Genome Mapping in Capsicum and the Evolution of Genome Structure in the Solanaceae

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Manuscript received December 11, 1998
Accepted for publication April 7, 1999

ABSTRACT

We have created a genetic map of Capsicum (pepper) from an interspecific F_2 population consisting of 11 large (76.2–192.3 cM) and 2 small (19.1 and 12.5 cM) linkage groups that cover a total of 1245.7 cM. Many of the markers are tomato probes that were chosen to cover the tomato genome, allowing comparison of this pepper map to the genetic map of tomato. Hybridization of all tomato-derived probes included in this study to positions throughout the pepper map suggests that no major losses have occurred during the divergence of these genomes. Comparison of the pepper and tomato genetic maps showed that 18 homeologous linkage blocks cover 98.1% of the tomato genome and 95.0% of the pepper genome. Through these maps and the potato map, we determined the number and types of rearrangements that differentiate these species and reconstructed a hypothetical progenitor genome. We conclude there have been 30 breaks as part of 5 translocations, 10 paracentric inversions, 2 pericentric inversions, and 4 disassociations or associations of genomic regions that differentiate tomato, potato, and pepper, as well as an additional reciprocal translocation, nonreciprocal translocation, and a duplication or deletion that differentiate the two pepper mapping parents.

THE field of comparative plant genomics was precipitated in 1988 by two studies in the Solanaceae relating the genetic maps of tomato and potato (Bonierbale *et al.* 1988) and tomato and pepper (Tanksley *et al.* 1988), each of which was constructed using common restriction fragment length polymorphism (RFLP) probes. The first study found that the tomato and potato genomes differed only by paracentric inversions, and further studies (Gebhardt *et al.* 1991; Tanksley *et al.* 1992) showed that five inversions differentiated these species. The tomato-pepper study also suggested that pepper had maintained genomic content similar to tomato, as defined by the presence of pepper sequences complementary to all tomato cDNAs tested, but that the pepper genome was rearranged substantially, with many pepper chromosomes containing three to four distinct tomato segments. A later, more detailed comparison of pepper and tomato concluded that pepper had lost regions homologous with the tomato genome and failed to change the perception that the homeologous linkage blocks in the pepper genome were fragmented substantially (Prince *et al.* 1993). As a result, the pepper-tomato comparison remains the frequently cited exception to numerous studies that have shown conservation of large linkage blocks between related genomes, even when comparing species that vary widely in DNA content and karyotype morphology (Gebhardt *et al.* 1991; Tanksley *et al.* 1992; Weeden *et al.* 1992; Menancio-Hautea *et al.* 1993; Kowalski *et al.* 1994; Bennetzen and Freeling 1997; Cheung *et al.* 1997; Bennetzen *et al.* 1998; Gale and Devos 1998a,b; Lagercrantz 1998).

There are other previously published and unpublished genetic maps of Capsicum based on either interspecific *Capsicum annuum* × *C. chinense* populations (Tanksley 1984; Tanksley *et al.* 1988; Prince *et al.* 1993; Kim *et al.* 1998a), intraspecific *C. annuum* doubled-haploid populations (Lefebvre *et al.* 1995, 1997; Lefebvre and Palloix 1996; Caranta *et al.* 1997a,b), other interspecific crosses (Zhang 1997; Y. Zhang, unpublished results), or an intraspecific *C. annuum* F_2 population (Massoudi 1995). To date, however, there is no map of Capsicum that achieves the goal of completely delineating and saturating the pepper chromosomes.

To answer questions remaining from these earlier studies of Capsicum, a complete genetic map with 12 linkage groups anchored in and completely representing the tomato genome is required. Toward this end, we have constructed a molecular map of Capsicum using randomly amplified polymorphic DNA (RAPD), isozyme, amplified fragment length polymorphism (AFLP), and RFLP analyses with tomato- and pepper-derived probes, which has allowed us to reexamine questions of genome structure and karyotypic evolution in pepper, tomato, and potato.

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MATERIALS AND METHODS

Population construction, DNA extractions, and cytological examination: The F_2 mapping population of 75 plants was developed by self-pollinating one F_1 hybrid plant of *C. annuum* cv. "NuMex RNaky" (Nakayama and Matta 1985) × *C. chinense* PI 159234, obtained from S. Tanksley (Cornell University, Ithaca, NY). The parents and F_1 and F_2 plants were grown in the greenhouse at Cornell University, and DNA was extracted from harvested leaf tissue following the procedure of Prince *et al.* (1997). Immature F_1 flower buds were collected and fixed in Carnoy's solution for cytological examination. The buds were transferred to a 70% ethanol solution and then squashed with gentle heating in a 2% acetocarmine:45% acetic acid solution. Metaphase I and anaphase I in pollen mother cells were then observed by light microscopy.

Molecular markers: *RFLP:* Pepper, tomato, and tobacco DNA fragments were used as probes for RFLP. Three types of tomato clones described in Tanksley *et al.* (1992) were supplied by S. Tanksley: tomato random genomic DNA (TG), tomato whole-leaf cDNA (CD), and tomato leaf epidermal cDNA (CT). Pepper leaf epidermal cDNA (PC) and pepper random genomic (PG) DNA clones, described by Prince *et al.* (1993) and Blauth (1994), were either supplied by S. Tanksley or generated in our lab. In addition to these, other clones used as probes included the following: the tomato genes *Prf*, supplied by G. Martin (Boyce Thompson Institute, Ithaca, NY), and *Sw-5*, supplied by S. Tanksley; four pepper genomic clones (UB) from U. Bonas (Martin Luther University, Domplatzl, Germany); and the tobacco *N* cDNA (pN18C) provided by B. Baker (U.S. Department of Agriculture, Albany, CA).

Survey filters were prepared to assess polymorphism between parental DNA digested with 12 different restriction enzymes: *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Bst*NI, *Taq*I, *Xba*I, *Bam*HI, *Bgl*II, *Bcl*I, *Sac*I, and *Stu*I. Mapping filter sets for each of the 12 restriction enzymes were also prepared, including both parents and all 75 F_2 progeny. All filters were prehybridized for at least 4 hr in 70 ml of hybridization buffer before use. The cloned inserts used as probes were amplified by PCR and then purified on Sephadex G50 spin columns equilibrated with Tris-EDTA to reduce background signal. These products were then radiolabeled using a protocol modified from Feinberg and Vogelstein (1983) as follows. The amounts of the following reagents were doubled to increase the signal strength when using heterologous probes: cloned DNA (200 ng instead of 100 ng DNA), labeling solution (22 μl instead of 11 μl), Klenow enzyme (8 units instead of 4 units), and [^{32}P]dCTP (0.1 mCi instead of 0.05 mCi). After a 90-min incubation at 37°, the labeled clones were purified on Sephadex G50 spin columns in 1% SDS and 25 mm EDTA spun at 2500 rpm for 8 min. The clones were denatured by heating at 100° for 10 min and then added to the hybridization buffer. Hybridization was carried out overnight at 65°. Filters were washed with one low-stringency (2× SSC, 0.1% SDS) wash and two moderate-stringency (1× SSC, 0.1% SDS) washes at 65° and placed on Kodak XAR-5 film for 3–12 days, depending on signal strength.

Isozyme analysis: The procedures of Loaiza-Figueroa *et al.* (1989) were carried out in the laboratory of N. Weeden (New York State Agricultural Experiment Station, Geneva, NY) to analyze *Idh-1*, *Gpi-1*, *Gpi-2*, *6-Pgdh*, *Pgm-1*, *Pgm-2*, and *Tk*.

PCR-based markers: RAPD markers were generated following the procedures of Prince *et al.* (1995). RAPD 10-mer primers were obtained from the National Science Foundation / Department of Energy / U.S. Department of Agriculture Plant Science Center at Cornell University and from Gilroy Foods (Gilroy, CA). AFLP markers were generated by Keygene n.v. (Wageningen, The Netherlands) using the procedure of Vos *et al.* (1995).

Genetic map construction: The genotypes of the RFLPs and RAPDs were scored independently to obtain a consensus genotype. If a probe produced multiple segregating bands, then each band was initially scored as a dominant band. Some of these genotypes were subsequently converted to codominant pairs if two dominant bands, one from each parent, mapped to the same position.

The initial RFLP data were then merged with isozyme, RAPD, and AFLP marker genotypes. A test of departure from expected Mendelian ratios was conducted where possible; however, a number of the AFLP genotypes included A, B, H, and C (=B or H) or D (=A or H) calls precluding this analysis. The markers were also divided into classes on the basis of a subjective measure of the certainty with which the genotype was ascertained. These classifications were used to find the RFLP markers that could be unambiguously read and whose deviations from the expected Mendelian frequencies were $P > 0.01$ for dominant markers and $P > 0.001$ for codominant markers. The more distorted markers were excluded from the initial framework construction phase to protect against mapping errors stemming from either pseudolinkages conjoining independent chromosomal regions or incorrect estimates of map distances (Lorieux *et al.* 1995; Cloutier *et al.* 1997).

The MAPMAKER / EXP v3.0b program (Lincoln *et al.* 1993) was used for map construction. The subset of markers defined above was divided into linkage groups using the "group" command (parameters: LOD > 3, θ < 20 cM), and then arranged into a framework map using the "order" command (parameters: LOD > 3 for linkage, θ < 20 cM, initial group size of 3, LOD > 3 *vs.* alternative orders). The resultant orders for each group were checked by multiple iterations of this process after scrambling the marker input order. When multiple markers cosegregated, codominant markers were given preference for inclusion in the framework. Framework orders were checked by manual examination of the "LOD table," the "ripple" command (parameters: 3 markers, reporting alternative orders if LOD < 3 compared to the set framework), and finally by checking the pairwise linkages between all framework markers from different linkage groups.

AFLP markers were added to the linkage groups via the "assign" command (parameter values LOD > 3 and θ < 20 cM), and they were added to the chromosomal frameworks using the "build" command if the placement of a marker into a particular interval was significantly better (LOD > 3) than all alternative placements. This addition procedure was then repeated for the remaining initial RFLP markers and the skewed markers. The resultant orders for each linkage group were then checked again by manual examination of the LOD table and the ripple command, as described above. Finally, the positions for all remaining markers were determined using the "try" command. Markers were given either a position between framework markers if 2 < LOD < 3 for the interval and all other placements were unlikely (LOD ≪ 1), or they were added into haplotype groups. Haplotype groups consisted of markers that were separated from framework markers by <5 cM and placement on either side of the framework marker was equally likely. These markers were assigned positions with the framework marker(s), but they are differentiated on the map (see Figure 1 legend). Some markers were placed beyond the framework ends of the linkage groups if they mapped to the end of a linkage group (2 < LOD < 3) and all alternative locations within the group were unlikely (LOD ≪ 1). Pairwise linkages with the terminal framework markers were checked to ensure correct placement. These markers were added to the map; however, they were not considered framework markers for further linkage group extension. All mapping was done without reference to the order of markers on the tomato map, and all distances were computed using the Kosambi mapping function (Kosambi 1944).

Construction of the synteny map: A second genetic map was constructed to maximize the number of tomato-derived markers for comparative analysis. For this map, all nontomato markers were removed, and positions for the remaining tomato-derived markers were estimated by reducing the stringency for addition to haplotype groups (±8 cM) or placement between framework loci (LOD > 1.5). Other markers that mapped into several contiguous intervals were placed at their most likely positions. The tomato map of Pillen *et al.* (1996) was then reduced to only the markers in common between the two maps.

RESULTS

Cytology of meiosis in (*C. annuum* NuMex RNaky × *C. chinense* PI 159234) F_1 plants: Cytological examination of meiotic chromosome spreads from pollen mother cells of *C. annuum* × *C. chinense* F_1 revealed multivalents and bridges extending across the metaphase plate at late anaphase I (M. Cadle, unpublished results). These data are consistent with the presence of at least one reciprocal translocation between these species, as described previously by Tanksley (1984), Kumar *et al.* (1987), and Lanteri and Pickersgill (1993).

Marker genotyping and analysis: *RFLP:* A total of 399 tomato probes selected to represent the tomato genome were assayed for polymorphism (270 TG, 89 CT, 33 CD, and 7 other tomato cDNAs). Furthermore, 184 PG clones, 4 PC clones, 4 other pepper genomic (UB) clones, and pN18C were also tested. All tomato clones tested hybridized to pepper genomic DNA, and 203 TG, 58 CT, 28 CD, 7 other tomato cDNA, 2 PC, 3 UB, pN18C, and 43 PG clones were used as probes. The lower percentage of PG clones used (23 *vs.* 75% for TG, 65% for CT, and 85% for CD) resulted from the number of PG clones (74 = 40.2%) that produced a smear of unresolvable bands. Some survey autoradiograms are available from the Solgenes database[2] (Paul *et al.* 1994). The probes produced 460 segregating RFLP; 227 (49.3%) were codominant.

Isozymes: Genotypes were obtained for all seven isozymes.

PCR-based markers: From the 116 RAPD primers tested, 59 produced 75 reproducible polymorphic bands (OPR, R, U, OPO, OPA, Q, and OPE markers). In addition, 20 primer combinations were used in AFLP. These reactions yielded 465 segregating bands (A markers), of which 340 could be scored as codominant by a proprietary algorithm. The primer sequences, fragment sizes, and parental lines showing amplification for all PCR-based markers are available from Solgenes.[3]

Segregation distortion: Of the 1007 markers generated, 665 could be tested for deviation from their expected single-locus Mendelian ratios. Slightly more than half of the tested subset (337 = 50.7%) showed deviation from the expected ratios ($P < 0.01$); 81 of these (12.2%) were severely distorted ($P < 0.001$), with P values as low as 2.69×10^{-25}. Two common causes of misclassified segregation distortion (residual heterozygosity and comigrating RFLP or PCR fragments) were ruled out because (1) all F_2 plants came from only one F_1 individual that could carry only one allele from each parent; and (2) within a distorted region, all RFLP and AFLP markers were distorted and comigration of multiple independent markers is extremely unlikely.

The distorted regions ($0.01 > P > 0.001$) included the middle of pepper linkage group (P)1 between TG70 and A211, distorted in favor of *C. annuum* homozygotes over *C. chinense* homozygotes; the middle of P3 between TG74 and TG290a, distorted in favor of *C. chinense* homozygotes over *C. annuum* homozygotes; the bottom of P6 near CT109, distorted in favor of heterozygotes over *C. annuum* homozygotes; and the middle of P11 between CT70 and CD127a, distorted in favor of *C. annuum* homozygotes over *C. chinense* homozygotes. Severely distorted regions ($P < 0.001$) included the upper end of P2 down to CT128a, with an excess of *C. chinense* homozygotes over *C. annuum* homozygotes; the upper end of P3 at CT220, with an excess of heterozygotes over *C. chinense* homozygotes; the upper end of P11 at CD127d and CD186d, again with an excess of heterozygotes over *C. chinense* homozygotes; all of P7, distorted in favor of *C. chinense* homozygotes, almost to the complete exclusion of *C. annuum* alleles in some regions; and the upper end of P12 down to A3, with an excess of *C. chinense* homozygotes over *C. annuum* homozygotes. The regions corresponding to P1, P2, P3, P6, P11, and P12 were also distorted in either one or both of the tomato populations of de Vicente and Tanksley (1993) or Bernacchi and Tanksley (1997), and markers from P7 and P12 were also distorted in the earlier pepper population of Prince *et al.* (1993) and the potato population of Tanksley *et al.* (1992).

Genetic map construction: A genetic map consisting of 11 large (76.2–192.3 cM) and 2 small (19.1 and 12.5 cM) linkage groups covering 1245.7 cM was constructed from the genotypic data (Figure 1). Although $2N = 2X = 24$ for these species, the reciprocal translocation in the parents would cause pseudolinkage between markers near the interchange breakpoints on the chromosomes involved (Burnham 1991). P1 contains *Idh-1* and *Pgm-2*, which are described by Tanksley (1984) as being near the exchange breakpoint in a similar interspecific cross; therefore, we propose that this linkage group represents the two pepper chromosomes involved in this rearrangement. The two small linkage groups in our map remained unlinked despite the use of clones from the corresponding regions of the tomato genome.

Of the 1007 marker genotypes generated, 677 (67.2%) were either given positions in the LOD 3 framework or placed in framework intervals at 2 < LOD <

[2] Go to http://probe.nalusda.gov:8300/ and choose "databases," then "browse" from the options of the Solgenes database, and look for "image" class objects with "ACF" in the title.

[3] Go to http://probe.nalusda.gov:8300/ and choose "databases," then "browse" from the options of the Solgenes database, and look for the marker under its name in the "locus" objects list.

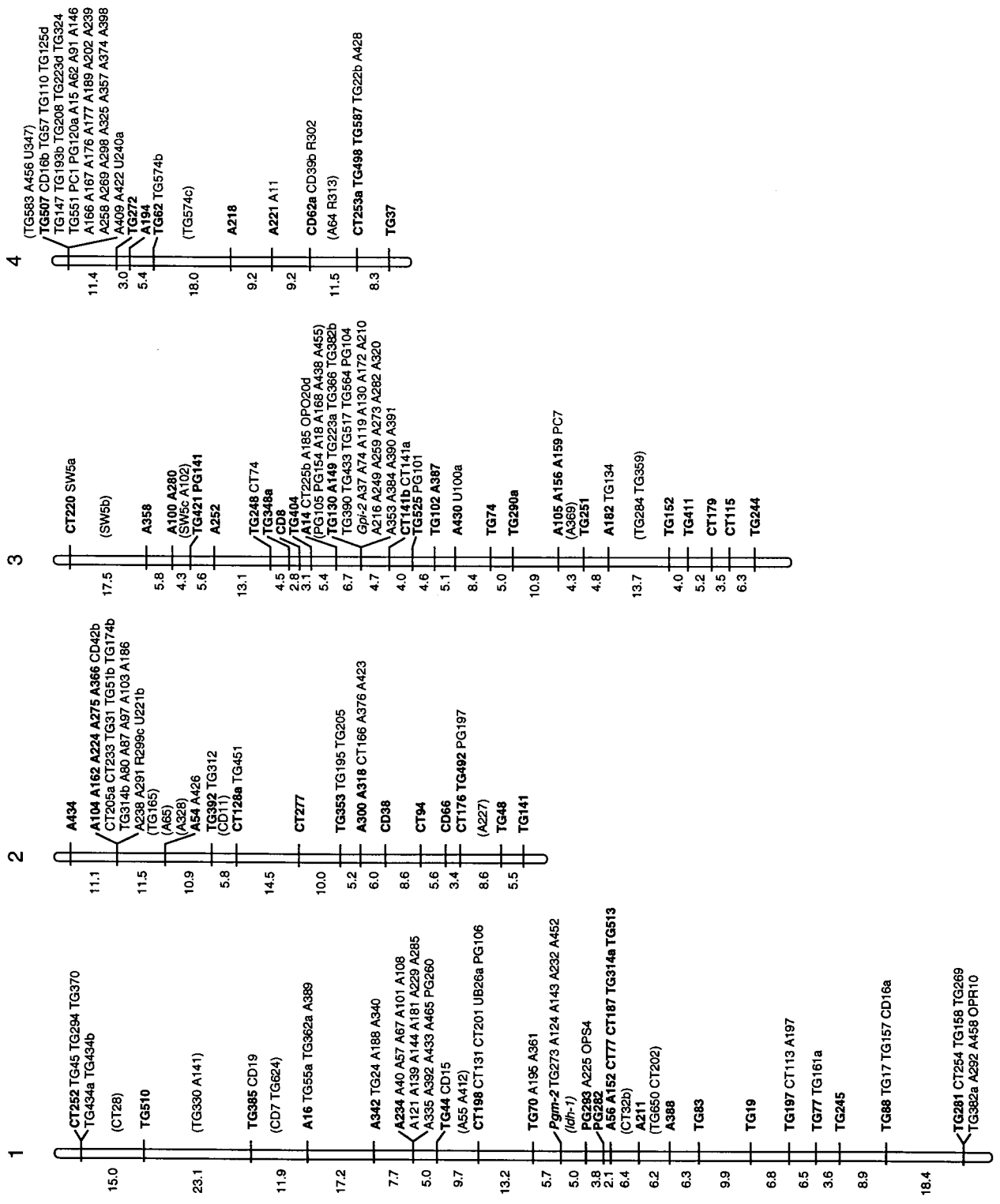


Figure 1.—A genetic map of Capsicum. Markers in boldfaced type at tick marks are framework markers ordered at LOD > 3, and multiple markers at a tick mark cosegregated. Markers listed in plain type after framework markers were closely linked to the framework markers (<5 cM), but placed equivalently on either side of the framework position at LOD > 2 when compared to other positions in the linkage group. Markers in parentheses placed between framework positions at 2 < LOD < 3. Marker types and designations are as follows: tomato genomic RFLP (TG); tomato cDNA RFLP (CD, CT, Sw5, and Prf); pepper genomic RFLP (PG and UB); pepper cDNA RFLP (PC); tobacco cDNA RFLP (pN18C); AFLP (A); RAPD (OP, U, R, Q); and isozyme (*Gpi-2*, *Idh-1*, and *Pgm-2*). Lowercase letters at the end of the marker names indicate that the marker is one of at least two segregating loci detected by a single assay. Distances between framework positions in centimorgans (Kosambi) are to the left of each chromosome.

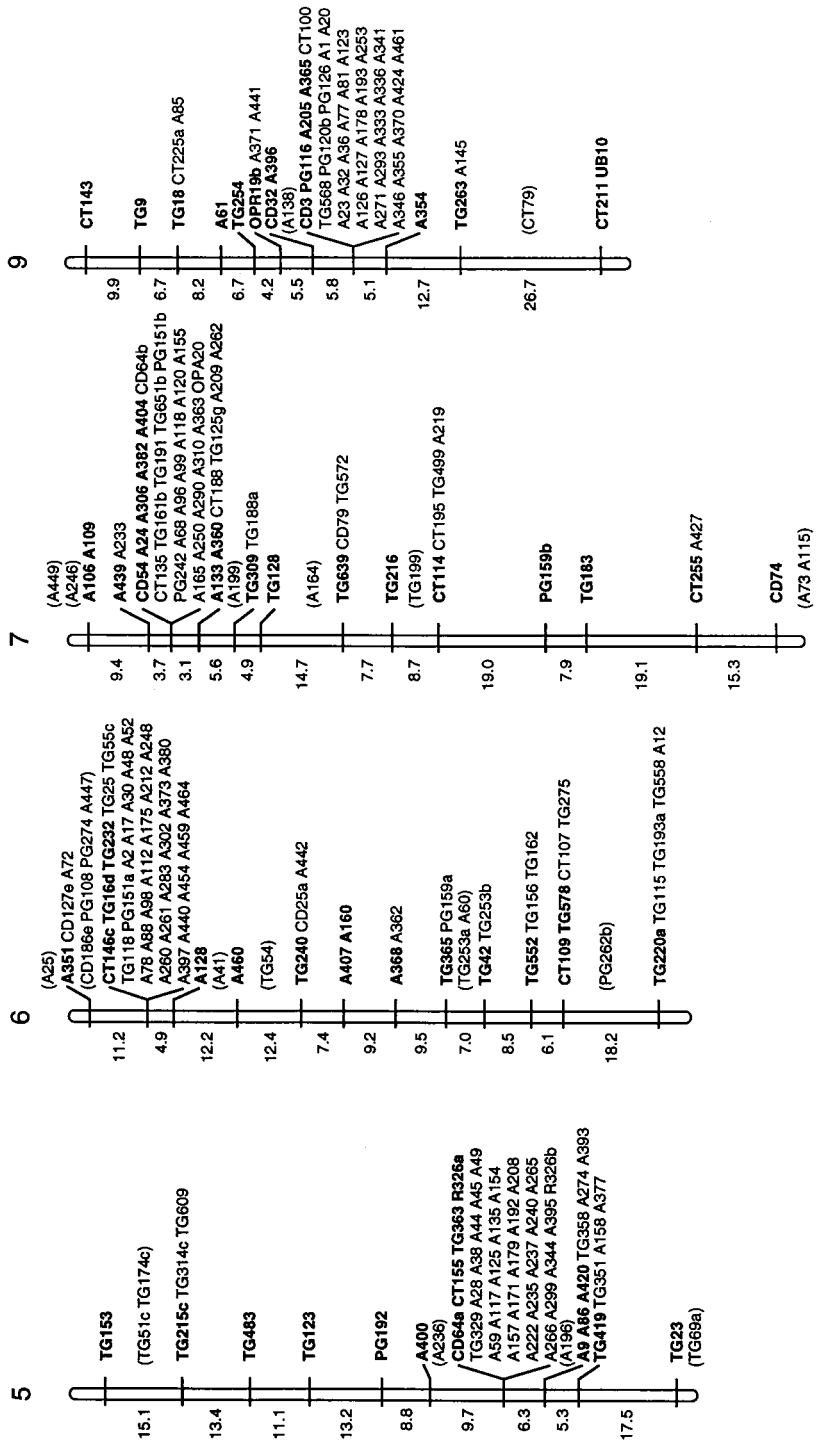
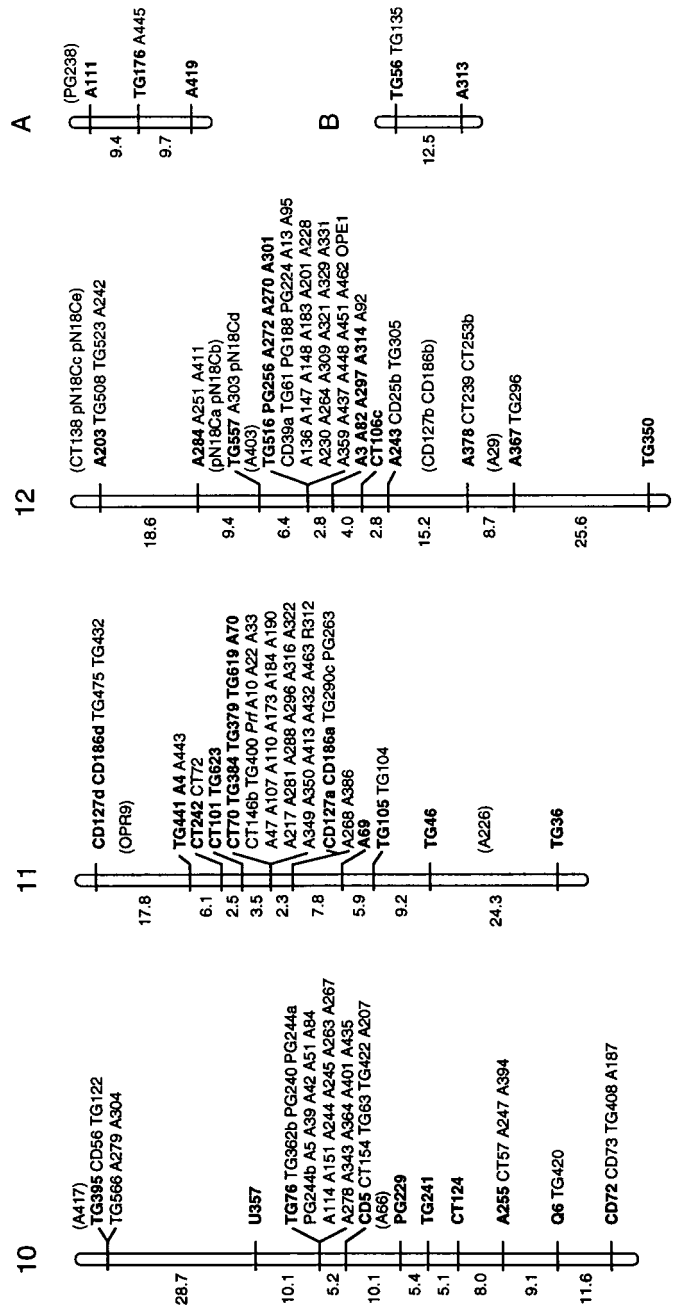


Figure 1.—*Continued.*

3, while 5 markers (1 isozyme, 1 TG, 1 CT, and 2 AFLP) remained unlinked. The remaining 325 markers grouped (LOD > 3) with the linkage groups but did not place well within the frameworks (multiple equivalent LODs). The number of these markers from each linkage group could be a function of the length of the linkage group ($r^2 = 0.77, P = 8.4 \times 10^{-5}$). Only groups 1 and 4 deviated significantly toward more of these loci than expected (data not shown). The majority of the markers that fit this classification placed equally well into a small number (3–5) of contiguous intervals in the framework areas with high marker densities. There were, however, 29 markers in this group that placed in a greater number of intervals all along the length of a particular chromosome. A hallmark of these loci was moderate pairwise linkage with a framework locus (LOD $\approx$ 3, 15–20 cM), but no linkage to the flanking framework markers.

The 677 markers across 1245.7 cM provide an average



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marker density of 1 marker per 1.8 cM; however, the mean distance between framework positions is 9.0 cM. There is great variability in these densities because many markers showed pronounced clustering (Figure 1). One region of marker clustering occurs on each of the large linkage groups and includes 44–66% of the markers in that linkage group. Overall, 54% of the markers are located in these clusters. This figure may be an overestimate, however, as some of the AFLP markers may be allelic pairs.

The nonrandom distribution of markers within linkage groups is similar to tomato, where cytogenetic data allowed Tanksley *et al.* (1992) to show that the clusters represented regions of suppressed recombination around tomato centromeres. Pepper centromeres have been mapped using C banding (Moscone *et al.* 1993); however, pepper lacks both the classical map and deletion studies to correlate our map with these data. Thus, while all the clusters on our map contain markers linked to centromeres in tomato, an observation also made for several of the linkage groups of the map in Prince *et al.* (1993), we can only presume that these regions correspond to the centromeric regions of each of the linkage groups.

Genome content: Most probes used (66%) produced 2–6 bands; 21% gave 6–10 bands, and 12% revealed segments of the genome that had been duplicated extensively (>10 bands) but still provided distinguishable polymorphisms. To examine the relative copy number of sequences detected by hybridization, we compared a subset of 30 pepper survey blots with tomato survey blots from the Tanksley laboratory using the same probe-enzyme combinations. Five of the tomato blots also included a lane with DNA from our *C. annuum* parent. In 2 of these 5 control comparisons, our surveys showed more bands than the Tanksley surveys (6 *vs.* 2 and 9 *vs.* 3). In the other three cases, results were more similar (3 *vs.* 1, 5 *vs.* 4, and 11 *vs.* 10), although in every case the larger number came from our laboratory. This limited sample suggests that some differences observed between tomato and pepper in the copy number of probes may result from specific laboratory procedures. When all 30 comparisons between pepper and tomato were considered, 14 showed more bands for a given probe in pepper than tomato, and 16 were approximately similar.

In our study, 43 clones (6 pepper genomic DNA, and 37 tomato probes, 17 cDNA, and 20 genomic clones) detected multiple segregating loci in pepper. Seven of the 37 tomato clones also detected multiple segregating loci in the tomato (Pillen *et al.* 1996), but the duplications in each species appeared to be distinct. The majority (93%) of the pepper duplications appeared to be unlinked duplications of single loci. There was only one set of multiple linked loci that revealed a duplication in the Capsicum genome: CD42, CD44, CT205, TG51, TG174, TG215, and TG314 produced loci on the middle of P1 in *C. annuum* and the top of P2 and P5 in *C. chinense.* The frequency of tandemly duplicated loci separable by recombination within intervals <10 cM was 0.87%.

Comparative map of pepper and tomato: The probes used detected 308 loci on the tomato map of Pillen *et al.* (1996) and 352 loci on our augmented pepper map (Figure 2). We then defined homologous segments containing common markers from contiguous regions of the genetic maps of both species. Breaks in homeology were not declared if the intervening markers not from the contiguous region of the other species were either from probes detecting multiple segregating loci or from more than one different chromosome.

There were only four regions in pepper and one region in tomato where homeology was either difficult to assign or not apparent: the top of P4, which included markers from throughout the tomato genome; the top of P5, which is duplicated in and represented only by dominant markers from the *C. chinense* parent; the top of P7, which contains a large number of markers that are spread throughout the tomato genome and some loci that were uniquely duplicated in pepper; the bottom of P7, which contained markers from different tomato chromosomes; and the top of tomato chromosome (T)7, which is represented in the pepper (all probes hybridized) but appears to be scattered throughout the pepper genome. It is impossible to tell whether these regions are the result of novel associations of markers in one of the genera or the disassociation of a group of markers in another. Overall, 98% of the tomato genome and 95% of the pepper genome were included in defined regions of homeology.

Within homeologous segments, ~30% of the loci mapped in only one genome, perhaps because no corresponding homolog was present in the other genus or the homolog was not segregating. We cannot distinguish between these two alternatives because nonsegregating bands were detected by most probes in at least one of the populations, but we can resolve two classes of loci. Some loci appear to be uniquely duplicated in one

Figure 2.—Homeologous relationships between the genetic maps of Capsicum and tomato. The genetic map of tomato was modified from the map of Pillen *et al.* (1996): only the markers in common between the two maps are presented, and the orientation of tomato chromosome 8 is reversed. For the tomato map, markers in plain type at tick marks are framework loci ordered at LOD > 3, markers in parentheses are placed in intervals at LOD < 3, and positions of underlined loci were approximated from other maps. The pepper map is based on Figure 1, but all markers except those in common to the two maps have been removed. Markers in bold represent framework markers ordered at LOD > 3. Markers in plain type listed with the framework markers may be up to ±8 cM from the framework marker, based on pairwise linkage estimates. Pepper markers enclosed in parentheses are placed at 1.5 < LOD < 3, and underlined markers are placed at their maximum likelihood position (LOD < 1.5). When a chromosome is homeologous to two chromosomes from the other species, one side of the chromosome has been widened out. Marker types and designations are as follows: tomato genomic RFLP (TG); tomato cDNA RFLP (CD and CT); pepper cDNA RFLP (PC); cloned genes [*Pto (=CD186), Fen (=CD127),* and *Prf*]; and isozymes *(Idh-1, Pgm).* Numbers in square brackets after locus names indicate the nonhomeologous chromosome in the other species where a map position was obtained for that particular probe. An asterisk after a locus name denotes a locus mapped uniquely in one species from a probe that produced multiple segregating loci, with the other loci mapping to syntenous positions; *e.g.*, if a probe produced two fragments in tomato and one fragment in pepper that mapped to a syntenous position, the second locus would be marked with an asterisk. A dagger indicates that a homolog groups with the syntenous linkage group, but a definitive position cannot be assigned. The markers that grouped (LOD > 3) but did not place well within the linkage group frameworks for each of the linkage groups are as follows (annotations are the same as above): P3–TG16b*; P4–CD55, CD64c*, CD64d*, CD64g*, CT205c *, TG503a [5], TG503b [5]; P7–CD39d*, CT32a [9], CT129a*, TG47 [11], TG220b*, TG418; the two unlinked markers are CT182 [11] and TG214 [3].

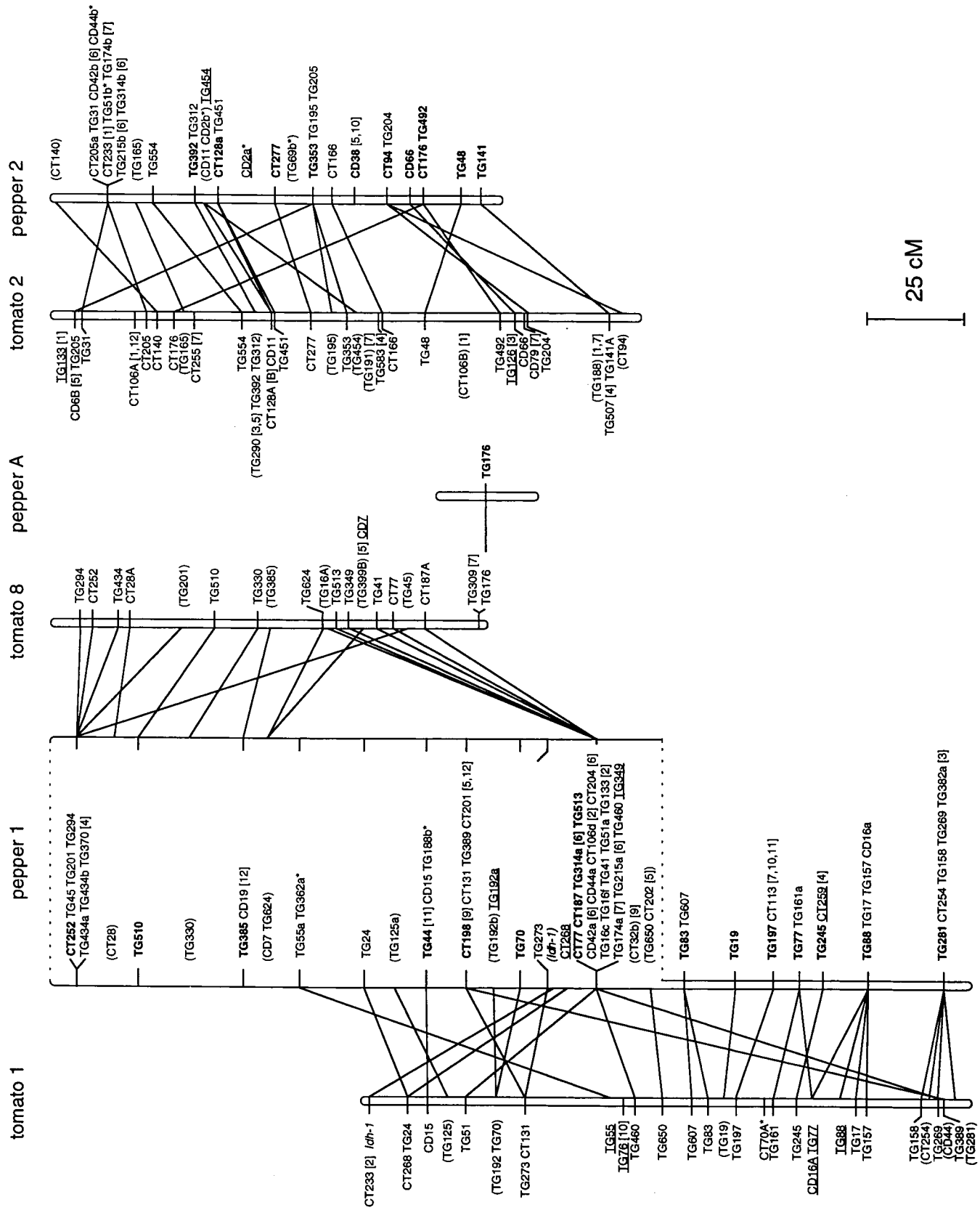


Figure 2.

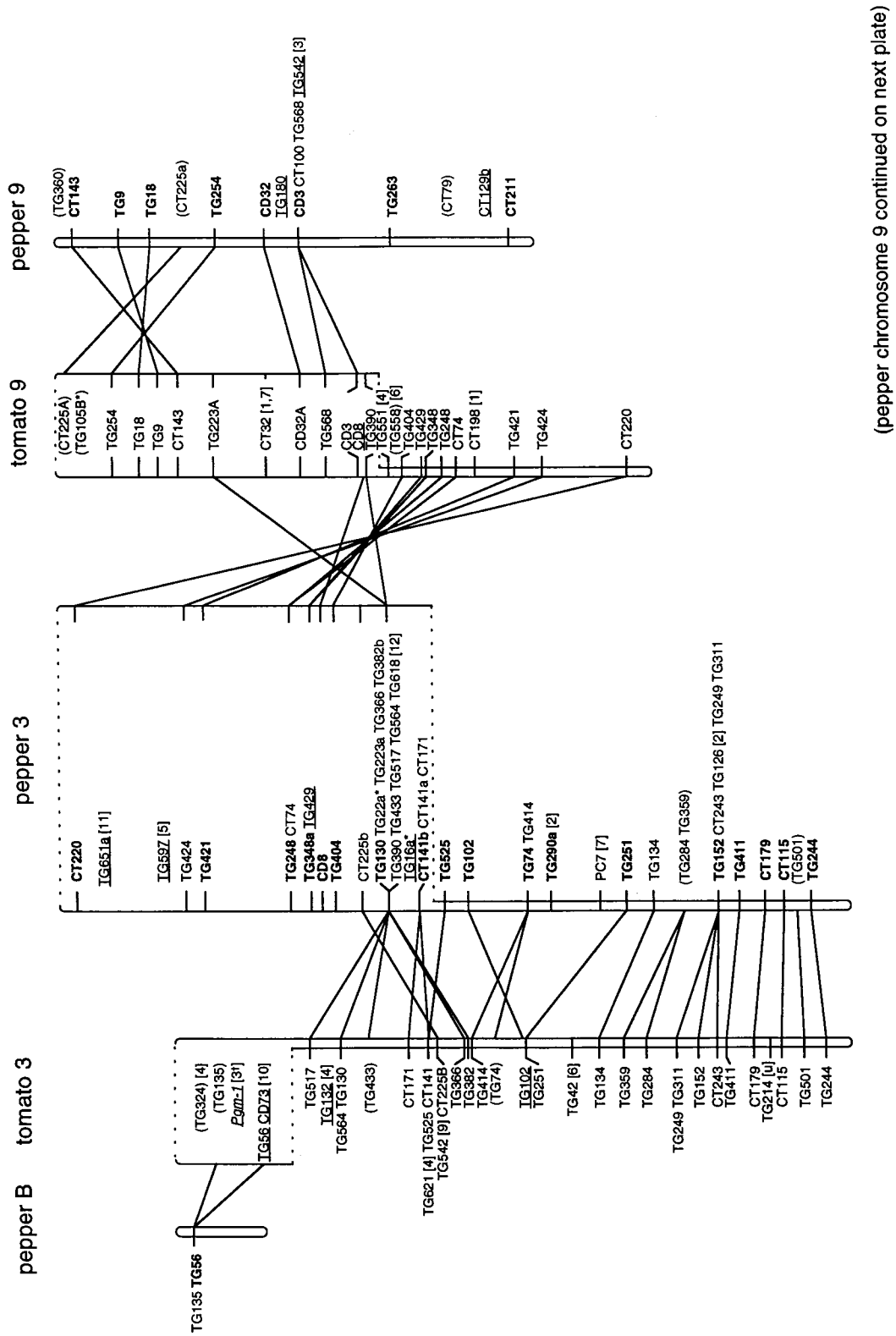
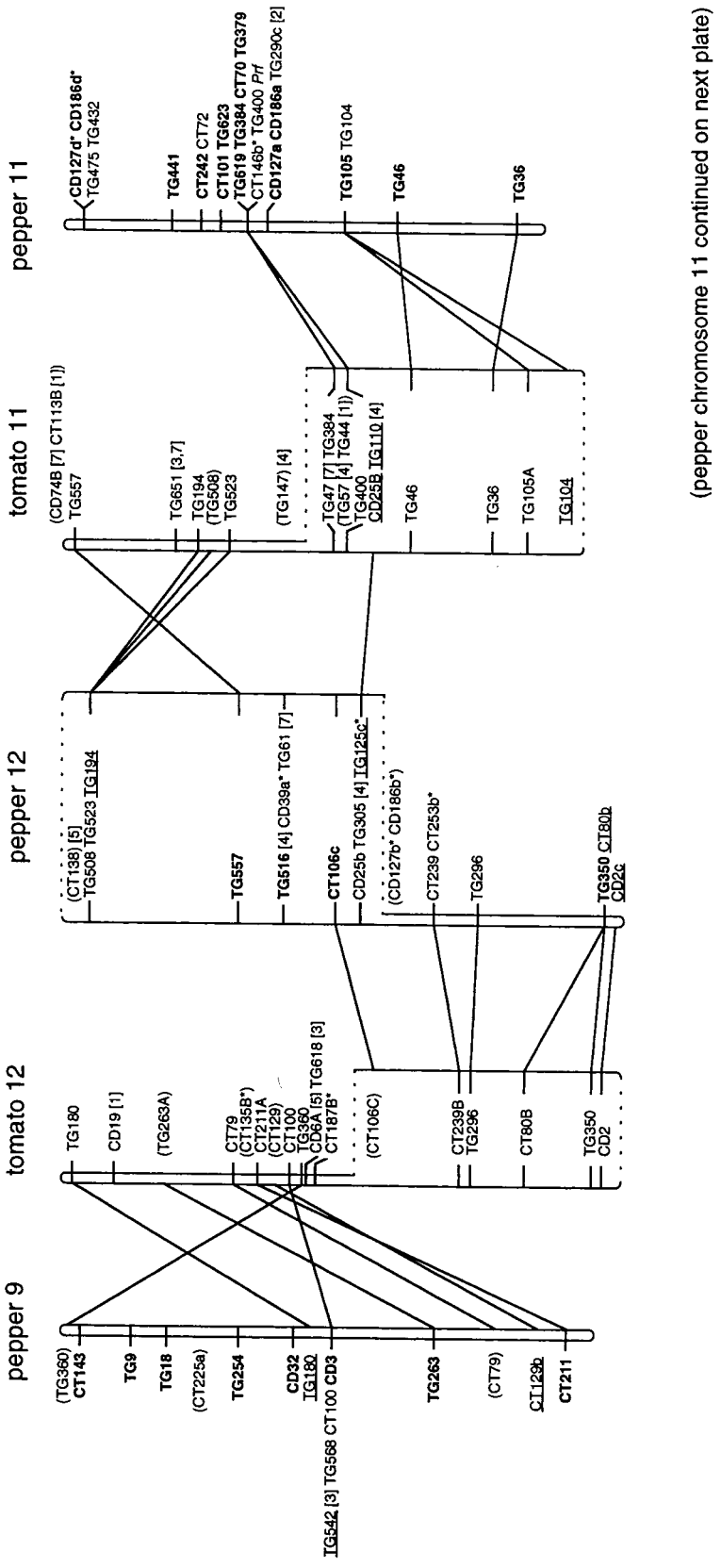


Figure 2.—*Continued.*

genus (a probe reveals a pair of loci that map to syntenous positions and an additional locus in one species); some loci are represented only once on each map not in homeologous segments. In tomato, 11 loci (3.6%) appear to be uniquely duplicated, while 67 loci (21.8%) lack a counterpart in the homeologous pepper segment. In pepper, 40 loci (11.4%) appear to be uniquely duplicated, and 70 loci (19.9%) lack a counterpart on the



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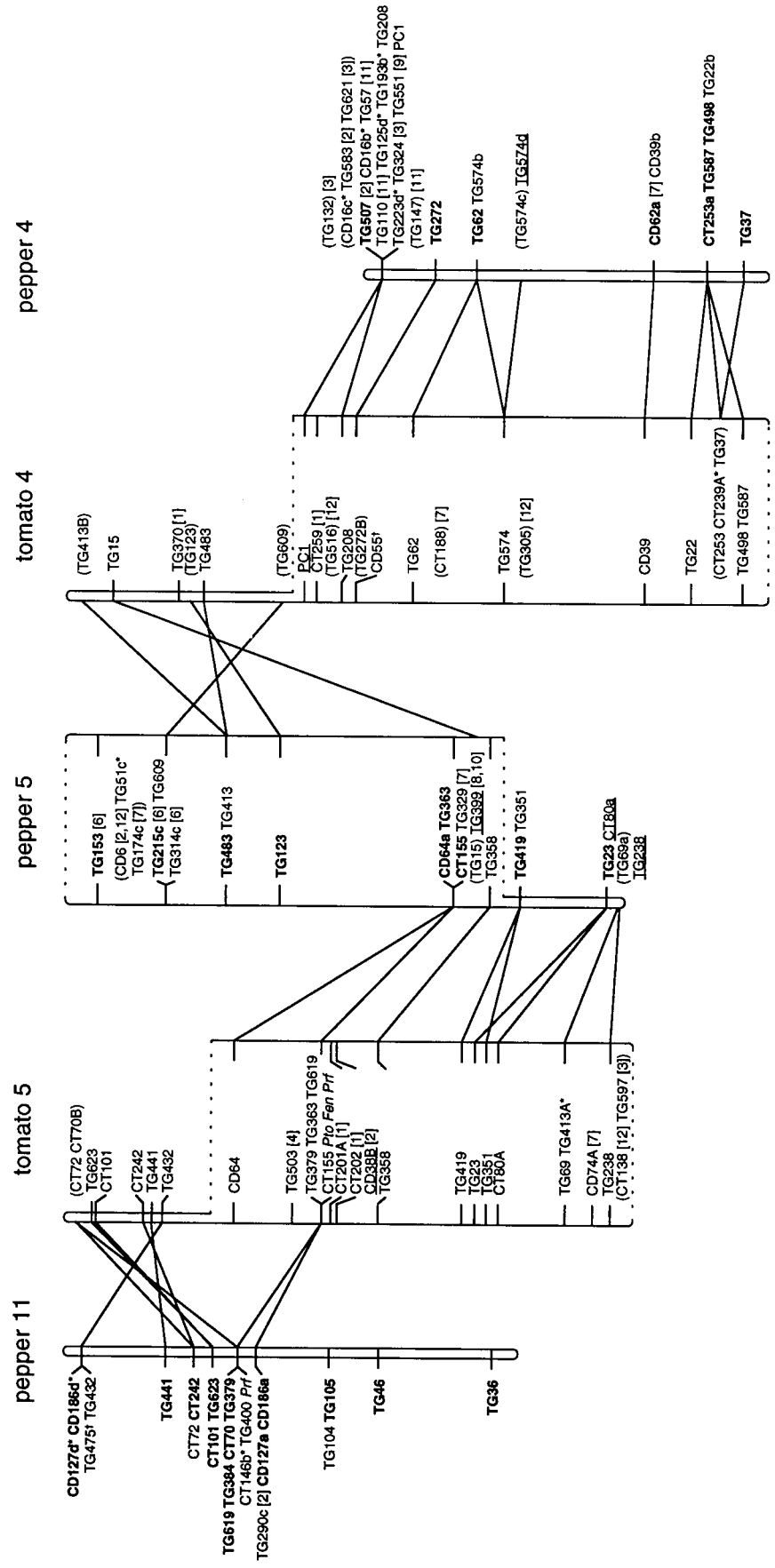

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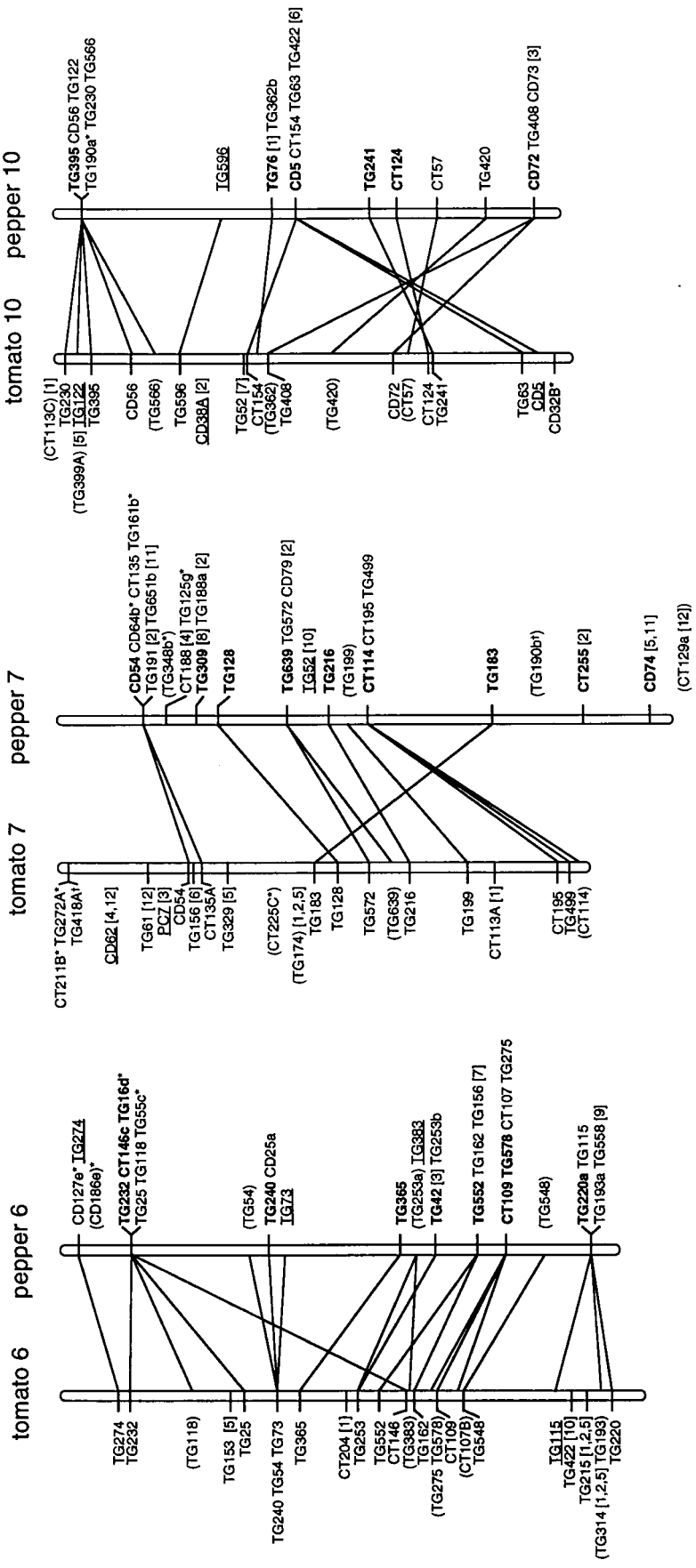

Figure 2.—*Continued.*

homeologous tomato segment. When all 660 loci are considered, 51 loci (7.7%) are uniquely duplicated on 1 map, and 137 loci (20.8%) lack a homolog in the homeologous segment of the other genome. This is comparable to the percentage of markers (20–40%) without meaningful synteny in comparisons among the grasses (Bennetzen and Freeling 1997).

To provide a concise representation of synteny between tomato and pepper, we modified Figure 2 as follows to create Figure 3: all marker names were omitted, indicators for markers with no homologs mapped in the homeologous region were omitted, the positions of the tomato centromeres and the regions of suppressed recombination on the pepper linkage groups were added, and the lines connecting pairs of loci were changed to dashed if one of the loci in a pair was not mapped at framework stringency, was inferred from another map, or was duplicated such that orthology was suspect.

Figures 2 and 3 show that the tomato and pepper genomes share large linkage blocks that have undergone various typical rearrangements during speciation[4]. Four pairs of chromosomes show conservation of content of entire chromosomes: T2-P2, T6-P6, T7-P7, and T10-P10. Both marker locus content and overall structure in the T6-P6 pair appears to have been maintained completely. Two of the other pairs show paracentric inversions, a small part of the lower end of T2/P2, and the lower arm of T10/P10. The T2-P2 pair also includes the region at the top of P2, which is unique to *C. chinense*, and three markers (TG205, CT140, and CT176) that map to nonsyntenous positions. The T7-P7 pair maintains a common core, but differs in the position of TG183 and unique linkages at both ends of P7 and one end of T7, as mentioned previously.

The rest of the genome appears to have been conserved in linkage blocks corresponding to chromosome arms rearranged by nonreciprocal translocations as well as pericentric and paracentric inversions. The complexity of the rearrangements ranges from a single nonreciprocal translocation, in the case of the T4-P4, to multiple nested translocations and inversions seen in the T9-P9-T12 comparison. The rearrangements must also have included multiple pericentric inversions in the P12-T11-P11-T5 chromosomes. The T1-P1-T8 comparison is unique because of the major translocation within Capsicum, but it also appears to conserve whole arms as linkage blocks. The possible exception is the upper arm of T1, although the observed lack of synteny could be caused by mapping artifacts arising from the translocation within pepper. Finally, Figure 3 illustrates the alignment of tomato centromeres with the regions of suppressed recombination in pepper.

The pepper and tomato genomes can then be represented by 18 homeologous segments with unique relationships in each genus. The majority of these segments maintain strict syntenic order throughout, while a few maintain locus content but have undergone paracentric inversions within the segment. Despite the number of rearrangements, chromosomes from both genera appear to contain at most only two conserved segments.

Genome structure in the Solanaceae: The pepper-tomato comparative map can be used with the phylogeny of Capsicum, Lycopersicon, and Solanum (Spooner *et al.* 1993) and the tomato-potato comparative map (Tanksley *et al.* 1992) to identify conserved linkage blocks, to reconstruct portions of the genome of the most recent ancestor of these species, and, in some cases, to determine in which lineage rearrangements occurred. Where pepper and tomato/potato differ, the number of *ad hoc* hypotheses is the same using either condition as the ancestral state, so only two alternative arrangements can be presented. We assumed that paracentric and pericentric inversions and translocations were the prominent mechanisms of structural change, and we did not equate nonsyntenous markers with breaks because no simple way exists to explain these loci concordantly with these typical chromosomal rearrangements.

A1: The ancestral homeologs of chromosome 1 (A1) in tomato and potato are identical (Figure 4). The pepper species differ by a reciprocal translocation with A8 and the small linkage block found on P1 in *C. annuum* and P2 and P5 in *C. chinense*, but not found in tomato/potato. We conclude that A1 was most like tomato/potato, with at least two breaks in the pepper lineages, one to create the A1-A8 translocation and the other to account for the position of the duplicated region in *C. annuum*.

A2: The tomato/potato A2 homeologs are identical, while pepper and tomato/potato differ by a paracentric inversion. The pepper species also differ in the distal region of P2; therefore, at least two breaks differentiate the pepper and tomato/potato lineages, with at least one more break in one of the pepper lineages.

A3: The tomato/potato homeologs are identical to each other and to a segment of pepper, but at least two breaks must have occurred in one of these lineages to account for the differences between pepper and tomato/potato.

A4: The tomato/potato A4 homeologs and most of pepper A4 occur as a single linkage block. The segment attached to tomato/potato A4 is attached to the A5 homolog in pepper in a reversed telomere-centromere orientation and is distinct in the two pepper species: *C. chinense* has an additional linkage block not found in *C. annuum*. As a result, we infer at least one break with a reversal of orientation between tomato/potato and pepper, as well as a break in one of the Capsicum species.

A5: The potato and tomato homeologs of A5 are identical for one arm, but differ by a paracentric inver-

[4] A more exhaustive description of the differences between the tomato and pepper genomes is also available (Livingstone 1999).

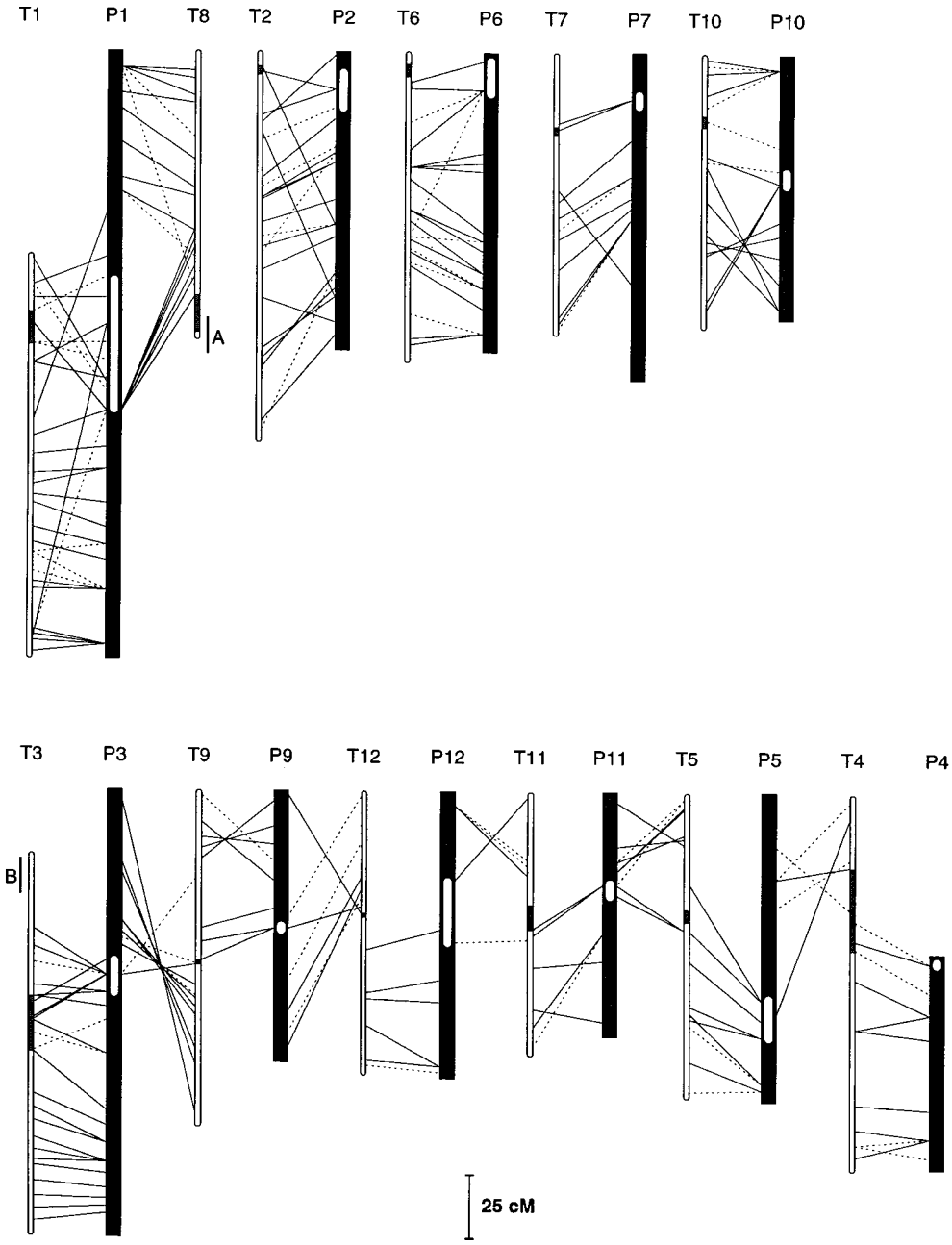

Figure 3.—Comparative genome structure of pepper and tomato. The genetic maps of Figure 2 [pepper chromosomes (P), gray bars; tomato chromosomes (T), white bars] have been reduced to only markers that map to homeologous segments of the genome. These points are connected by solid lines, except where either a clone produced multiple segregating loci in one or both of the species with loci mapping to nonsyntenous positions, making the assignment of paralogous pairs questionable, or one or both of the marker(s) mapped at LOD < 2 or was mapped in a different population. These marker pairs are connected by dashed lines. The positions of the tomato centromeres are indicated by shaded bars or points on the tomato chromosomes. The regions of suppressed recombination on the pepper chromosomes are indicated by white circles or bars. The locations of pepper linkage groups A and B are indicated by bars next to tomato chromosomes 8 and 3.

sion for the other. The noninverted arm of the A5 homeolog is also conserved in pepper, while the other arm is attached to the A11 homeolog. The orientation of this arm in pepper and potato is superficially similar; however, CD64 homologs occur in different linkage blocks of pepper and tomato/potato, indicating that the inversions are independent and that a pericentric inversion occurred early in either the tomato/potato or pepper lineage. We conclude that there was an early pericentric inversion followed by independent paracentric inversions in the potato and pepper lineages.

A6: The structure of this homeolog is maintained in all three species.

A7: The structure is conserved, except for the unique distal linkage blocks.

A8: This chromosome is identical in tomato/potato, but is part of a reciprocal translocation pepper. The other difference between pepper and tomato/potato is the small piece distal to the centromere in tomato/potato that is unlinked in pepper. At least one break occurred in one of the pepper species, and another may have occurred in either the tomato/potato or pepper lineage.

A9: The tomato and potato homeologs of this chromosome differ by a paracentric inversion. Tomato and pepper also differ by a similar paracentric inversion; however, the positions of the breakpoints are different. These data are consistent with either tomato or pepper being closest to the ancestral condition, as both configurations require two breaks and two new telomeres.

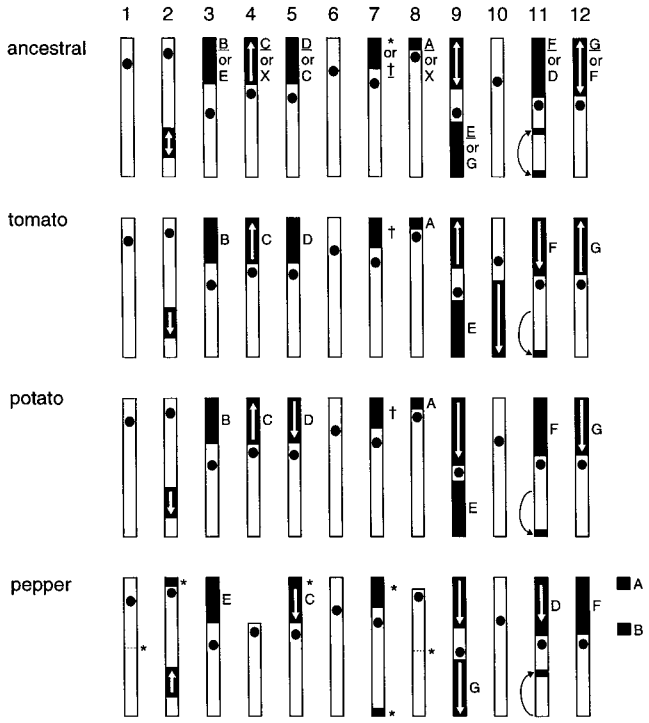

Figure 4.—Overview of genome structure within the Solanaceae. The genetic maps of the 12 chromosomes of tomato, potato, pepper (P), and an inferred configuration for their most recent common ancestor (A) are represented, along with the deviations from this common ancestor within each species. White regions are conserved between the three species and define the ancestral condition. Other white regions are conserved between pepper and either potato or tomato, but not both, indicating a derived condition in the nonidentical species. Gray regions in the ancestral genome denote areas where the ancestral condition cannot be defined. Gray regions in the other genomes show actual or potential differences between that species and the proposed ancestral genome. Inversions are indicated by white arrows within gray bars. An upward direction is defined as the ancestral condition, except for areas on A2, A9, and A12, where orientation could not be determined on the basis of parsimony. On these segments, the arrows in the A genome are double headed, and the direction of the arrows in potato, tomato, and pepper indicate relative orientation only. Rearranged segments are identified by a letter to the right of the segment. In the ancestral genome, all possibilities for a region are indicated (an X indicates absent), and the region present in potato/tomato is underlined. Asterisks denote areas unique to pepper (the extensions at the ends of P7) and differences that have occurred between Capsicum species (the extensions in *C. chinense* at the ends of P2 and P5 and the reciprocal translocation between P1 and P8 in one species). Regions marked with a dagger denote linkage blocks unique to tomato/potato. The curved arrows to the left of A11 homeologs indicate possible movements within the linkage blocks.

Furthermore, the location and arrangement of linkage block G is distinct in all three species; therefore, we cannot ascertain the ancestral position or orientation of G, but all scenarios require at least four breaks. In conclusion, we infer at least one break in the potato lineage and a series of at least four breaks to account for the position and arrangements of the G block.

A10: The potato/pepper and tomato homeologs differ by one paracentric inversion, indicating the paracentric inversion and break has occurred in the tomato lineage.

A11: The tomato and potato homeologs differ by a paracentric inversion. The corresponding pepper arm is similar to that of potato, although attached to a different chromosome. We presume the pepper/potato orientation of this arm is ancestral. The position of TG104/TG105 also differs in tomato/potato and pepper. We conclude that one break and a paracentric inversion occurred in the tomato lineage, and another three breaks and a pericentric inversion occurred in either the tomato/potato or pepper lineage that moved TG104/TG105 and changed the attached linkage blocks.

A12: One arm of the A12 homeolog has been conserved in pepper, tomato, and potato. Tomato and potato differ by a paracentric inversion of the other arm that requires one break in either tomato or potato to account for the inversion and at least one break in either the tomato/potato or pepper lineages to change the attached blocks.

In total, we conclude that there have been at least 2 breaks in the tomato genome, 2 breaks in the potato genome, 1 break in either the tomato or potato genome, 1 break in the pepper genome that differentiates it from tomato/potato, and another 5 breaks to account for the differences between the 2 pepper species. Nineteen other breaks must have occurred in either the pepper lineage or in the common ancestor of tomato/potato. These breaks were part of 5 translocations, 10 paracentric inversions, 2 pericentric inversions, and 4 disassociations or associations of genomic regions that produced the observed genome variation among genera. An additional reciprocal translocation, nonreciprocal translocation, and a duplication or deletion differentiates the two pepper mapping parents.

DISCUSSION

The Capsicum genetic map: The variability between our mapping parents maximized intralocus polymorphism, but also included major structural rearrangements. As a result, we have defined more precisely the differences between the genomic structures of *C. annuum* and *C. chinense*, knowledge that is important to plant breeders because *C. chinense* is a significant source of characters for *C. annuum* breeding. The major reciprocal translocation reduced this map to 11 large linkage groups; however, through comparative mapping, we could establish the locus order for two of the four linkage blocks of the two chromosomes involved. The marker order for the other two blocks will require intraspecific populations genotyped with those markers.

This map contains two small, unlinked groups: P(A),

which is homeologous to the telomeric region of T8, and P(B), which is homeologous to the telomeric region of T3. In the two other interspecific maps constructed in our lab using a *C. frutescens* BG 2814-6 × *C. chinense* PI 159234 F_2 and a *C. frutescens* BG 2814-6 × *C. annuum* RNaky F_2, linkages are seen between TG176 on P(A) and CT252 from the opposite end of T8, and between P(B) and the top end of P4 (Zhang 1997; Y. Zhang, unpublished results). The linkages between P(B) and P4 through TG132 are detectable in the *C. annuum* × *C. chinense* map; however, neither was strong enough (LOD < 2.5, > 30 cM) in this population to assert linkage. The absence of linkage between P(A) and CT252 in our population is puzzling; this linkage is clear when either parent is crossed to *C. frutescens*, but it disappears when the *C. annuum* and *C. chinense* parents are intercrossed. Perhaps *C. annuum* and *C. chinense* differ in the location of P(A), and the strength of the linkage in the *C. frutescens* parent is causing the association in one of the other maps, or P(A) may be separated enough from other detected markers in the linkage group in both *C. annuum* and *C. chinense* that no linkage is apparent. There is support for both hypotheses: Kumar *et al.* (1987) and Lanteri and Pickersgill (1993) have both reported that at least two translocations differentiate these species, and Moscone *et al.* (1993) observed heterochromatic segments at the telomeres of all *C. annuum* chromosomes.

Several Capsicum maps have related linkage groups to chromosomes through a series of primary trisomics generated by Pochard (1977). Unfortunately, this series is no longer complete or available. Because of inconsistencies between markers and their chromosomal associations among recently published maps (Lefebvre and Palloix 1996; Caranta *et al.* 1997a,b; Lefebvre *et al.* 1997), and some significant differences in observed linkages between these maps and our map, we have not assigned our linkage groups to named chromosomes. Moscone *et al.* (1993) have succeeded in differentiating the pepper chromosomes using C banding, indicating that fluorescent *in situ* hybridization could be used with single-copy probes identified in this study to relate the genetic map to Capsicum chromosomes.

Genome size, structure, and evolution in the Solanaceae: *Genome size and content:* The 2C DNA content of pepper is two- to fourfold greater than that of tomato (Arumuganathan and Earle 1991). We observed that all tomato clones tested hybridized to pepper DNA and covered the pepper genetic map. Our results conflict with Prince *et al.* (1993), who observed that clones from regions of tomato chromosomes 1, 2, 6, and 9–12 failed to hybridize with pepper DNA, perhaps because these clones were either more diverged from their pepper homologs or representative of intergenic sequences unique to tomato (Ganal *et al.* 1988). In the case of the former, perhaps different hybridization conditions in our RFLP analysis revealed these more weakly related loci, whereas in the latter case, cross-hybridization would not be expected, as demonstrated by Hulbert *et al.* (1990), Barakat *et al.* (1997), and Bennetzen *et al.* (1998).

Differences between the tomato and pepper genomes with regard to the number of both homologous and segregating loci detected by a probe were apparent, with pepper generally showing a higher copy number. The greater number of probes detecting multiple loci in pepper relative to tomato could be a consequence of the detection of more loci per probe in pepper, or perhaps evidence of a higher degree of interspecific polymorphism within Capsicum compared to the interspecific cross used to construct the tomato map. Regardless, these differences in copy number between the species lacked the patterns that would be associated with systematic duplications. Overall, our observations concur with results from previous work in this system (Tanksley *et al.* 1988) and the grasses (Bennetzen and Freeling 1997) that have shown only limited species-specific duplications and deletions. The absence of systematically duplicated or deleted genomic regions or individual loci effectively rules out paleopolyploidy, duplication, or deletion as explanations for the differences in genome size between pepper and tomato.

Increases in the amount of repeated DNA have been established as a cause of genome expansion in plants (Flavell *et al.* 1974), and recent work in the Gramineae has shown a pattern of increases in retroelements between the genes of large-genome species relative to smaller-genome species (SanMiguel *et al.* 1996; Chen *et al.* 1997; Panstruga *et al.* 1998). A study of repetitive DNA in the pepper genome by An *et al.* (1996) estimated that 5% of the pepper genome was composed of elements with copy number >10,000, 26% with copy number >150, and 65% single-copy sequences. This estimate of the single-copy fraction of the pepper genome appears high in light of our results, unless some of the single-copy sequences are also unique to pepper, but these data do show a significant amount of both high- and medium-copy-number sequences. Our observation that a high percentage of pepper genomic DNA clones detected repeated sequences also points to repeated sequences in the pepper genome. Moreover, retrotransposons are well documented in Capsicum (Flavell *et al.* 1992; Pozueta-Romero *et al.* 1995; K. Livingstone, unpublished results). The extra DNA in pepper relative to tomato cannot all be accounted for by the blocks of constitutive heterochromatin (7% of the total karyotypic length) seen exclusively at the telomeres of *C. annuum* chromosomes (Moscone *et al.* 1993). Therefore, it is probable that the retrotransposons interspersed equally across both the gene-rich and gene-poor regions of the genome, as seen in the Gramineae (Barakat *et al.* 1997), will account for differences in nuclear DNA content between pepper and tomato.

Conservation of linkages: Our comparison of the pepper

and tomato genomes demonstrates overwhelmingly the conservation of marker order from whole-tomato chromosome arms, and even entire chromosomes, in pepper. There were, however, seemingly random interruptions in synteny. These markers may have been what led Tanksley *et al.* (1988) to conclude that many of the pepper chromosomes were comprised of many independent tomato segments.

One observation emerging from other comparative studies is that these rogue markers seem to be concentrated in centromeric and telomeric regions (Moore *et al.* 1997). This same result is manifest in our comparison at the centromeres of P1, P2, P4, P7, P12, T4, T5, T9, and T12, and the telomeres of P2, P4, P5, T3, T8, and T11. This phenomenon may be a function of the concentration of breaks at these sites; however, the specific mechanisms that account for marker accumulation and loss at these breakpoints remains unknown. Another general mechanism that could possibly explain the appearance of nonsyntenous markers is excision via intrachromosomal recombination of direct repeats, followed by integration at distant sites. This process has been shown to move transgenes to distant sites in the genome of transformed tobacco (Peterhans *et al.* 1990). If retrotransposons, which have been associated with duplications and rearrangements in the genome of *Saccharomyces cerevisiae* (Kim *et al.* 1998b), acted as direct repeats in this process, it is not difficult to see how the present situation could have developed.

Genome rearrangement: Our estimate of the minimum number of breaks that differentiate tomato and pepper (22) is 7 greater than that reported by Prince *et al.* (1993), but 10 less than that reported by Tanksley *et al.* (1988). The probable cause of the differences in these estimates is the extent of coverage of the tomato genome in each study. By looking at the entire tomato genome as it is represented in pepper, we have been able to both see the larger patterns of genome reorganization and refine the estimate of the overall number of events that have occurred since divergence.

This study joins with others that begin to provide glimpses into the complex nature and mechanisms of genome structural rearrangements. Similar to our results, inversions were also a major contributor to the extensive rearrangement of the *Brassica nigra* genome relative to Arabidopsis (Lagercrantz 1998). Clues to the biology underlying these rearrangements include this and other studies of intraspecific karyotype diversity (Gill *et al.* 1980; Badaeva *et al.* 1994) and comparative mapping (Moore *et al.* 1997) that have shown translocation breakpoints to occur more frequently at centromeres. Although to the best of our knowledge no comparable study has been done for inversions, the analogous localization of inversion breakpoints to regions of the genome would make similar independent inversions more likely, such as those we observed in the upper arms of T5 and T9 in pepper and potato and those seen by Caccone *et al.* (1998) in mosquitoes.

In the recent comparison of Arabidopsis and *B. nigra*, Lagercrantz (1998) reported that interstitial telomeric repeats colocalized with rearrangement breakpoints. Telomeric sequences have been mapped to the centromeric regions of eight tomato chromosomes (Presting *et al.* 1996), and all but one of the translocation breakpoints between tomato/potato and pepper appear at the centromere or putative centromere of the respective chromosome. Tomato telomeric sequences appear at tomato centromeres, where we believe the chromosomes have been truncated in pepper (T4 and T8); at the centromeres of T5, T9, T11, and T12, which are breakpoints; and at the centromeres of T3 and T7, which may or may not be sites of rearrangement. No telomeric sequences were mapped, however, to the breakpoints that we concluded were unique to tomato, T10 and T11, although this may result from lack of polymorphism or the pericentric inversion we believe occurred on either T11/P11. These results, therefore, support both the observation that centromeres are important sites for chromosomal rearrangement and the hypothesis that interstitial telomeric repeats may be a critical if not a causal link between centromeres and these events.

Three interrelated lines of evidence imply that the majority of the unplaced chromosomal rearrangements probably occurred in the pepper lineage. (i) We have observed at least five differences between the parental pepper species that are equal to the number of differences between potato and tomato (Tanksley *et al.* 1992); therefore, propensity toward rearrangement may be an inherent property of the pepper genome, a trend noticed earlier by Lanteri and Pickersgill (1993). (ii) The increase in the size of the pepper genome, without apparent increases in gene content, implicates expansion of heterochromatin in this genome. Heterochromatin has been positively correlated with the amount of rearrangement in a genome (Prokofieva-Belgovskaya 1986). (iii) Retrotransposons probably make up the bulk of the extra DNA in the pepper genome, and retroelements have been associated with chromosomal rearrangements in plants (Robbins *et al.* 1989; Belzile and Yoder 1994), yeast (Kim *et al.* 1998b), mosquitoes (Mathiopoulos *et al.* 1998), and Drosophila (Engels and Preston 1984; Lim 1988; Lyttle and Haymer 1992; Sheen *et al.* 1993; Eggleston *et al.* 1996; Ladeveze *et al.* 1998; O'Hare *et al.* 1998).

Segregation distortion factors: Many of the markers in our population displayed significant deviations from their expected Mendelian ratios. While this observation has been made in many interspecific plant populations (*e.g.*, Zamir and Tadmor 1986), comparisons of the amount and direction of distortion across different genera are limited. We have identified regions that display consistent segregation distortion both within a genus

and across genera. Understanding of the loci controlling this behavior has important practical applications for breeders and biological implications for the evolution of these genera. The recent cloning of the *Segregation distorter* locus from Drosophila (Merrill *et al.* 1999) shows that molecular study of these loci is feasible. Loci with conserved distortion functionality apparent in multiple genera would make appropriate initial targets for molecular characterization in plants.

Genome size, genetic length, and implications for recombination: The lengths of the tomato and pepper genetic maps are almost identical: 1275 cM in tomato *vs.* 1246 cM in pepper. Figures 2 and 3 illustrate that most of the intervals between adjacent syntenous markers are approximately equal in pepper and tomato despite comparisons of dual interspecific maps. The similar lengths of the genetic maps, together with the difference in DNA content, indicate that the average recombination rate per unit of physical distance is not the same in pepper and tomato. This is what would be expected if recombination was restricted only to homologous genes, as originally proposed by Thurieaux (1977) and shown in maize (Dooner *et al.* 1985; Dooner 1986; Brown and Sundaresan 1991; Civardi *et al.* 1994; Eggleston *et al.* 1995; Patterson *et al.* 1995; Dooner and Martínez-Férez 1997).

Conclusions: We have produced a moderate-density map of the Capsicum genome using tomato-derived clones distributed across the tomato genome. Comparison of this map with the tomato map shows that: (i) insofar as has been analyzed, the genic contents of tomato and pepper are equivalent; (ii) virtually all the tomato and pepper genomes are covered by conserved linkage blocks; (iii) the overall genetic lengths of pepper and tomato are approximately equal despite at least a twofold increase in the 2C content of the pepper genome; (iv) a typical number of common types of chromosomal rearrangements differentiate these genomes. This study unites the tomato-pepper comparison with results from other dicotyledonous genera (*cf.* Tanksley *et al.* 1992; Weeden *et al.* 1992; Menancio-Hautea *et al.* 1993; Kowalski *et al.* 1994; Cheung *et al.* 1997; Lagercrantz 1998) and the grasses (reviewed recently in Bennetzen and Freeling 1997; Bennetzen *et al.* 1998; Gale and Devos 1998a,b), which all show conservation of content within large linkage blocks, but not associations between blocks, as the primary consequences of chromosomal evolution.

The ability to compare phenotypes genetically mapped to orthologous positions of the different genomes will shed light on genes that operate across the Solanaceae. This map will also contribute to the understanding of pepper at the molecular level through comparative genomics between pepper and tomato, and between pepper and Arabidopsis via tomato-Arabidopsis comparisons now underway (S. Tanksley, personal communication). The Solanaceae, with whole-genome comparisons available across three genera, now stands as the broadest and most thoroughly characterized comparative genetic system in dicotyledonous plants.

The authors acknowledge J. Prince, E. Radwanski, J. Jantz, M. Cadle, L. Landry, and G. Moriarty for their assistance. We thank Drs. S. Tanksley for clones and access to unpublished data; B.-D. Kim, U. Bonas, M. Massoudi, K. Welch, A. Palloix, and V. Lefebvre for markers and unpublished data; and N. Weeden for isozyme analyses. We thank R. Grube, A. Matern, L. Landry, M. Sorrells, S. Tanksley, and two anonymous reviewers for critical reading and suggestions that improved the manuscript. K.D.L. and J.R.B. were supported in part by a Department of Energy / National Science Foundation / United States Department of Agriculture grant to the Research Training Group in Molecular Mechanisms of Plant Processes and in part by Marie Lavallard. Financial and material support was provided by Seminis Vegetable Seeds, the California Pepper Commission / California Pepper Improvement Foundation, Gilroy Foods, Mr. C. Werly, USDA NRICGP awards 91-37300-6564 and 94-37300-0333, and BARD award IS-2389-94.

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Communicating editor: J. A. Birchler