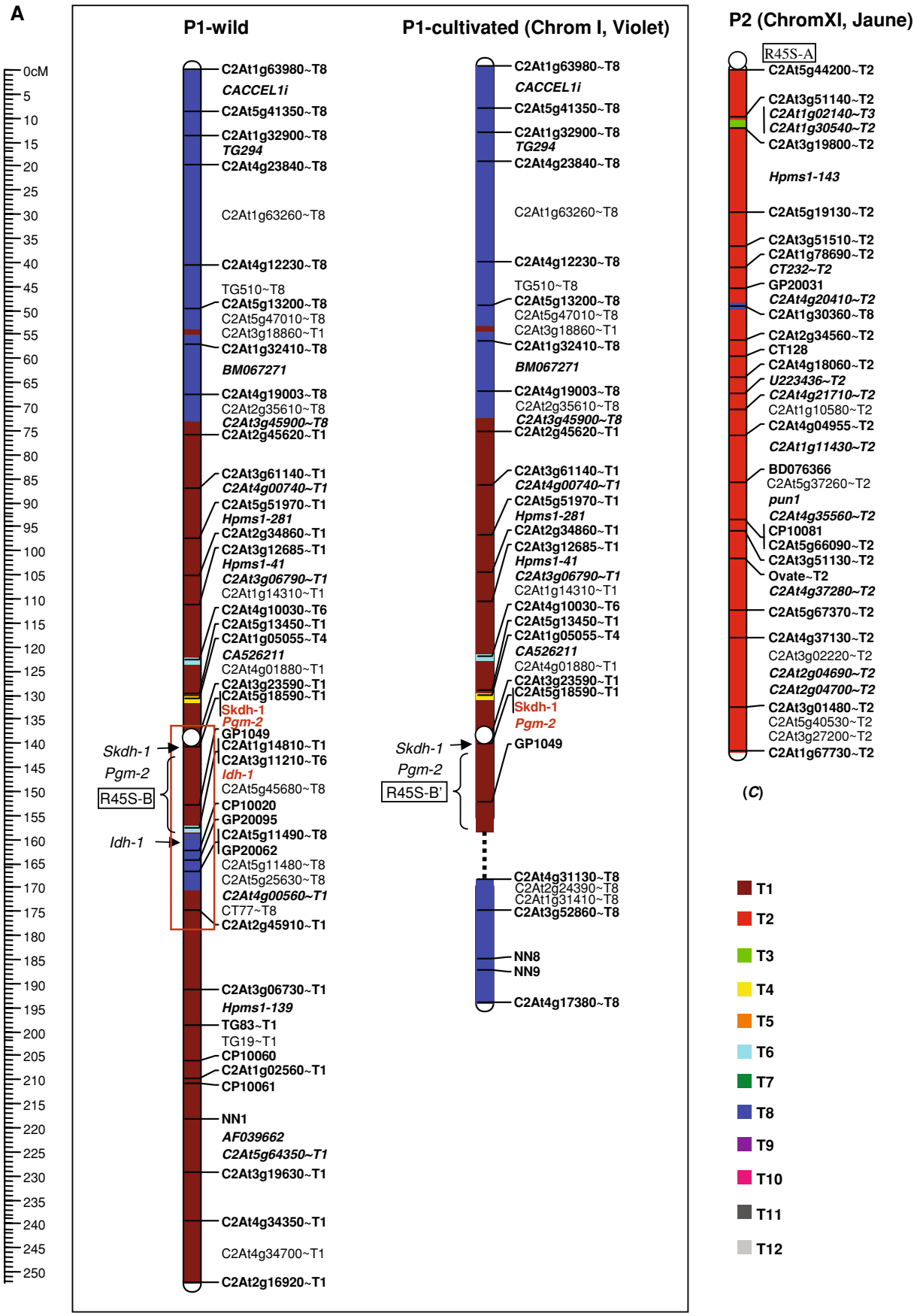
Over 400 COSII markers were tested in both mapping parents—*C. annuum* cv. NuMex RNaky and *C. frutescens* var. BG 2814-6. In 22 cases, the two parents had different amplicon sizes that were detectable (>30 bp) on agarose gels (supplementary Table S1); in 241 of the other cases, it was feasible to design CAPS or dCAPS assays based on the SNPs detected in the amplicon sequences of the two parents. A subset of 214 COSII markers in the latter category, which have a minimum of 200 bp sequenced exon and/or intron, was subjected to further analysis (supplementary Table S3; sequence and sequence alignments available at ftp://ftp.sgn.cornell.edu/COSII/pepper_mapping/). The intron positions of the COSII markers had been predicted previously based on comparison with the *Arabidopsis* orthologs (Wu et al. 2006). Analysis of these amplicon sequences (171 introns and 43 exons) further confirmed the conserved intron positions between the family Solanaceae and Arabidopsis. Not surprisingly, the average SNP frequency is significantly higher in intron (128 bp/SNP) than exon (182 bp/SNP). INDELs were identified in 71 introns (42%) but only 1 exon (2%, supplementary Table S3). In addition, the first 20 highly polymorphic markers are distributed on ten linkage groups except for P5 and P9 (supplementary Table S3), therefore they can potentially be useful for breeders to assess germplasm collections.

Genetic map construction

Of the above more than 400 COSII markers tested, 263 gave usable polymorphism for genetic mapping—including amplicon size difference, CAPS and dCAPS, and also provided a good coverage of the tomato genetic map (one marker every 5–10 cM). These 263 COSII markers were then scored in the pepper mapping population and combined with 36 single copy, orthologous RFLP/CAPS/dCAPS markers derived from the tomato genome as well as 74 miscellaneous, non-orthologous RFLP/SSR/CAPS markers to generate a linkage map of the pepper genome (Fig. 1). The framework map (markers ordered at LOD ≥ 3) comprises 230 markers, of which 169 are COSII markers. The remaining markers were mapped into framework marker intervals at LOD < 3 (see "Materials and methods"). The map is composed of 12 linkage groups (named P1-12 based on synteny with tomato chromosomes T1-12) that correspond to the 12 chromosomes in the haploid pepper genome. The entire pepper genetic map totals 1,613 cM, with an average density of one framework marker for every 6.9 cM (Fig. 1; Table 1).

As most markers were selected at an intervals of 5 ~ 10 cM for a nearly uniform coverage of the tomato genome, the phenomenon of clustering of markers around centromeric regions wasn't as evident as observed in tomato (Tanksley et al. 1992) or in pepper with random markers (Livingstone et al. 1999). The earlier pepper map by Livingstone et al. (1999) identified regions of AFLP and RAPD marker clusters, which are known to preferentially amplify repeated sequences in centromeric heterochromatin (Grandillo and Tanksley 1996). Comparison with that map allowed the localization of putative centromeres in the

Fig. 1 A genetic linkage map of the pepper genome. The pepper linkage groups are designated as P1–P12 based on synteny with the tomato chromosomes T1-12 (Frary et al. 2005; Fulton et al. 2002). P1-cultivated, P2, P4, P8-cultivated, P10 and P12 were each associated with a chromosome (its name and/or corresponding color via trisomic analyses by Pochard 1970 listed in the parentheses) by comparison with the previous pepper maps (Livingstone et al. 1999; Pochard 1970; Prince et al. 1993; Tanksley 1984; Tanksley et al. 1988), and the markers used for association were listed below the linkage groups. Markers in *bold* are framework markers (LOD > 3); marker in *bold*, *italic* are internal markers with 2 ≤ LOD < 3; others are interval markers with LOD < 2; co-segregating markers are denoted by *vertical bars* beside the marker names. "~Tx" following the name of a marker indicates its chromosome location on the tomato map. Each tomato chromosome is assigned one *color* (see color code below P2) and the corresponding pepper chromosome segment(s) are painted with the same color. Putative centromere location of each pepper chromosome is indicated by a *white dot* based on comparison with the previous pepper map (RAPD/AFLP marker clustering regions) by Livingstone et al. (1999). P1-wild and P8-wild were constructed by Mapmaker, while P1-cultivated and P8-cultivated were deduced based on the proposed model (see "Results"). For the translocation related markers, *Idh-1*, *Skdh-1* and *Pgm-2* were mapped in the current population by CAPS (*arrows pointing* to their map positions), while R45S-B was placed at its approximate position based on comparison with the previous pepper map (Tanksley et al. 1988). Markers in the *red box* of P1-wild have a weak linkage with markers of P8-wild (18 ~ 30 cM in pairwise distance). The *vertical broken line* in P1-cultivated indicates an unknown map distance between R45S-B' cluster and the marker C2At4g31130


A
P1-wild
P1-cultivated (Chrom I, Violet)
P2 (ChromXI, Jaune)
0cM
5
10
15
20
25
30
35
40
45
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55
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150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
C2At1g63980~T8
CACCEL1i
C2At5g41350~T8
C2At1g32900~T8
TG294
C2At4g23840~T8
C2At1g63260~T8
C2At4g12230~T8
TG510~T8
C2At5g13200~T8
C2At5g47010~T8
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CP10081
C2At5g66090~T2
C2At3g51130~T2
Ovate~T2
C2At4g37280~T2
C2At5g67370~T2
C2At4g37130~T2
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C2At3g27200~T2
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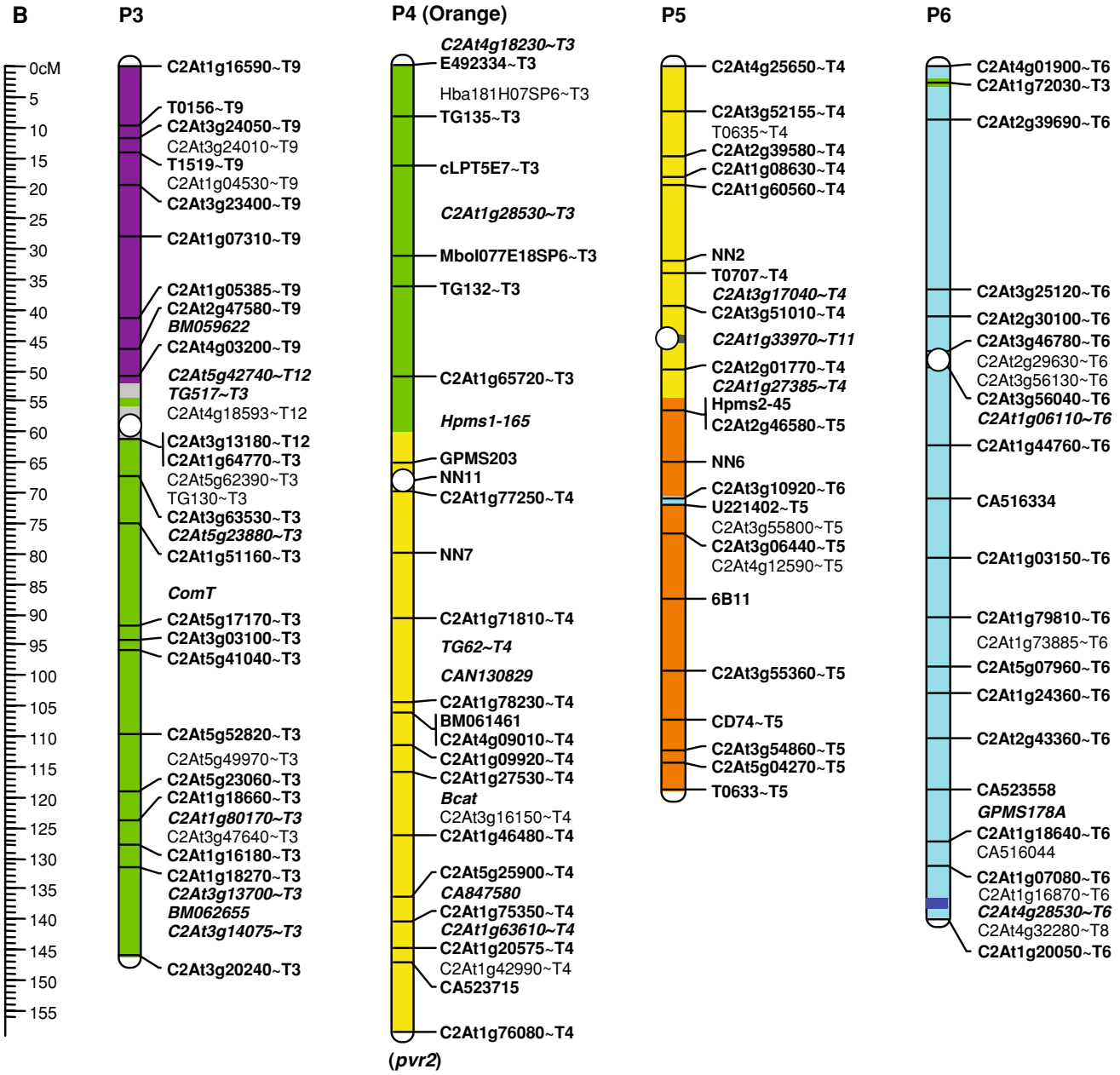

Fig. 1 continued

current linkage groups (Fig. 1). These predictions were largely supported by the synteneous position of centromeres in the tomato genome—a conclusion consistent with that observed by Livingstone et al. (1999).

Characterization of a reciprocal translocation differentiating the genome of cultivated *C. annuum* from that of wild *C. annuum* and other *Capsicum* species

As introduced earlier, we hypothesized that the translocation between cultivated *C. annuum* and other *Capsicum* species were caused by illegitimate pairing between R45S-B and R45S-C clusters. Tanksley (1984) mapped the site of the translocation event to the interval between the isozyme encoding loci—*Idh-1* and *Pgm-2*/*Skdh-1*. One cluster of R45S genes (R45S-B) mapped to the same location (Tanksley et al. 1988). That study also showed that Chromosome XII (Pochard 1970) terminates near the *Idh-1* locus. In this current work, the above three markers were mapped to linkage group P1-wild (Fig. 1). The markers near these three markers have a weak linkage (18 ~ 30 cM in pairwise distance; see markers in the red box of Fig. 1) to a small linkage group P8-wild, although P8-wild stands as a separate linkage group because none of the markers from P8-wild

Fig. 1 continued

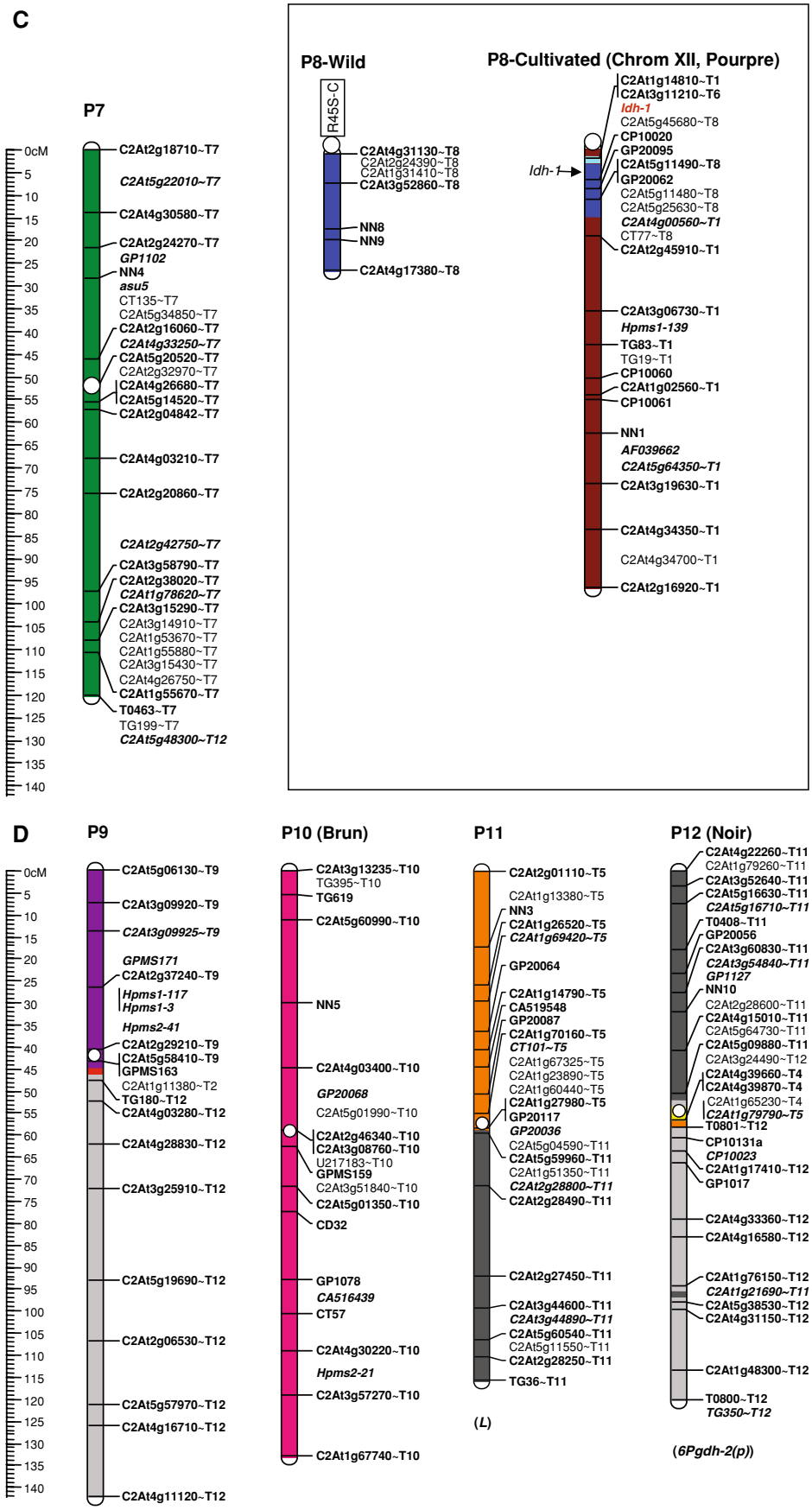

Table 1 Statistics of the pepper genetic map and its comparison with the tomato map

Pepper linkage group	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	Sum
Map distance (cM)	252	142	146	159	119	139	120	26	142	133	116	120	1613
Number of framework markers	36	21	22	22	21	18	16	5	15	15	17	22	230
Number of markers	64	39	38	33	27	27	32	7	21	22	29	34	373
Number of COSII markers	42	30	31	17	18	24	26	5	15	11	20	24	263
Number of synteny markers	47	33	35	23	23	23	29	5	16	14	23	28	299
Number of SMP[a]	11	10	13	13	6	3	11	1	7	5	6	9	95
Number of CSS[b]	5	6	2	2	3	3	1	1	2	2	4	4	35
Min./Max. size of CSS(cM)	13/69	9/26	52/61	31/86	9/42	17/38	117/117	20/20	34/67	52/60	6/35	14/46	–
Mean size of CSS (cM)	26	15	56.5	59	25	25	117	20	51	56	18	24	–
Sum of CSS sizes (cM)	131	91	113	117	76	74	117	20	101	111	71	95	1117
Number of translocations	1	0	2	1	1	0	0	0	1	0	1	2	–
Number of inversions	4	3	1	1	1	1	0	0	1	1	5	1	19

[a] SMP synteny marker pair

[b] CSS conserved syntenic segment

could be mapped to P1-wild. Given the reciprocal translocation between the two mapping parents, *C. annuum* and *C. frutescens*, this phenomenon may be explained by pseudolinkage of loci near the breakpoints in the reciprocal translocation (Burnham 1962).

Combining all of the information above, we proposed a model in which illegitimate pairing and crossing over occurred at or near non-homologous R45S clusters between two non-homologous, metacentric chromosomes in the ancestral genome shared by *C. frutescens*, *C. chinense* and wild *C. annuum*. These two chromosomes correspond to P1-Wild and P8-Wild in Fig. 1. We conjecture that these two metacentric chromosomes underwent an unequal exchange at or near their corresponding R45S clusters (Fig. 2). The outcome of the reciprocal exchange corresponds to Chromosome I (submetacentric) and Chromosome XII (acrocentric) in the genome of modern, cultivated *C. annuum*. Thus, the cultivated *C. annuum* genome now contains two acrocentric chromosomes versus a single acrocentric chromosome observed in *C. chinense*, *C. frutescens* and wild *C. annuum*. This same exchange event also presumably resulted in the loss of the R45S-C cluster in cultivated *C. annuum*, which is possessed by other *Capsicum* species as shown on P8-wild (Tanksley et al. 1988). The remaining ten pepper chromosomes (referred to as P2–P7 and P9–P12) were not affected by this translocation and presumably have the same gene content and gene order in all *C. annuum*, *C. frutescens* and *C. chinense*. Based on this model, we predict the genetic map of the two chromosomes resulting from the translocation event in cultivated *C. annuum*, which were referred to as P1-cultivated (submetacentric, Violet) and P8-cultivated (acrocentric, Pourpre) (Fig. 1).

This model is consistent with all data available thus far from cytogenetic, mapping and geographical/taxonomic studies (Koompai 1976; Lanteri and Pickersgill 1993; Livingstone et al. 1999; Pickersgill 1971, 1979; Pochard 1970; Prince et al. 1993; Tanksley et al. 1988). However, we recognize that rigorous testing of this model will depend on results from future studies—including genetic mapping within wild and within cultivated accessions and analysis of cytogenetic stocks (Pochard 1970). As the karyotype of cultivated *C. annuum* is apparently recently derived (due to the translocation events), we will use the karyotype and the genetic map for *C. frutescens* (including P1-wild and P8-wild) as the point of comparison in discussion about synteny with tomato and other solanaceous species (see next section). It should be noted here that Livingstone et al. (1999) reported a pepper linkage group comprising large portions of tomato chromosomes 1 and 8 (similar to P1-wild in Fig. 1). However, that linkage group was interpreted to be an artifact (pseudolinkage group) attributable to the translocation difference between the mapping parents (*C. annuum* and *C. chinense*). It was therefore proposed that the chromosome, which was ancestral to both tomato and pepper chromosome 1, had a similar arrangement to tomato chromosome 1. Likewise, it was proposed that the chromosome, ancestral to both tomato and pepper chromosome 8, was similar to tomato chromosome 8. The current study differs from that earlier study, in that we find that the large linkage group corresponding to P1-wild (comprising the majority of tomato chromosomes 1 and 8) is not an artifact of the translocation difference between the mapping parents. Rather than that the P1-wild configuration represents an ancestral condition for the clade of *Capsicum* species containing *C. frutescens*, *C. chinense* and *C. annuum*

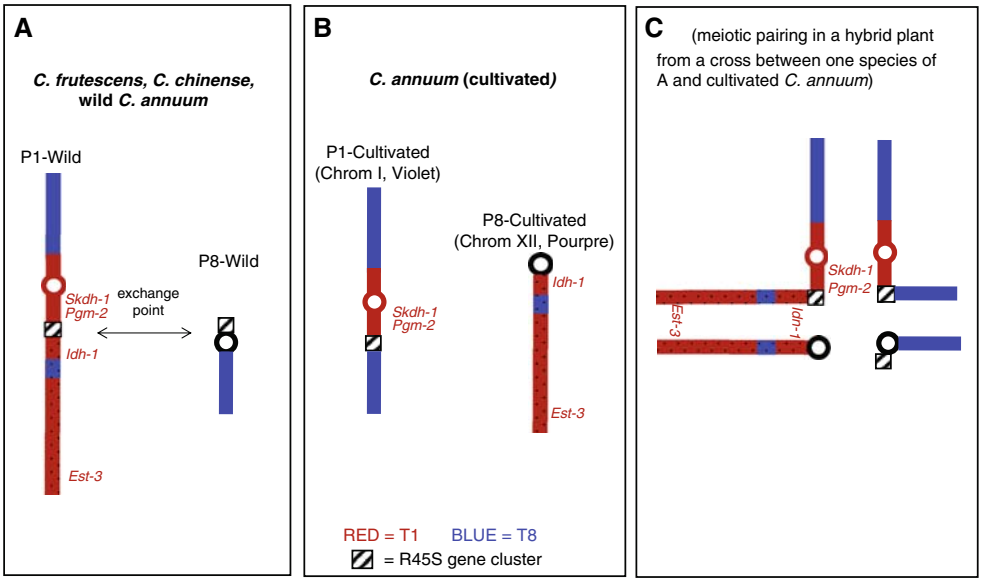

Fig. 2 A model to depict the translocation between cultivated *C. annuum* and related *Capsicum* species including wild *C. annuum*, *C. chinense* and *C. frutescens*

(Fig. 1). Thus, it is not possible at this time to deduce whether the ancestor of tomato and pepper was similar to the tomato karyotype for chromosomes 1 and 8 as previously proposed. Clarification of this point must await comparative mapping in outgroup species (e.g. tobacco or petunia).

Syntenic relationship of pepper and tomato genomes

Deductions concerning the syntenic relationships of the pepper and tomato genome were based on the 263 COSII markers (Wu et al. 2006) plus the additional 36 single copy tomato derived RFLP/CAPS markers (Frary et al. 2005; Fulton et al. 2002). Hereafter, these orthologous markers are referred to as "synteny markers". The first step in deducing syntenic relationships between the two genomes was to identify synteny marker pairs (SMP). A SMP is defined as a pair of orthologous markers that are adjacent to each other in both genomes. To minimize erroneous results, we searched for SMPs only within the subset of synteny markers that had been mapped and ordered in both genomes with a confidence of LOD > 2. The resultant 95 SMPs were then coalesced into conserved syntenic segments (CSSs) defined as of shared blocks of genes/markers with preserved order between genomes (Nadeau and Taylor 1984). Markers ordered at LOD < 2 on either map were included in the analysis only in reference to interchromosomal translocations or a single gene transposition (see the following paragraphs describing pepper-tomato synteny). The result was the identification of 35 CSSs shared between the pepper and tomato genomes (supplementary Fig. S2). The CSSs ranged in size from 6 to 117 cM with an average size of 32 cM (cM values based on the pepper map, Table 1). They covered from 52% (P1) to 97% (P7) of different pepper linkage groups and totaled 1,117 cM corresponding to 69% of the pepper map.

The following discussion will focus on deciphering the chromosomal rearrangements that differentiate the genomes of pepper and tomato. To declare a disruption in synteny between two genomes, two criteria have to be met. First, a structural difference was inferred only if two or more linked markers (in at least one genome) confirmed the rearrangement (an inversion involving one end synteny marker and one interval synteny marker was also accepted); second, for inversions, the involved markers should be ordered at LOD $\geq$ 2 on both maps. This method is less likely to declare false-positive rearrangements, but may result in some rearrangements not being deciphered. For the purposes of comparison, we describe how the pepper genome differs with respect to the tomato genome that is used as a standard of reference. However, we do not wish to imply that any of the discussed structural differences in pepper are derived or ancestral, unless additional information can be brought to bear from a third genome (e.g. potato or eggplant). The syntenic relationships between pepper and tomato are depicted with pepper linkage groups (P1-12 based on synteny with tomato) and their homologous tomato chromosomes/segments (T1–T12 as described in Frary et al. 2005; Fulton et al. 2002) side by side. A schematic depiction of pepper–tomato synteny is presented in Fig. 3 and a detailed close-up picture of each pepper linkage group is in supplementary Fig. S2. The following paragraphs will discuss the pepper–tomato synteny in the order of pepper linkage groups (P1-12).

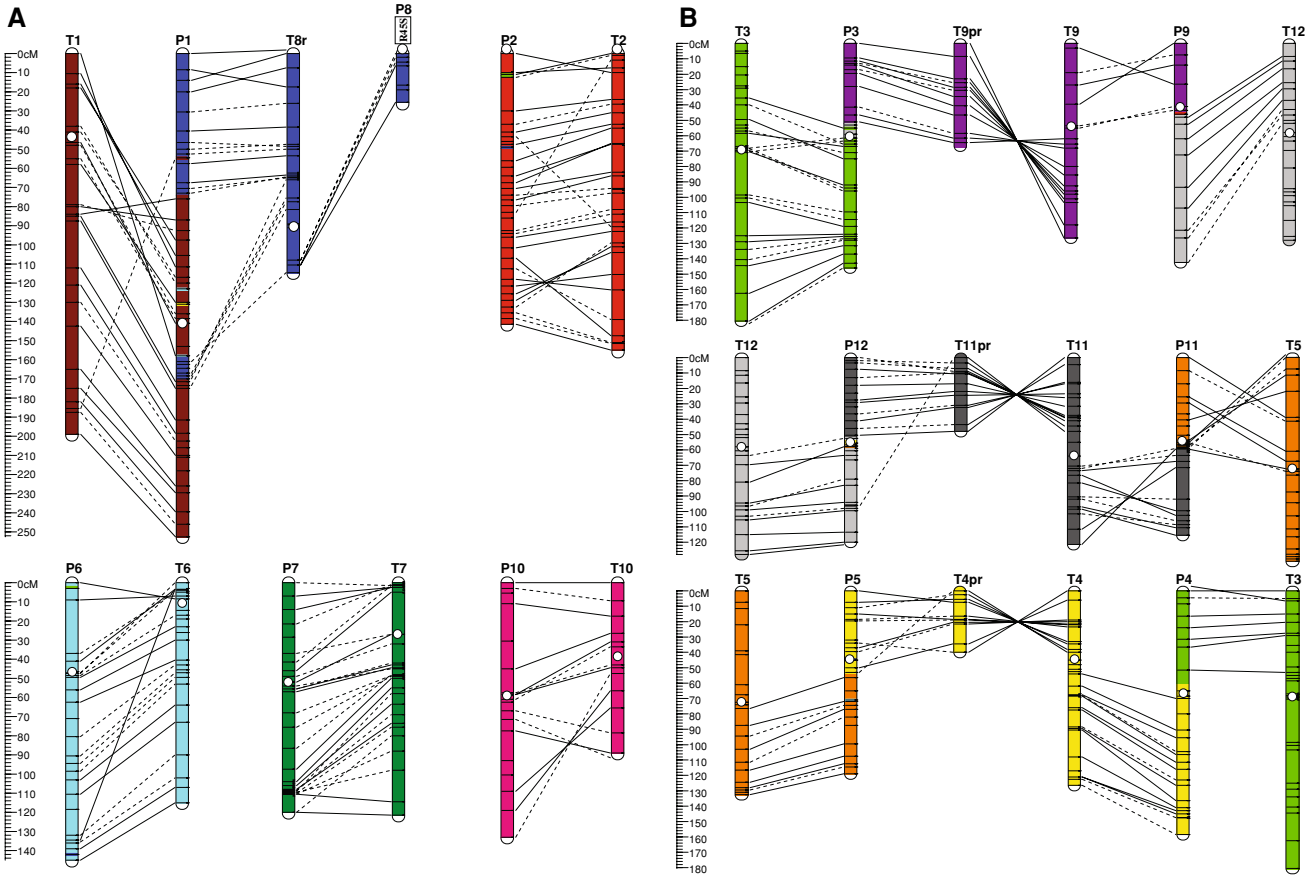

Fig. 3 Comparative maps between pepper and tomato (see close-up in supplementary Fig. S2). Color coding and chromosome designation follow Fig. 1. T4pr, T8r, T9pr and T11pr indicate that reversed and/or partial chromosomes are presented for a better depiction of synteny. Orthologous markers are connected by *lines*, in which a *dash line* indicates that either or both markers are mapped at LOD < 2 and thus are not used for deduction of inversions. *White dots* indicate centromere locations of tomato chromosomes (Frary et al. 2005) and that of pepper chromosomes (as shown in Fig. 1)

P1-wild and P8-wild versus T1 and T8

As discussed earlier, P1-wild and P8-wild represent the condition shared by most *Capsicum* species except for cultivated *C. annuum* (a derived condition); therefore, P1-wild and P8-wild should be used for comparison between the pepper and tomato genomes. P1-wild is 252 cM long and thus the longest linkage group in the pepper genome. It comprises largely of markers from T1 and T8. Subsequently, at least two paracentric and two pericentric inversions would be required to further explain the shuffle of the markers from T1 and T8. Some of the inversions may have included T8 markers so that they have been divided into three segments and interspersed with T1 markers. Two separate T6 markers (20 cM away) have been mapped to P1 (36 cM away) and so did one T4 marker—a phenomenon quite common in comparison of the pepper and tomato genomes and to be discussed in Section "The apparent occurrence of single gene transpositions". The putative centromere of P1 Livingstone et al. (1999) is positioned in a region corresponding to the centromere on T1.

P8-wild, comprising 7 markers (26 cM), is the smallest linkage group in the pepper map. It is synteneous to a 2 cM segment near the top T8. As discussed earlier, the genetic map of P8-wild possibly represent only one arm of the submetacentric chromosome while the other arm and the centromere may largely be occupied by the R45S gene cluster.

P2 versus T2

P2 has three paracentric inversions relative to T2. The lower inversion is in common with eggplant (Wu et al. 2009) and potato (C. Gebhardt, MPI for Plant Breeding Research, Köln, Germany, personal communication), suggesting that pepper/eggplant/potato represents the ancestral gene arrangement and that the inversion occurred in the lineage leading to tomato. The other two inversions occurred after *Capsicum–Solanum* divergence but require an outgroup to decide which lineage represents the ancestral condition. Similar to the phenomenon in P1, two single markers from T3 and T8, respectively, mapped to P2. Both T2 and P2 are acrocentric chromosomes and have a R45S

gene cluster at the top of each chromosome (Doganlar et al. 2002; Tanksley et al. 1992).

P3 versus T3, T9 and T12

P3 combines markers from three tomato chromosome segments—lower T3, upper T9 and a small segment of T12. A paracentric inversion differentiates the P3 and the T3 segments. The putative centromeric region of P3 is synteneous with that of T12.

P4 versus T3 and T4

P4 combines upper T3 and lower T4. Gene order and gene content are largely conserved except for an inversion near top P4 and top T3. The syntenic region of the putative P4 centromere is close to the T4 centromere.

P5 versus T4 and T5

P5 comprises upper T4 and lower T5, but the position of a centromere is not obvious on either of them. Instead, the P5 centromere may be homologous to that of T11 since C2At1g33970 close to T11 centromere was mapped to P5 and inserted into the T4 segment. One inversion differentiates the T4 segment from P5. While gene order is conserved between the T5 segment and lower P5, P5 has lost one marker to P12 but gained one from T6.

P6 versus T6

P6 differs from T6 in an inversion that corresponds to almost the entire short arm of T6. However, pepper is likely to have preserved the ancestral condition in that both eggplant and potato have the same inversion relative to tomato (Tang et al. 2008; Wu et al. 2009). Therefore, the inversion must have occurred in the lineage leading to tomato after it diverged from potato. Putative single marker transpositions are quite common in this chromosome, resulting in the movement of markers from T3 and T8 to P6 and from T6 to P1 and P5. The syntenic region of the putative P6 centromere is close to T6 centromere.

P7 versus T7

Both gene order and content are conserved between P7 and T7 except for one T12 marker mapped to P7. Interestingly, P7 seems to have a second marker clustering area besides the one synteneous to T7 centromere. We cannot rule out the possibility that the second cluster is the result of a paracentric inversion between the two pepper mapping parents—a phenomenon that would result in suppression of recombination and hence marker clustering.

P9 versus T9 and T12

P9 combines upper T9 and upper T12, the former of which includes the centromere. An inversion exists between P9 and T9, which likely occurred in the tomato lineage since both potato and eggplant have the same marker order as pepper (Tanksley et al. 1992; Wu et al. 2009). In addition, one T2 marker mapped to P9. On the other hand, lower P9 agrees with upper T12 in both gene order and gene content (except for a T12 marker mapped to P7).

P10 versus T10

P10 contains all of the T10 markers. One paracentric inversion differentiates P10 from T10, which occurred in the lineage leading to tomato since potato and eggplant share the same marker order with pepper (Tanksley et al. 1992; Wu et al. 2009).

P11 versus T5 and T11

P11 comprises upper T5 and lower T11. The location of the T5 centromere is synteneous with that of the putative P11 centromere. At least five inversions differentiate P11 from its T5 and T11 counterparts. Interestingly, further comparison with potato and eggplant (Tanksley et al. 1992; Wu et al. 2009) revealed that majority of the inversions occurred relatively recently—either in the tomato lineage or in the common ancestor of tomato and potato.

P12 versus T11 and 12

P12 is composed of upper T11, lower T12 and a small T4 segment, the last of which contains a centromere apparently synteneous with that of P12. Two putative single marker transpositions were also observed—one marker from T11 and the other from T5. In addition to the translocations, an inversion exists between P12 and lower T12.

The use of synteny to predict the position of additional COSII markers in the pepper map

The detailed synteny between the pepper and tomato genomes, as described above, can generally be used to infer the relatively precise map positions of additional COSII markers on the pepper map—thereby facilitating mapping studies in pepper and permitting comparisons between pepper and tomato QTL studies. As described earlier, 95 SMPs were identified between the two genomes. Gene content and gene order between an SMP in both pepper and tomato are likely to have been preserved since pepper–tomato divergence. Thus, we searched for COSII markers from

tomato that are located within SMPs, but not yet mapped on pepper. To be more conservative, this analysis was only applied on the mapped tomato COSII markers, which have been confirmed to be single copy and have pepper orthologs (Wu et al. 2006). We referred to these as "inferred pepper COSII markers". As a result, an additional set of 288 COSII markers could be integrated into the pepper map—bringing the total COSII markers in the pepper map to 551 (Fig. S3). Subsequently, a random subset of eight inferred pepper COSII markers were selected and subjected to actual mapping in the pepper genome. Seven of them were mapped to the same SMP interval as predicted, and the other one to an interval flanking the SMP. As a conclusion, it is a fairly precise and applicable approach to predict the map position of COSII markers on the pepper map based on the pepper–tomato synteny.

Discussion

Structural differences between the pepper and tomato genomes

Comparative mapping between pepper (*Capsicum*) and tomato (*Solanum*) revealed that inversions and translocations (especially with break points at or near the centromeres) are the major rearrangement types that have differentiated these two genomes, which is consistent with the finding of Livingstone et al. (1999). A minimum of 19 inversions, 1.6 per chromosome on an average, differentiates the pepper and tomato genomes. Of these, only two (P1) are likely to be pericentric inversions. Further comparison with the potato and eggplant maps (Tang et al. 2008; Tanksley et al. 1992; Wu et al. 2009; C. Gebhardt, MPI for Plant Breeding Research, Köln, Germany, personal communication) suggested that some inversions in lower P11 occurred in the lineage leading to tomato and potato while four others (lower P2, upper P6, upper P9 and lower P10) apparently occurred more recently after tomato–potato divergence in the lineage leading to tomato. Without an outgroup it remains uncertain when and where the other inversions occurred. An ongoing *Nicotiana* mapping project, using the same set of COSII markers, may eventually allow this issue to be resolved (Wu et al. unpublished results).

Eight pepper chromosomes (except for P2, P6, P7 and P10) were involved in one or more translocation events. The majority of those translocations were reciprocal except for the one that combined the majority of T1 and T8 markers into P1-wild. Besides the non-reciprocal translocation, transition from the tomato karyotype (T3, T4, T5, T9, T11 and T12) to the pepper karyotype (P3, P4, P5, P9, P11 and P12) requires a minimum number of five reciprocal translo cations (Fig. 4), however, the order and timing of these events remains uncertain.

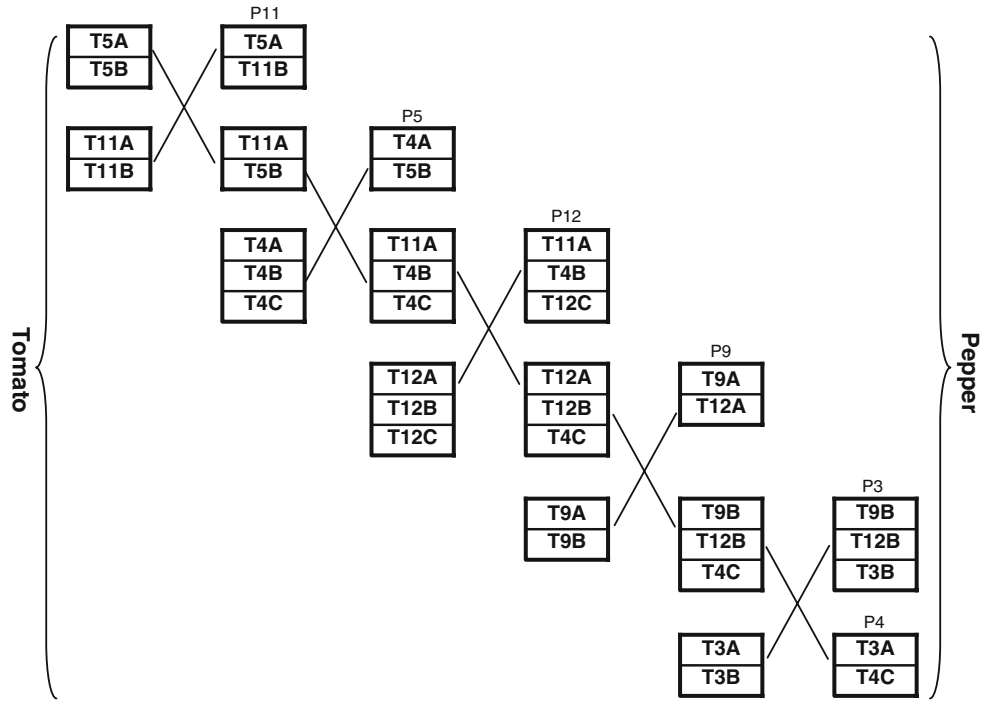

Fig. 4 One possible evolutionary pathway with five reciprocal translocations between the pepper and tomato genomes. **a**, **b** and **c** are segments of a chromosome (from top to bottom), which represent either a chromosome arm or only a small segment (see supplementary Fig. S2 for details). A cross represents a reciprocal translocation but the direction is unknown

The apparent occurrence of single gene transpositions

In addition to the translocations and inversions, a significant number of single marker transpositions have disrupted the synteny between tomato and pepper genomes. Twelve single tomato markers were mapped to a non-homologous chromosome in pepper while some others mapped to a location quite distant from its synteneous position, e.g. C2At1g07080 near top T6 was mapped to the bottom of P6 (supplementary Fig. S2). Since these COSII markers have been confirmed as single copy markers with orthologs in both pepper and tomato genomes, it's unlikely that all cases were due to erroneous mapping of paralogs. This raises the possibility that a third mechanism (other than translocation and inversion) may have played a role in gene movements. To test this hypothesis, we examined the distribution of all putative single marker transpositions in the pepper genome. To facilitate this analysis, the pepper map was divided into putative conserved syntenic segments (PCSSs). A PCSS is defined as a segment that has no translocations or inversions within the segment between the pepper and tomato maps. Like CSSs, markers at LOD < 2 were only considered in reference to a translocation but not an inversion. But the end markers of a PCSS can be at LOD < 2 so that all the markers in the pepper map could be assigned into PCSSs. The resultant 86 PCSSs, 35 of which contained only one single marker, were then categorized according to their size (cM) (Fig. 5a). Apparently, there was a significant difference in distribution between multiple-marker PCSSs and single-marker PCSSs, in that the small size (≤10 cM) category was largely occupied by single marker PCSSs (33 out of 46) and only two single-marker PCSSs were bigger than 10 cM (12 and 17 cM, respectively).

The goal was to determine whether PCSSs comprises single markers which occur at a frequency higher than those comprised of two or more markers, assuming that classical cytogenetic inversions and translocations were the only mechanisms underlying disruption of synteny. If single-marker PCSSs were to occur in a higher-than-expected frequency, it would provide evidence for additional mechanism(s) (e.g. transposition). Since each rearrangement (inversion or translocation) added two to the total number of PCSSs and there were a minimum number of 12 PCSSs (equal to the chromosome number), the 86 PCSSs would require 37 rearrangements between the two genomes. From the analysis of pepper–tomato synteny, there were a minimum number of six translocations each involving multiple-marker PCSSs; however, 11 single-marker PCSSs, which were mapped to non-homologous chromosomes (C2At5g48300 was mapped beyond the P7 framework and thus could not be assigned as a PCSS), may suggested either additional translocations or single gene transpositions. Therefore, from 6 up to 17 translocations

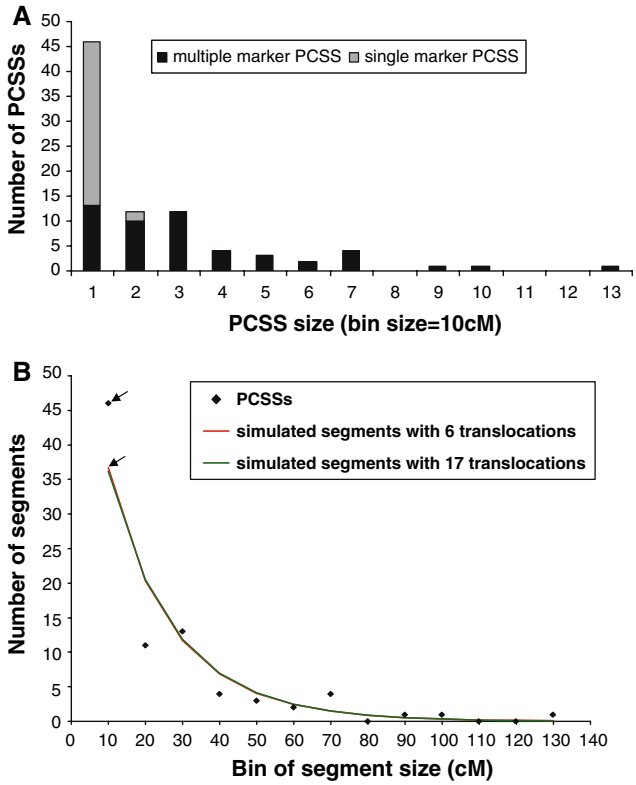

Fig. 5 The distribution of putative conserved syntenic segments (PCSSs) between the pepper and tomato genomes. The segment sizes (cM) were based on the pepper map. **a** A bar chart of multiple-marker and single-marker PCSSs in bins of 10 cM. **b** The two simulated curves depict the expected segment number (based on 100,000 replicates) under the conditions of 6 and 17 translocations, respectively, which suggests an excess of PCSSs under 10 cM. *Arrows* point to the most significant difference between the actual data and the simulated data

differentiated the pepper and tomato genomes. Simulations with 100,000 replicates were performed for scenarios of both 6 and 17 translocations, respectively. The result suggested that, for both scenarios, 37 is the average number of segments with length ≤ 10 cM (Fig. 5b) and only less than 1% simulated cases ($p < 0.01$) were consistent with the actual data, i.e. the number of segments with length ≤ 10 cM is 46 or more. We thus conclude that at least some of the single-marker transposition events may not be attributed to the same mechanisms that create multi-marker PCSSs (e.g. chromosome translocations and inversions); instead, a different mechanism (e.g. transposon-mediated transposition) may also be operating in the decay of synteny that followed the divergence of the pepper and tomato genomes from their last common ancestor.

This conclusion is consistent with our current knowledge of plant transposons which are known to have played an important role in the contraction and expansion of chromosome size, as well as movement of genes between non-homologous chromosomal regions (Bennetzen 2005, 2007; Morgante et al. 2007). Moreover, it has also been shown

that transposon activity can also lead to unlinked duplications through transduplication or retrotransposition (Morgante et al. 2007). It is interesting to note that in the current pepper–tomato study, the 12 gene markers, which were found to have moved to a non-homologous chromosome (possible through transposon-mediated activity), are all located at or near the centromere of either tomato or pepper chromosome (supplementary Fig. S2). It is known that most of the transposons in tomato (especially retrotransposons) are located in pericentric heterochromatin, thus providing a possible explanation for this observation (Wang et al. 2006).

Conclusions

A genetic linkage map of the pepper genome has been constructed based on 299 single copy orthologous markers (primarily conserved ortholog set II or COSII markers) mapped directly onto the genome and 288 markers whose positions were inferred via conserved pepper–tomato synteny. This is the first complete map of the pepper genome, in which 12 contiguous linkage groups correspond to the 12 chromosomes in cultivated *C. annuum*, as well as wild *C. annuum*, *C. chinense* and *C. frutescens*. Combination of this genetic map, earlier genetic maps and cytogenetic evidence has led to a model that depicts the recent reciprocal translocation that differentiates the genome of cultivated *C. annuum* from those of wild *C. annuum*, *C. chinense* and *C. frutescens*. Furthermore, mapping of COSII markers in both pepper and tomato genomes permits us to infer a detailed syntenic relationship between the two genomes—shedding new light on chromosome evolution in the family Solanaceae. A minimum of 19 inversions and 6 translocations have occurred to differentiate the pepper and tomato genomes; transposable elements may have also played a role in single locus transpositions that interrupt synteny. Nonetheless, we were able to identify 35 CSSs—defining majority of the pepper and tomato genomes within which gene/marker order have been well preserved. Because of the high-resolution synteny, the map will provide a platform for cross-reference of genetic and genomic information between pepper and tomato (including the tomato genome sequence) and therefore facilitate both applied and basic research in pepper.

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