Synteny Among Solanaceae Genomes

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Abstract

The Solanaceae was among the first plant families to be analyzed via comparative mapping and thus was a pioneer in the realm of synteny studies. Analyses of chromosome content and organization have employed a range of techniques, including linkage mapping of genes and molecular markers, physical mapping via fluorescence in situ hybridization, and sequencing of relatively small genomic segments as well as the complete sequencing of the tomato genome. Early comparisons in the family involved tomato and its close relative potato and have extended outward to include eggplant, pepper, tobacco, and petunia. Not surprisingly, the degree of synteny among these species is a function of the time since their divergence, with inversion, translocation, and transposition being the chief mechanisms of chromosome rearrangement. The results of this work provide important insight into the modes and tempo of plant genome evolution while serving a practical purpose as well: knowledge of genome synteny and colinearity makes it easier to leverage resources from one species to another in this agronomically important family.

Keywords

Tomato · Eggplant · Pepper · Synteny · Solanaceae

Introduction

The term 'synteny' was originally used in genetics to describe the presence of two or more genes on the same chromosome, however, its meaning has evolved with changes in the discipline (McCouch 2001). Today the terms 'synteny,' 'conserved synteny,' and 'shared synteny' are all used to indicate co-localization of genes or

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M. Causse et al. (eds.), *The Tomato Genome*, Compendium of Plant Genomes,
DOI 10.1007/978-3-662-53389-5_12

markers on chromosomes of two or more species derived from a common ancestor (Abrouk et al. 2010). The terms 'colinear' (var. collinear) and 'conserved syntenic segments' (CSSs; Nadeau and Taylor 1984) are more specific and indicate the shared order of loci in syntenic regions (Abrouk et al. 2010). 'Macrosynteny' describes synteny for a large number of loci over a whole chromosome, while 'microsynteny' describes the detailed relationships between smaller CSSs.

Examination of shared synteny in plant genomes followed soon after the appearance of the first molecular linkage map in tomato (Bernatzky and Tanksley 1986). Only 3 years later, maps comparing the tomato, potato, and pepper genomes were published (Bonierbale et al. 1988; Tanksley et al. 1988). Since this pioneering work in Solanaceae, comparative genome mapping of molecular markers and genes has revealed much about macrosynteny in plant genomes. Another technique used in synteny studies is fluorescence in situ hybridization (FISH) which involves localization of specific probes on pachytene chromosomes. FISH analyses can reveal chromosomal rearrangements such as inversions and translocations. DNA sequencing projects including the complete sequencing of the tomato genome (Tomato Genome Consortium 2012) have allowed comparison of genomes on a finer, microsyntenic, scale.

The study of shared synteny can shed light on the evolution of individual chromosomes and whole genomes. As synteny is the result of descent from a common ancestor, disruption in CSSs can be used to deduce the mechanisms of chromosome rearrangement that accompanied species divergence. Examination of synteny also helps to identify orthologous regions in different species' genomes. This can be useful for determining gene function or for isolating genes in non-model plant species. Shared synteny is also important in the study of paleogenomics, the use of extant species to reconstruct ancestral genomes (Abrouk et al. 2010). More practical applications of shared synteny include the ability to map genes or markers in silico and to leverage resources developed for model species in lesser-studied genomes.

Tomato (*Solanum lycopersicum*) is the model species of the Solanaceae. As a result, most studies of synteny in this family have entailed comparisons with tomato. The species discussed in the following review of synteny research are thereby organized according to their relationship to tomato, beginning with comparisons between tomato and its wild relatives and moving to more distant species within the Solanaceae.

Cultivated Tomato

Examination of synteny within *S. lycopersicum* is extremely limited. Asamizu et al. (2012) compared bacterial artificial chromosome (BAC) end sequences from the cultivar 'Micro-Tom' with the sequence of 'Heinz 1706.' 'Heinz 1706' is the inbred tomato cultivar whose genome was sequenced by the Tomato Genome Consortium (2012). 'Micro-Tom' is a dwarf cultivar which is used as a model because of its small size, relatively short lifecycle, and ease of genetic transformation. Examination of microsynteny between the two cultivars indicated two possible rearrangements. Chromosome 2 contains an inversion of 20–220 kb, its size depending on the orientation of the inversion. Chromosome 3 contains an intrachromosomal translocation and inversion. The presence of a putative reverse transcriptase within the region allowed the authors to hypothesize that the rearrangement was due to retrotransposon activity.

Wild Tomato

The closest relatives of domesticated tomato include nine wild tomato species that can be crossed with *S. lycopersicum*. These species are a rich source of genetic diversity (Tanksley and McCouch 1997; Bai and Lindhout 2007) and have been widely exploited for improvement of tomato including the introgression of over 40 disease resistance alleles from wild germplasm to cultivated tomato (Hajjar and Hodgkin 2007). Moreover, by providing DNA polymorphism which is limited within *S. lycopersicum*,

interspecific populations derived from the wild species have allowed identification and mapping of many qualitative and quantitative traits (Lippman et al. 2007). Fine mapping of disease resistance and morphological genes in interspecific populations of tomato have also revealed genomic rearrangements that distinguish cultivated tomato from its closest wild relatives. Reduced recombination within introgressed segments is often a preliminary indicator of altered synteny. For example, in fine mapping the *Cf-4*/*Cf-9* leaf mold resistance gene cluster on chromosome 1 of tomato, Bonnema et al. (1997) noted that a *S. pennellii*-derived population had a highly suppressed recombination rate as compared to a *S. peruvianum*-derived one. The authors surmised that small inversions in the region might be responsible for this discrepancy.

Lack of recombination in a *S. pennelli*-derived population also hindered high resolution mapping of the *sun* locus on the short arm of chromosome 7 (van der Knaap et al. 2004). This led to the identification of a paracentric inversion in *S. pennellii* relative to cultivated tomato. The same inversion was not detected in *S. pimpinellifolium* (van der Knaap et al. 2004), *S. peruvianum* (van Heusden et al. 1999) or potato (Gebhardt et al. 1991) but is present in eggplant (Doganlar et al. 2002). These results were confirmed by FISH analysis of chromosome 7S which suggested that the *S. pennelli*/eggplant arrangement is ancestral (Szinay et al. 2012). Thus, the inversions occurred independently in the *S. pennellii* and eggplant lineages suggesting that this region of the genome may be subject to frequent rearrangements during evolution. Interestingly, the region containing *sun* is 30 kb shorter in *S. pimpinellifolium* than in *S. lycopersicum* (van der Knaap et al. 2004). Further investigation indicated that the size discrepancy is due to a 24.7 kb duplication at the *sun* locus in cultivated tomato which confers an elongated phenotype to fruit (Xiao et al. 2008). This duplication was attributed to the activity of a long terminal repeat retrotransposon, *Rider*. Thus, a lack of microsynteny between two genomic regions helped to elucidate the identity and mechanism of the *sun* locus in tomato.

Another inversion distinguishing cultivated and wild tomato was detected on chromosome 6 in the region of a root knot nematode resistance gene (*Mi-1*) (Seah et al. 2004). The region contains two clusters of homologous genes which are arranged similarly in both *S. lycopersicum* and *S. peruvianum*, the original source of *Mi-1*. Physical mapping revealed that the clusters are inverted relative to each other in the two species. Examination of microsynteny in the region indicated that simple inversion alone could not explain the arrangement and sequence identity of homologues (Seah et al. 2007). Instead the authors proposed the occurrence of several rearrangements (inversion and/or intra- or interchromosomal recombination) as well as gene conversion but did not specify the events or their timing during evolution. Interestingly this chromosome 6 inversion in *S. peruvianum* was not detected by Szinay et al. (2012) using BAC-FISH. However, they did identify an inversion at the top of 6S in *S. pennelli*. This research also showed that a portion of *S. chilense* chromosome 12S is inverted relative to tomato and wild tomato species.

Physical mapping and sequence analysis were also used to compare large portions of the *S. lycopersicum* and *S. pennellii* genomes (Kamenetzky et al. 2010). With QTLs for metabolic traits as the starting point for their comparisons, the authors examined five regions of the genome and produced a detailed physical map of 1 % of the wild species' genome. *S. pennellii* and cultivated tomato were found to be mostly colinear in these regions. In addition, over 1 million bp of DNA were sequenced and functionally annotated. Examination of the microsynteny in this region revealed that gene order, orientation and exon/intron structure were conserved between the two species with small differences in transposable element insertion and the size of intergenic regions. A divergence time

of 2.7 million years ago (MYA) was estimated based on the rate of amino acid substitution for *S. lycopersicum* and *S. pennellii*.

Tomato-Like Nightshades

Nightshade is often used as a general term to refer to any member of the Solanaceae. However a more specific definition, 'tomato-like nightshades,' includes only those species closely related to tomato: *S. ochranthum*, *S. juglandifolium*, *S. sitiens*, and *S. lycopersicoides* (Rick 1979). These species are of interest because both morphological and molecular phylogenetic studies place them between tomato and potato (Peralta and Spooner 2001; Albrecht and Chetelat 2009). In addition, the tomato-like nightshades are expected to contain more diversity for useful traits such as biotic and abiotic stress tolerance than their domesticated relatives (Albrecht and Chetelat 2009). Some species of tomato-like nightshades can be hybridized, albeit with some difficulty, to tomato, therefore, these species represent a potential genepool of novel traits for tomato improvement.

Molecular genetic mapping in *S. lycopersicum* × *S. lycopersicoides* BC1 and BC2 populations revealed a high degree of synteny between the two species' genomes (Chetelat et al. 2000; Chetelat and Meglic 2000). A total of 139 RFLP, isozyme and morphological markers previously mapped in tomato indicated complete colinearity with the tomato genome except on chromosome 10L. These results suggested an inversion of this arm in *S. lycopersicoides* relative to tomato, a paracentric inversion that is also observed in potato. The same rearrangement of 10L was detected in a pseudo F2 population derived from a cross between two related nightshades, *S. lycopersicoides* and *S. sitiens*, and mapped with 101 RFLP markers (Pertuze et al. 2002). Because this arrangement is common to these tomato-like nightshades, potato, pepper, and eggplant, the inversion must have occurred during the divergence of tomato from these other species. However, it is important to note that BAC-FISH analysis in the same species did not confirm the 10L inversion, instead inversions were detected on chromosomes 6S and 7S of the *S. lycopersicoides* genome relative to tomato. (Szinay et al. 2012). Thus, more detailed analysis of these regions is merited.

The chromosome 10L inversion was also not detected in an F2 population derived from the cross *S. ochranthum* × *S. juglandifolium* (Albrecht and Chetelat 2009). Mapping using 132 tomato COS, COSII, RFLP, and SSR markers revealed overall synteny between these nightshades and tomato with a shared arrangement of chromosome 10L. This finding was confirmed by FISH analysis of 10L in *S. ochranthum* (Szinay et al. 2012). Overall, these results agree with the molecular phylogeny which indicates that the section Juglandifolia nightshades (*S. ochranthum* and *S. juglandifolium*) are more closely related to tomato than the section Lycopersicoides nightshades (*S. sitiens* and *S. lycopersicoides*) (Peralta and Spooner 2001). Szinay et al. (2012) also described an inversion on 6S of *S. ochranthum* relative to tomato which is larger than that in *S. lycopersicoides* and is also shared by potato. The *S. ochranthum* and *S. juglandifolium* genomes were found to differ by a reciprocal translocation of chromosomes 8 and 12. Although other inversions were detected, they might be artifacts as they were only supported by single marker deviations from colinearity.

In other work, the first linkage map for a non-tomato-like nightshade was constructed (D'Agostino et al. 2013). *S. dulcamara*, also known as bittersweet or climbing nightshade, is native to Europe and may be a source of useful abiotic and biotic stress resistances for related crop species. D'Agostino et al. (2013) compared this species' genome with those of tomato, potato, and eggplant. Five *S. dulcamara* chromosomes (1, 3, 6, 8, and 9) were completely colinear with the respective tomato chromosomes indicating that the tomato/bittersweet chromosomes represent the ancestral arrangement. Chromosomes 2, 5, 7, and 10 of *S. dulcamara* contain inversions relative to their tomato counterparts with some of these inversions also observed in potato, eggplant, and/or pepper. Translocations were seen on chromosomes 4, 11, and 12 as has also been observed in solanaceous

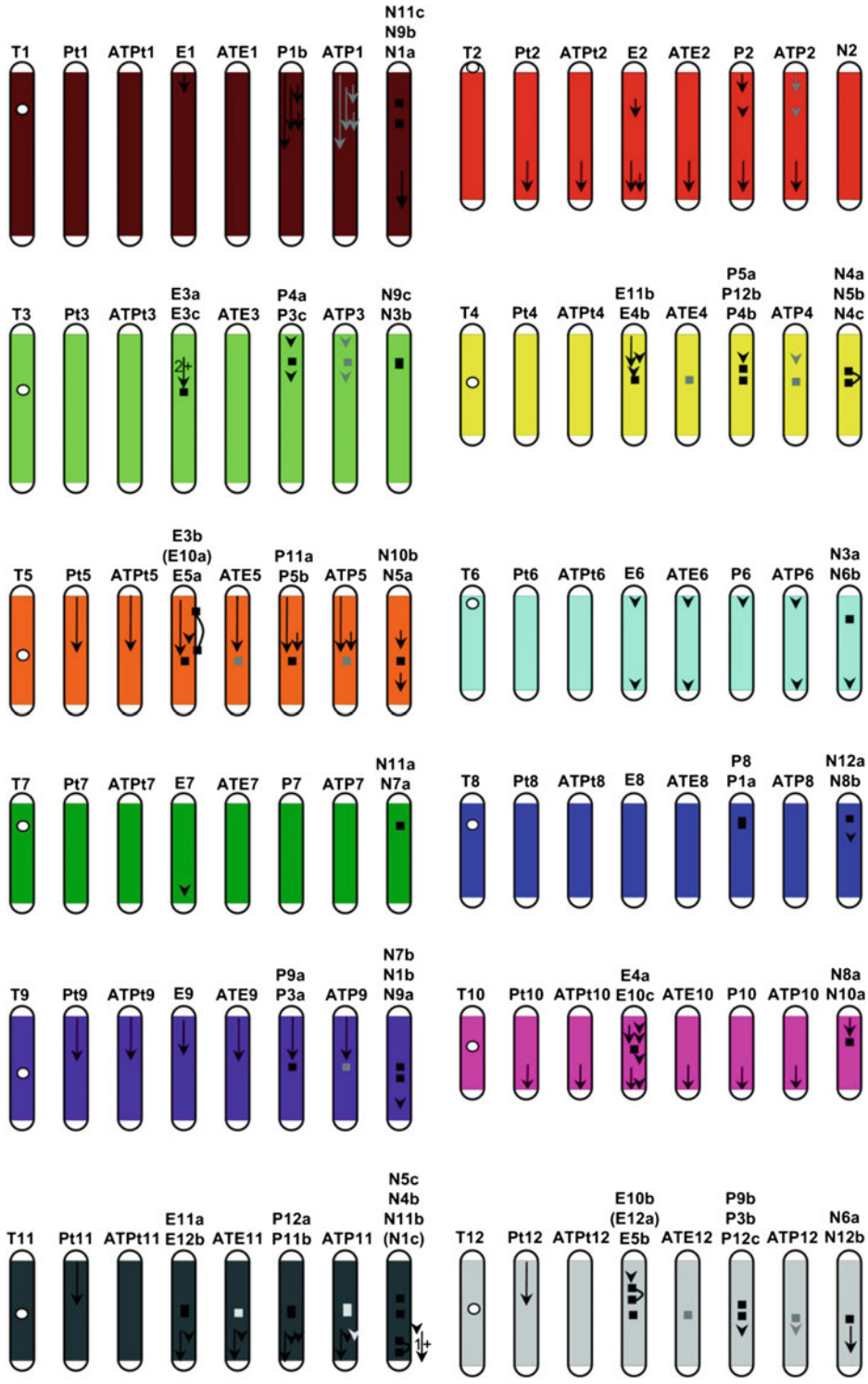
crop species but with different combinations of chromosome arms. This re-use of chromosome breakpoints suggests that certain chromosomes are unstable and have been rearranged more than once over evolutionary time.

Potato

In economic and agricultural terms, potato (*Solanum tuberosum*) is the most important member of the Solanaceae. The genetics of potato is more complex than tomato, owing to its autotetraploid nature. The existence of diploid wild relatives as well as potato's close kinship with tomato provided an essential foundation for molecular genetic analyses in the crop. The construction of molecular genetic linkage maps for potato using genomic and cDNA clones derived from tomato permitted some of the first explorations of synteny in dicot plant genomes (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992). The initial molecular map of potato was developed by examining the segregation of 134 RFLP and isozyme markers in offspring from an interspecific cross of diploid *Solanum* parents: *S. phureja* × (*S. tuberosum* × *S. chacoense*) (Bonierbale et al. 1988). The use of common markers revealed homologous relationships between the 12 linkage groups of potato and tomato and demonstrated that marker content and order are highly conserved between the two species. Four paracentric inversions were identified as disrupting the karyotypic similarity between the species. Three of these chromosomal rearrangements were confirmed and an additional two inversions were discovered as a result of the subsequent parallel construction of high-resolution maps of the tomato and potato genomes (Tanksley et al. 1992). Based on a cross between *S. tuberosum* and *S. berthaultii*, this potato map provided evidence that the entire short arms of chromosomes 5, 9, 11, and 12 and the long arm of chromosome 10 are inverted relative to tomato. Synteny and colinearity of markers between the two species was otherwise strongly conserved leading the authors to surmise that chromosome breakage followed by inversion was the principle mechanism of genome rearrangement during divergence of the two lineages.

Wu and Tanksley (2010) performed a comprehensive review of the data from COSII marker and other comparative mapping studies to ascertain the nature of structural changes distinguishing the genomes of tomato, potato, eggplant, pepper, and *Nicotiana* (Fig. 12.1). This work confirmed the positions of six inversions in potato relative to tomato and deduced that two of these inversion events had occurred along the potato line whereas the rest were specific to tomato (Fig. 12.2). They estimated that the last common ancestor (LCA) of potato–tomato lived 7.3 MYA and that the karyotype of the ancestral genome resembled the following extant chromosomes (where T = tomato and Pt = potato): T1/Pt1, Pt2, T3/Pt3, T4/Pt4, Pt5, T6/Pt6, T7/Pt7, T8/Pt8, Pt9, Pt10, T11, T12 (Wu et al. 2010).

Another approach to elucidating the synteny of the potato and tomato genomes has involved comparative mapping of disease resistance and pathogen recognition genes (Grube et al. 2000; Huang et al. 2004, 2005). In a genome-wide survey of resistance genes (R genes) in tomato, potato, and pepper, Grube et al. (2000) discovered that clustering of R genes at homologous positions is a common phenomenon: four such clusters were found on potato–tomato chromosomes 6, 9, 10, and 12. The authors point out that it would, however, be difficult to exploit this synteny for the purposes of isolating orthologous R genes as corresponding R gene clusters typically contain genes with different pathogen specificity. Nevertheless, Huang et al. (2004, 2005) found strong conservation in marker content and order between the *I2* region of tomato (which confers *Fusarium* wilt resistance) and the *R3* region of potato (confers late blight resistance) that proved useful in isolating *R3a*. Based on their protein sequences, *I2* and *R3a* belong to the same gene family and may have evolved from an R gene locus present in their LCA. Interestingly, the tomato *I2* region is half the size of the potato *R3* region. Thus in addition to the change in pathogen specificity, a greater number of R genes have evolved at this particular locus in potato than in tomato (Huang et al. 2005).


T1
Pt1
ATPt1
E1
ATE1
P1b
ATP1
N11c
N9b
N1a
T2
Pt2
ATPt2
E2
ATE2
P2
ATP2
N2
T3
Pt3
ATPt3
E3a
E3c
ATE3
P4a
P3c
ATP3
N9c
N3b
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Pt4
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P12a
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ATP11
N5c
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N11b
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Pt12
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E10b
(E12a)
E5b
ATE12
P9b
P3b
P12c
ATP12
N6a
N12b

◀ **Fig. 12.1** Comparative maps of tomato (T), potato (Pt), eggplant (E), pepper (P), and tobacco (N) chromosomes and their most recent ancestors (chromosomes with AT prefixed to name) as determined by COSII mapping. *White circles* indicate positions of tomato centromeres. *Black arrows* and bars indicate inversions and breakpoints relative to tomato. *Grey symbols* indicate uncertain chromosomal rearrangements (used with permission from Wu and Tanksley 2010)

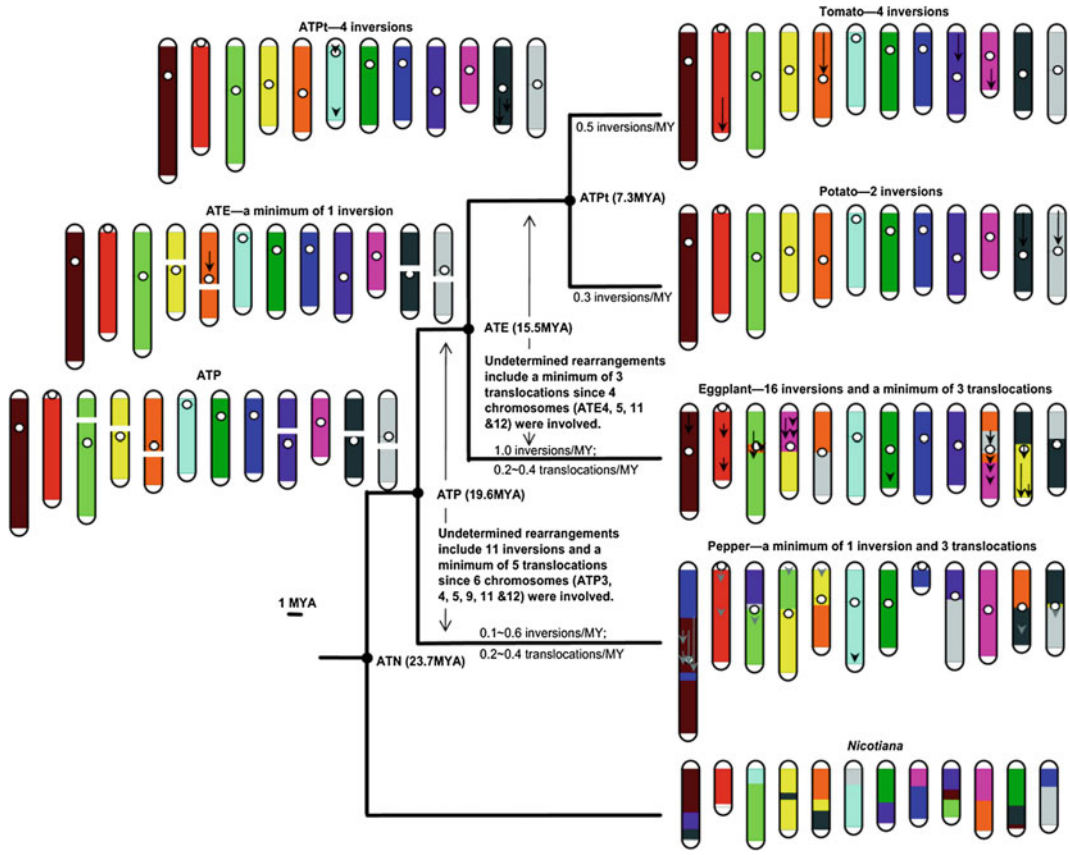

Fig. 12.2 Karyotypes of tomato, potato, eggplant, pepper, and tobacco and their most recent ancestors as determined by COSII mapping. Tomato chromosomes are *color-coded*. *Symbols* and abbreviations are as described for Fig. 12.1. Chromosome breaks indicate areas that require further study (used with permission from Wu and Tanksley 2010)

With the availability of the complete genome sequence of tomato (Tomato Genomics Consortium 2012), it has become easier to conduct genome-wide surveys and phylogenetic analyses of disease-resistance genes. An analysis of the bHLH transcription factor family uncovered 152 members distributed across the entire tomato genome, with evidence suggesting that one was upregulated in a resistant line following infection by tomato yellow leaf curl virus (Wang et al. 2015). Comparison with potato enabled the identification of over 160 orthologous gene pairs, each single copy tomato gene being represented in potato by up to four genes, reflecting the polyploid origins of *S. tuberosum*. Physical localization of these gene pairs revealed a high degree of synteny between the potato and tomato genomes. Similarly, Andolfo et al. (2013) performed a comprehensive analysis of pathogen recognition genes (specifically, nucleotide-binding site, receptor-like protein, and receptor-like kinase genes) in the tomato genome, in an effort to

localize the genes and trace their evolutionary origins via gene duplication. More than 300 putative orthologs of the pathogen recognition genes were obtained from potato, using synteny (comparative genome positions) as a key criterion for orthology. Slight differences in the genomic positions of many of the orthologous pairs were noticed in addition to differences in gene number between tomato and potato, suggesting that these genes are evolving independently in the two lineages.

Although comparative genetic mapping has revealed a great deal about the syntenic relationship between genomes in the Solanaceae, suppressed recombination in heterochromatic genomic regions limits the ability of genetic maps to fully resolve genome structure. Recognizing this shortcoming, physical mapping approaches have become increasingly popular especially now that extensive BAC libraries have been made available by the work of the tomato and potato genome sequencing consortia (http://www.sgn.cornell.edu/; http://potatogenome.net). Thus localization of BACs on pachytene chromosomes of tomato and potato using FISH uncovered two structural differences between tomato and potato chromosome 6 (Iovene et al. 2008; Tang et al. 2008). Iovene et al. (2008) reported that, while the pachytene chromosomes of tomato and potato are morphologically similar, an interstitial heterochromatic knob is specific to potato 6L. BAC colinearity on 6L is, however, conserved. In addition, both studies (Iovene et al. 2008; Tang et al. 2008) confirmed the existence of a large inversion encompassing the euchromatic portion of 6S that had been suggested by a previous molecular genetic analysis of chromosome 6 (van Wordragen et al. 1994) but was not apparent on the high-density tomato–potato map (Tanksley et al. 1992).

Comparative sequencing offers an avenue for elucidating microsyntenic relationships between potato and tomato. Wang et al. (2008) included potato in their sequence analysis of a 105 kb CSS in five solanaceous species (tomato, potato, eggplant, pepper, petunia). Of the 17 genes contained within this region, two showed a reversed orientation in potato as compared with tomato (Fig. 12.3). Because the potato orientation was also seen in eggplant and petunia, it was judged to be the ancestral condition. These authors also calculated an approximate date of 6.2 MYA for the divergence of potato and tomato (Wang et al. 2008). Zhu et al. (2008) generated almost 90 Mb of potato genomic sequence from 77,000 BAC ends and 22 BACs. BLAST searches in Genbank and the SGN database (solgenomics.net) were then used to

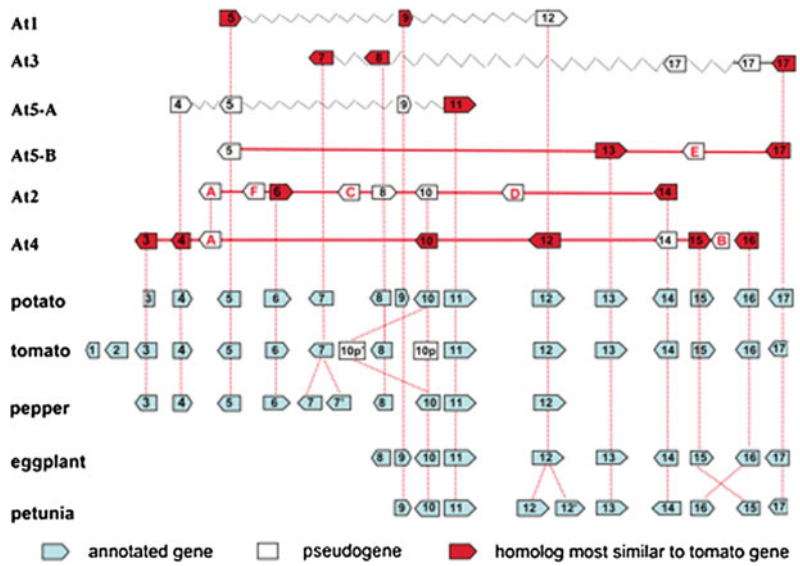

Fig. 12.3 Organization of a 105 kb conserved syntenic segment (CSS) in potato, tomato, pepper, eggplant, and petunia containing 17 annotated genes. Positions of the genes in the arabidopsis (At) genome are also shown. Putative orthologs are connected by *dashed red lines* (used with permission from Wang et al. 2008)

identify segments syntenic to tomato. In some instances, the conserved segments spanned more than 100 kb and sequence coverage ranged from 13 to 73 %. Although macrosynteny between potato and tomato was apparent, evidence of small-scale rearrangements such as insertions/deletions and micro-inversions were also seen. Nevertheless, protein sequence alignments as well as a comparison of the length of genes, exons, and introns indicated that genic synteny was maintained. A comprehensive analysis of repeated DNA sequences within the BACs suggests that transposition, alongside chromosome inversion, is a key contributor to genome restructuring between potato and tomato (Zhu et al. 2008).

In a multipronged approach employing cross-species BAC-FISH and comparative sequencing, Peters et al. (2012) analyzed 7 Mb of the euchromatic portion of the long arm of chromosome 2. Six major rearrangements including inversions ranging in size from 20 kb to 3 Mb as well as several translocations were identified. These structural changes appear to have occurred along the lineage leading to tomato as they are absent from pepper, eggplant, and potato. This work also revealed that the rearrangements affecting 6S, 10L, and 11L are more complex than previously suspected. The inversion on 6S involved several reversals, deletions, and translocations. A second inversion was pinpointed on 10L. In addition, three inversions, three deletions, and an inverted translocation occurred on 11L. Microsynteny in potato and tomato was also explored by examining the adjacency of orthologous genes on 2L. Within 664 ortholog groups, the vast majority (96 %) consisted of homologous gene pairs that mapped to corresponding colinear positions. However, gene adjacencies were not conserved between potato and tomato for 46 % of these ortholog pairs. In many cases, the insertion of putative retrotransposons appears to have disrupted microcolinearity. Sequences similar to transposable elements were also found near rearrangement junctions suggesting that repeat-mediated recombination is a plausible mechanism for genome reorganization. Accordingly, the authors hypothesized that a series of intra-strand and ectopic recombination events transformed 2L from the ancestral state found in potato to that found in tomato (Peters et al. 2012). Thus, what has emerged from this and other physical mapping studies is a far clearer picture of chromosome evolution in *Solanum* as well as the importance of examining synteny and colinearity on a finer scale.

Synteny studies in potato have extended beyond tomato to encompass a number of other *Solanum* species. In a comparison of tuber-bearing *Solanums*, a BC1 population was derived from a cross between two Mexican diploid species *S. pinnatisectum* (a source of late blight resistance) and *S. cardiophyllum* ssp. *cardiophyllum* (Kuhl et al. 2001). The resulting molecular map, albeit low resolution (99 markers derived from tomato) and incomplete (13 linkage groups), showed good overall synteny and colinearity with previously published potato linkage maps (Bonierbale et al. 1988; Tanksley et al. 1992; Perez et al. 1999). Interestingly, despite the morphological similarity among potato and its non-tuber-bearing relatives (section *Etuberosum*) (Contreras-M and Spooner 1999), a wide range of evolutionary mechanisms has operated to distinguish the A genome of cultivated potato from the E genome of section *Etuberosum* species (Perez et al. 1999). Using established tomato/potato markers on a F2 population derived from an interspecific cross between *S. palustre* and *S. etuberosum*, Perez et al. (1999) placed 80 loci in 19 linkage groups. While the excess of linkage groups indicates that the E genome was not completely mapped, this work did reveal general synteny in that markers usually mapped to homeologous chromosomes in both genomes. However, the linear order of markers was frequently disrupted by putative translocations, inversions, and occasional transpositions. Thus, it is not surprising that attempts to cross A and E genome *Solanum* species have not been successful: the extent of chromosome rearrangement could explain the lack of chromosome pairing and hybrid sterility that are typically observed (Ramanna and Hermsen 1979; Watanabe et al. 1995). The comparative mapping

results of Perez et al. (1999) provide additional support for the phylogenetic placement of *S. tuberosum* and *S. lycopersicum* as sister groups on a lineage separate from section *Etuberosum* (Spooner et al. 1993). However, more recent research (Szinay et al. 2012; described below) places *S. etuberosum* closer to the tomato clade.

Research in potato has also examined synteny within the species. Tang et al. (2008) extended the analysis of the chromosome 6S inversion across six potato genotypes and found evidence of a single minor structural rearrangement of 6S in one potato line. This, combined with their failure to observe the 6L interstitial knob identified by Iovene et al. (2008) in any of their lines, led them to speculate that a certain degree of chromosomal rearrangement has occurred within *S. tuberosum* (Tang et al. 2008). Lou et al. (2010) broadened the cytogenetic comparison of chromosome 6 to include a total of seven *Solanum* species: cultivated potato (A genome), two wild potato species (*S. bulbocastanum* and *S. chromatophilum* representing the B and P genomes, respectively), the E genome species *S. etuberosum*, as well as tomato, eggplant, and its relative *S. caripense*. Synteny in BAC position and orientation was found across all species with the exception of the aforementioned paracentric short arm inversion in tomato and a large pericentric inversion in *S. etuberosum*. The paracentric 6S inversion was deemed to have occurred after the divergence of tomato from the other *Solanum* species. The pericentric inversion is noteworthy as being the first such inversion identified in the genus. Interestingly, Perez et al. (1999) failed to detect this inversion in their cross-species comparison of the A and E genomes, once again highlighting the shortcomings of linkage analysis as the sole approach to synteny studies.

In addition to revealing hidden structural changes in genomes, the BAC-FISH approach has been useful as a means of understanding evolutionary relationships within *Solanum*. Szinay et al. (2012) used BAC-FISH signal order to perform a phylogenetic analysis of 18 *Solanum* species/accessions. BACs specific to seven chromosome arms known to harbor inversions among the selected species (5S, 6S, 7S, 9S, 10L, 11S, 12S) were isolated and mapped via FISH. Two syntenic species groups (composed of species with identical hybridization patterns) emerged: group A comprising potato and its relatives within section *Petota* and group B comprising several members of section *Lycopersicon*, including tomato. The genome of *S. etuberosum* differed from that of syntenic species group A due to inversions on three of the studied chromosome arms: 7S, 9S, and 10L (this latter inversion is apparently shared with group B). As a result, the phylogenetic tree based on these results places *S. etuberosum* closer to tomato and its relatives than to potato (Szinay et al. 2012), a topology that differs slightly from that deduced by Perez et al. (1999) based on their comparison of the A and E genomes. Finally, the authors hypothesize that cultivated and wild potato species must have diverged in the recent past as no structural differences among their genomes were detected (Szinay et al. 2012). In a broader comparison within the clade, over 300 COSII markers were assessed in eight potato accessions (including the wild species *S. berthaultii*, *S. chomatophilum,* and *S. paucissectum* as well as two diploid landraces of *S. tuberosum*) (Lindqvist-Kreuze et al. 2013). Only a small number of the COSII markers did not map in their predicted locations based on the established synteny between potato and tomato.

In contrast, by aligning diversity arrays technology (DArT) marker sequences derived from the wild tuber-bearing species *S. commersonii* and *S. bulbocastanum* with genome sequences from cultivated potato and tomato, Traini et al. (2013) discovered a greater amount of variability between the genomes. The failure of a proportion of the markers to align with the potato (8 %) or the tomato (21 %) genome sequences was taken as evidence of this heterogeneity. The existence of gaps between the markers provided additional support of small-scale structural divergence between the genomes of wild and cultivated potato. The use of DArT markers to construct medium-density genetic linkage maps for *S. bulbocastanum* has shed additional light on the degree of divergence between the A genome of

S. tuberosum and the B genome of *S. bulbocastanum* (Iorizzo et al. 2014). The wild potato genome shows the same nine chromosomal rearrangements previously described as distinguishing tomato and cultivated potato. Moreover, two additional, albeit small (5–10 cM), inversions on 2S and 8S appear to be specific to *S. bulbocastanum*. The results of these two studies are suggestive of the sorts of microscale, lineage-specific rearrangements that have accompanied the diversification of potato as a clade and which should become more and more visible as new strategies for mining whole genome sequence data are developed.

Eggplant

Eggplant (*S. melongena*), unlike many other solanaceous species, was domesticated in the Old World and its current importance as a crop is primarily limited to the Mediterranean Basin and Asia. In the past decade, genetic studies in eggplant have been facilitated by genome similarity with tomato. Synteny between eggplant and tomato was first investigated by Doganlar et al. (2002) who mapped 233 single copy tomato RFLP markers in an interspecific (*S. linnaeanum* × *S. melongena*) eggplant population. This work indicated that the eggplant and tomato genomes share large colinear regions and differ by 28 rearrangements encompassing 23 inversions, 4 reciprocal, and 1 non-reciprocal translocation. A total of 36 CSSs were identified in the genomes with an average size of 34 cM. Two chromosomes, 1 and 8, were found to be completely syntenic between eggplant and tomato. The use of tomato RFLP markers that had also been mapped in potato allowed comparisons between eggplant and potato and revealed similar high levels of synteny with 24 rearrangements differentiating these two genomes. Examination of the synteny between eggplant and these two species indicated that eggplant and tomato are five to six times more diverged than tomato and potato in terms of numbers of rearrangements. The work also provided insight into mechanisms of chromosome evolution in the Solanaceae. Results indicated a moderate rate of chromosome evolution (0.19 rearrangements per chromosome per million years) and that paracentric inversions of CSSs were the primary mechanism of rearrangement. Translocations were of secondary importance in divergence of eggplant and tomato/potato. Translocations and inversions generally occurred at or near the centromeres as indicated by the presence of telomeric sequences at the centromeres of affected chromosomes (Presting et al. 1996).

Further work with the same population by Wu et al. (2009a), added 110 COSII markers to the eggplant map. Their results were very similar to those of Doganlar et al. (2002) indicating that the eggplant and tomato genomes share 37 CSSs and differ by 24 inversions and five translocations with some differences detected in the locations of the inversions. Wu et al. also identified five single markers with altered positions suggesting possible transposable element activity during the divergence of eggplant and tomato from their LCA. The authors took advantage of the high degree of synteny of the species to infer the locations of 522 additional COSII markers thus producing a virtual eggplant map containing 869 markers. Comparison of this map with potato and pepper maps indicated that several rearrangements are shared by eggplant and pepper only and not by tomato and potato. These results indicated that the eggplant-pepper arrangements seen at the bottoms of chromosomes E2, E10, and E12 and at the tops of chromosomes E6 and E9 are ancestral.

In more recent work, Doganlar et al. (2014) mapped an additional 192 RFLP, 6 COSII, and 400 AFLP markers on the interspecific eggplant population. This work confirmed the established syntentic relationships between eggplant and tomato with 33 CSSs identified. However, the higher resolution map indicated more translocations (19) and fewer inversions (14) than previously hypothesized. Thus, translocation appears to be a more common mechanism of chromosome evolution in the Solanaceae than previously thought. Eleven marker transpositions were also detected confirming the role of transposable

elements in genome evolution of the family as suggested by Wu et al. (2009a).

Wu and Tanksley (2010) compared COSII maps for eggplant and four other solanaceous species to deduce ancestral chromosome arrangements (Fig. 12.1) and their timing. Based on this work, they hypothesized that 16 inversions and 3 translocations occurred along the eggplant lineage (Fig. 12.2). They calculated the divergence time of tomato and eggplant as 15.5 MYA. This value allowed them to estimate the rate of chromosomal evolution in the eggplant lineage. Thus, they determined that this lineage experienced 1 inversion and 0.2–0.4 translocations every MY. This rate of inversion is higher than those calculated for the potato and pepper lineages, however, the authors cautioned that the number of inversions in the eggplant lineage may have been overestimated due to difficulties in assigning some inversions to specific lineages. The rate of translocation was the same as that estimated for the pepper lineage.

In the past, mapping in intraspecific populations of eggplant was constrained by limited polymorphism. However, this has changed with the advent of SNP and InDel markers. Fukuoka et al. (2012) integrated results from two intraspecific eggplant populations to obtain a map with 952 markers. Of these, 469 were SNP and InDel markers derived from *Solanum* ortholog gene sets (SOL markers). These are orthologous unigene markers identified in the eggplant, tomato and potato genomes. As 70 % of these markers had also been mapped in the tomato genome, the authors were able to observe synteny between the two genomes. Although detailed comparisons were not made, the results indicated several rearrangements with overall agreement with the findings of Wu et al. (2009a).

Gramazio et al. (2014) have recently developed an interspecific genetic linkage map for a BC1 population generated by backcrossing a *S. melongena* × *S. incanum* F_1 to the *S. melongena* parent. Of the molecular markers mapped (a combination of COSII, SSR, AFLP, CAPS and SNPs), 123 had been positioned on the maps of Nunome et al. (2009), Wu et al. (2009a), Barchi et al. (2012) and Fukuoka et al. (2012). These anchor loci revealed good correspondence in marker order between the new map and the four previous eggplant maps (Fig. 12.4). In addition, comparing the map positions of 130 markers shared with tomato (Fulton et al. 2002) uncovered regions of chromosome shuffling that were consistent with those reported by Wu et al. (2009a).

The well-established synteny between the eggplant and tomato genomes has been used to infer gene position and to identify candidate genes controlling quantitative traits. Thus, Gramazio et al. (2014) mapped a number of genes involved in chlorogenic acid synthesis and phenol oxidation using orthologous sequences from tomato. In all cases, the genes mapped to the eggplant linkage groups in regions corresponding to their syntenic positions on the tomato map. Similarly, synteny with tomato was used to identify candidate genes in genomic regions harboring QTL for anthocyanin levels and fruit color (Cericola et al. 2014) and a range of agronomic traits (Portis et al. 2014) in eggplant.

Synteny in eggplant and other Solanaceae has also been examined with FISH mapping. Using this technique, Lou et al. (2010) localized 17 BAC clones on chromosome 6 of tomato, eggplant, potato, and wild potatoes. Based on this analysis, the authors concluded that the arrangement of chromosome 6 in eggplant represents the ancestral condition. Moreover, the paracentric inversion of 6S, which was detected by Doganlar et al. (2002) in their comparison of tomato and eggplant, was not identified in any of the other species and suggesting that it only occurred in the tomato lineage. More extensive BAC-FISH analysis examined seven previously identified inversions in the *Solanum* genome (Szinay et al. 2012). This work indicated that the *S. melongena* genome represents the ancestral state for chromosomes 6S, 7S, 9S, and 11S. Therefore, the inversions in these regions occurred in the tomato–potato lineage. In contrast the inversion described on chromosome 10L is derived and occurred only in the eggplant lineage.

To date, limited insight has been gained from sequencing analyses of eggplant. Wang et al. (2008) included eggplant in their comparison of a

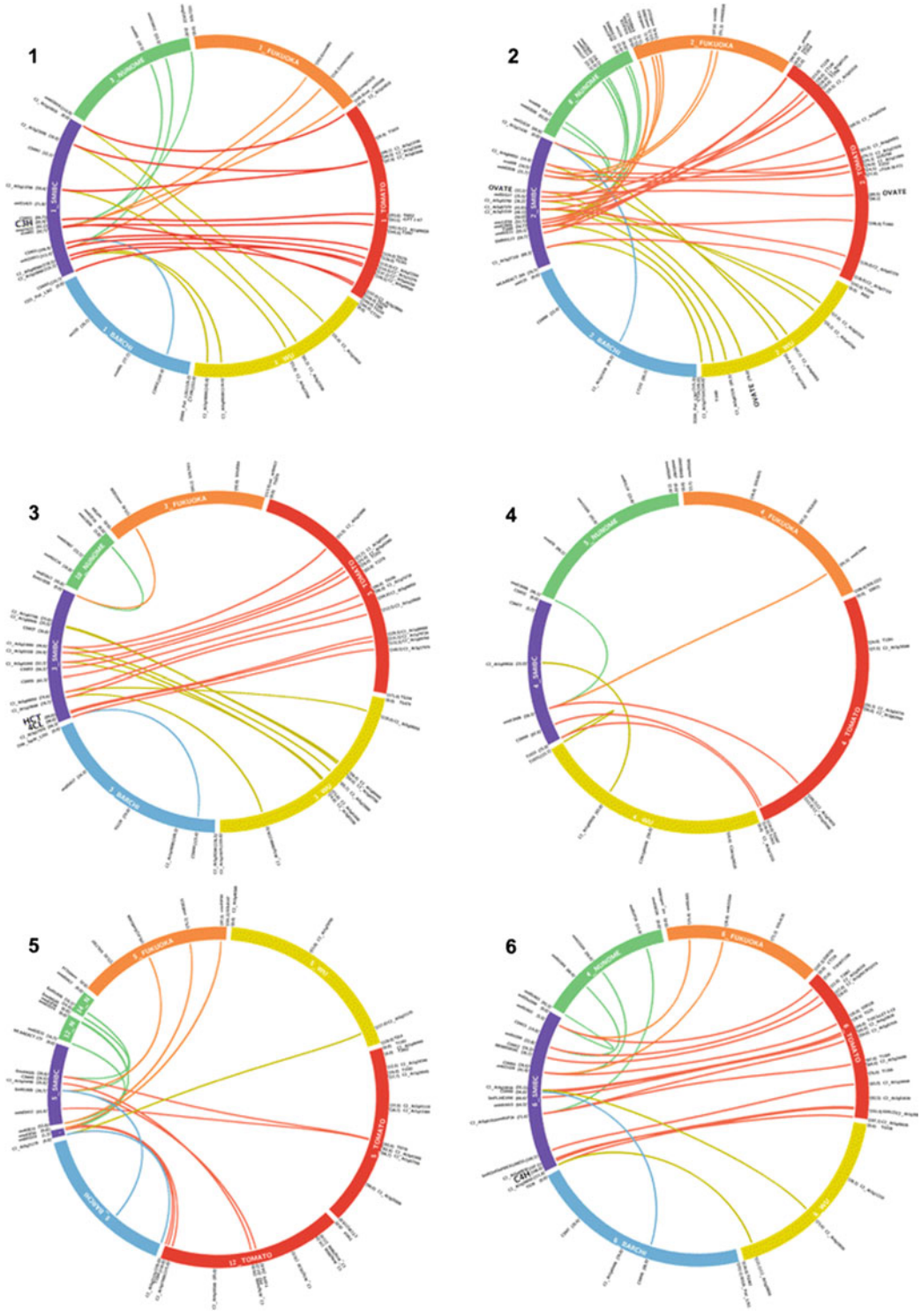

Fig. 12.4 Macro-syntenic relationships between five eggplant maps and tomato for linkage groups E1–E6. Each eggplant linkage map is *color-coded*: Gramazio et al. (2014) in *purple*, Barchi et al. (2012) in *blue*, Fukuoka et al. (2012) in *orange*, Nunome et al. (2009) in *green* and Wu et al. (2009a) in *yellow*. The tomato map (Fulton et al. 2002) is in *red*. Marker names and positions appear on the outside of the *circles* (used with permission from Gramazio et al. 2014)

105 kb CSS in solanaceous species. They found that gene orientation and position in the segment was very similar with only two differences between eggplant and tomato (Fig. 12.3). In the case of one gene, tomato had a reverse orientation which was not shared by any of the other species studied (potato, pepper, petunia, eggplant). In the other example, eggplant, potato, and petunia all contained a gene which was absent in tomato and pepper. Based on their examination of rates of evolution in the CSS, the authors hypothesized that 13.7 MY separate eggplant and tomato from their LCA which is similar to the value estimated by Wu and Tanksley (2010).

The recent release of a draft genome of eggplant that covers an estimated 74 % of the genome promises to reveal much more about chromosomal evolution in the Solanaceae (Hirakawa et al. 2014). Mapping nearly 10,000 eggplant sequence super-scaffolds over >98 % of the tomato genome revealed 56 conserved synteny blocks and 44 synteny break points between the genomes of the two species. Newly identified rearrangements included inversions on chromosomes 1 and 8. Given its higher resolution, it is not surprising that whole genome sequence analysis has enabled detection of a greater number of syntenic blocks and chromosome rearrangements than genetic linkage analysis (Doganlar et al. 2002, 2014). Refinement of the draft genome therefore promises to reveal much more about how the eggplant and tomato genomes have diverged.

Pepper

The genus *Capsicum* contains five domesticated species, *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum,* and *C. pubescens* (Heiser and Pickersgill 1969), collectively referred to as peppers. Of these, *C. annuum* is the most commonly cultivated and has the most characterized genome. In fact, some of the first comparative molecular mapping done in plants was performed between *C. annuum* and tomato (Tanksley et al. 1988). In this early work, an interspecific map (85 tomato RFLPs and isozyme loci) constructed from a cross between *C. annuum* and *C. chinense* indicated that at least 32 chromosome breaks distinguished the tomato and pepper genomes. Using a similar *C. annuum* × *C. chinense* population, Prince et al. (1993) mapped nearly 200 RFLP markers which showed that 32 % of marker order was conserved between the two species. The map also indicated that far fewer breaks, at least 15, could explain the differences between the pepper and tomato genomes. Livingstone et al. (1999) extended this map to include 352 markers that could be used for comparisons of synteny between the pepper and tomato genomes. With these markers, they identified 13 linkage groups and 18 homeologous segments which corresponded to 95 and 98 % of the pepper and tomato genomes, respectively. Four chromosome pairs were entirely syntenic between tomato and pepper: T2/P2, T6/P6, T7/P7, and T10/P10. The remaining linkage groups were substantially rearranged with at least 30 breaks required to explain the differences between the two genomes. Livingstone et al. (1999) proposed the occurrence of 5 translocations, 10 paracentric inversions, 2 pericentric inversions, and 4 other changes since divergence of tomato and pepper from their LCA.

Synteny between pepper and tomato was further examined by Wu et al. (2009b) who primarily used COSII markers (263 COSII, 36 RFLP markers). For the first time, the number of linkage groups (12) corresponded to pepper's base chromosome number. These linkage groups included 35 CSSs which covered 67 % of the pepper map and had an average length of 32 cM. Based on this work, at least six translocations, 19 inversions, and many single gene transpositions were required to explain the genomic differences between tomato and pepper. As in other comparisons, most of the rearrangements seemed to involve breakpoints at or near centromeres. Moreover, individual markers that moved among non-homologous chromosomes tended to be located near the centromere. Because pericentromeric regions of the tomato genome have been found to be rich in retrotransposons (Wang et al. 2006), this can be taken as evidence of

transposon activity. Comparison of the pepper and tomato maps with those of potato and eggplant allowed estimation of the timing of some rearrangements (Wu et al. 2009b). Thus it was found that inversions in the lower part of P11 occurred in the tomato/potato lineage while four other inversions most likely occurred in the tomato lineage after divergence of potato and tomato.

Further comparisons of the pepper COSII map with those of tomato, potato, eggplant, and tobacco allowed determination of the timing of other chromosomal rearrangements (Wu and Tanksley 2010). According to this work, only one inversion and three translocations are specific to the pepper lineage (Figs. 12.1 and 12.2). The divergence time between tomato and pepper was estimated as 19.6 MYA, allowing the rate of chromosomal rearrangements in the pepper lineage to be estimated as 0.1–0.6 inversions and 0.2–0.3 translocations per MY.

Use of interspecific populations for comparative mapping has also allowed rearrangements in the genomes of different *Capsicum* species to be identified. Namely, two translocations (one reciprocal, one nonreciprocal) and a duplication/deletion were found to distinguish *C. annuum* and *C. chinense* (Livingstone et al. 1999). In addition, the work of Wu et al. (2009b) proposed a model to explain the karyotypic differences between *C. annuum* and *C. chinense*/*C. frutescens*. Cultivated *C. annuum* has 2 acrocentric and 10 metacentric chromosomes while wild *C. annuum*, *C. chinense,* and *C. frutescens* have only 1 acrocentric and 11 metacentric chromosomes (Lanteri and Pickersgill 1993). According to Wu et al. (2009b) this difference can be explained by illegitimate recombination between ribosomal RNA (R45S) gene clusters on chromosomes 1 and 8 in the wild *C. annuum* genome resulting in a reciprocal translocation that altered chromosome arm length as seen in chromosomes I and XII in cultivated *C. annuum*.

Synteny of gene, rather than marker, location was examined by Grube et al. (2000) in their study of the genomic organization of disease resistance genes in tomato, pepper, and potato. They found that homologues of the tomato *N*, *Pto*, *Prf*, *Sw-5,* and *I2C* genes had syntenic positions in the pepper and tomato genomes. This work also showed that resistance genes clustered at homeologous positions in tomato, pepper, and potato on chromosomes T3, T4, T9, and T11. Resistance gene clusters on T1 and T7 were syntenic between tomato and pepper while a cluster on T8 had synteny between pepper and potato. (Tomato–potato synteny is discussed in the section on potato.)

Mazourek et al. (2009) focused on the orthologous disease resistance genes *Bs2* and *Rx/Gpa2* in pepper and potato, respectively. They demonstrated that the orthology between *Bs2* and the potato genes was disrupted by recombination, duplication, and deletion events, at least some of which involved retrotransposons. *Bs2* was found to map to chromosome P9 in a region syntenic to the top of potato chromosome 12 (XII) which contains *Rx* and *Gpa2*. This region is colinear in tomato (T12), pepper,and potato; however, this part of chromosome 12 in potato is inverted. Moreover, although *Rx* and *Gpa2* are tightly clustered in the potato genome, the resistance genes in the syntenic regions of the pepper and tomato genomes are more numerous and more dispersed. In fact, an examination of the entire pepper and tomato genomes indicates a close correspondence between the locations of R genes and chromosome breakpoints (Fig. 12.5). These results reinforce previous work which showed that chromosome breakpoints were associated with resistance gene duplication and dispersal in arabidopsis and *Medicago truncatula* (Baumgarten et al. 2003; Ameline-Torregrosa et al. 2008).

BAC-FISH analysis was also used to examine macrosynteny in pepper, tomato, and potato (Peters et al. 2012). FISH analysis on chromosomes 2L, 6S, 10L, and 11L revealed that the pepper arrangement differs by inversion from tomato but is colinear to potato on 2L, 6S, and 10L. In contrast, tomato and potato share an arrangement of 11L which is interrupted by an inverted translocation in pepper (Yang et al. 2009; Peters et al. 2012). These results disagree with those of Livingstone et al. (1999) who found complete colinearity between pepper and tomato

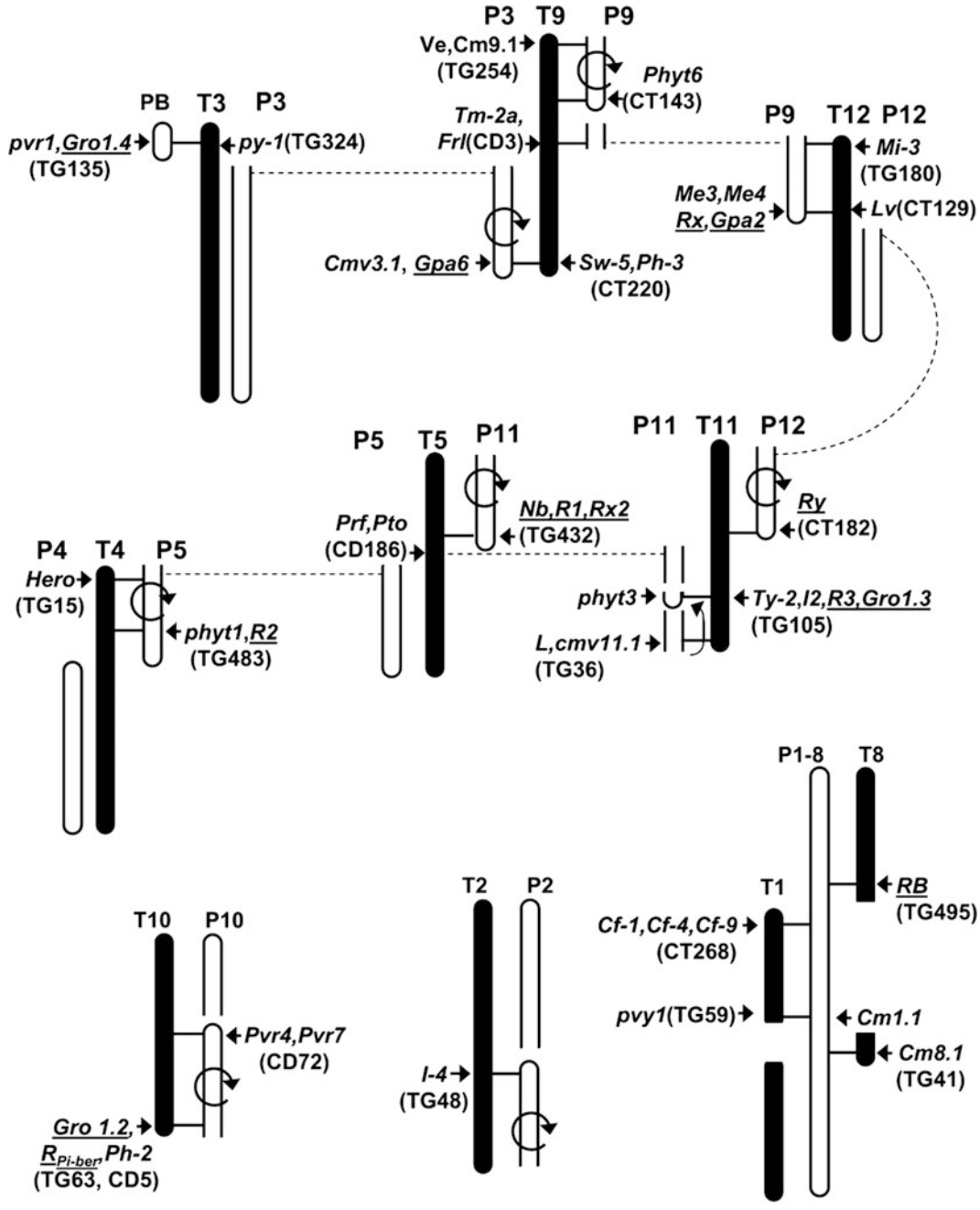

Fig. 12.5 Locations of selected resistance (R) genes in the tomato (T) and pepper (P) genomes. Potato genes are *underlined*. *Circular arrows* indicate putative inversions while *dotted lines* indicate translocations between chromosomes (used with permission from Mazourek et al. 2009)

chromosomes 2, 6, and 10. This discrepancy highlights the limitations of mapping for detailed analyses of synteny. While molecular mapping and FISH analyses allow examination of gross chromosomal rearrangements, other techniques are required to study microsynteny. One such

technique is sequencing. As previously mentioned, Wang et al. (2008) sequenced a 105 kb CSS in tomato, pepper, potato, eggplant, and petunia. They detected only two differences between pepper and tomato in gene arrangement in the region (Fig. 12.3). One gene had a reverse orientation in tomato as compared to all of the other species indicating that an inversion occurred along the tomato lineage. In addition, pepper had a duplication of one of the genes which was hypothesized to have occurred by tandem duplication. Based on their analyses, Wang et al. (2008) calculated that pepper and tomato diverged from their LCA approximately 19.1 MYA, which is nearly identical to the divergence time (19.6 MYA) calculated by Wu and Tanksley (2010).

Although gene order and repertoire are conserved in the Solanaceae, genome size is variable. The pepper genome contains fourfold more DNA than the tomato genome (Kim et al. 2014). To determine the cause of this difference, Park et al. (2011) compared nearly 36 Mb of euchromatic pepper DNA sequence with its orthologous region in tomato. They found that the number and identities of predicted genes in the genomic sequences were similar. Gene length differences were mainly due to longer introns in pepper (1815 bp on average as compared to 1459 bp in tomato). In addition, pepper contained many more transposons between genes. These were mostly *Ty3/Gypsy*-like LTR retrotransposons. FISH with one of these transposons, Tat, showed that this element is primarily located in heterochromatic regions of the tomato genome while it is dispersed in both euchromatin and heterochromatin in pepper. Thus, as found in other plant and animal species, transposable elements play a major role in genome size determination in pepper with a lesser, but still significant role played by intron size (reviewed by Gregory 2005).

A more comprehensive comparison of the pepper and tomato genomes was made possible by sequencing the pepper genome (Kim et al. 2014; Qin et al. 2014). Sequencing of hot pepper cultivar CM334 (Kim et al. 2014) and nonpungent cultivar Zunla-1 (Qin et al. 2014) provided nearly full coverage of pepper's 3.48 Gb genome. In both studies, predicted gene number was similar to that for tomato, approximately 35,000 protein-coding sequences with nearly 18,000 orthologous gene sets shared by the pepper and tomato genomes. Both genomes were found to have many large blocks syntenic with tomato. However, the pepper genome is fourfold larger due to the accumulation of repetitive sequences which make up 81 % of the genome (Qin et al. 2014). As in previous work (Park et al. 2011), these repetitive sequences were found to be mostly *Gypsy*-like LTR retrotransposons which are not seen to such an extent in tomato (Kim et al. 2014; Qin et al. 2014). Based on these results and an estimate of the timing of transposon activity, the authors hypothesized that the accumulation of transposable elements in the pepper genome was quite recent (0.3 Mya) (Qin et al. 2014) and that the concomitant alteration and increase in heterochromatin were involved in pepper speciation (Kim et al. 2014). Qin et al. (2014) also report that translocations were the main drivers of chromosomal rearrangement in the Solanaceae with 612 and 430 translocations differentiating pepper from the tomato and potato genomes, respectively. Extensive inversions also occurred with 468 and 367 inversion events distinguishing pepper from tomato and potato, respectively.

Kim et al. (2014) used the whole genome sequence and microsynteny to identify capsaicinoid pathway orthologs in the pepper, tomato, and potato genomes. The orthologs were found to be expressed during placenta development in pepper but in tomato or potato. Microsynteny was also used to analyze the region surrounding the capsaicin synthase gene in hot pepper and the corresponding area in tomato. The region was found to contain seven acyltransferase genes in pepper but only four in tomato. Phylogenetic analyses of these genes indicated that the capsaicin synthase gene emerged after speciation. More dramatic gene family expansion was observed for the *Bs2*-containing subclass of NBS-LRR (nucleotide-binding site-leucine-rich repeat) disease resistance genes. Hot pepper contains 82 such genes in its genome while tomato and potato have only three and one,

respectively. The expansion of this gene family has resulted in a loss of colinearity in the affected genomic regions of the three species. Thus, both retrotransposon amplification and gene family expansion were found to be significant factors in the divergence and speciation of hot pepper.

Nicotiana

Very few comparative genetic mapping studies have been published in *Nicotiana*, a genus of 66 species that includes the agricultural commodity and model organism for genetic engineering, cultivated tobacco (*N. tabacum*). While tobacco is an allotetraploid with a base chromosome number of 12, karyotypic variability is found within section *Alatae*. Chromosome numbers of nine and ten are found in several species, including *N. alata*, *N. bonariensis*, *N. forgetiana* and *N. langsdorffii* (x = 9) as well as *N. longiflora,* and *N. plumbaginifolia* (x = 10). While this variability in chromosome number makes *Nicotiana* an attractive system for chromosome evolution and synteny studies, the late development (within the last decade) of genetic maps in the genus means that such work remains to be done.

The first analysis of genome synteny in *Nicotiana* was made possible by the construction of a RFLP/RAPD linkage map for an interspecific (*N. plumbaginifolia* × *N. longiflora*) population (Lin et al. 2001). Only nine linkage groups were obtained, thus genome coverage was not complete. Nevertheless, comparison of linkage group assignments of 20 RFLP markers derived from *N. sylvestris* (Suen et al. 1997) revealed a lack of synteny between the mapped portions of the *plumbaginifolia* and *sylvestris* genomes. Given the difference in chromosome number between these species (x = 10 vs. x = 12 in *sylvestris*), this indication of chromosome disruption was not unexpected. Unfortunately, the positions of the markers relative to one another were unknown in *sylvestris*. Thus, colinearity between the two genomes could not be explored. However, based on evidence that several duplicate and triplicate loci in *sylvestris* were single copy in *plumbaginifolia*, the authors hypothesized that gene loss from multigene families may have contributed significantly to chromosome evolution and reduction in the *Nicotiana* genome.

While its large genome size (4500 Mbp; Arumuganathan and Earle 1991) and polyploid nature make *N. tabacum* less amenable to genomic research than other *Nicotiana* species, a concerted effort has been made to overcome these difficulties in recent years. A microsatellite map comprising 293 loci was published for a cross between the varieties 'Hicks Broadleaf' and 'Red Russian' (Bindler et al. 2007). The authors deemed the initial map incomplete due to the presence of large gaps and unlinked markers and subsequently published a high resolution map containing 2317 microsatellite markers (Bindler et al. 2011). The tenfold increase in marker number was accomplished by screening EST sequences generated by the Tobacco Genome Initiative (Gadani et al. 2003). As the authors suggest, this strategy of targeting single copy sequences for SSR marker development should facilitate the localization of homologous regions in other solanaceous genomes. However, explorations of tobacco-tomato synteny based on these markers have not yet been reported.

An allotetraploid that behaves as a diploid, tobacco is thought to have arisen as an interspecific hybrid between *N. sylvestris* and *N. tomentosiformis* (Kenton et al. 1993; Lim et al. 2004). Thanks to the availability of the aforementioned tobacco map (Bindler et al. 2011) as well as COSII/SSR maps (Wu et al. 2010) chromosome evolution within the two genomes of tobacco (the S- and T-genomes) is starting to be revealed. The mapping study conducted by Wu et al. (2010) compared the genomes of *N. tomentosiformis* and *N. acuminata* to each other and to that of *N. tabacum*. Extremely low polymorphism prevented mapping of *N. sylvestris*. *N. acuminata* was chosen as a substitute because it is evolutionarily closer to *N. sylvestris* than is *N. tomentosiformis*. Comparative analysis of COSII marker positions indicated that a minimum of seven chromosomal inversions and one reciprocal translocation distinguish the *tomentosiformis* (Tmf) and *acuminata* (Acn) genomes.

Chromosomes 6, 7, 9, and 11 show conservation of gene content and order in the diploid *Nicotiana* genomes. The timing of these structural changes relative to the polyploidization event leading to tobacco was determined by using a set of SSR markers from the Bindler et al. (2007) map. Mapping the SSR markers in the Tmf and Acn genomes also allowed the 24 tobacco linkage groups to be assigned to their respective ancestral genomes (T-genome for those markers that mapped to Tmf and S-genome for those in Acn). Since the divergence of the Tmf and Acn genomes, four inversions occurred in the lineage leading to *tomensiformis*, with the majority (3 of 4) pre-dating the tetraploidization event leading to tobacco. Of the two inversions specific to the Acn genome, one occurred before its split from *sylvestris* and one after. Based on these changes, it was estimated that the rate of chromosome evolution in *N. tomentosiformis* has varied from 0.5 to 2.1 rearrangements/MY before tetraploidization to 0.6 rearrangements/MY after that event. Similarly, a slower rate of evolution is evident in *N. acuminata* since its divergence from *N. sylvestris*: 0.4–1.3 rearrangements/MY before to 0.2 rearrangements/MY after divergence.

Comparison of SSR marker positions between the diploid *Nicotiana* species and tobacco revealed a minimum of 12 rearrangements: 9 inversions and single occurrences of chromosome breakage, fusion, and reciprocal translocation (Wu et al. 2010). The inversions were split almost equally between the T- and S- genomes (4 and 5 events, respectively). Since the speciation event, which the authors estimated as occurring less than one MY ago, the sub-genomes of tobacco are evolving at a faster rate than their diploid relatives: six changes in the T-genome as compared to just one in the Tmf genome. Estimated chromosomal evolution rates provide another measure of accelerated evolution following interspecific hybridization and polyploidization: 3.5 rearrangements/MY in the T-genome (as compared to 0.6 in *N. tomentosiformis*) and as many as 1.2 rearrangements/MY in the S-genome (vs. 0.2 in the Acn genome). Bindler et al. (2011) also found evidence for chromosomal rearrangement in tobacco, so much so that they were unable to identify homeologous chromosomes: more than 90 % of their SSR markers were specific to just one of the ancestral genomes. Nevertheless, the fact that markers specific to *N. sylvestris* and *N. tomentosiformis* mapped to the same linkage group in tobacco was interpreted as evidence of translocation between homeologous chromosomes. Thus the findings of Wu et al. (2010) and Bindler et al. (2011) provide valuable insight into how plant genomes reorganize after polyploidization.

Wu et al. (2010) also used COSII marker position to investigate the synteny of the *Nicotiana* and tomato genomes. Colinearity of markers was observed in 25 CSSs. With an average size of 15 cM, these CSSs spanned 34 % of the Tmf map. Outside of these regions of conservation, at least 11 reciprocal translocations and three (and perhaps as many as 10) inversions have occurred since the divergence of tomato and the LCA of the *Nicotiana* species. The relative frequency of each type of structural change was a bit of a surprise; previous analyses in the Solanaceae had prepared the authors to expect a greater number of inversions relative to translocations. Given the length of evolutionary time separating tomato and *Nicotiana* (some 27.7 MYA as calculated in this study), a number of inversions may have been obscured by subsequent changes in chromosome structure. In addition, they also noted that comparing tomato to the extant species *N. tomentosiformis* shows a slightly different picture: 14 inversions and 11 translocations, supporting the hypothesis that inversion is the predominant mode of rearrangement. As with the analysis of the ancestral genomes of tobacco, numerous instances of single marker transpositions were found between tomato and the *Nicotiana* species (36 in Tmf and 13 in Acn), suggesting that this is another important mechanism contributing to loss of synteny as genomes diverge.

Rapid progress has been made in sequencing *Nicotiana* genomes as evidenced by the recent publication of draft genome sequences of the diploid species *N. sylvestris* and

N. tomentosiformis (Sierro et al. 2013) as well as the allotetraploid species *N. benthamiana* (Bombarely et al. 2012) and *N. tabacum* (Sierro et al. 2014). These sequences should provide considerable insight into synteny within the genus and the family, however such analyses remain in their infancy. Thus, comparison of the diploid species genomes with those of other solanceous species was limited to localizing COSII markers from the *N. acuminata* and *N. tomentosiformis* genetic maps on the genome assemblies of *N. sylvestris* and *N. tomentosiformis*, respectively (Sierro et al. 2013). Only one-third of COSII markers could be mapped, highlighting the fragmented and incomplete nature of these assemblies. The lack of a genetic map for *N. benthamiana* has restricted comparative genomic studies with this species despite its popularity as a model system for plant–pathogen interations. However analysis of the draft *N. benthamiana* genome sequence revealed microsynteny with tomato in the *Pto-Prf* gene cluster, suggesting that this region evolved before the divergence of *Nicotiana* and *Solanum* (Bombarely et al. 2012).

The sequencing of three varieties of commercial tobacco (*N. tabacum*) has provided the greatest insight into genome evolution within the genus (Sierro et al. 2014). Comparisons with the genetic maps generated by Wu et al. (2009b) and Bindler et al. (2011) show the extent of chromosomal rearrangements that have occurred within the diploid and allotetraploid species. Moreover, use of the sequence data provided by the *sylvestris* and *tomentosiformis* draft genomes (Sierro et al. 2013) helped verify these species as the putative ancestors of *N. tabacum* and revealed that only 4–8 % of the ancestral genomes was lost subsequent to the hybridization event that created *N. tabacum* (Sierro et al. 2014). Mapping protein sequences of tomato and potato onto the tobacco genome sequence revealed considerable genome reorganization, however syntenic regions do exist and gene content is strongly conserved across the ~ 30 MY of evolution separating these solanaceous species (Fig. 12.6).

Petunia

Analyses of synteny between the horticultural plant *Petunia* and tomato have been hampered by the fact that genome mapping efforts in petunia have been limited. Until recently, the most comprehensive genetic linkage map for petunia contained just 36 RFLP markers spread across the plant's seven chromosomes (Strommer et al. 2000). For this reason, early reports of synteny between petunia and tomato arose from research focused on specific genomic regions.

It is not surprising that the first report of synteny between the petunia and tomato genomes originated from an analysis of the self-incompatibility (SI) locus. Members of the Solanaceae, including petunia, have served as model organisms for the analysis of gametophytic SI for several decades. While mapping the self-incompatibility locus (*S* locus), ten Hoopen et al. (1998) established a syntenic relationship between petunia chromosome III and chromosome 1 of tomato and potato. The position of the *S* locus was initially determined through T-DNA tagging and further substantiated by the cosegregation of an *S*-linked potato RFLP marker (CP100) with a peroxidase isozyme locus (*PrxA*) that had been previously mapped to chromosome III. Citing similar linkage between the *S*-locus and a peroxidase isozyme in *Nicotiana alata* (Labroche et al. 1983), the authors suggested that synteny of the self-incompatibility locus may be conserved in the Solanaceae (ten Hoopen et al. 1998).

In contrast, a region of the *Petunia* genome in which five floral traits (color, UV absorption, scent, and pistil and stamen length) are tightly linked appears not to be conserved in the family (Hermann et al. 2013). BLAST searches revealed that homologs of ten *Petunia* markers spanning this pollination syndrome gene cluster were widely distributed in the tomato and potato genomes, mapping to six chromosomes in tomato and five in potato. The authors speculate that this difference between *Petunia* and *Solanum* species may reflect different evolutionary pressures on these genes in the two genera. *Solanum*

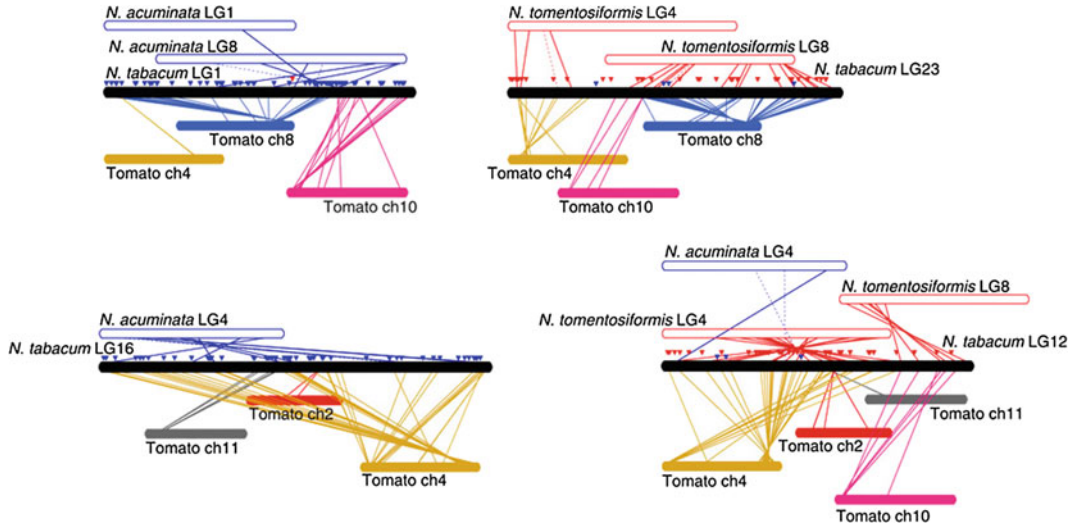

Fig. 12.6 Synteny between *Nicotiana* species and tomato for selected tobacco linkage groups. The comparison of *N. tabacum* and tomato is based on mapping tomato proteins. Links between tobacco and *N. acuminata* and *N. tomentosiformis* are derived from shared SSR (*solid lines*) and COSII (*dotted lines*) markers (used with permission from Sierro et al. 2014)

species tend to be bee-pollinated, whereas *Petunia* species exhibit frequent pollinator changes which could be facilitated by tight linkage of the genes underlying pollinator attraction.

On a broader scale, the conservation of a 17-gene region in the Solanaceae was revealed in sequence analysis of a 105 kb CSS in five species including petunia (Wang et al. 2008). While gene order and orientation were largely maintained, a small number of petunia-specific evolutionary changes were identified including a relatively recent tandem duplication of gene 12 and a 20 kb inversion involving genes 15 and 16 (Fig. 12.3). Comparison of gene structure revealed that ORFs and exon/intron positions in four out of seven genes were conserved across lineages. The degree of conservation of gene content was somewhat surprising given that homologous regions of the Arabidopsis genome have evolved at a considerably faster rate (Ku et al. 2000). However, the authors attributed this difference to the fact that no whole genome duplication events have occurred within the solanaceous lineage over the $\sim$ 30 million years since the divergence of petunia and tomato.

The recent construction of linkage maps for wild *Petunia* species (Bossolini et al. 2011) has made it possible to compare synteny within *Petunia* as well as between petunia and tomato on a genome-wide basis. Such comparative mapping studies provide valuable insight into patterns of chromosomal evolution throughout the Solanaceae. A question of particular interest which the work of Bossolini et al. (2011) begins to answer is how chromosome number in the family was reduced from an ancestral value of x = 12 to x = 7 in the lineage leading to petunia. Bossolini et al. (2011) mapped a total of 207 CAPs and AFLP markers in two interspecific populations: *P. exserta* × *P. parodii* and *P. axillaris* × *P. inflata*. Thirty-seven shared markers revealed complete preservation of marker order between the two petunia maps. In order to compare the petunia and tomato genomes, BLASTN searches were used to position the petunia marker sequences on the physical map of tomato. A large amount of rearrangement was uncovered, with the degree of macrosynteny varying depending on the chromosome. Petunia chromosomes 5 and 7 were found to be syntenic with tomato chromosomes T12 and T8, respectively. Petunia chromosomes 1 and 6 were composite in nature. Petunia chromosome 1 has segments specific to T5 and T6 while chromosome 6 carries markers shared with T1 and

T9. While portions of chromosomes 3 and 4 are syntenic with T3 and T4, the synteny is limited to a segment of the long arms of the tomato chromosomes. An even more complex pattern is seen in the make up of chromosome 2 as it comprises markers found on T2, T7, T8, and T10. Thus, as compared to the localized synteny revealed by ten Hoopen et al. (1998) and Wang et al. (2008), the genomes of petunia and tomato show evidence of extensive structural differentiation when viewed on a larger scale. This loss of synteny makes it difficult to use the abundant genomic resources of tomato to assist petunia genetics but is indicative of the complex patterns of evolutionary change that occur during genome evolution.

Conclusions

The Solanaceae has been the subject of pioneering work in studies of genome synteny. By investigating the differences in genome size, content, and organization, this work has provided insight into the ways in which the structural rearrangement of chromosomes can lead to reproductive isolation and, ultimately, speciation. Moreover, it has helped to extend the utility of the extensive genomic resources of tomato to other members of this economically important family of plants.

Both DNA content and chromosome number vary in the Solanaceae. It has been hypothesized that the primary mechanisms responsible for genome size variation in plants and animals are transposable element replication, polyploidy, intron size, gene, and chromosome loss (Gregory 2005). Species in the Solanaceae provide examples of each of these mechanisms. There is ample evidence of transposable element, especially retrotransposon, activity in the genomes of tomato (Asamizu et al. 2012), wild tomato (Xiao et al. 2008), potato (Peters et al. 2012), eggplant (Wu et al. 2009a) and pepper (Mazourek et al. 2009; Park et al. 2011). For example, the pepper genome is three times larger than that of tomato and large scale (35.6 Mb) sequence comparisons indicated that many LTR retrotransposons (primarily Tat and Athila) have been inserted in the pepper genome while gene order and content are conserved across the two species (Park et al. 2011). Transposable element activity is also evident in the displacement of single markers/genes in genomes that are otherwise syntenic as observed in potato (Perez et al. 1999), eggplant (Wu et al. 2009a; Doganlar et al. 2014), pepper (Mazourek et al. 2009; Wu et al. 2009b), and tobacco (Wu et al. 2010). Examination of repetitive DNA at rearrangement junctions in potato indicates that transposition has played an important role in larger breaks from synteny including translocation and inversion (Zhu et al. 2008; Peters et al. 2012). In addition, transposable element activity was found to be responsible for a duplication resulting in the *sun* locus phenotype in tomato (Xiao et al. 2008). Polyploidy is observed in both the potato and tobacco genomes. Studies of synteny in these species provide insight into how genomes are rearranged after polyploidization (Bindler et al. 2011; Wu et al. 2010). Intron size has not yet been examined extensively in the Solanaceae, however, preliminary work in pepper indicates that increased gene size in pepper is mainly due to the presence of longer introns as compared to tomato (Park et al. 2011). Gene and chromosome loss are observed in some species of tobacco which have fewer members within multigene families and only ten chromosomes (Suen et al. 1997; Lin et al. 2001). Chromosome loss is also apparent in the petunia lineage as this species has only seven chromosomes (Bossolini et al. 2011).

While genome size is affected by a number of factors, only a few mechanisms are responsible for restructuring chromosomes: inversions, translocations, and transpositions (discussed in the previous paragraph). In the Solanaceae, paracentric inversions are usually reported as the most common type of rearrangement in potato, pepper, nightshade, and eggplant (Tanksley et al. 1992; Livingstone et al. 1999; Chetelat et al. 2000; Doganlar et al. 2002). A definite pericentric inversion was reported for *S. etuberosum* (Lou et al. 2010) and others have been hypothesized for pepper (Livingstone et al. 1999; Wu et al. 2009a, b). The preponderance of paracentric as compared to pericentric inversions supports the hypothesis that these inversions have fewer harmful effects

on fertility than pericentric inversions (Burnham 1962), and therefore, may be less detrimental to organism fitness. Similarly, translocations may be more likely than inversions to interfere with chromosome pairing and are usually less frequent than inversions in the Solanaceae (Wu and Tanksley 2010). Interestingly, during the evolution of the Solanaceae, a few recurrent chromosomal breakpoints seem to have been primarily responsible for genome restructuring (Wu and Tanksley 2010). These breakpoints are commonly found in pericentromeric regions (Wu and Tanksley 2010), areas of the tomato genome that are known to be rich in retrotransposons (Wang et al. 2006) and other repetitive sequences (Presting et al. 1996). Thus, these observations in Solanaceae are in accordance with the general finding that rearrangements involving repetitive DNA are important in plant speciation (Raskina et al. 2008).

Examination of synteny among tomato, potato, eggplant, pepper, and tobacco using COSII markers has allowed rates of rearrangement and divergence times along these lineages to be calculated (Fig. 12.2). Such estimates allow comparison of rates of evolution within the family, as well as with other plant families. Thus it has been hypothesized that the eggplant lineage has undergone the more frequent rearrangements than the tomato, potato or pepper lineages (Wu and Tanksley 2010). The same work also indicates that genomes in the Solanaceae are evolving at similar rates as genomes in the Poaceae (the true grasses, including all the major cereals), Malvaceae (the mallows, including cotton and cacao) and Brassicaceae (the crucifers including rapeseed and cabbage) (Wu and Tanksley 2010).

In addition to impacting our understanding of the modes and tempo of plant genome evolution, synteny studies in solanaceous species have had several practical applications. Discovery of shared synteny in the family has allowed the use of RFLP and COSII markers originally developed for tomato (Bernatzky and Tanksley 1986; Wu et al. 2006) in related species (Tanksley et al. 1992; Livingstone et al. 1999; Doganlar et al. 2002; Albrecht and Chetelat 2009; Wu et al. 2009a, b, 2010; Doganlar et al. 2014). This has been a tremendous advantage for studies of the less important solanaceous species such as eggplant and nightshade. Shared synteny also enables in silico mapping of markers (Wu et al. 2009a, b) and facilitates gene cloning. For example, late blight resistance genes in both potato (Huang et al. 2005) and wild potato (Pel et al. 2009) were isolated with the help of comparative genomic analyses made possible by conserved genome organization in these species. In addition, knowledge of syntenic relationships between donor and recipient genomes can be extremely useful when attempting to minimize linkage drag while introgressing traits (Peters et al. 2012). Thus, synteny studies in the Solanaceae have provided fruitful results in both basic and applied plant genetics and will continue to do so as genome sequence data become more readily available.

Acknowledgments We are grateful to The Scientific and Technological Research Council of Turkey (Project No. 104T224) for support of our work in eggplant.

References

Abrouk M, Murat F, Pont C, Messing J, Jackson S, Faraut T, Tannier E, Plomion C, Cooke R, Feuillet C, Salse J (2010) Paleogenomics of plants: synteny-based modelling of extinct ancestors. Trends Plant Sci 15:479–487

Albrecht E, Chetelat RT (2009) Comparative genetic linkage map of *Solanum* sect. Juglandifolia: evidence of chromosomal rearrangements and overall synteny with the tomatoes and related nightshades. Theor Appl Genet 118:831–847

Ameline-Torregrosa C, Wang B-B, O'Bleness MS, Deshpande S, Zhu H, Roe B, Young ND, Cannon SB (2008) Identification and characterization of nucleotide-binding site-leucine-rich repeat genes in the model plant *Medicago truncatula*. Plant Physiol 146:5–21

Andolfo G, Sanseverino W, Rombauts S, Van de Peer Y, Bradeen JM, Carputo D, Frusciante L, Ercolano MR (2013) Overview of tomato (*Solanum lycopersicum*) candidate pathogen recognition genes reveals important *Solanum* R locus dynamics. New Phytol 197:223–237

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Asamizu E, Shirasawa K, Hirakawa H, Sato S, Tabata S, Yano K, Ariizumi T, Shibata D, Ezura H (2012) Mapping of Micro-Tom BAC-end sequences to the reference tomato genome reveals possible genome rearrangements and polymorphisms. Int J Plant Genomics. doi:10.1155/2012/437026

Bai Y, Lindhout P (2007) Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? Ann Bot 100:1085–1094

Barchi L, Lanteri S, Portis E, Val G, Volante A, Pulcini L, Ciriaci T, Acciarri N, Barbierato V, Toppino L (2012) A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. PLoS ONE 7:e43740

Baumgarten A, Cannon S, Spangler R, May G (2003) Genome-level evolution of resistance genes in *Arabidopsis thaliana*. Genetics 165:309–319

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bindler G, van der Hoeven R, Gunduz I, Plieske J, Ganal M, Rossi L, Gadani F, Donini P (2007) A microsatellite marker based linkage map of tobacco. Theor Appl Genet 114:341–349

Bindler G, Plieske J, Bakaher N, Gunduz I, Ivanov N, van der Hoeven R, Ganal M, Donini P (2011) A high density genetic map of tobacco (*Nicotiana tabacum* L.) obtained from large scale microsatellite marker development. Theor Appl Genet 123:219–230

Bombarely A, Rosli HG, Vrebaliv J, Moffett P, Mueller LA, Martin GB (2012) A draft genome sequence of *Nicotiana benthamiana* to enhance molecular plant-microbe biology research. Mol Plant Microbe Interact 25:1523–1530

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Bonnema G, Schipper D, van Heusden S, Zabel P, Lindhout P (1997) Tomato chromosome 1: high-resolution genetic and physical mapping of the short arm in an interspecific *Lycopersicon esculentum* × *L. peruvianum* cross. Mol Gen Genet 253:455–462

Bossolini E, Klahre U, Brandenburg A, Reinhardt D, Kuhlemeier C (2011) High resolution linkage maps of the model organism *Petunia* reveal substantial synteny decay with the related genome of tomato. Genome 54:327–340

Burnham CR (1962) Discussions in cytogenetics. Burgess, Minneapolis

Cericola F, Portis E, Lanteri S, Toppino L, Barchi L, Acciarri N, Pulcini L, Sala T, Rotino GL (2014) Linkage disequilibrium and genome-wide association analysis for anthocyanin pigmentation and friut color in eggplant. BMC Genom 15:896–911

Chetelat RT, Meglic V (2000) Molecular mapping of chromosomes segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*). Theor Appl Genet 100:232–241

Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on BC1 *Lycopersicon esculentum* × *Solanum lycopersicoides* reveals overall synteny but supressed recombination between these homeologous genomes. Genetics 154:857–867

Contreras-M A, Spooner DM (1999) Revision of *Solanum* section *Etuberosum* (subgenus *Potatoe*). In: Nee M, Symon DE, Lester RN, Jessop JP (eds) Solanaceae IV. Royal Botanic Gardens, Kew, pp 227–245

D'Agostino N, Golas T, van der Geest H, Bombarely A, Dawood T, Zethof J, Driedonks N, Wijnker E, Bargsten J, Nap J-P, Mariani C, Rieu I (2013) Genomic analysis of the native European *Solanum* species, *S. dulcamara*. BMC Genom 15:356–370

Doganlar S, Frary A, Daunay M-C, Lester RN, Tanksley SD (2002) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711

Doganlar S, Frary A, Daunay M-C, Huvenaars K, Mank R, Frary A (2014) High resolution map of eggplant (*Solanum melongena*) reveals extensive chromosome rearrangement in domesticated members of the Solanaceae. Euphytica 198:231–241

Fukuoka H, Miyatake K, Nunome T, Negoro S, Shirasawa K, Isobe S, Asamizu E, Yamaguchi H, Ohyama A (2012) Development of gene-based markers and construction of an integrated linkage map in eggplant by using *Solanum* orthologous (SOL) gene sets. Theor Appl Genet 125:47–56

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Gadani F, Hayes A, Opperman CH, Lommel SA, Sosinski BR, Burke M, Hi L, Brierly R, Salstead A, Heer J, Fuelner G, Lakey N (2003) Large scale genome sequencing and analysis of *Nicotiana tabacum*: the tobacco genome initiative. In: Proceedings, 5èmes Journées Scientifiques du Tabac de Bergerac—5th Bergerac Tobacco Scientific Meeting, Bergerac, pp 117–130

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufman H, Thompson RD, Bonierbale MW, Ganal MW, Tanskley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Gramazio P, Prohens J, Plazas M, Andjar I, Herraiz FJ, Castillo E, Knapp S, Meyer RS, Vilanova S (2014) Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. BMC Plant Biol 14:350–365

Gregory TR (2005) The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. Ann Rev Bot 95:133–146

Grube RC, Radwanski ER, Jahn M (2000) Comparative genetics of disease resistance within the Solanaceae. Genetics 155:873–887

Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1–13

Heiser CB, Pickersgill B (1969) Names for the cultivated *Capsicum* species (Solanaceae). Taxon 18:277–283

Hermann K, Klahre U, Moser M, Sheehan H, Mandel T, Kuhlemeier C (2013) Tight genetic linkage of prezygotic barrier loci creates a multifunctional speciation island in *Petunia*. Curr Biol 23:873–877

Hirakawa H, Shirasawa K, Miyatake K, Nunome T, Negoro S, Ohyama A, Yamaguchi H, Sato S, Isobe S, Tabata S, Fukuoka H (2014) Draft genome sequence of eggplant (*Solanum melongena* L.): the representative *Solanum* species indigenous to the Old World. DNA Res doi:10.1093/dnares/dsu027

Huang S, Vleeshouwers VGAA, Werij JS, Hutten RC, van Eck HJ, Visser RGF, Jacobsen E (2004) The *R3* resistance to *Phytophthora infestans* in potato is conferred by two closely linked R genes with distinct specificities. Mol Plant Microbe Interact 17:428–435

Huang S, van der Vossen EAG, Kuang H, Vleeshouwers VGAA, Zhang N, Borm TJA, van Eck HJ, Baker B, Jacobsen E, Visser RGF (2005) Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. Plant J 42:251–261

Iorizzo M, Gao L, Mann H, Traini A, Chiusano ML, Kilian A, Aversano R, Carputo D, Bradeen JM (2014) A DArT marker-based linkage map for wild potato *Solanum bulbocastanum* facilitates structural comparisons between *Solanum* A and B genomes. BMC Genet 15:123–132

Iovene M, Wielgus SM, Simon PW, Buell CR, Jiang J (2008) Chromatin structure and physical mapping of chromosome 6 of potato and comparative analyses with tomato. Genetics 180:1307–1317

Kamenetzky L, Asis R, Bassi S. de Godoy F, Bermudez L, Fernie AR, Van Sluys MA, Vrebalov J, Giovannoni JJ, Rossi M, Carrari F (2010) Genomic analysis of wild tomato introgressions determining metabolism- and yield-associated traits. Plant Physiol 152:1772–1786

Kenton A, Parokonny AS, Gleba YY, Bennett MD (1993) Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Mol Gen Genet 240:159–169

Kim S, Park M, Yeom S-I, Kim Y-M, Lee JM et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nat Genet 46:270–279

Ku H-K, Vision T, Liu J, Tanksley SD (2000) Comparing sequenced segments of the tomato and Arabidopsis genomes: large-scale duplication followed by selective gene loss creates a network of synteny. Proc Natl Acad Sci USA 97:9121–9126

Kuhl JC, Hanneman RE, Havey MJ (2001) Characterization and mapping of *Rpi1*, a light-blight resistance locus from diploid (1EBN) Mexican *Solanum pennatisectum*. Mol Genet Genomics 265:977–985

Labroche P, Poirier-Hamon S, Pernes J (1983) Inheritance of leaf peroxidase isozymes in *Nicotiana alata* and linkage with the *S*-incompatibility locus. Theor Appl Genet 65:163–170

Lanteri S, Pickersgill B (1993) Chromosomal structural changes in *Capsicum annuum* L. and *C. chinense* Jacq. Euphytica 67:155–160

Lim KY, Matyasek R, Kovarik A, Leitch AR (2004) Genome evolution in allotetraploid *Nicotiana*. Biol J Linn Soc 82:599–606

Lin TY, Kao YY, Lin S, Lin RF, Chen CM, Huang CH, Wang CK, Lin YZ, Chen CC (2001) A genetic linkage map of *Nicotiana plumbaginifolia/Nicotiana longiflora* based on RFLP and RAPD markers. Theor Appl Genet 103:905–911

Lindqvist-Kreuze H, Cho K, Portal L, Rodriguez F, Simon R, Mueller LA, Spooner DM, Bonierbale M (2013) Linking the potato genome to the conserved ortholog set (COS) markers. BMC Genom 14:51–63

Lippman ZB, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev 17:545–552

Livingstone KD, Lackney VK, Blauth JR, van Wijk R, Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. Genetics 152:1183–1202

Lou Q, Iovene M, Spooner DM, Buell CR, Jiang J (2010) Evolution of chromosome 6 of *Solanum* species revealed by comparative fluorescence in situ hybridization mapping. Chromosoma 119:435–442

Mazourek M, Cirulli ET, Collier SM, Landry LG, Kang B-C, Quirin EA, Bradeen JM, Moffett P, Jahn MM (2009) The fractionated orthology of *Bs2* and *Rx/Gpa2* supports shared synteny of disease resistance in the Solanaceae. Genetics 182:1351–1364

McCouch (2001) Genomics and synteny. Plant Physiol 125:152–155

Nadeau JH, Taylor BA (1984) Lengths of chromosomal segments conserved since divergence of man and mouse. Proc Natl Acad Sci USA 81:814–818

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119:1143–1153

Park M, Jo SH, Kwon J-K, Park J, Ahn JH, Kim S, Lee Y-H, Yang T-J, Hur C-G, Kang B-C, Kim B-D, Choi D (2011) Comparative analysis of pepper and tomato reveals euchromatin expansion of pepper genome caused by differential accumulation of *Ty3/Gypsy*-like elements. BMC Genom 12:85

Pel MA, Foster SJ, Park T-H, Rietman H, van Arkel G, Jones JDG, Van Eck HJ, Jacobsen E, Visser RGF, Van der Vossen EAG (2009) Mapping and cloning of

late blight rsistance genes from *Solanum venturii* using an interspecific candidate gene approach. Mol Plant Microb Interact 22:601–615

Peralta I, Spooner DM (2001) Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum L.* section *Lycopersicon* (Mill.) Wettst. subsection *Lycopersicon*). Am J Bot 88:1888–1902

Perez F, Menendez A, Dehal P, Quiros CF (1999) Genomic structural differentiation in *Solanum*: comparative mapping of the A and E genomes. Theor Appl Genet 98:1183–1193

Pertuze RA, Ji Y, Chetelat RT (2002) Comparative linkage map of the *Solanum lycopersicoides* and *S. sitiens* genomes and their differentiation from tomato. Genome 45:1003–1012

Peters SA, Bargsten JW, Szinay D, van de Belt J, Visser RGF, Bai Y, de Jong H (2012) Structural homology in the Solanaceae: analysis of genomic regions in support of synteny studies in tomato, potato and pepper. Plant J 71:602–614

Portis E, Barchi L, Toppino L, Lanteri S, Acciarri N, Felicioni N, Fusari F, Barbierato V, Cericola F, Vale G, Rotino GL (2014) QTL mapping in eggplant reveals clusters of yield-related loci and orthology with the tomato genome. PLoS ONE 9:e89499

Presting G, Frary A, Pillen K, Tanksley SD (1996) Telomere-homologous sequences occur near the centromeres of many tomato chromosomes. Mol Gen Genet 251:526–531

Prince JP, Pochard E, Tanksley SD (1993) Construction of a molecular linkage map of pepper and comparison of synteny with tomato. Genome 36:404–417

Qin C, Yu C, Shen Y, Fang X et al (2014) Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. Proc Natl Acad Sci 111:5135–5140

Ramanna M, Hermsen J (1979) Unique meiotic behavior in F1 plants from a cross between non-tuberous and tuberous *Solanum* species in section *Petota*. Euphytica 28:9–15

Raskina O, Barber JC, Nevo E, Belyayev A (2008) Repetitive DNA and chromosomal rearrangement: speciation-related events in plant genomes. Cytogenet Genome Res 120:351–357

Rick CM (1979) Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the Solanaceae. Academic Press, New York, pp 667–678

Seah S, Yaghoobi J, Rossi M, Gleason CA, Williamson VM (2004) The nematode-resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistance tomato. Theor Appl Genet 108:1635–1642

Seah S, Telleen AC, Williamson VM (2007) Introgressed and endogenous *Mi-1* gene clusters in tomato differ by complex rearrangements in flanking sequences and show sequence exchange and diversifying selection among homologues. Theor Appl Genet 114:1289–1302

Sierro N, Battey JND, Ouadi S, Bovet L, Goepfert S, Bakaher N, Peitsch MC, Ivanov NV (2013) Reference genomes and transcriptomes of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. Genome Biol 14:R60

Sierro N, Battey JND, Ouadi S, Bakaher N, Bovet L, Willig A, Goepfert S, Peitsch MC, Ivanov NV (2014) The tobacco genome sequence and its comparison with those of tomato and potato. Nat Commun 5:3833

Spooner D, Anderson G, Jansen R (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes and pepinos (Solanaceae). Am J Bot 80:676–688

Strommer J, Gerats AGM, Sanago M, Molnar SJ (2000) A gene-based RFLP map of *Petunia*. Theor Appl Genet 100:899–905

Suen DF, Wang CK, Lin RF, Kao YY, Lee FM, Chen CC (1997) Assignment of DNA markers to *Nicotiana sylvestris* chromosomes using monosomic alien addition lines. Theor Appl Genet 94:331–337

Szinay D, Wijnker E, van den Berg R, Visser RGF, de Jong H, Bai Y (2012) Chromosome evolution in *Solanum* traced by cross-species BAC-FISH. New Phytol 195:688–698

Tang X, Szinay D, Lang C, Ramanna MS, ven der Vossen EAG, Datema E, Lankhorst RK, de Boer J, Peters SA, Bachem C, Stiekema W, Visser RGF, de Jong J, Bai Y (2008) Cross-species bacterial artificial chromosome-fluorescence in situ hybridization painting of the tomato and potato chromosome 6 reveals undescribed chromosomal rearrangements. Genetics 180:1319–1328

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu R, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

ten Hoopen R, Harbord RM, Maes T, Nanninga N, Robbins TP (1998) The self-incompatibility (*S*) locus in *Petunia hybrida* is located on chromosome III in a region syntenic for the Solanaceae. Plant J 16:729–734

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Traini A, Iorizzo M, Mann H, Bradeen JM, Carputo D, Frusciante L, Chiusano ML (2013) Genome microscale heterogeneity among wild potatoes revealed by diversity arrays technology marker sequences. Int J of Genomics 2013:257218

van der Knaap E, Sanyal A, Jackson SA, Tanksley SD (2004) High-resolution fine mapping and fluorescence *in situ* hybridization analysis of *sun*, a locus

controlling tomato fruit shape, reveals a region of the tomato genome prone to DNA rearrangements. Genetics 168:2127–2140

van Heusden AW, Koornneef M, Voorrips RE, Bruggemann W, Pet G, Vrielink-van Ginkel R, Chen X, Lindhout P (1999) Three QTLs from *Lycopersicon peruvianum* confer a high level of resistance to *Clavibacter michiganensis* ssp. *michiganensis*. Theor Appl Genet 99:1068–1074

van Wordragen MF, Weide R, Liharska T, Vandersteen A, Koornneef M, Zabel P (1994) Genetic and molecular organization of the short arm and pericentromeric region of tomato chromosome 6. Euphytica 79:169–174

Wang J, Hu H, Zhao T, Yang Y, Chen T, Yang M, Yu W, Zhang B (2015) Genome-wide analysis of bHLH transcription factor and involvement in the infection by yellow leaf curl virus in tomato (*Solanum lycopersicum*). BMC Genom 16:39–53

Wang Y, Tang X, Cheng Z, Mueller L, Giovannoni J, Tanksley SD (2006) Euchromatin and pericentromeric heterochromatin: comparative composition in the tomato genome. Genetics 172:2529–2540

Wang Y, Diehl A, Wu F, Vrebalov J, Giovannoni J, Siepel A, Tanksley SD (2008) Sequencing and comparative analysis of a conserved syntenic segment in the Solanaceae. Genetics 180:391–408

Watanabe K, Orrillo M, Vega S, Valkonen J, Pehu E, Hurtado A, Tanksley S (1995) Overcoming crossing barriers between non-tuber-bearing and tuber-bearing Solanum species: towards potato genome enhancement with a broad spectrum of solanaceous genetic resources. Genome 38:27–35

Wu F, Eannetta NT, Xu Y, Tanksley SD (2009a) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor Appl Genet 118:927–935

Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009b) 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*. Theor Appl Genet 118:1279–1293

Wu F, Eannetta NT, Xu Y, Plieske J, Ganal M, Pozzi C, Bakaher N, Tanksley SD (2010) COSII genetic maps of two diploid *Nicotiana* species provide a detailed picture of synteny with tomato and insights into chromosome evolution in tetraploid *N. tabacum*. Theor Appl Genet 120:809–827

Wu F, Mueller LA, Crouzillat D, Petiard V, Tanksley SD (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics 174:1407–1420

Wu F, Tanksley SD (2010) Chromosomal evolution in the plant family Solanaceae. BMC Genom 11:182–193

Xiao H, Jiang N, Schaffner E, Stockinger EJ, van der Knaap (2008) A retrotransposon mediated gene duplication underlies morphological variation of tomato fruit. Science 319:1527–1530

Yang H-B, Liu WY, Kang W-H, Jahn M, Kang B-C (2009) Development of SNP markers linked to the *L* locus in *Capsicum* spp. By comparative genetic analysis. Mol Breed 24:433–446

Zhu W, Ouyang S, Iovene M, O'Brien K, Vuong H, Jiang J, Buell CR (2008) Analysis of 90 Mb of the potato genome reveals conservation of gene structure and order with tomato but divergence in repetitive sequence composition. BMC Genom 9:286–300