A Comparative Genetic Linkage Map of Eggplant (*Solanum melongena*) and Its Implications for Genome Evolution in the Solanaceae

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Manuscript received November 9, 2001
Accepted for publication May 23, 2002

ABSTRACT

A molecular genetic linkage map based on tomato cDNA, genomic DNA, and EST markers was constructed for eggplant, *Solanum melongena*. The map consists of 12 linkage groups, spans 1480 cM, and contains 233 markers. Comparison of the eggplant and tomato maps revealed conservation of large tracts of colinear markers, a common feature of genome evolution in the Solanaceae and other plant families. Overall, eggplant and tomato were differentiated by 28 rearrangements, which could be explained by 23 paracentric inversions and five translocations during evolution from the species' last common ancestor. No pericentric inversions were detected. Thus, it appears that paracentric inversion has been the primary mechanism for chromosome evolution in the Solanaceae. Comparison of relative distributions of the types of rearrangements that distinguish pairs of solanaceous species also indicates that the frequency of different chromosomal structural changes was not constant over evolutionary time. On the basis of the number of chromosomal disruptions and an approximate divergence time for Solanum, ~0.19 rearrangements per chromosome per million years occurred during the evolution of eggplant and tomato from their last ancestor. This result suggests that genomes in Solanaceae, or at least in Solanum, are evolving at a moderate pace compared to other plant species.

THE Solanaceae is a very large plant family containing 2300 species, nearly one-half of which belong to a single genus, Solanum (D'Arcy 1991). Most species within Solanum are endemic to the Americas; however, ~20% are Old World species. The common name eggplant encompasses three closely related cultivated species that belong to Solanum subgenus Leptostemonum: *Solanum melongena* L., brinjal eggplant or aubergine; *S. aethiopicum* L., scarlet eggplant; and *S. macrocarpon* L., gboma eggplant (Daunay *et al.* 2001b). Although most Leptostemonum species are of New World origin (Daunay *et al.* 1999), all three eggplant species as well as their wild relatives are endemic to the Old World (Lester 1998). Phylogenetic classification of species in Solanum using chloroplast DNA restriction site variation reveals that, within Leptostemonum, the Old World and Australian species form a monophyletic clade (Olmstead and Palmer 1997). *S. aethiopicum* and *S. macrocarpon* were domesticated in Africa from their wild relatives, *S. anguivi* and *S. dasyphyllum*, respectively (Lester 1998). The cultivation of these two species for their fruits and leaves is still primarily limited to Africa (Daunay *et al.* 2001b). The precise origin of the brinjal eggplant, *S. melongena,* is uncertain; however, it may have been indirectly derived from the wild African species *S. incanum* (Daunay *et al.* 2001a) and was domesticated in India and southeast China. Cultivation of the crop for its fruit gradually spread to the Mediterranean during the Arab conquests of the area starting in the seventh century. Today, brinjal eggplant (hereafter referred to as simply eggplant) is cultivated throughout the world (Daunay *et al.* 2001b).

Based on production statistics, eggplant is the third most important crop in the Solanaceae, after potato and tomato (FAO 2000). Although considered somewhat of an exotic ingredient in the United States, eggplant is a major and inexpensive component of many people's daily diet in the developing world, especially in China and India where it is considered the "king of vegetables." This is also reflected in the fact that 21 million metric tons (t) of eggplant were produced in 2000 in developing countries while only 1.3 million t were produced in developed countries. More than 90% of the world's eggplant is produced in Asia with China growing 54% of the supply (12 million t). Other important producers are India (6 million t) and Turkey (850,000 t). In contrast, the European Union and United States grow a mere 2.7 and 0.35%, respectively, of the world's eggplant crop. In addition to its nutritional value, which is

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similar to other common vegetables (Aubert 1971), eggplant is believed to have numerous beneficial medicinal qualities (Khan 1979), including antioxidant properties (Cao *et al.* 1996) and the ability to reduce serum cholesterol levels (Kayamori and Igarashi 1994).

Like tomato and pepper, eggplant is an autogamous diploid with 12 chromosomes ($2n = 24$). The eggplant nuclear genome is slightly larger than that of tomato and contains 1100 Mb of DNA (2.4 pg/2C; Arumuganathan and Earle 1991). Despite eggplant's similarities to the other major solanaceous crops, there is a dearth of molecular genetic information for the species as compared to tomato, potato, and pepper. These crops have all been the subjects of extensive molecular genetic analyses and high density genetic linkage maps have been constructed for all three species (Tanksley *et al.* 1992; Livingstone *et al.* 1999). A molecular genetic linkage map for *S. melongena* is essential for the identification, localization, marker-assisted selection, and isolation of qualitative and quantitative traits in the crop. Moreover, a map will be a valuable addition to the biotechnological tools currently available for eggplant improvement: somaclonal variation, somatic hybridization, haploid production, and genetic transformation (reviewed in Collonnier *et al.* 2001). The combination of these techniques and the molecular linkage map will facilitate eggplant breeding and advance our understanding of eggplant genetics.

Comparative genome analysis is a well-developed area of study in the Solanaceae and rivals work done in the cereals (reviewed in Gale and Devos 1998) and Cruciferae (Kowalski *et al.* 1994; Lagercrantz 1998). Early work showed that the tomato and potato genomes differ by only five paracentric inversions (*i.e.*, inversions that did not involve the centromere; Tanksley *et al.* 1992) but that the tomato and pepper genomes differ by numerous rearrangements (Tanksley *et al.* 1988; Prince *et al.* 1993). A more recent comparison of tomato and pepper suggests that the differences between their two genomes can be accounted for by 22 rearrangements including several translocations as well as both pericentric (*i.e.*, inversions that involved the centromere) and paracentric inversions (Livingstone *et al.* 1999). The goal of the present research was to develop a restriction fragment length polymorphism (RFLP) linkage map for eggplant using primarily single-copy tomato cDNA, genomic DNA, and expressed sequence tag (EST) probes. The use of these clones allowed comparisons of chromosomal organization among the four major crops of the Solanaceae and provided insight into genome evolution in this family. The map also allowed comparative mapping of qualitative and quantitative traits among tomato, potato, pepper, and eggplant as described in the accompanying article (Doganlar *et al.* 2002, this issue). It is hoped that the availability of an RFLP map for eggplant will encourage the use of the wealth of genetic knowledge developed for the other solanaceous crops to benefit our understanding of this species.

MATERIALS AND METHODS

Plant material: A population of 58 F_2 plants derived from an interspecific cross made by M. C. Daunay at the Institut National de la Recherche Agronomique, France, between *S. linnaeanum* Jaegaer & Hepper MM195 and *S. melongena* L. MM738 was used for mapping. *S. linnaeanum,* the female parent, is a spiny, wild relative of *S. melongena* and bears small, green, striped, round fruit. MM738 is a nonspiny commercial-type eggplant line that bears large, purple, unstriped, oblong fruit. An interspecific cross was selected for mapping as preliminary work indicated that DNA polymorphism in a *S. melongena* intraspecific population was inadequate. The population was grown in the greenhouse in Ithaca, New York.

RFLP analysis: Procedures for DNA extraction, restriction enzyme digestion, and Southern blotting were as described for tomato by Bernatzky and Tanksley (1986). In most cases, DNA from the two parents was surveyed for polymorphism using eight enzymes: *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Sca*I, *Xba*I, *Hae*III, and *Bst*NI. In some cases, markers that were not polymorphic for any of these enzymes were also surveyed with *Taq*I, *Bcl*I, *Dpn*II, and *Hin*cII. Single-copy tomato cDNA, genomic DNA (Tanksley *et al.* 1992), and tomato conserved orthologous set (COS; Fulton *et al.* 2002) RFLP markers were surveyed. The COS markers are EST clones that show homology with Arabidopsis ESTs. Polymorphic markers were labeled by primer extension (Feinberg and Vogelstein 1983) and probed on DNA from the F_2 population digested with the appropriate restriction enzyme. Hybridization and washing to a stringency of 0.5× SSC were performed at 65°.

Genetic map construction: The Mapmaker v. 2.0 computer program (Lander *et al.* 1987) was used to construct the linkage map. The group and order commands were used to establish linkage groups and linear orders for the markers. The ripple command was used to protect against map order errors by confirming marker order at LOD $\geq$ 3.0. These markers comprised the framework map. Additional markers were placed in the intervals between framework markers, using the try command with a LOD $\geq$ 2.0 threshold. These markers are shown in parentheses. All genetic distances were computed using the Kosambi mapping function (Kosambi 1944).

RESULTS AND DISCUSSION

Marker analysis: A total of 413 tomato cDNA, genomic DNA, and COS RFLP markers were selected to provide complete genome coverage using the tomato map as a guide (Tanksley *et al.* 1992; Fulton *et al.* 2002). To facilitate comparative mapping and prevent the confounding of orthologous and paralogous markers, primarily single-copy markers were chosen. The markers were assayed for polymorphism on DNA from the two parental lines: *S. melongena* and *S. linnaeanum.* Overall, 81% of the markers revealed DNA polymorphisms between the parents. There were no significant differences for percentage of polymorphism for the different types of markers surveyed (cDNA *vs.* genomic DNA *vs.* EST; data not shown).

Most of the markers segregated with the 1:2:1 Mendelian ratio expected for an F_2 population; however, 16%

(33/207) of the framework markers deviated significantly ($P \leq 0.01$) from that ratio. Although many of these skewed markers were dispersed throughout the genome, four clusters containing three or more markers with distorted segregation were identified. These clusters were located on the upper ends of eggplant linkage group 2 (E2; R45S to CT244) and E7 (CT52 to cLED21J7) and in the middle portions of E3 (TG564 to CP116A) and E11 (CT97 to GP180). Of these four regions, one was distorted in favor of *S. linnaeanum* homozygotes (E2), two were skewed toward *S. melongena* homozygotes (E3 and E7), and one displayed an excess of heterozygotes (E11). Unequal segregation of marker loci is a common feature of interspecific plant populations and may be attributed to structural differences or loci that affect gamete transmission in the region with distorted segregation (Zamir and Tadmor 1986).

Genetic map construction: A total of 334 polymorphic markers were genotyped for the interspecific F_2 population. Of these markers, 233 (70%) met the minimum requirement for linkage at LOD $\geq$ 2.0 and were used for map construction. The resulting map is composed of 12 linkage groups covering 1480 cM (Figure 1). Because the basic chromosome number in eggplant is 12, it is likely that these 12 linkage groups correspond to eggplant's 12 chromosomes. Of the 233 markers mapped, 207 (89%) were considered to be framework markers as they were positioned with a LOD $\geq$ 3.0. The remaining 26 markers mapped to the intervals between framework markers at 2.0 $\leq$ LOD $<$ 3.0. Linkage groups ranged in size from 99 cM (E2) to 163 cM (E1) and contained between 12 (E12) and 21 (E1) framework markers. Distances between framework markers varied from 0 to 24 cM with 75% of the markers $<$12 cM apart. The 207 framework markers provide an average marker density of 1 marker every 7.6 $\pm$ 0.4 cM. Pronounced clustering of markers, which occurred in tomato (Tanksley *et al.* 1992) and pepper (Livingstone *et al.* 1999), was not evident in eggplant most likely because the mapped markers were not randomly selected but were expressly chosen for full genome coverage using the tomato map as an indicator of possible genomic location.

The use of common markers for the tomato and eggplant maps allowed a comparison of meiotic recombination frequency between specific pairs of markers in the two species. Although recombination frequencies between specific pairs of markers were generally higher in eggplant than in tomato, a paired *t*-test indicated that this difference was not statistically significant ($P = 0.06$). Regression analysis indicated a significant ($P < 0.0001$) positive correlation ($r = 0.51$) between recombination frequencies for specific pairs of markers in eggplant and tomato.

Comparative mapping in the Solanaceae: Because the eggplant map was constructed primarily with single-copy tomato RFLP markers, it was possible to identify homeologous regions of the two genomes and to determine the sizes of segments conserved during the evolution of eggplant and tomato from a common ancestor. Conserved segments were defined by colinearity of the markers within a given region containing at least two contiguous markers. Markers that did not map to the framework in either eggplant or tomato (*i.e.*, those in parentheses on the maps) were excluded from this analysis. In all, 36 conserved segments were identified that encompassed the entire eggplant and tomato genomes. These segments ranged in size from 3 to 163 cM with an average size of 34 $\pm$ 6 cM. Although most (72%) of the conserved segments were 40 cM or less in length, in two cases, the marker orders of entire chromosomes were conserved. Linkage groups 1 and 8 of eggplant were completely colinear to chromosomes 1 and 8, respectively, of tomato (Figure 1). Linkage groups 2, 3, 10, and 12 showed the most breaks from colinearity. E2, E10, and E12 each consisted of 5 conserved segments while E3 showed six breaks from colinearity with tomato. Colinearity of large tracts of contiguous markers appears to be a feature of genome evolution in the Solanaceae as it was also observed in comparisons of potato (Tanksley *et al.* 1992) and pepper (Livingstone *et al.* 1999) with tomato. Conservation of colinear linkage blocks has also been seen in the genomes of the Poaceae (reviewed in Devos and Gale 2000), the Fabaceae (Weeden *et al.* 1992), and the Brassicaceae (Kowalski *et al.* 1994).

Comparison of the eggplant linkage map with that of tomato (Figure 1) reveals the number and types of rearrangements that occurred during the evolution of these two species from a common ancestor. Because extensive comparative mapping has also been done between tomato and potato (Bonierbale *et al.* 1988; Gebhardt *et al.* 1991; Tanksley *et al.* 1992) and tomato and pepper (Tanksley *et al.* 1988; Prince *et al.* 1993; Livingstone *et al.* 1999), it is possible to include these species in a consideration of the chromosomal rearrangements that differentiate these four crops. By comparing the chromosomal organization of each species with reference to the phylogenetic relationships among the species as determined by Olmstead and Palmer (1997) (Figure 2), it is also possible to hypothesize about the genome arrangement of the most recent ancestor of tomato, potato, eggplant, and pepper. The following discussion is based upon the tomato and potato linkage maps of Tanksley *et al.* (1992) and the pepper map of Livingstone *et al.* (1999).

E1: Linkage group 1 of eggplant is homeologous to chromosome 1 of tomato and potato and all three species have retained a conserved marker order. Pepper also has a conserved order for these markers; however, they are linked to a segment of the genome that is homeologous to tomato chromosome 8. Therefore, a translocation differentiates pepper from the other three species. Because the homeologs of chromosome 1 in tomato, potato, and eggplant are identical while only

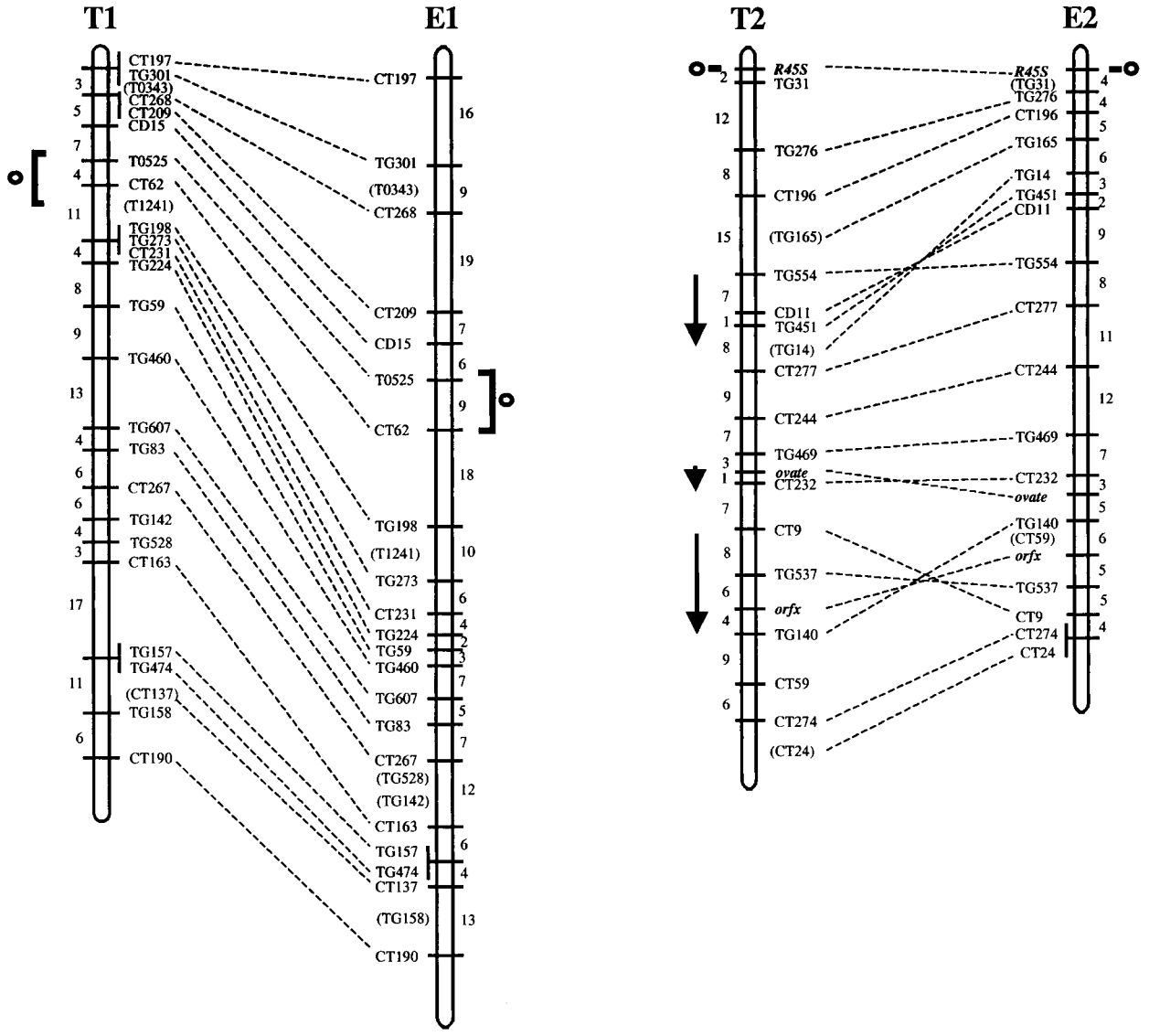


FIGURE 1.—Molecular linkage map of the eggplant genome and comparison with the homeologous regions of the tomato genome. Eggplant linkage groups are designated E1–E12, tomato chromosomes are designated T1–T12. Chromosome arms are labeled S (short arm) and L (long arm). Markers by tick marks on the eggplant map are framework markers and have been ordered at LOD ≥ 3. Cosegregating markers are denoted by vertical solid bars next to tick marks. Markers enclosed in parentheses have been located to the intervals between framework markers at $2 < \text{LOD} < 3$ as described in MATERIALS AND METHODS. Map distances are in centimorgans. Approximate positions of centromeres are indicated by solid bars and circles and, for eggplant, were inferred from locations in tomato. Dashed lines link each eggplant framework marker to its tomato counterpart. Arrows adjacent to tomato chromosomes indicate the locations of inversions that distinguish the eggplant and tomato genomes.

pepper is different, the ancestral condition of this chromosome may be represented by either the eggplant/tomato/potato or the pepper arrangement.

E2: E2 shows conservation of marker content with the homeologous tomato, potato, and pepper chromosomes but marker order is somewhat rearranged with respect to the order found on tomato/potato chromosome 2. This rearrangement of markers can be explained by three paracentric inversions. Interestingly, pepper also provides evidence of an inversion in the region surrounding *fw2.2.* These results suggest that this inversion may represent the ancestral state for this portion of chromosome 2. The ancestral arrangement of the rest of the chromosome cannot be deduced.

E3: The marker content and order of much of E3 is the same as that seen for tomato and potato chromosome 3; however, four markers on E3 are found on tomato/potato chromosome 5. This indicates that a translocation has occurred after the divergence of these species from a common ancestor. A translocation also occurred in the pepper lineage such that pepper 3 is composed of segments that are homeologous to chromosome 3 and chromosome arm 9L (long) of tomato. The breakpoint of the putative translocations in the

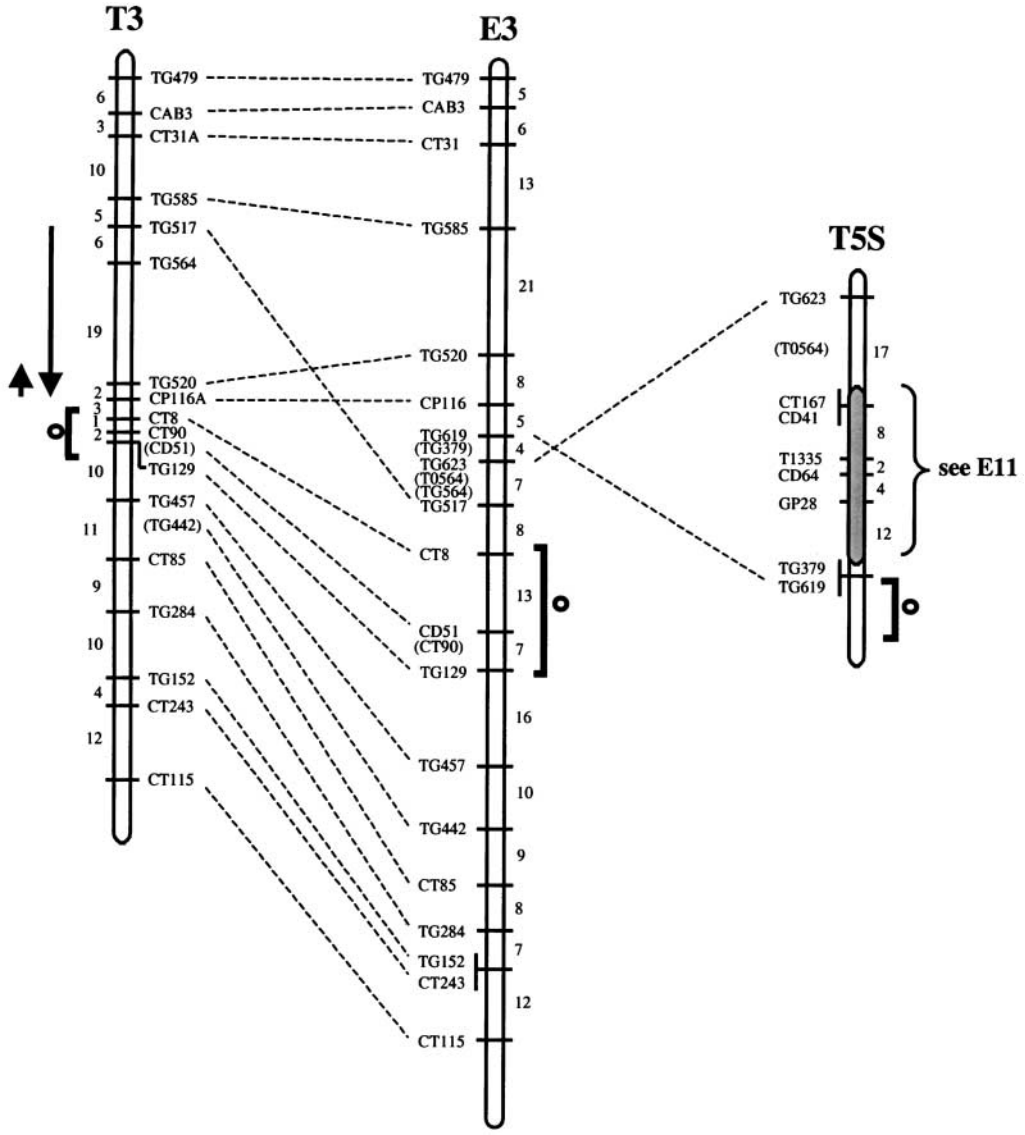
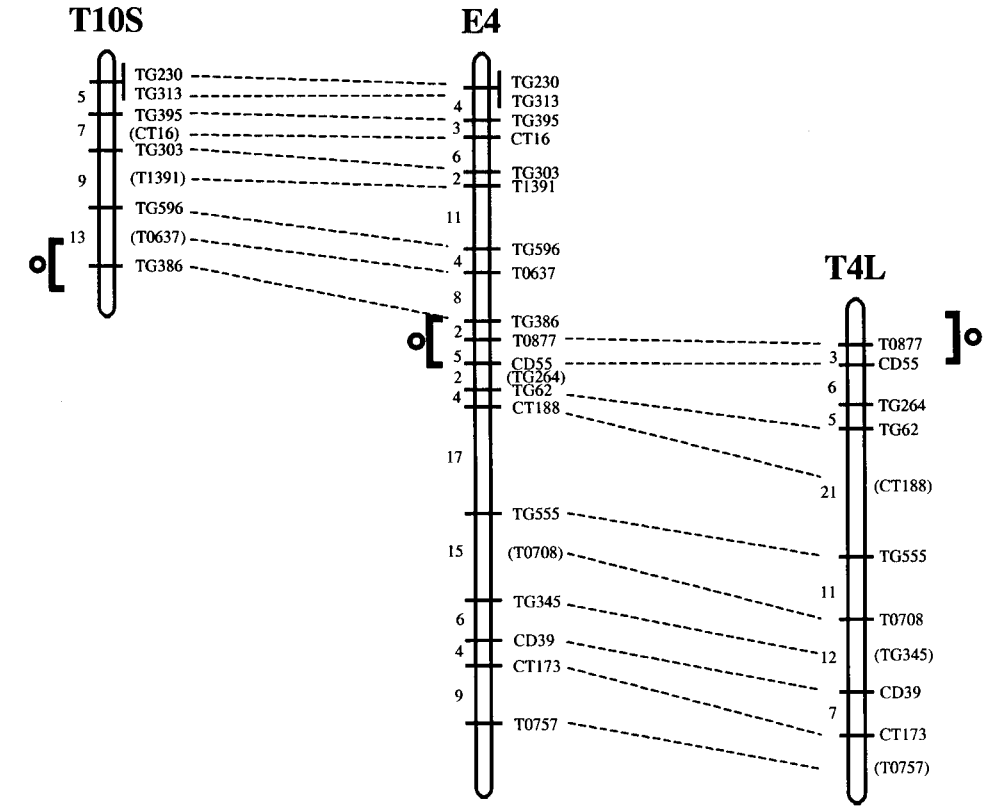


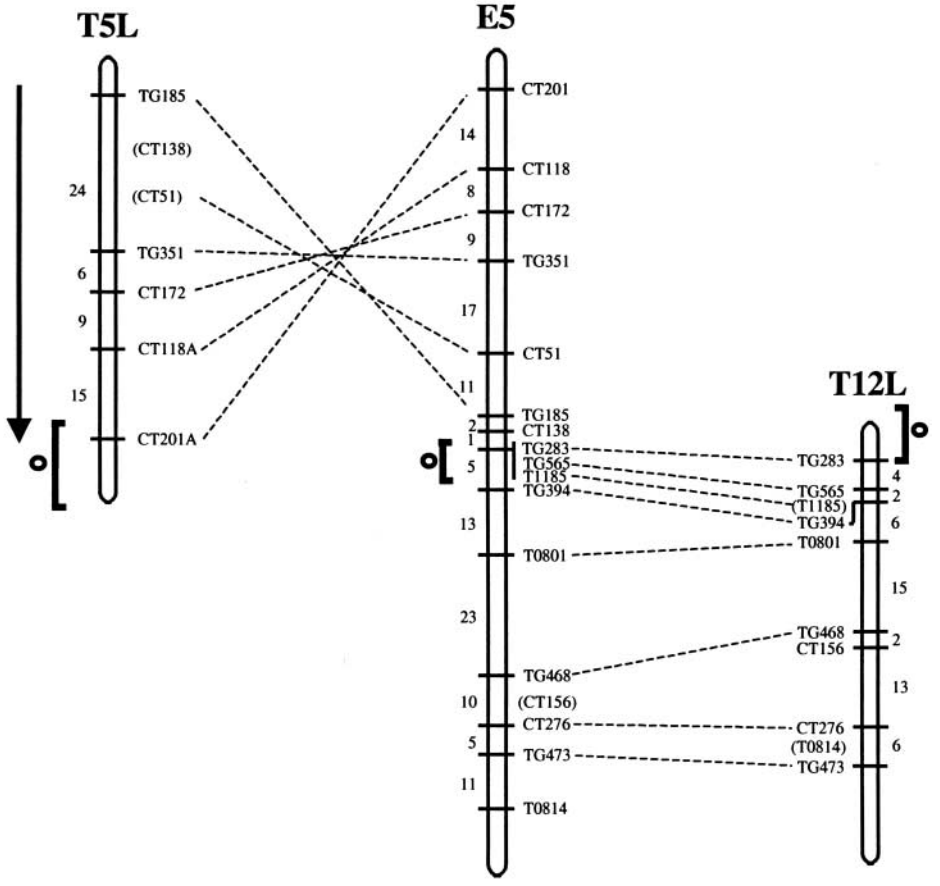
FIGURE 1.—*Continued.*

eggplant and pepper lineages is approximately the same and both species show evidence of multiple inversions in the same region (*i.e.*, the translocation breakpoint). These observations suggest that this was a particularly unstable region of the genome during evolution of the Solanaceae. Overall, the differences between E3 and tomato chromosome 3 can be explained by one translocation and three paracentric inversions (two in the lineage of E3 and one prior to the translocation). Because eggplant and pepper differ from each other and from tomato and potato by several translocations and inversions, it is not possible to determine their ancestor's chromosome 3 composition.

E4: Eggplant linkage group 4 corresponds to homeologous segments of tomato chromosome arms 10S (short) and 4L, thereby indicating that a translocation has occurred during the evolution of these species. Additional evidence for translocation between these chromosome arms is found in the pepper genome. In pepper, the markers associated with tomato 4L comprise their own linkage group while those associated with 4S are linked to a region homeologous to tomato 5L. Despite the differences in the four genomes, the marker order for E4 and its tomato, potato, and pepper counterparts is colinear. Thus, the differences between E4 and the corresponding chromosomal regions in tomato can be accounted for by a single translocation event. Whether the translocation occurred in the tomato/potato or eggplant lineage cannot be determined from the available data. Thus, it is not possible to deduce the chromosome arrangement of their putative ancestor.

E5: The arrangement of E5 indicates that a translocation also occurred during the evolution of this linkage group. This translocation involved portions of the genome homeologous to tomato chromosome arms 5L and 12L. The instability of linkages involving these chromosome arms is also seen in the pepper genome where one linkage group is composed of markers from tomato





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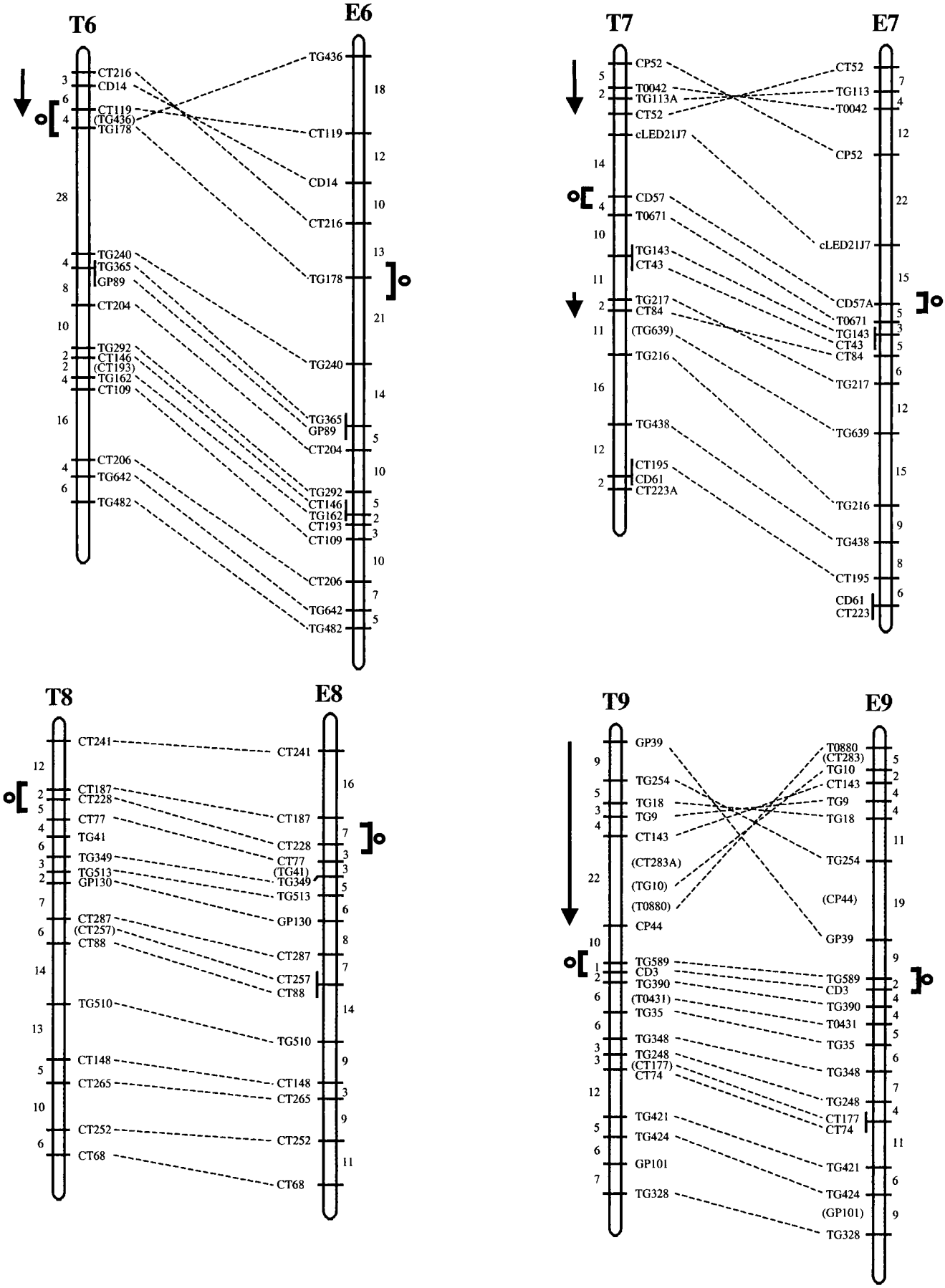

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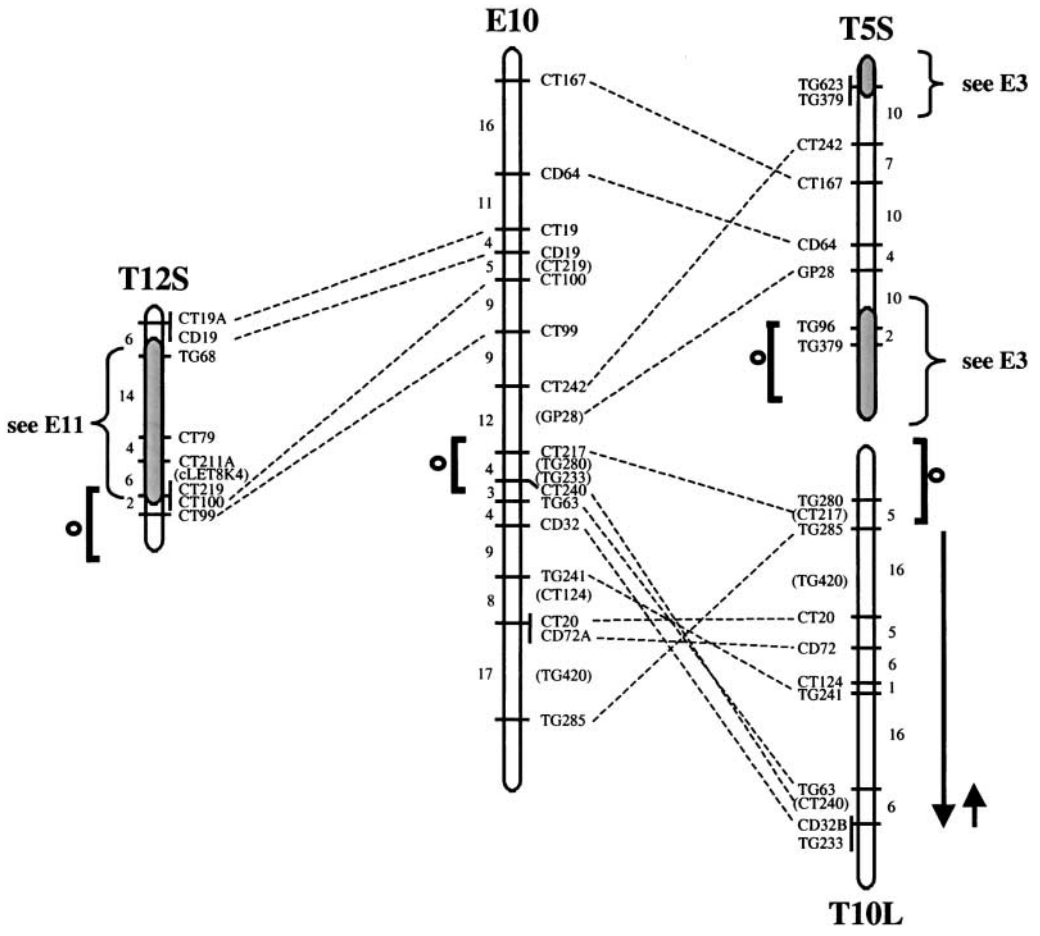

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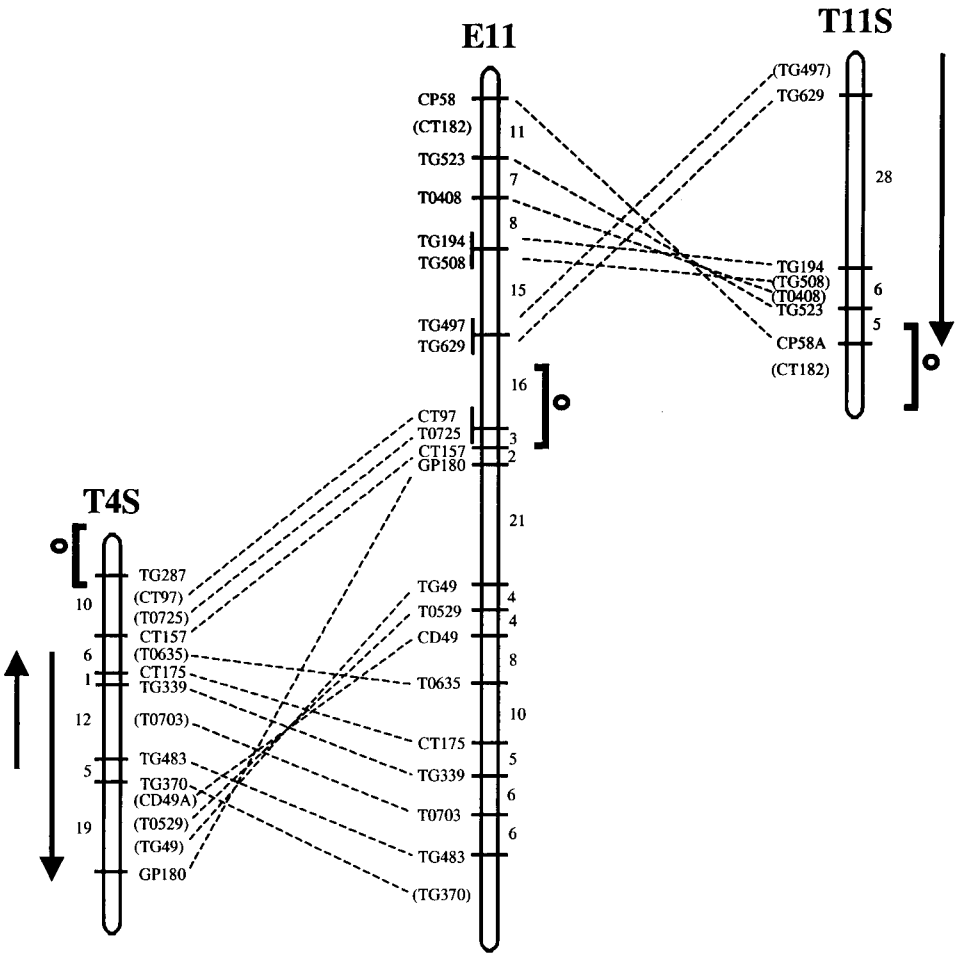

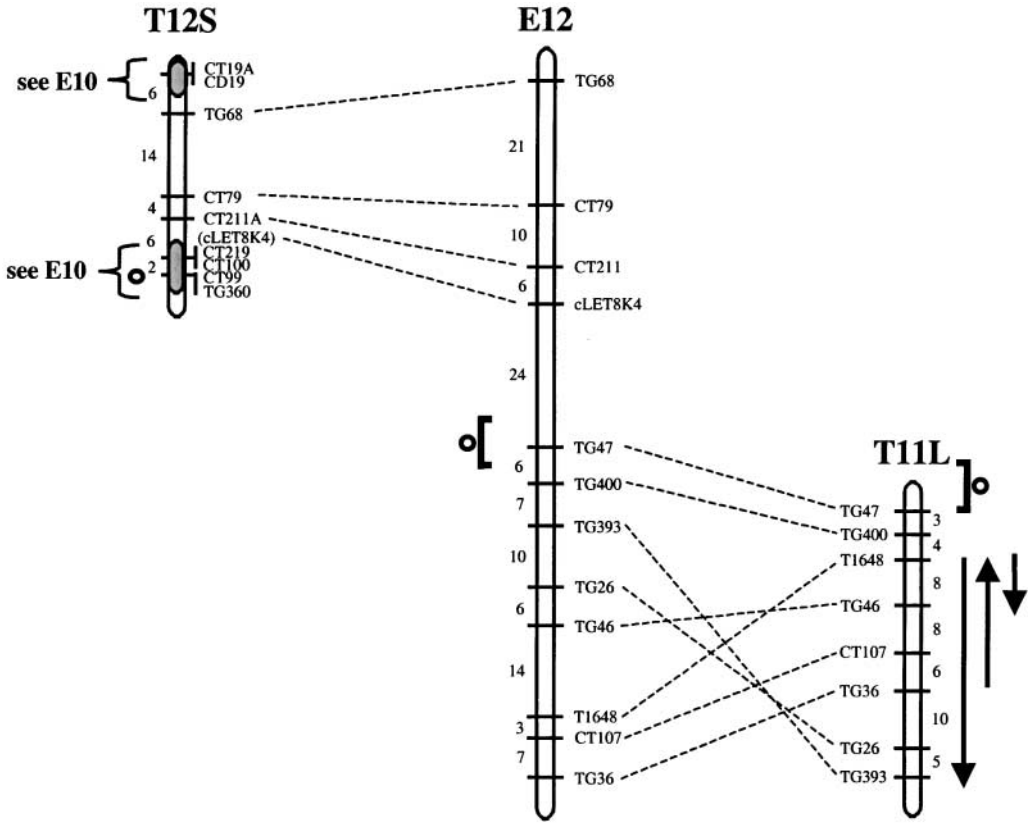

FIGURE 1.—*Continued.*

5L and 4S and another contains markers from 12L and 11S. Although these chromosome arms have been shuffled during evolution in the Solanaceae, the order of markers on the arms has remained highly conserved. The only exception is the region of E5 that is homeologous to tomato 5L. This portion of the genome is inverted in eggplant as compared to tomato, potato, and pepper. Because eggplant and pepper have undergone different translocation events, it is not possible to determine which combination of chromosome arms is ancestral. However, the order of markers within the ancestral arm 5L most likely resembled that of tomato/potato/pepper. The inversion of 5S seen in potato is also apparent in eggplant (see E3 and E10) and pepper; however, these events appear to have different breakpoints and are likely to be independent. Therefore, it is not possible to determine the marker order of the ancestral homeolog of 5S.

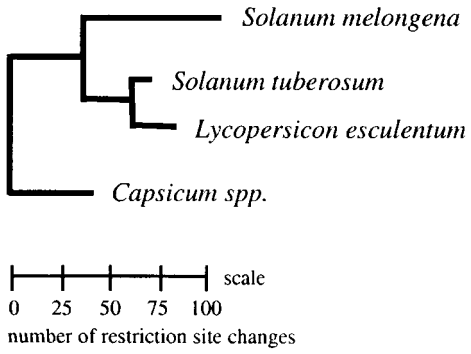

FIGURE 2.—Dendrogram of solanaceous crop plants. Tree is based on chloroplast DNA restriction site variation and is simplified from OLMSTEAD and PALMER (1997). Branch lengths correspond to the approximate number of inferred restriction site changes.

E6: Eggplant linkage group 6 is homeologous to tomato and potato chromosome 6 and pepper 6. However, eggplant is distinct in that the upper portion of E6 has undergone a paracentric inversion that is not apparent in any of the other species. Because only the eggplant homeolog of chromosome 6 has a different organization, it is assumed that the ancestor of the four species had an arrangement similar to tomato/potato/pepper and that the inversion occurred in the eggplant lineage.

E7: The marker content of E7 is homeologous to tomato chromosome 7. The linear order of markers is mostly conserved between eggplant and tomato; however, two segments of E7 are inverted with respect to tomato. The corresponding pepper linkage group is very similar to tomato in marker content and order except that the markers that comprise the upper portion of chromosome 7 in tomato are scattered throughout the pepper genome. Because it is certain only that tomato and potato are identical, it is not possible to deduce the ancestral arrangement of this chromosome.

E8: Eggplant linkage group 8 is homeologous to tomato chromosome 8. The entire content and order of the markers that comprise E8 have been conserved during the evolution of eggplant, tomato, potato, and pepper. The only difference is found in the pepper lineage and involved a translocation of segments homeologous to tomato chromosomes 1 and 8 as already mentioned. Because the only difference is found in the pepper lineage, it appears that the tomato/potato/eggplant homeolog of chromosome 8 most closely resembles their ancestor.

E9: Eggplant linkage group 9 is very similar to potato chromosome 9. Both species show an inversion of the upper portion of the chromosome as compared to tomato. Pepper also contains this inverted order and has indications of additional chromosomal rearrangements. The fact that potato, eggplant, and pepper all share the same order of markers within the homeologous chromosome arm 9S suggests that their ancestor had an arrangement of chromosome 9 similar to these three species and that the inversion of 9S occurred in the tomato lineage.

E10: E10 is exceptional in that it provides evidence of two separate translocation events in the eggplant lineage. These translocations have resulted in the combination of regions homeologous to tomato chromosome arms 5S, 12S, and 10L. Such a combination of conserved linkage blocks representing portions of three different chromosomes in tomato and potato has not been previously reported in the Solanaceae. The arrangement of markers on E10 also suggests that five paracentric inversions occurred during the divergence of eggplant and tomato. The inversion of the lower portion of E10 (corresponding to tomato 10L) is also evident in the potato and pepper genomes. Like eggplant, the pepper linkage groups show translocations involving the regions of the genome corresponding to these chromosome arms. Because eggplant and pepper have undergone different translocation events, it is not possible to determine the ancestral combination of chromosome arms for the chromosome 10 homeolog. However, the fact that eggplant, potato, and pepper all have the same arrangement of markers for the arms homeologous to 10L indicates that the tomato arrangement is the derived condition for this arm.

E11: Eggplant linkage group 11 contains conserved blocks of markers homeologous to tomato chromosome arms 4S and 11S, indicating that a translocation has occurred during the divergence of these two species. The portion of the eggplant genome corresponding to 11S has the same marker order as potato, which is inverted relative to tomato. Pepper also provides some evidence of inversion in this region of the genome. In pepper, however, this segment is linked to a segment homeologous to tomato 12L, indicating that different translocation events occurred in the lineages of pepper and eggplant. The marker order for the lower portion of E11 indicates that two inversion events occurred during the divergence of eggplant and tomato. Multiple inversions are also apparent in this segment of the pepper genome. Overall, the differences between the tomato and eggplant genomes for this linkage group can be explained by one translocation and three paracentric inversions. The fact that different translocations have occurred in the lineages of E11 and its pepper counterparts makes it impossible to deduce the ancestral combination of these chromosome arms. However, the inversion of 11S in potato, eggplant, and possibly pepper as compared to tomato suggests that their ancestor also had this marker order and that the inversion occurred in the tomato lineage.

E12: E12 combines a portion of the homeologous tomato chromosome arm 12S with the entire arm of 11L. Translocations involving these arms are also evident in pepper. The upper portion of E12 has a conserved order with respect to tomato and pepper; however, the region is inverted in potato. The arrangement of markers in the lower part of E12 as compared to tomato and potato indicates that three paracentric inversions have occurred in this region. Examination of the pepper map indicates that the pepper lineage has also undergone multiple inversions in the same area. Overall, E12 can be differentiated from tomato by one translocation and three paracentric inversion events. Because inversion of marker order for 12S is apparent only in potato, it is assumed that the arrangement of the ancestral arm 12S is best represented by the tomato and eggplant homeologs. As with E3, E4, E5, E10, and E11, it is not possible to make further hypotheses about the ancestral arrangement of this chromosome.

Comparison of the genetic linkage maps of eggplant and tomato indicates that the chromosomal differences between these two species can be explained by a total of 22 paracentric inversions and seven translocations. In an attempt to determine the relative order of these inversions and translocations, the genome-wide rearrangements required to convert the chromosome organization of eggplant into tomato and vice versa were modeled. A schematic depiction of the events involved for linkage groups E3, E4, E5, E10, E11, and E12 is shown in Figure 3. The other linkage groups are not included because simple inversions alone can be used to explain their organization. Modeling of these genome-wide rearrangements invokes 23 paracentric inversions and only five translocations, including four reciprocal and one nonreciprocal translocation. Because tomato and potato differ by 5 inversions and 4 of these (on E9, E10, E11, and E12) are also apparent in eggplant, it is assumed that the differences in the eggplant and potato genomes can be accounted for by 19 paracentric inversions and five translocations. Thus, 28 rearrangements have occurred since the divergence of eggplant and tomato from a common ancestor while only 24 rearrangements have occurred since the divergence of

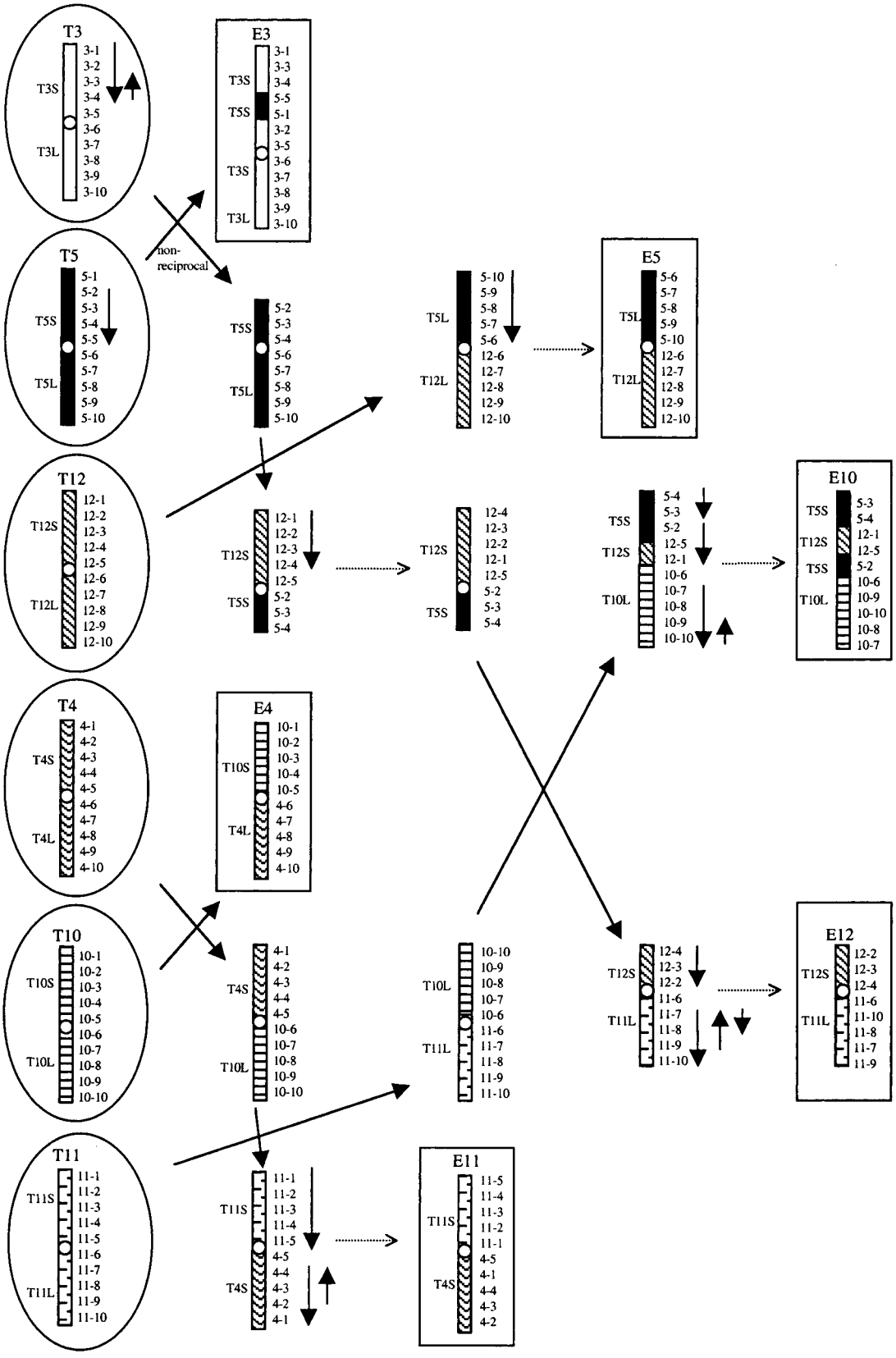

FIGURE 3.—Depiction of the putative translocation and inversion events that distinguish the eggplant and tomato genomes. Tomato chromosomes are circled, eggplant linkage groups are boxed. Each chromosome arm is labeled as in tomato (S, short arm; L, long arm). Markers are coded: chromosome no.-marker no. Thus, markers 3-1 to 3-5 are found on the short arm of tomato chromosome 3 while markers 3-6 to 3-10 are found on the long arm. Translocations are indicated by intersecting arrows, and inversions are designated by arrows parallel to the chromosomes. Dashed arrows indicate transitions due to inversion events. Changes may have occurred in either direction; therefore, directionality of events should not be inferred from the figure.

TABLE 1

Types of rearrangements that differentiate solanaceous species

Rearrangement	Comparison		
	Eggplant/tomato	Eggplant/potato	Tomato/potato
Paracentric inversions			
Whole arm	5/23 (22%)	1/19 (5%)	5/5 (100%)
Partial arm, centromeric break	2/23 (9%)	2/19 (11%)	0/5 (0%)
Partial arm, noncentromeric break	16/23 (69%)	16/19 (84%)	0/5 (0%)
Total	23	19	5
Pericentric inversions	0	0	0
Total no. of inversions	23	19	5
Reciprocal translocations			
Centromeric break	3/4 (75%)	3/4 (75%)	0
Noncentromeric break	1/4 (25%)	1/4 (25%)	0
Total	4	4	0
Nonreciprocal translocations	1	1	0
Total no. of translocations	5	5	0

eggplant and potato. Livingstone *et al.* (1999) calculated that 22 chromosomal disruptions occurred in the tomato-pepper lineages. These findings agree well with a phylogeny of the Solanaceae based on chloroplast restriction site variation (Figure 2; Olmstead and Palmer 1997). This phylogeny indicates that tomato and potato are most closely related and are in the same clade while eggplant and pepper are more distantly related to tomato and potato and belong to different clades.

Modes of chromosome evolution: Comparison of the eggplant and tomato linkage maps reveals that chromosomal evolution since their divergence from a common ancestor has proceeded primarily through inversion of otherwise colinear segments of the genome (Table 1). It has been proposed that inversions are more frequent than translocations in wild populations because chromosomal interchanges usually have negative effects on an organism's fertility (Burnham 1962). Interestingly, all of the inversions that differentiate eggplant and tomato appear to be paracentric. Previous comparative mapping in the Solanaceae also supports this finding. Tanksley *et al.* (1992) determined that the tomato and potato genomes are virtually identical except for five paracentrically inverted regions. Livingstone *et al.* (1999) found that 12 of the 22 rearrangements that distinguish tomato and pepper were the result of inversions and that most of these inversions (83%) were paracentric. Although comparative mapping of Arabidopsis and *Brassica nigra* indicates that the Brassicaceae have a rate of chromosomal evolution higher than that of the Solanaceae and all other plant families studied so far, the majority of these rearrangements have also been attributed to inversion events (Lagercrantz 1998). The prevalence of paracentric inversion as an important mechanism of chromosome evolution is not restricted to plants. In Drosophila, most of the chromosomal rearrangements that have accompanied evolution are attributed to paracentric inversions (Vieira *et al.* 1997; Ranz *et al.* 2001). The Drosophila genome has the highest rate of chromosomal evolution identified to date in the eukaryotes and only small segments of conserved marker order were identified between homeologous *D. repleta* and *D. melanogaster* chromosomes that differed by >100 paracentric inversions (Ranz *et al.* 2001). On the basis of these results, it has been hypothesized that the Drosophila genome has a modular organization that is apparently unconstrained by a necessity for conservation of gene order. Such extreme genome flexibility has not yet been observed in pairs of related plant species.

The infrequency of pericentric inversions in the Solanaceae, other plants, and Drosophila is probably related to the fertility effects of crossing over within the inverted portion of the genome. These effects were summarized by Burnham (1962), who reported that crossing over in a pericentrically inverted chromosome region results in high levels of both ovule and pollen abortion in plants and frequent zygote abortion in Drosophila. In contrast, crossing over in a paracentrically inverted region reduces only pollen viability in plants and has little or no effect on zygote survival in Drosophila. Thus, individuals that harbor paracentric inversions in their genomes are more likely to transmit this chromosome rearrangement to the next generation than are those that harbor pericentric inversions.

Of the 23 paracentric inversions that differentiate eggplant and tomato, most (69%) involved partial chromosome arms and breaks that did not occur at the centromere (*i.e.*, noncentromeric breaks; Table 1). Similarly, eggplant and potato are distinguished primarily by noncentromeric, partial arm inversions. In contrast, nearly equal numbers of centromeric and noncentro-

meric paracentric inversions distinguish tomato and pepper and only whole arm inversions differentiate tomato and potato (Tanksley *et al.* 1992; Livingstone *et al.* 1999). Thus, it appears that a certain type (*i.e.*, whole arm *vs.* partial arm, centromeric break *vs.* noncentromeric break) of paracentric inversion was not predominant during evolution in the Solanaceae. Pairwise genome comparisons in the grasses also indicate that their evolution did not favor a particular type of paracentric inversion. In some cases, species are primarily differentiated by paracentic inversions with centromeric breakpoints [*e.g.*, rice and maize (Wilson *et al.* 1999) and rice and foxtail millet (Devos *et al.* 1998)] while other species pairs are characterized by noncentromeric paracentric inversions [*e.g.*, diploid wheat and barley (Dubcovsky *et al.* 1996)].

Chromosomal interchanges or translocations have been a secondary mechanism of chromosome evolution in the Solanaceae. When these translocations as well as the inversions are taken into account, it is apparent that a significant number of the rearrangements that occurred during evolution of the Solanaceae involved breaks at or close to the putative locations of centromeres. A similar observation has been made in other plant species (Lagercrantz 1998; Devos and Gale 2000). Telomere-telomere fusions have also been proposed as an important factor in genome evolution (Lagercrantz 1998). Telomeric sequences have been identified at the centromeres of eight tomato chromosomes (Presting *et al.* 1996) and were hypothesized to have a role in many of the rearrangements that occurred in the pepper genome (Livingstone *et al.* 1999). By comparison of the eggplant and tomato maps, it appears that rearrangements have occurred at the centromeres of the homeologous tomato chromosomes 3–6 and 9–12. Moreover, a comparison of the tomato, potato, eggplant, and pepper genomes indicates that the chromosome arms corresponding to tomato chromosomes 5, 9, 11, and 12 most frequently underwent translocation or inversion during evolution of the Solanaceae as different arrangements of these arms are seen in all four species. Telomeric sequences have been mapped to the centromeres of all but two of these chromosomes (6 and 10). Thus, there may indeed be a connection between telomeric sequences at the centromere and instability of the associated chromosome arms.

An important question raised by comparative genome analysis is whether the profile of the different types of chromosomal disruptions that accompany divergence is constant through evolutionary time. Detailed classification of the rearrangements that occurred during the evolution of eggplant, tomato, and potato from a common ancestor (Table 1) provides the data to answer this question. A χ^2 test of homogeneity for these data shows that the relative proportions of the different types of rearrangements that occurred during the divergence of eggplant/tomato and tomato/potato are not the same ($0.025 \leq P \leq 0.01$). These results indicate significant variation in the types of chromosomal changes that occur during evolution and suggest that different events are favored at different times during evolution. Thus, the fact that tomato and potato are differentiated by only whole arm paracentric inversions while eggplant and tomato are distinguished by translocations and a variety of different types of paracentric inversions cannot be explained by chance alone.

Rates of chromosomal evolution: The dearth of fossils for the Solanaceae makes calculations of divergence times in the family problematic. However, a recent study suggests that the genus Solanum, which includes Lycopersicon, diverged from its closest relative ~12 million years (myr) ago (Wikstrom *et al.* 2001). Thus, this value can be used as an estimate of the maximum divergence time for eggplant and tomato/potato. Given that 28 rearrangements differentiate eggplant and tomato, it appears that these species underwent ~0.19 rearrangements/chromosome/myr or 0.002 rearrangements/Mb of DNA/myr. This rate of chromosomal evolution is higher than that observed in many of the grasses (Paterson *et al.* 1996) but much lower than that seen in some lineages of the Brassicaceae, which are reported to have the fastest evolving genomes identified to date in plants (Lagercrantz 1998). Thus, compared with other species, Solanum appears to have a moderate rate of chromosomal evolution. Because it has been reported that certain lineages in the Poaceae are evolving more quickly than other lineages (Paterson *et al.* 2000), it is not known if the rate of chromosomal evolution observed for the tomato-eggplant comparison is characteristic for the entire Solanaceae family.

If chromosomal rearrangements are occurring at a relatively constant rate, the numbers of chromosomal rearrangements that differentiate eggplant, tomato, and potato can also be used as a gauge of their relative divergence. Because tomato and potato are distinguished by only 5 rearrangements while eggplant and tomato differ by 28, it is apparent that eggplant and tomato are diverged five- to sixfold more than tomato and potato. To determine if the amount of chromosomal evolution in eggplant, tomato, and potato is consistent with the extent of change at the nucleotide level, a rough estimate of DNA sequence divergence between pairs of the three species was calculated. According to estimates that included both synonymous and nonsynonymous nucleotide changes, eggplant and tomato are diverged ~2.5 (determined from sequences for the chloroplast *ndhf* gene and a portion of the nuclear *waxy* gene; L. Bohs, personal communication) up to 3.5 (calculated from 13 COS marker sequences; data not shown) times more than tomato and potato. It is interesting that these values are of the same order of magnitude as those obtained at the chromosomal level.

Conclusions: Despite ~12 myr of chromosomal evolution, the eggplant and tomato genomes have remained

largely colinear. All of the rearrangements that distinguish eggplant, tomato, and potato can be attributed to two common mechanisms of chromosomal evolution: translocation and paracentric inversion. Although comparison of the types of rearrangements that differentiate the chromosomes of the solanaceous species indicates that their evolution favored paracentric inversions, whole arm or partial arm inversions with a breakpoint at the centromere were not more common than partial arm inversions with noncentromeric breakpoints. Examination of the relative distribution of the types of paracentric inversions and translocations that occurred during the evolution of eggplant, tomato, and potato from a common ancestor also indicates that different rearrangements occurred at different evolutionary times. Thus, the profile of rearrangements that characterizes the divergence of eggplant and tomato is not the same as the profile that characterizes the divergence of tomato and potato. In addition, it was observed that the numbers of chromosomal rearrangements that differentiate these three species are consistent with the numbers of nucleotide changes in orthologous coding sequences of the species. Overall, these results indicate that eggplant and tomato are diverged three- to sixfold more than tomato and potato.

The construction of a molecular genetic linkage map of eggplant and the identification of the chromosomal changes that differentiate the solanaceous crop species provide a bridge to the utilization of the genomic information and genetic resources of tomato, potato, and pepper for eggplant studies. Thus, in addition to shedding light on chromosomal evolution in the Solanaceae, it is hoped that this work will have practical implications for the advancement of eggplant breeding and genetics.

We thank Dr. Lynn Bohs for calculation of relative divergence for the *ndhf* and *waxy* genes. This project was supported by grants from the U.S. Department of Agriculture National Research Initiative Cooperative Grants Program (no. 96-35300-3646), the Binational Agricultural Research and Development Fund (no. US 2427-94), and the National Science Foundation (no. 9872617) to S.D.T.

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Communicating editor: V. L. Chandler