High Density Molecular Linkage Maps of the Tomato and Potato Genomes

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ABSTRACT

High density molecular linkage maps, comprised of more than 1000 markers with an average spacing between markers of approximately 1.2 cM (*ca.* 900 kb), have been constructed for the tomato and potato genomes. As the two maps are based on a common set of probes, it was possible to determine, with a high degree of precision, the breakpoints corresponding to 5 chromosomal inversions that differentiate the tomato and potato genomes. All of the inversions appear to have resulted from single breakpoints at or near the centromeres of the affected chromosomes, the result being the inversion of entire chromosome arms. While the crossing over rate among chromosomes appears to be uniformly distributed with respect to chromosome size, there is tremendous heterogeneity of crossing over within chromosomes. Regions of the map corresponding to centromeres and centromeric heterochromatin, and in some instances telomeres, experience up to 10-fold less recombination than other areas of the genome. Overall, 28% of the mapped loci reside in areas of putatively suppressed recombination. This includes loci corresponding to both random, single copy genomic clones and transcribed genes (detected with cDNA probes). The extreme heterogeneity of crossing over within chromosomes has both practical and evolutionary implications. Currently tomato and potato are among the most thoroughly mapped eukaryotic species and the availability of high density molecular linkage maps should facilitate chromosome walking, quantitative trait mapping, marker-assisted breeding and evolutionary studies in these two important and well studied crop species.

IN 1980 BOTSTEIN *et al.* proposed the construction of a genetic linkage map in humans based on restriction fragment length polymorphisms (RFLPs) (BOTSTEIN *et al.*, 1980). The success of their idea has been verified by the fact that RFLP linkage maps have already been constructed, not only for humans, but for a wide variety of other organisms (see O'BRIEN 1990). Most of the RFLP maps published to date have been of low or moderate density (*i.e.*, average marker spacing >5 cM) and have included 50–300 markers. While these maps are useful tools for many genetic endeavors, they have inherent limitations that would be overcome by the development of high density maps in which the markers are spaced at very close intervals throughout the genome.

High density maps can serve a number of purposes in basic and applied research. First, they are a key tool for chromosome walking. In order to clone a gene by chromosome walking, it is necessary to identify molecular marker(s) closely linked to the gene of interest to provide a starting point for the walk (WICKING and WILLIAMSON 1991). High density linkage maps can provide starting points for chromosome walks to virtually any gene in the genome. Second, high density maps have direct application in plant and animal breeding since they virtually assure that any gene of interest will be tightly linked to at least one molecular marker. Such tight linkages can be exploited for marker-based selection of desirable genes in breeding programs (BURR *et al.* 1983; TANKSLEY *et al.* 1989). Finally, high density linkage maps provide a greater probability that the entire genome is completely covered with molecular markers (*i.e.*, there are no large, markerless gaps in the map). This last point is especially important when using molecular linkage maps to detect and characterize loci underlying quantitative traits where one needs to be assured that the entire genome has been uniformly surveyed (PATERSON *et al.* 1988; LANDER and BOTSTEIN 1989).

To facilitate map-based cloning in crop plants and establish a general tool for marker-enhanced breeding, we have constructed a high density map of the tomato/potato genomes comprised of more than 1000 markers (mainly RFLPs). A number of morphological and isozyme markers have also been mapped with respect to RFLP markers making it possible to orient the molecular linkage map with both the classical morphological and cytological maps of tomato. This level of marker saturation offers new opportunities for genome research in tomato and potato and reveals a number of interesting features of chromosome structure and evolution that were not apparent from previous linkage maps of the tomato/potato genomes (BERNATZKY and TANKSLEY 1986; BONIERBALE, PLAISTED and TANKSLEY 1988).

MATERIALS AND METHODS

Tomato: A population of 67 F_2 plants derived from the interspecific cross *Lycopersicon esculentum* cv VF36-*Tm2a* × *Lycopsersicon pennellii* LA716 served as the mapping population for tomato. The two species used in this cross have the same chromosome constitution ($2n = 2x = 24$) and all evidence suggests that their genomes are homosequential (KHUSH and RICK 1963). Moreover, interspecific hybrids between the two species are highly fertile and demonstrate near normal levels of meiotic pairing and crossing over (KHUSH and RICK 1963; RICK 1969).

Potato: A population of 155 plants, derived from the cross *Solanum tuberosum* ($2x = 24$) × *Solanum berthaultii* backcrossed to *S. berthaultii*, constituted the mapping population for potato. *S. tuberosum* and *S. berthaultii* are closely related taxonomically and were previously known to give rise to fertile hybrids (MEHLENBACHER, PLAISTED and TINGEY 1983).

Probes and markers: Random single and low copy RFLP probes were obtained from three cDNA libraries [CD = derived from total mRNA from tomato leaves, (BERNATZKY and TANKSLEY 1986); CT = derived from mRNA from tomato epidermal tissue (YU *et al.*, in preparation); CP = derived from total mRNA of potato (GEBHARDT *et al.* 1989)] and four genomic libraries [TG = derived from *Pst*I or *Eco*RI size-selected tomato genomic fragments (MILLER and TANKSLEY 1990); size-selected sheared tomato genomic clones (ZAMIR and TANKSLEY 1988); GP = *Pst*I size-selected potato genomic fragments (GEBHARDT *et al.* 1989)]. A number of clones corresponding to known genes were also used as probes for RFLP mapping and are listed in Table 1. Probes maintained at Cornell University are available upon request.

Methods used for probe preparation, Southern hybridization and autoradiography were the same as previously published with the exception that clone inserts were polymerase chain reaction (PCR) amplified before radiolabeling (BERNATZKY and TANKSLEY 1986).

Isozyme markers segregating in the tomato population were scored using previously described methods (VALLEJOS 1983). The positions of morphological markers on the molecular map (including many disease resistance genes) were determined from previously published work or were obtained by direct mapping against RFLP markers in segregating populations (see Table 1 for references).

Map construction: All autoradiographs from segregating populations were scored at least twice by different individuals. Ambiguous genotypes were treated as missing data for map construction. Both the tomato and potato maps were constructed using MapMaker software on a Sun II workstation (LANDER *et al.* 1987). All pairs of linked markers were first identified using the 'group' command LOD > 5, R_F = 0.20. Cosegregating markers (*e.g.*, markers showing no recombination with one another) were identified by scanning two-point linkage data. Framework maps were constructed using only one marker from each set of cosegregating markers. The "orders" command was used to establish the framework order of markers within groups and the "ripples" command was used to verify the order. Markers were retained within the framework map only if the LOD value for ripples was >3 (probability of deduced sets of triplet orders 1000 times more likely than alternative orders). All remaining markers were assigned to intervals within the LOD 3 framework using the "try" command. Map units (centimorgans, cM) between markers were calculated using the KOSAMBI (1944) function. The chromosomal affiliation of each linkage group was established by identifying, within each group, markers of known chromosomal position based on previously published work (BERNATZKY and TANKSLEY 1986).

RESULTS AND DISCUSSION

Tomato map

Marker distribution: A total of 1030 molecular markers were mapped onto the tomato genome and together these markers cover 1276 map units (Figure 1). The haploid DNA content of tomato is estimated to be approximately 950 Mbp (ARUMUGANATHAN and EARLE 1991) which means that, on average, 1 cM equals approximately 750 kb. However, this is only an average value and probably varies tremendously depending on which portion of the map is being considered (see next section). While each chromosome has at least 50 markers, the markers are not uniformly distributed across the chromosomes (Figure 2). The tomato chromosomes differ considerably in size (and presumably DNA content) and these differences may account for much of the differential distribution of markers. For example, the three largest chromosomes (chromosomes *1–3*) together contain 38% of the markers, whereas the three smallest chromosomes (chromosomes *10–12*) contain only 18% (Figures 1 and 2). A plot of the pachytene length of each chromosome *vs.* the number of total markers per chromosome reveals a tight association between these two parameters ($r = 0.95$, Table 2). The length of mitotic metaphase chromosomes is also highly correlated with the number of markers per chromosome ($r = 0.90$) (Table 2). The only chromosome for which the marker content is not well predicted by the chromosome length is chromosome *5* (Figure 2). Chromosome *5* is the fifth largest chromosome according to measurement of pachytene chromosomes (BARTON 1950), yet it ranks 10th in terms of its marker content and has substantially fewer markers than chromosome *6* which has a pachytene length nearly identical to chromosome *5* (Figure 2). RAMANNA and PRAKKEN (1967) proposed reclassifying chromosome *5* as one of the shortest chromosomes (also based on pachytene and somatic lengths). Similar conclusions were reached by SHERMAN and STACK (1992) based on two-dimensional spreads of synaptonemal complexes. The results presented here also support this proposal.

The relative number of loci corresponding to putative coding regions (*i.e.*, loci detected with cDNA probes) *vs.* that corresponding to random genomic clones also differs considerably from chromosome to chromosome (Figure 2). For example, the largest chromosomes (*1–3*) have more total markers but a lower proportion of loci corresponding to cDNAs than do the smaller chromosomes (Figure 2). A chi-square contingency test reveals that the differences in proportion of cDNA markers among the different chromosomes are significant ($P = 0.02$).

Distribution of map units: The number of map

TABLE 1

Genes of known function or phenotype that have been mapped onto the molecular map of tomato/potato (morph = morphological marker)

Gene	Type	Product/phenotype	Chromosome	Reference
6Pgdh-1	Isozyme	6-Phosphogluconate dehydrogenase	*4*	TANKSLEY and KUEHN (1985); BERNATZKY and TANKSLEY (1986)
6Pgdh-2	Isozyme	6-Phosphogluconate dehydrogenase	*12*	TANKSLEY and KUEHN (1985)
6Pgdh-3	Isozyme	6-Phosphogluconate dehydrogenase	*5*	TANKSLEY and KUEHN (1985); BONIERBALE, PLAISTED and TANKSLEY (1988)
a	Morph	Anthocyaninless	*11*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
ae	Morph	Entirely anthocyaninless	*8*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
af	Morph	Anthocyanin free	*5*	RICK (1980); G. B. MARTIN and S. D. TANKSLEY (unpublished data)
ag	Morph	Anthocyanin gainer	*10*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
alb	Morph	Albescent	*12*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
ACC1	RFLP	ACC synthase	*8*	ROTTMAN *et al.* (1991)
ACC2	RFLP	ACC synthase	*1*	ROTTMAN *et al.* (1991)
ACC3	RFLP	ACC synthase	*2*	ROTTMAN *et al.* (1991)
ACC4	RFLP	ACC synthase	*5*	ROTTMAN *et al.* (1991)
Aco-1	Isozyme	Aconitase	*12*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Aco-2	Isozyme	Aconitase	*7*	TANKSLEY and RICK (1980) BERNATZKY and TANKSLEY (1986)
Adh-1	Isozyme	Alcohol dehydrogenase	*4*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Adh-2	Isozyme	Alcohol dehydrogenase	*6*	TANKSLEY and JONES (1981); this report
Aps-1	Isozyme	Acid phosphatase	*6*	TANKSLEY and RICK (1980); this report
Aps-2	Isozyme	Acid phosphatase	*8*	TANKSLEY and RICK (1980); this report
B	Morph	Beta carotene	*6*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
CAB1	RFLP	Chlorophyll *a/b* binding polypeptide	*2*	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
CAB2	RFLP	Chlorophyll *a/b* binding polypeptide	*8*	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
CAB3	RFLP	Chlorophyll *a/b* binding polypeptide	*3*	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
CAB4	RFLP	Chlorophyll *a/b* binding polypeptide	*7*	PICHERSKY *et al.* (1987a); this report
CAB5	RFLP	Chlorophyll *a/b* binding polypeptide	*12*	PICHERSKY *et al.* (1987a); S. D. TANKSLEY, unpublished data
CAB6	RFLP	Chlorophyll *a/b* binding polypeptide	*5*	PICHERSKY *et al.* (1987b); this report
CAB7	RFLP	Chlorophyll *a/b* binding polypeptide	*10*	PICHERSKY *et al.* (1988); this report
CAB8	RFLP	Chlorophyll *a/b* binding polypeptide	*10*	PICHERSKY *et al.* (1989); this report
CAB11	RFLP	Chlorophyll *a/b* binding polypeptide	*6*	SCHWARTZ *et al* (1991)
CAB12	RFLP	Chlorophyll *a/b* binding polypeptide	*3*	SCHWARTZ *et al.* (1991)
Cf-2	Morph	Resistance to Cladosporium fulvum	*6*	JONES, DICKINSON and JONES (1991)
Cf-9	Morph	Resistance to Cladosporium fulvum	*1*	JONES, DICKINSON and JONES (1991)
CHS1	RFLP	Chalcone synthase	*6*	A. DREWS and R. GOLDBERG, personal communication
CHS3	RFLP	Chalcone synthase	*5*	A. DREWS and R. GOLDBERG, personal communication
CHS4	RFLP	Chalcone synthase	*9*	A. DREWS and R. GOLDBERG, personal communication
E4	RFLP	Ethylene inducible polypeptide	*3*	LINCOLN *et al.* (1987); this report
E8A	RFLP	Ethylene inducible polypeptide	*9*	LINCOLN *et al.* (1987); this report
E8B	RFLP	Ethylene inducible polypeptide	*3*	LINCOLN *et al.* (1987); this report
Est-1	Isozyme	Esterase	*2*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Est-2	Isozyme	Esterase	*9*	TANKSLEY and RICK (1980); this report
Est-4	Isozyme	Esterase	*12*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Est-5	Isozyme	Esterase	*2*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Est-6	Isozyme	Esterase	*2*	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)

TABLE 1—*Continued*

Gene	Type	Product/phenotype	Chromosome	Reference
Est-7	Isozyme	Esterase	2	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Got-1	Isozyme	Glutamate oxyaloacetate transaminase	4	TANKSLEY and RICK (1980)
Got-2	Isozyme	Glutamate oxyaloacetate transaminase	7	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Got-3	Isozyme	Glutamate oxyaloacetate transaminase	7	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
h	Morph	Hairs absent	10	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpub-
hl	Morph	Hairless	11	RICK (1980); KINZER, SCHWAGER and MUTSCHER (1990)
HMG2	RFLP	HMG CoA reductase	2	J. NARITA and W, GRUISSEM (in preparation)
HMG3	RFLP	HMG CoA reductase	3	J. NARITA and W. GRUISSEM (in preparation)
HOX7A	RFLP	Homeobox gene (tomato)	2	K. SCHARF (personal communication)
HOX7B	RFLP	Homeobox gene (tomato)	2	K. SCHARF (personal communication)
HOX7C	RFLP	Homeobox gene (tomato)	10	K. SCHARF (personal communication)
HSF8	RFLP	Heat shock transcription factor	2	SCHARF *et al.* (1990)
HSF24	RFLP	Heat shock transcription factor	8	SCHARF *et al.* (1990)
HSF30	RFLP	Heat shock transcription factor	8	SCHARF *et al.* (1990)
hy	Morph	Homogeneous yellow	10	RICK (1980); KINZER, SCHWAGER and MUTSCHER (1990); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
I-2	Morph	Resistance to *Fusarium oxysporum* race 2	11	SAFARTTI *et al.* (1989)
I-3	Morph	Resistance to *F. oxysporum* race 3	7	BOURNIVAL VALLEJOS and SCOTT (1989): TANKSLEY and COSTELLO (1991)
Idh-1	Isozyme	Isocitrate dehydrogenase	1	BERNATZKY and TANKSLEY (1986)
j	Morph	Jointless	11	RICK (1980); R. A. WING and S. D. TANKSLEY (unpublished data)
L-2	Morph	Lutescent-2	10	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
Mdh-3	Isozyme	Malate dehydrogenase	7	S. D. TANKSLEY (unpublished data)
Mi	Morph	Resistance to root know nematodes	6	MESSEGEUR *et al.* (1991)
nor	Morph	Nonripening	10	RICK (1980); J. GIOVANNONI and S. D. TANKSLEY (unpublished data)
Nr	Morph	Never ripe	9	RICK (1980); J. GIOVANNONI and S. D. TANKSLEY (unpublished data)
PGAL	RFLP	Polygalaturonidase	10	KINZER, SCHWAGER and MUTSCHER (1990); this report
Pgi-1	Isozyme	Phosphoglucoisomerase (cytosolic)	12	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Pgm-1	Isozyme	Phosphoglucomutase (plastid)	3	BERNATZKY and TANKSLEY (1986)
Pgm-2	Isozyme	Phosphoglucomutase (cytosolic)	4	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
PPO	RFLP	Polyphenol oxidase	8	S. NEWMAN (submitted)
Prx-1	Isozyme	Peroxidase	1	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Prx-2	Isozyme	Peroxidase	2	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Prx-3	Isozyme	Peroxidase	2	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
Prx-7	Isozyme	Peroxidase	3	TANKSLEY and RICK (1980); BERNATZKY and TANKSLEY (1986)
PTC	RFLP	Phytochrome	10	LISSEMORE, COLBERT and QUAIL (1987)
PTN	RFLP	Patatin (tuber storage protein)	8	BONIERBALE, PLAISTED and TANKSLEY (1988); GANAL *et al.* (1990)
Pto	Morph	Resistance to Pseudomonase syringae	5	MARTIN, WILLIAMS and TANKSLEY (1991)
rin	Morph	Ripening inhibitor	5	J. GIOVANNONI and S. D. TANKSLEY (unpublished data)
R45s	RFLP	45S ribosomal RNA	2	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
R5s	RFLP	5S ribosomal RNA	1	LAPITAN, GANAL and TANKSLEY (1991)
RBCS1	RFLP	ss ribulose bisphosphate carboxylase	2	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)

TABLE 1—*Continued*

Gene	Type	Product/phenotype	Chromosome	Reference
RBCS2	RFLP	ss ribulose bisphosphate carboxylase	*3*	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
RBCS3	RFLP	ss ribulose bisphosphate carboxylase	*2*	VALLEJOS, TANKSLEY and BERNATZKY (1986); BERNATZKY and TANKSLEY (1986)
Skdh-1	Isozyme	Shikimic acid dehydrogenase	*1*	BERNATZKY and TANKSLEY (1986)
Sm	Morph	Resistance to Stemphilium	*11*	BEHARE *et al.* (1991)
Sod-2	Isozyme	Superoxide dismutase	*1*	D. ZAMIR (unpublished data)
sp	Morph	Self-pruning	*6*	PATERSON *et al.* (1988)
spa	Morph	Sparsa	*8*	RICK (1980); S. GRANDILLO and S. D. TANKSLEY (unpublished data)
tf	Morph	Trifoliate	*5*	RICK (1980); G. B. MARTIN and S. D. TANKSLEY (unpublished data)
T311	RFLP	5′ patatin class I promoter	*3*	GANAL, LAPITAN and TANKSLEY (1991)
Tm-1	Morph	Resistance to tobacco mosaic virus	*2*	LEVESQUE *et al.* (1990)
Tm-2a	Morph	Resistance to tobacco mosaid virus	*9*	YOUNG *et al.* (1988)
TOM25A	RFLP	Ripening related	*6*	KINZER, SCHWAGER and MUTSCHER (1990)
TOM25B	RFLP	Ripening related	*6*	KINZER, SCHWAGER and MUTSCHER (1990)
Tpi-2	Isozyme	Triose phosphate isomerase	*4*	TANKSLEY and RICK (1980), BERNATZKY and TANKSLEY (1986)
u	Morph	Uniform ripening	*10*	PATERSON *et al.* (1988); KINZER, SCHWAGER and MUTSCHER (1990)
Ve	Morph	Resistance to Verticillium	*7*	JUVICK, BOLKAN and TANKSLEY (1991)
Wx	RFLP	Waxy	*8*	GEBHARDT *et al.* (1989)
y	Morph	Yellow flesh (potato)	*3*	BONIERBALE, PLAISTED and TANKSLEY (1988)

References are for mapping of loci onto molecular linkage map and/or source of probe used for such mapping.

units per chromosome is tightly correlated with both number of markers per chromosome and pachytene length ($r = 0.84$ and 0.89, respectively) (Table 2). To test the nature of the relationships among these variables, slopes from linear regressions were calculated for normalized plots between pachytene length and cM/number of markers per chromosome. The slope for pachytene length *vs.* cM/chromosome was 0.89 ± 0.15 and the slope for pachytene length *vs.* number of markers/chromosome was 0.95 ± 0.10. The fact that both of these slopes are very close to 1.0 indicates that, in general, as the pachytene length of the chromosomes increases, the number of map units and number of markers increases in direct linear proportion. These findings are in contrast with earlier studies in tomato which suggested a nonlinear relationship between the cytological and genetic maps (KHUSH and RICK 1968). The reason for this discrepancy is unknown but may be due to the fact that the earlier analyses were based on incomplete genetic maps–a variable of uncertainty acknowledged by authors of that earlier work.

Heterogeneity of marker density along the genetic linkage map: While the number of markers *among* linkage groups appears to be uniformly distributed according to chromosome size, the distribution of map units *within* chromosomes varies dramatically, depending on which part of the linkage group is being examined (Figure 1). Good examples are chromosomes *3* and *12* where a preponderance of markers occur toward the middle of the linkage groups (Figure 1). To better evaluate the heterogeneity of marker density along the map, a series of histograms were produced in which linkage maps for each chromosome were divided into 2-cM segments and the density of markers (per 2-cM interval) were plotted along the chromosome (see Figure 3 for sample histograms). For these plots, only those markers positioned at a LOD > 3 were included. Based on these histograms, regions of high marker density could be identified in all chromosomes and a comparison with the pachytene karyotype of each chromosome suggests that the regions of high marker density correspond to centromeric areas and, in some instances, telomeric regions (Figure 3). In some cases the changes in marker density are sudden and dramatic. For example, the average density for most regions of chromosome *3* is one marker per 2-cM interval. However, in a region of approximately 10 cM near the center of the chromosome, the marker density increases more than 10-fold, to as high as 16 markers per 2-cM interval (Figure 3). Another good example is chromosome *4* (Figure 3) and similar, but less striking, changes in marker density could be seen for each of the other chromosomes (histograms not shown).

Chromosomes *1* and *2* are submetacentric chro-

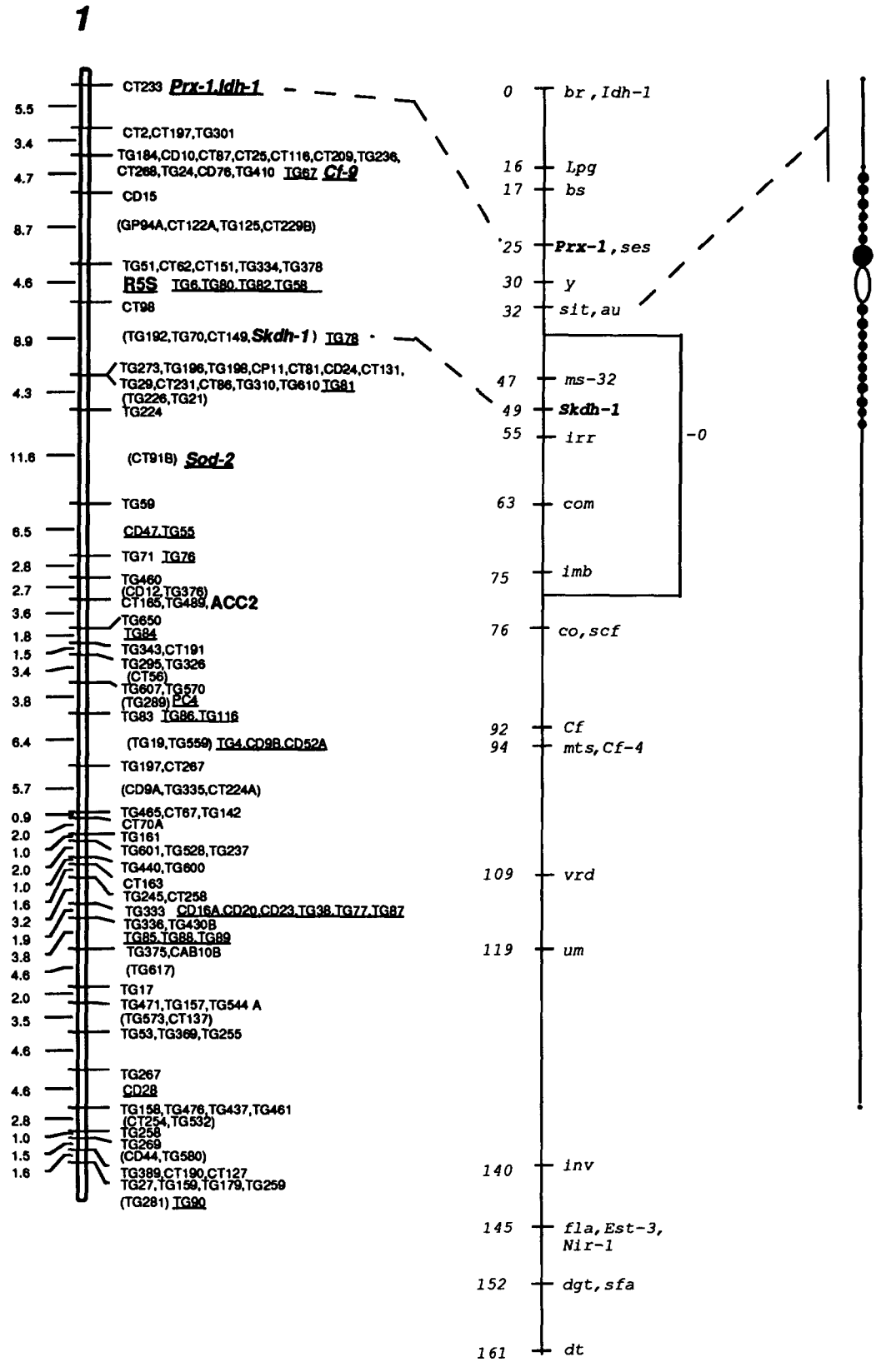
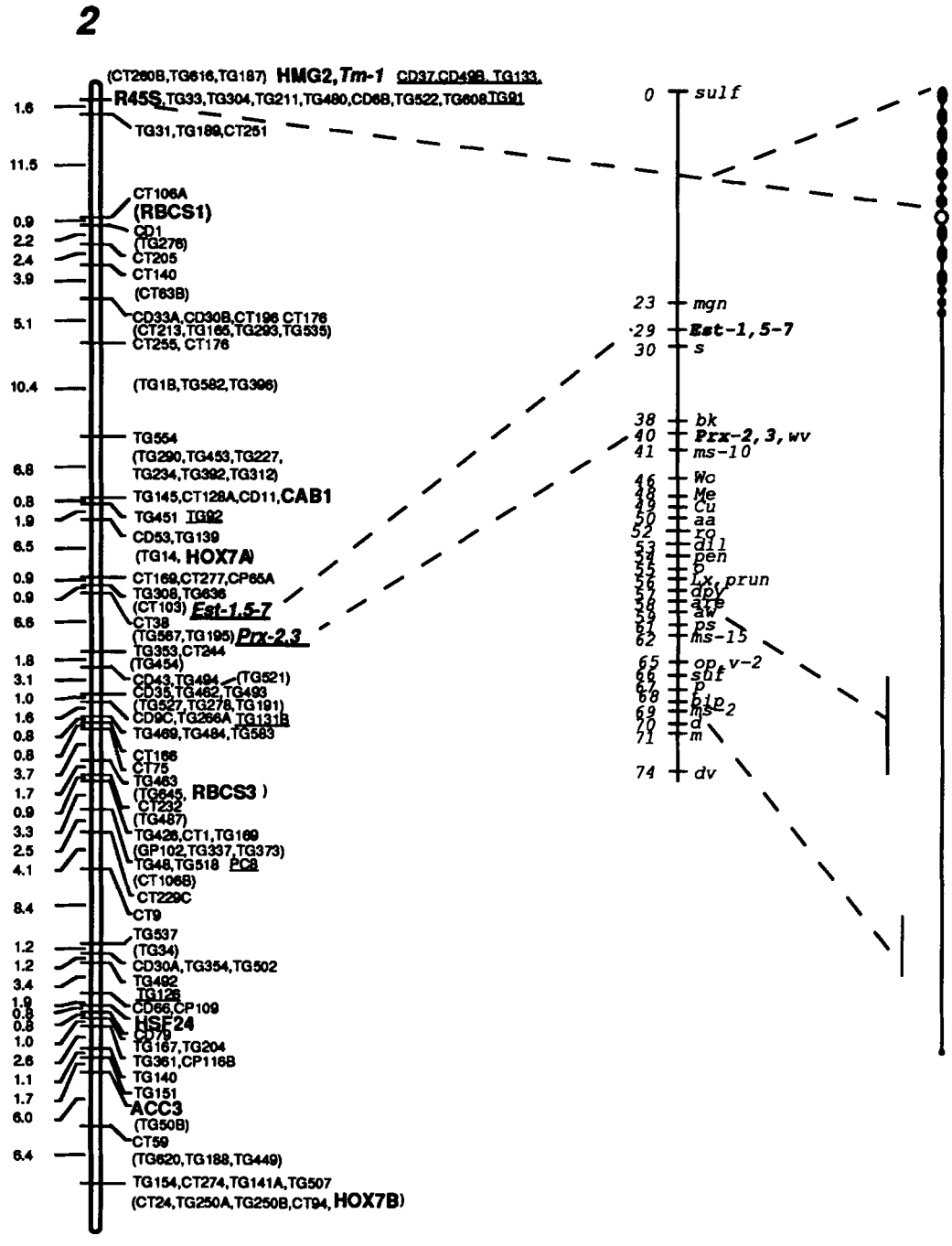


FIGURE 1.—Molecular linkage map of the tomato genome (left) and comparison with classical map (center) and cytological (pachytene) map (right). Loci by tick marks on molecular map have been ordered with LOD > 3. Loci in bold correspond to known genes (see Table 1 for details). Loci following commas cosegregate. Markers enclosed in parentheses have been located to corresponding intervals with LOD < 3. Position of underlined loci approximated from placement on previously published maps. All other loci mapped directly on F_2 population of 67 plants from *L. esculentum* × *L. pennellii* (see MATERIALS AND METHODS for details). Approximate positions of selected markers (bold) from classical map on both the molecular and cytological maps are shown by dashed lines. Positions of markers from classical map on pachytene chromosomes are based on deletion mapping (KHUSH and RICK 1968). Placement of markers from classical map onto molecular map based on direct linkage studies with RFLP markers (see Table 1 for details).


2
(CT260B,TG616,TG187) HMG2,Tm-1 CD37,CD49B, TG133,
1.6 R45S, TG33,TG304,TG211,TG480,CD68,TG522,TG608,TG91
TG31,TG189,CT251
11.5
CT106A
0.9 (RBCS1)
2.2 CD1
(TG276)
2.4 CT205
3.9 CT140
(CT63B)
5.1 CD33A,CD30B,CT196 CT176
(CT213,TG165,TG293,TG535)
CT255, CT176
10.4 (TG18,TG582,TG396)
TG554
(TG290,TG453,TG227,
6.8 TG234,TG392,TG312)
0.8 TG145,CT128A,CD11,CAB1
1.9 TG451 TG92
CD53,TG139
6.5 (TG14, HOX7A)
0.9 CT169,CT277,CP65A
0.9 TG308,TG636
(CT103) Est-1,5-7
6.6 CT38
(TG567,TG195) Prx-2,3
1.8 TG353,CT244
(TG454)
3.1 CD43,TG494 (TG521)
1.0 CD35,TG462,TG493
(TG527,TG278,TG191)
1.6 CD9C,TG266A,TG131B
0.8 TG469,TG484,TG583
0.8 CT166
3.7 CT75
1.7 TG463
(TG645, RBCS3)
0.9 CT232
(TG487)
3.3 TG426,CT1,TG169
2.5 (GP102,TG337,TG373)
4.1 TG48,TG518 PC8
(CT106B)
CT229C
8.4 CT9
1.2 TG537
(TG34)
1.2 CD30A,TG354,TG502
3.4 TG492
1.9 TG126
0.8 CD66,CP109
0.8 HSP24
1.0 CD70
2.6 TG167,TG204
1.1 TG361,CP116B
1.7 TG140
6.0 TG151
ACC3
(TG50B)
CT59
6.4 (TG620,TG188,TG449)
TG154,CT274,TG141A,TG507
(CT24,TG250A,TG250B,CT94, HOX7B)
0 sulf
23 mgn
29 Est-1,5-7
30 s
38 bk
40 Prx-2,3,wv
41 ms-10
46 Wc
48 Me
49 Cu
50 aa
52 ro
53 dil
54 pen
55 p
56 Lx,prun
57 dpy
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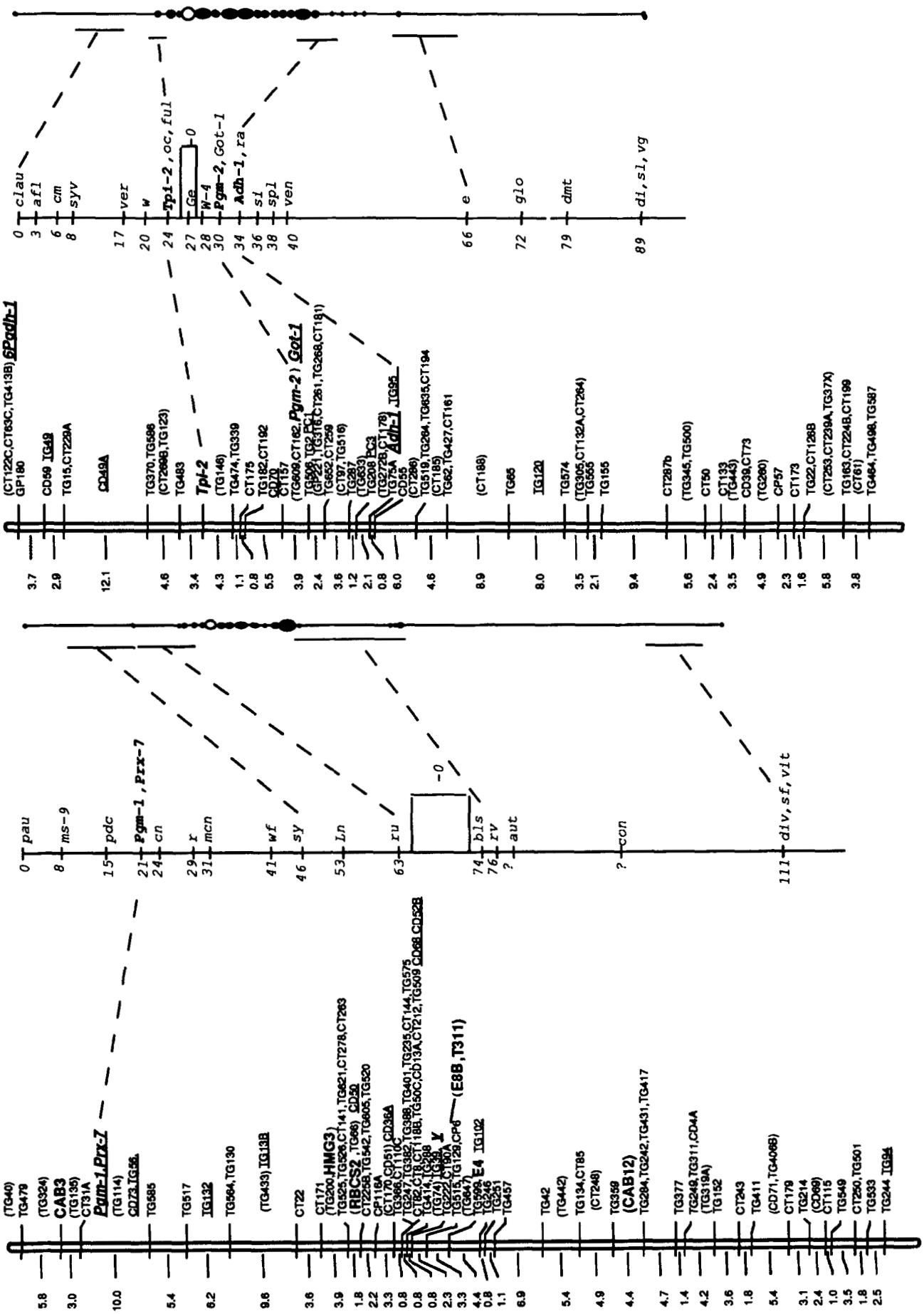
74 dv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Got-1
Adh-1
(E8B,T311)
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pau
ms-9
pdc
cn
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mcn
wf
sy
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bls
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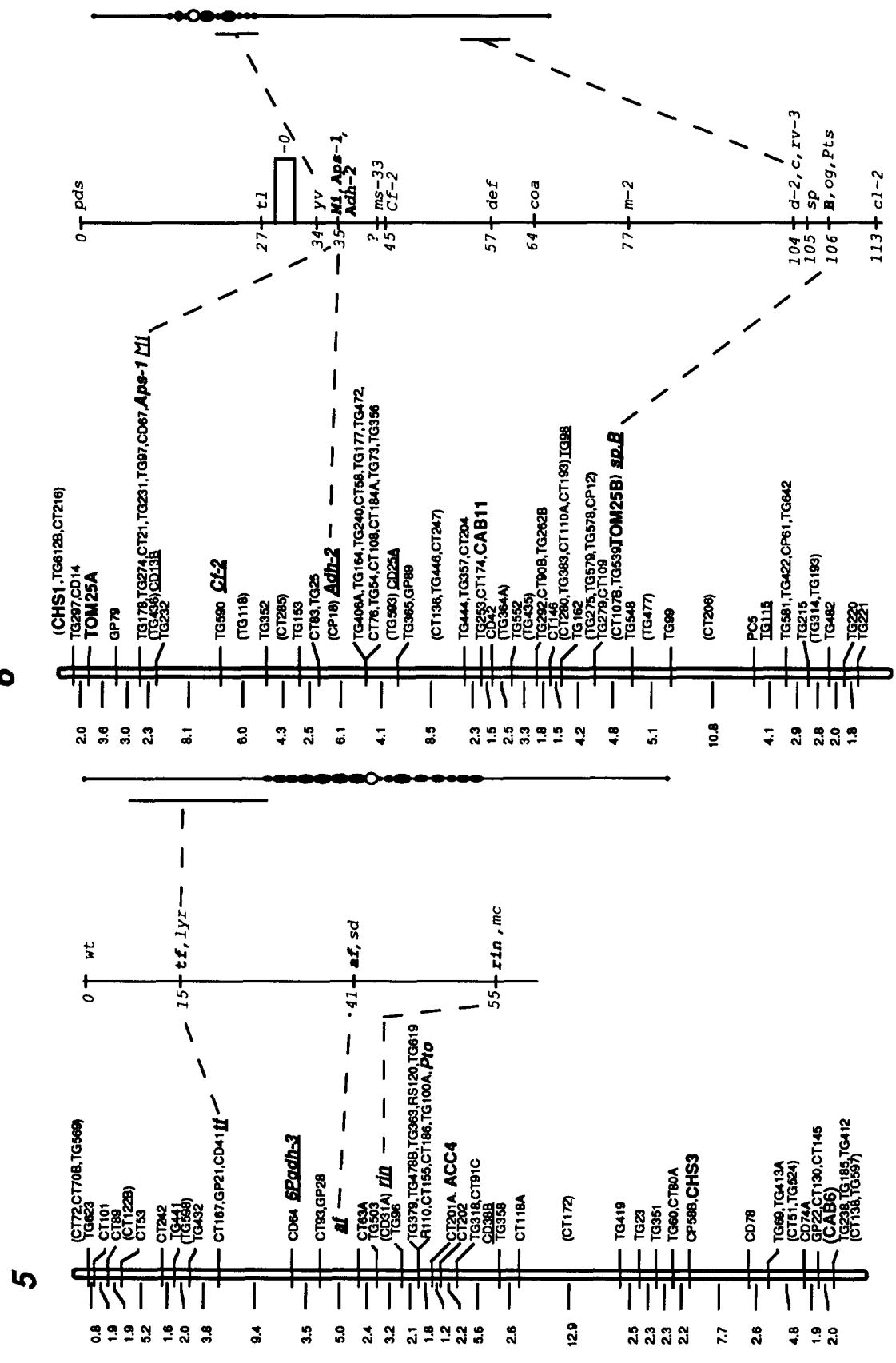
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0 pds
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35 M1, Aps-1, Adh-2
? 45 ms-33 Cf-2
57 def
64 coa
77 m-2
104 d-2, c, rv-3
105 sp
106 B, og, Pts
113 c1-2
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CT101 3.6
CT89 3.0
(CT122B) CT53 2.3
CT242 8.1
TG441 (TG596) TG432
CT167,GP21,CD41 tf 9.4
CD64 6Pgdh-3 3.5
CT83,GP28 af 5.0
CT63A 2.4
TG503 3.2
(CD31A) tin TG96 2.1
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R110,CT155,CT186,TG100A, Pto 1.2
CT201A ACC4 2.2
CT202 5.6
TG318,CT91C CD38B
TG358 2.6
CT118A 12.9
(CT172)
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TG23 2.3
TG351 2.3
TG60,CT80A 2.2
CP588,CHS3 7.7
CD78 2.6
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TG162 10.8
(TG275,TG579,TG578,CP12)
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(CT1078,TG539)TOM25B sp-B
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(TG477) 2.9
TG99 2.8
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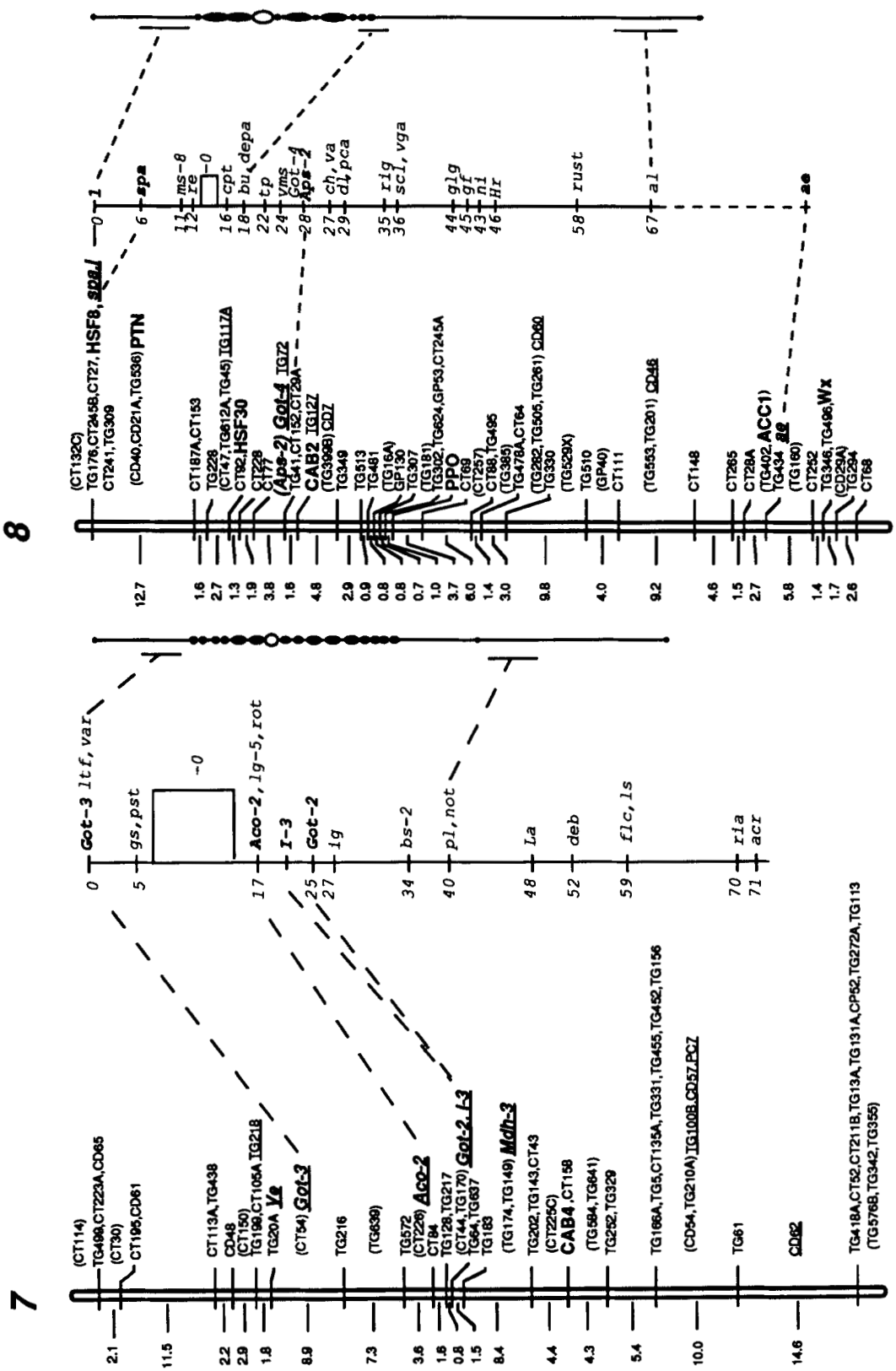
TG221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tp
vms
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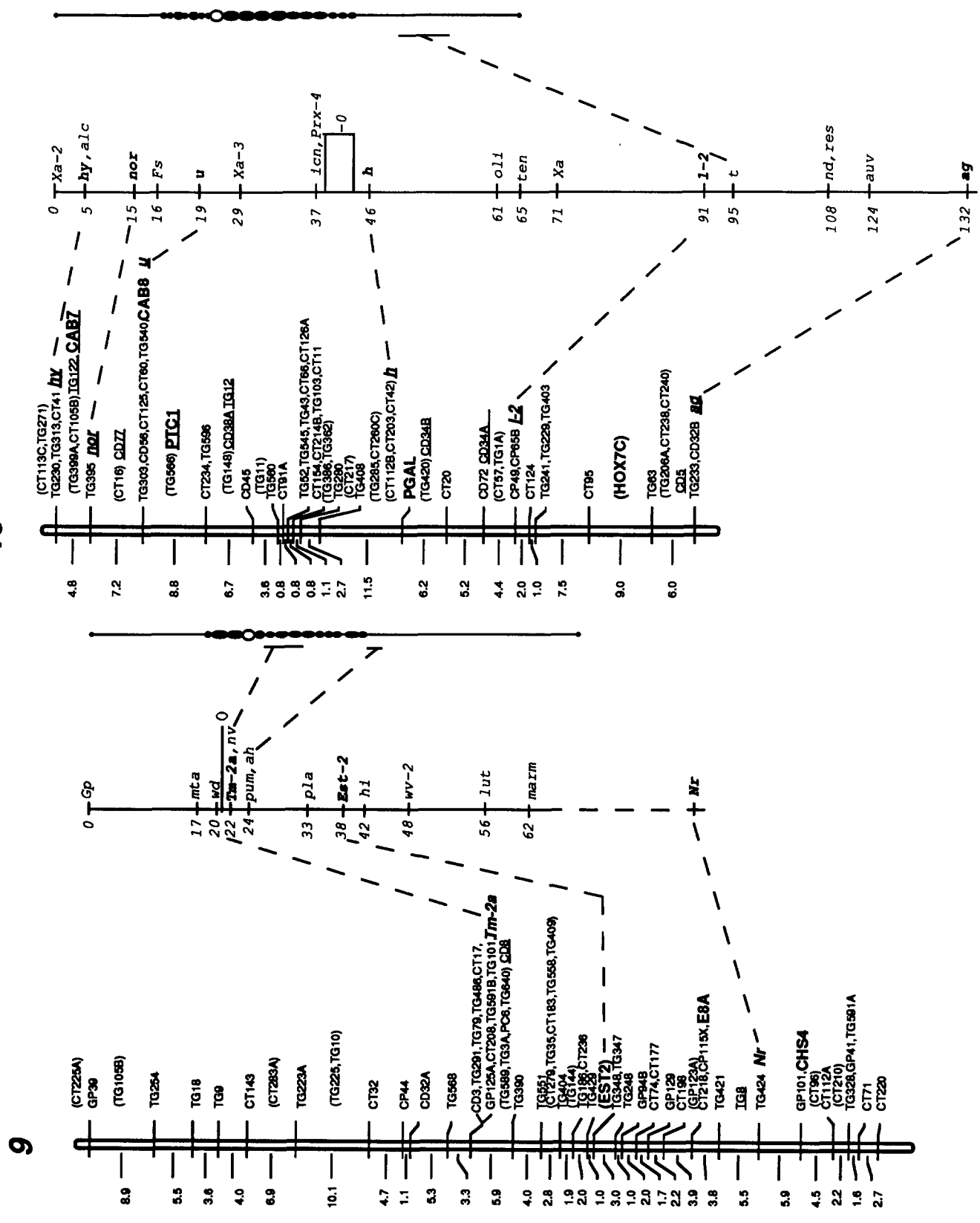
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Fs
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h
oli
ten
Xa
I-2
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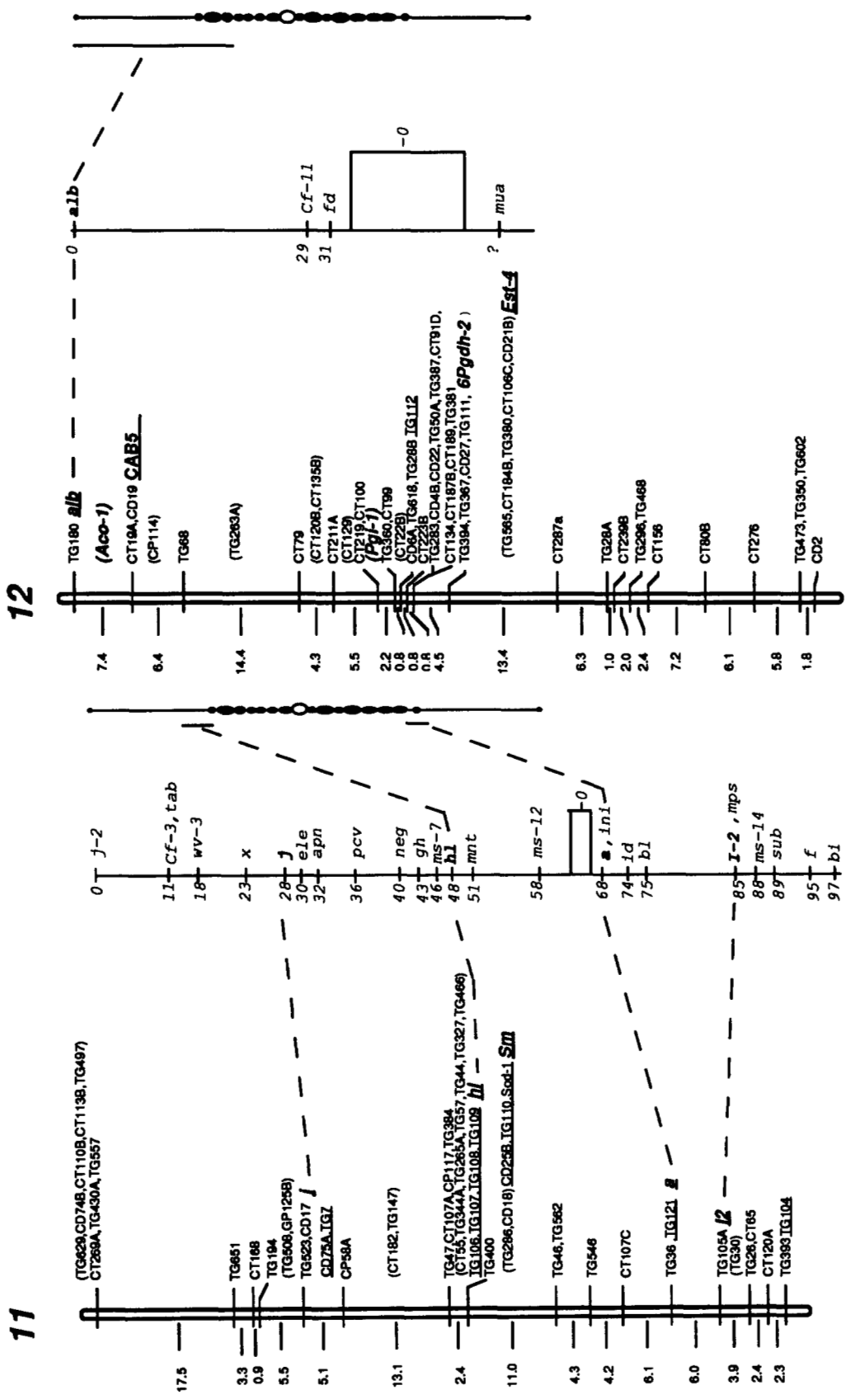
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(CT182,TG147)
TG47,CT107A,CP117,TG384
(CT55,TG344A,TG265A,TG57,TG44,TG327,TG486)
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TG46,TG562
TG546
CT107C
TG36 TG121
TG105A
(TG30)
TG26,CT65
CT120A
TG393 TG104
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Cf-3,tab
wv-3
x
j
ele
apn
pcv
neg
gh
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mnt
ms-12
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f
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(CP114)
TG68
(TG263A)
CT79
(CT1208,CT1358)
CT211A
(CT129)
CT219,CT100
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(CT228)
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CT134,CT1878,CT189,TG381
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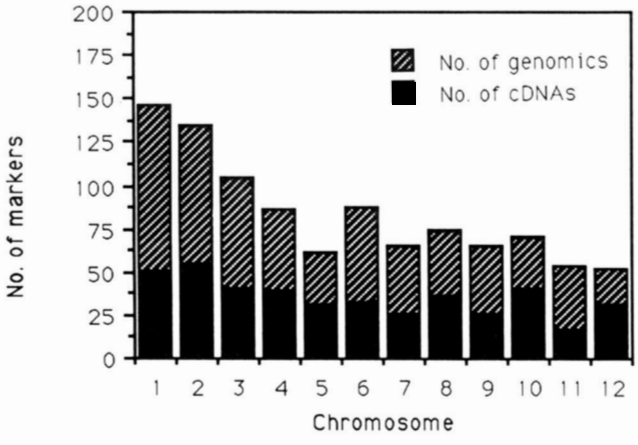

FIGURE 2.—Histogram depicting the number of loci detected with genomic clones and cDNA clones for each of the tomato chromosomes.

TABLE 2

Correlation coefficients (*r*) for pairwise combinations among various chromosomal/map variables in tomato

	cDNA	Genomics	Total	Pachytene	Mitotic
cM	0.71	0.83	0.84	0.89	0.91
cDNA		0.74	0.88	0.79	0.71
Genomics			0.97	0.95	0.91
Total				0.95	0.90
Pachytene					0.97

cM = # centimorgans per chromosome; cDNA = # loci per chromosome corresponding to cDNA clones; genomics = # loci per chromosome corresponding to single copy genomic clones; total = loci corresponding to both cDNA and genomic clones; pachytene = relative length of pachytene chromosomes (BARTON 1950); mitotic = relative length of mitotic (metaphase) chromosomes (LAPITAN, GANAL and TANKSLEY 1989).

mosomes and the high density areas occur near the end of the linkage groups. These two chromosomes are unique in that clusters of ribosomal genes are located adjacent to the centromeres (5S rDNA in chromosome *1* and 45S rDNA in chromosome *2*). These ribosomal sequences have been localized both by RFLP mapping (Figure 1) and by *in situ* hybridization (TANKSLEY *et al.* 1988, LAPITAN, GANAL and TANKSLEY 1991) and provide an approximate location of the centromere in the molecular linkage map for these chromosomes. In both instances the position of the centromere coincides very closely to the areas of high marker density supporting the proposal that much of the clustering of markers on the linkage maps is around centromeres (Figure 3). For a few chromosomes (*4L*, *7L*, *8S* and *11S*), areas of high marker density occur at the ends of the map and may correspond to telomeric areas which are reduced in recombination (Figure 1 and 3).

To estimate the percentage of the total markers on the map located in areas of high marker density we summed the number of markers in high density areas (defined as intervals with >5 markers/2 cM) for each chromosome and divided by the number of total markers located on that chromosome. The values ranged from 15% for chromosomes *4* and *5* to 40% for chromosome *12* with a mean of 28%. The areas of high density included markers corresponding to both cDNA clones and genomic clones in a relative frequency not significantly different from markers in the rest of the genome.

The clustering of markers at centromeric and possibly telomeric areas could be due to the interspecific nature of the cross used to construct the map. Inversions, or other chromosomal variations that differentiate species, are known to cause regional suppression of meiotic recombination (and hence clustering of markers on a linkage map) (BURNHAM 1962). However, two lines of evidence argue against this explanation. First, cytological and genetic studies, using the same interspecific cross, have failed to detect any significant structural differences in the chromosomes of the two species (KHUSH and RICK 1963; RICK 1969). In fact, all evidence thus far suggests that the chromosomes of *L. esculentum* and *L. pennellii* are conserved in gene order and share homology in both single copy and repetitive sequences (ZAMIR and TANKSLEY 1988). Second, subsets of the molecular markers reported in this study have also been mapped in other populations derived from crosses involving more closely related tomato species, as well as in populations derived from intraspecific crosses. While the overall levels of recombination may be higher in these more related crosses, the distribution pattern of crossing over is similar (PATERSON *et al.* 1988, 1991; M. W. GANAL, unpublished data). Similar observations have been made in potato when comparing maps made from different crosses (M. W. BONIERBALE, unpublished data).

Centromeric suppression of recombination: Assuming a random distribution of markers, low levels of meiotic recombination would cause markers, that are physically well separated, to cluster on a linkage map. Therefore, the higher density of markers in centric regions may be an effect of lower levels of meiotic recombination. In Drosophila, up to a 40-fold suppression of recombination has been reported near the centromeres (ROBERTS 1965). There is evidence that two factors contribute to the suppression in Drosophila. First, the centromere is believed to exert a direct, negative effect on crossing over in flanking chromosomal sequences, a phenomena termed the "centromere effect" or "spindle fiber effect" (BEADLE 1932; MATHER 1938). The direct effect of centromeres on suppressing recombination has been recently

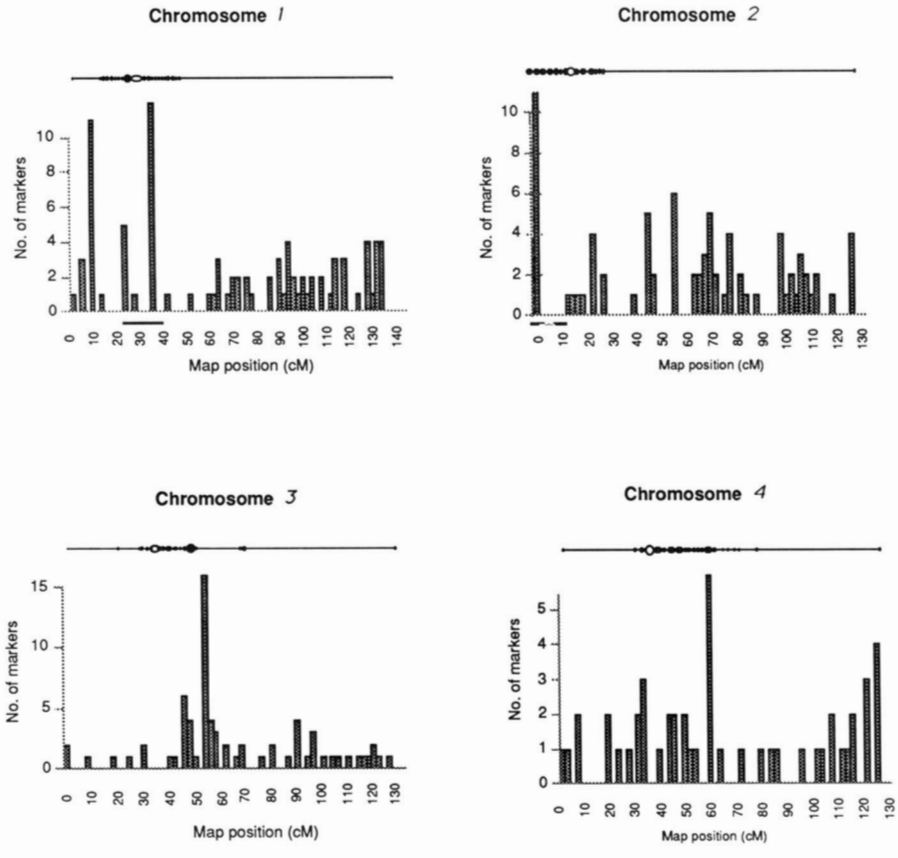

FIGURE 3.—Histograms showing density of markers (per 2-cM intervals) along chromosomes *1–4* of tomato. Pachytene chromosomes depicted above graph for each chromosome. Bar under *x*-axis for chromosomes *1* and *2* show position of centromere (see text for details).

demonstrated in yeast where a cloned centromere from the third chromosome (CEN3) has been shown to decrease recombination when it is artificially integrated into new sites in the genome (LAMBIE and ROEDER 1986). The second factor reducing recombination around centromeres in Drosophila is attributed, not to the centromere, but to heterochromatin which is proximal to the centromeres (ROBERTS 1965). Heterochromatin experiences reduced levels of recombination compared with euchromatin, presumably due to the more condensed state of heterochromatin in meiosis at the time of crossing over (ROBERTS 1965).

Tomato chromosomes, like those of Drosophila, contain centromeric heterochromatin and it has been shown that, at least for some chromosomes, recombination is suppressed in the heterochromatic region around the centromeres and that this effect can be enhanced in wide crosses (KHUSH and RICK 1967, 1968; RICK 1969, 1972). The suppression of recombination in tomato may therefore result from both a centromeric effect and/or inherently lower levels of recombination in the heterochromatin around centromeres. The hypothesis that high density clustering of markers in centromeric regions is due to suppressed recombination, and not other factors, is supported by physical mapping data around one of the tomato centromeres. GANAL, YOUNG and TANKSLEY (1989) constructed a partial restriction map, using pulsed field gel electrophoresis, around the *Tm-2a* gene (resistance to tobacco mosaic virus) which is known to be near the centromere on chromosome *9*. Results from that study indicate that 1 cM in this region of the map corresponds to more than 4 Mb–a value 6-fold greater than expected. In contrast, physical mapping around the *I2* gene (conferring resistance to *Fusarium* race 3), which is located in euchromatin and distant from the centromere on chromosome *11*, indicates a base pair to cM relationship more than 10-fold less than expected, suggesting much higher levels of recombination (SEGAL *et al.* 1992).

As previously mentioned, the areas of the map putatively affected by reduced centromeric recombination contain both cDNA and genomic clones in a ratio not significantly different from other regions of the map (Figure 1). Centromeric heterochromatin in tomato has been considered to be the "silent" portion of the genome and to be deficient in active genes (SNOAD 1963). If reduced crossing over is restricted to sequences in centric heterochromatin, then the finding that "suppressed" areas contain an average frequency of cDNA-detected loci would suggest that the heterochromatin has a gene content similar to that of euchromatin. Deletion mapping in tomato has pro-

vided direct evidence suggesting that genes can reside in the centromeric heterochromatin; however, these cases are rare (KHUSH, RICK and ROBINSON 1964). Alternatively (and perhaps more likely), if heterochromatin is deficient in genes, then the suppressive effects around centromeres must extend into adjacent euchromatin. Studies in Drosophila suggest that this is likely to be the case (MATHER 1938).

Telomeric suppression of recombination? Centromeric effects cannot explain all of the areas of high marker density on the map since some chromosomes (*e.g.*, *7*, *8* and *11*) have more than one high density area (Figures 1 and 3). In most cases, the additional high density regions occur at the end of the linkage group and may correspond to telomeric regions. This finding is consistent with studies in Drosophila that show suppressed recombination at the telomeres of some, but not all chromosomes (LEFEVRE 1970).

The occurrence along the chromosome of sequences that are hot spots for recombination may explain heterogeneities in marker densities along the map which are independent of major cytological features like centromeres, telomeres and heterochromatin (LINDAHL 1991). Elucidation of these factors in tomato await further research.

Practical and theoretical implications of heterogeneity in recombination: Results from this study allow the estimation of two genomic parameters previously not well known in plants. First, it is possible to estimate the degree to which recombination varies from one region of the chromosome to another. In regions of suppressed recombination (*e.g.*, areas proximal to the centromeres) meiotic crossing over occurs at a rate 5–10-fold less than in regions distal to the centromeres. Second, it is estimated that approximately 28% of the loci in the tomato genome map in these regions of suppressed recombination. Therefore, not only does recombination vary greatly from one portion of the chromosome to another, but a large portion of the genes are contained in regions of the chromosome in which recombination is greatly suppressed. This finding has both practical and theoretical implications.

High resolution genetic maps, a prerequisite for chromosome walking, will be much easier to generate in regions of higher recombination. For regions of suppressed recombination, much larger progeny sizes will be needed in order to recover the crossovers necessary for constructing detailed genetic maps.

With respect to plant breeding, suppression of recombination will likely enhance the effects of "linkage drag." Linkage drag is the phenomenon by which loci linked to a selected target gene are carried along during the breeding process due to their tight association with the target gene (STAM and ZEVEN 1981). If a target gene is in an area of suppressed recombination, large segments of linked DNA (and the genes contained in that DNA) will likely be carried along with the target gene (YOUNG and TANKSLEY 1989). As more genes are "dragged" along with the target locus, the probability increases that one or more of those associated genes will have a deleterious effect on the final variety (STAM and ZEVEN 1981). Knowledge of gene position along the map may be useful in adjusting population sizes to compensate for expected rates of recombination.

Finally, the extreme heterogeneity in recombination along the chromosomes has evolutionary implications. Recent studies in Drosophila suggest that the level of allelic variation at a locus is correlated with the rate of recombination in the region of the chromosome in which that locus resides. Loci in regions of low recombination are predicted to maintain, on average, fewer alleles than those in regions of higher recombination due to "genetic hitchhiking" (BEGUN and AQUADRO 1992). More than one-quarter of the genes in tomato are estimated to map in regions of suppressed recombination, and thus may be more susceptible to the hitchhiking process. In addition, the paracentric inversions that differentiate tomato and potato are likely to have moved genetic loci from regions of low recombination (*e.g.*, centromeres) to regions of higher recombination (and vice versa) and therefore have changed the evolutionary outlook for those loci.

Correspondence between molecular and classical genetic linkage maps: Tomato has one of the best classical genetic linkage maps of any plant species (RICK 1975, 1980). Located on this map are many genes of biological interest and economic importance, including genes coding for disease resistance, male sterility, fruit ripening and abscission. This classical map has served as the foundation for many of the previous genetic and cytological studies in tomato. For these reasons, it is important to establish a linear correspondence between the molecular and the classical linkage maps. Toward this goal, a number of morphological and isozyme markers found on the classical map were mapped with respect to RFLP markers. The results of these analyses are displayed in Figure 1. Since some of the markers on the classical map have been located via deletion mapping on the cytological map (pachytene chromosomes, KHUSH and RICK 1968) it is additionally possible to establish an approximate relationship between the molecular and cytological maps of tomato (Figure 1).

All of the morphological markers tested could be located within the molecular linkage map. However, there are substantial regions of the molecular map that apparently have no counterpart in the classical map. For example, the molecular map extends chromosomes *6* and *12* by approximately 30% and 50%,

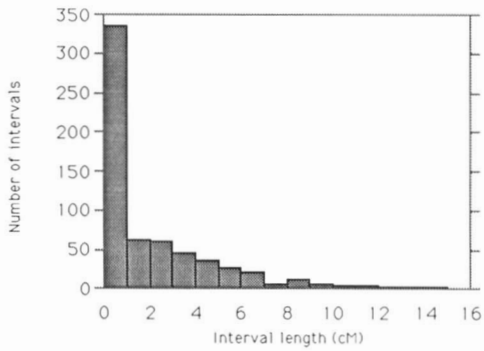

FIGURE 4.—Distribution of interval sizes (in centiMorgans, cM) between adjacent markers on tomato molecular map.

respectively. Chromosome *5* is even more extreme as the classical map can account for only approximately 25% of the length of the molecular map. Summed over all chromosomes, it is estimated that the molecular map extends the classical map by at least 300 cM–an increase of more than 20%. The full extent of the expansion of the molecular map relative to the classical map will only be known when more morphological markers are mapped with respect to markers on the molecular map.

Genome coverage: Despite the large number of markers mapped, gaps still remain in some regions of the linkage map (see Figures 1 and 4). The longest gap corresponds to a 17-cM interval on chromosome *11*, and there are a total of 14 gaps that exceed 10 cM (Figure 4). The expected maximum gap size for a genome the size of tomato with 1000 markers, among random marker distribution, is approximately 15 cM (TANKSLEY *et al.* 1988). This is close to the size of the gaps observed in the map reported here. However, while the size of the gaps is not unexpected, the number of such gaps does exceed expectations (TANKSLEY *et al.* 1988). These gaps may represent areas which are deficient in genes and/or low copy sequences, or that are hot spots for recombination. The later explanation is suggested by data from yeast and mice where certain sequences can enhance recombination by a factor of 5 or more (COLEMAN *et al.* 1986; LINDAHL 1991). While mapping additional markers may eventually fill larger gaps in the map, a more directed approach is to target markers to specific gaps in the map using the recently developed RAPD technique (WILLIAMS *et al.* 1990) on pooled DNA from selected individuals of the mapping population. Recently the RAPD strategy has been used in tomato to partially fill a large gap in the linkage map of chromosome 11 (GIOVANNONI *et al.* 1991).

Evaluating the completeness of the map: Three lines of evidence suggest that the tomato molecular map is basically complete (*i.e.*, most if not all chromosomal regions are represented by well mapped counterparts in the molecular linkage map). The first piece of evidence is that all RFLP loci could be placed within the 12 linkage groups. In other words, no loci failed to link up with the map. Second, all classical genetic markers could be placed in the molecular map (Figure 1). Finally, four of the tomato telomeres have been recently mapped genetically onto the molecular map using satellite clones known to represent the virtual end of the tomato chromosomes (GANAL, BROUN and TANKSLEY 1992). In all four of these cases the telomeres were found to be very closely linked (on average approximately 5 cM) to the respective ends of the molecular linkage groups presented in this report.

Potato map

As previously shown, the genetic content of potato chromosomes is nearly identical to that of tomato (*i.e.*, there is no evidence of chromosomal translocations differentiating the species) (BONIERBALE, PLAISTED and TANKSLEY 1988). However, five inversions of marker order within chromosomes can be identified by comparing the two maps (Figure 5). Affected are chromosomes *5*, *9*, *10*, *11* and *12*, of which four (*5*, *9*, *11*, *12*) involve the short arm of the chromosome, while one (chromosome *10*) affects the long arm (Figure 5). Previously we had reported inversions on three of these chromosomes (*5*, *9*, *10*) (BONIERBALE, PLAISTED and TANKSLEY 1988). The additional two inversions (*11*, *12*) were not observed earlier due to the smaller number of markers available for mapping the genomes at that time.

All inversions appear to be paracentric and involve entire chromosome arms–a conclusions consistent with an earlier study by BONIERBALE, PLAISTED and TANKSLEY (1988). Moreover, only one breakpoint can be identified per chromosome and these occur in regions of the genetic map at or near the centromere. In no instances could a second breakpoint for an inversion be seen in the distal part of the arm, indicating that entire arms have been inverted (Figure 5). For all except chromosome *11*, the position of the putative centric breaks can be narrowed down to very small intervals (ca. 5 cM). The precision with which the inversion breakpoints have been located and the overall high level of conservation of chromosome content and gene order between potato and tomato, make precise predictions possible for the position of most molecular markers from the tomato map on the potato map – including those that were mapped only in tomato.

In contrast to tomato, the potato map is comprised of 684 cM, approximately one-half that of tomato (1276 cM). Reductions in crossing over affect all potato chromosomes, although some chromosomes (*e.g.*, chromosome *2*) are affected more dramatically than others. The finding of reduced recombination in potato, compared with tomato, is consistent with earlier findings (BONIERBALE, PLAISTED and TANKSLEY 1988).

Chromosome inversions that differentiate tomato

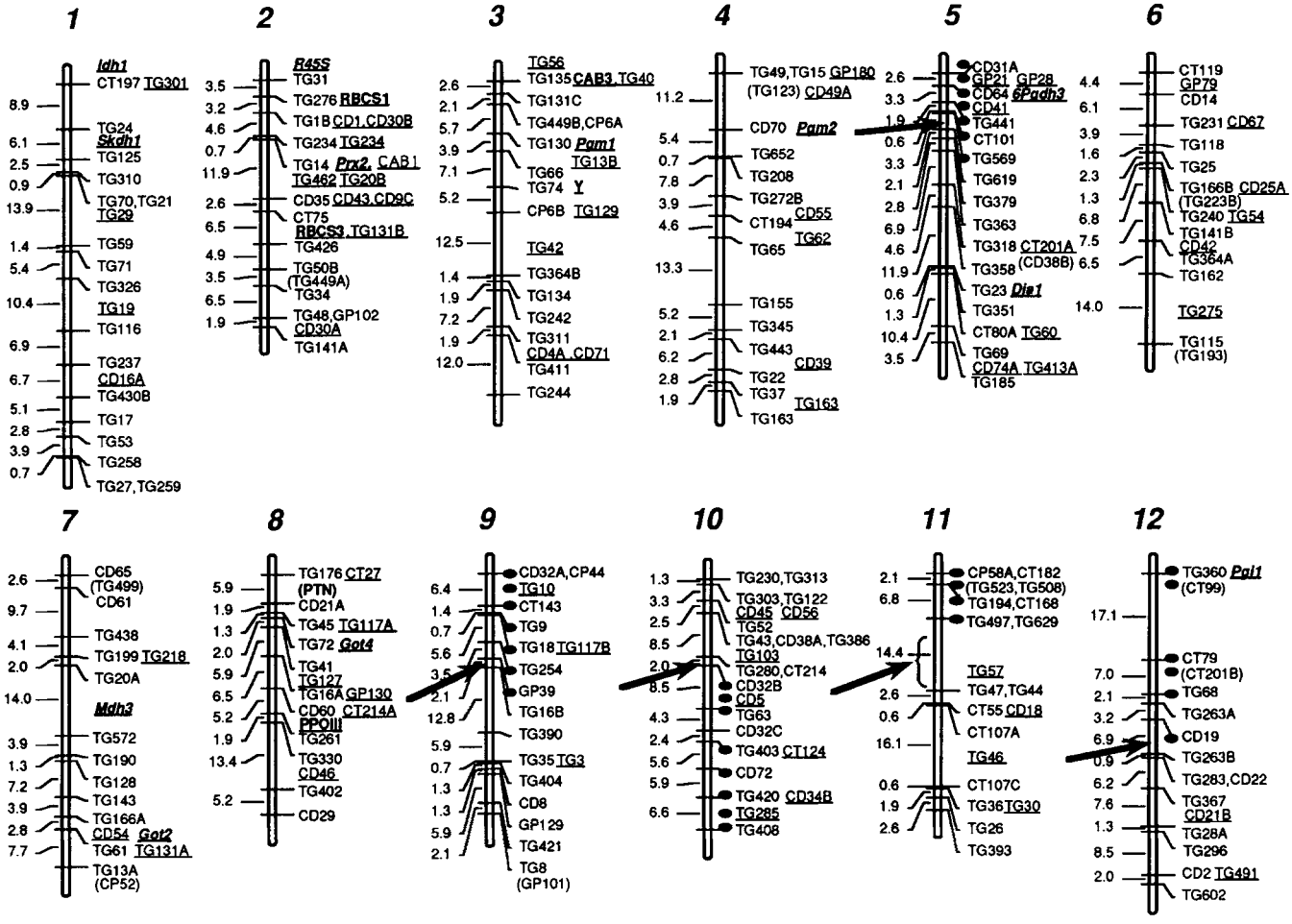

FIGURE 5.—Molecular linkage map of the potato genome. Loci by tick marks ordered with LOD > 2. Loci following commas cosegregate. Markers enclosed in parentheses have been located to corresponding intervals with LOD < 2. Position of underlined loci approximated from placement on previously published maps. All other loci mapped directly on backcross family N263 of 155 plants (*S. tuberosum* USW2230 ($2n = 24$) × *S. berthaultii* PI473331 ($2n = 24$) × *S. berthaultii* PI473331. Dots indicate markers involved in inversions that differentiate potato from tomato. Arrows indicate approximate break points for inversions (see text for details).

and potato did not leave obvious karyotypic footprints: All of the inversions differentiating tomato and potato appear to involve an entire chromosome arm and single putative breakpoint near the predicted position of the centromere (Figure 5). We deduce from these results that two events (sequential or simultaneous) were involved in the formation of the inversions. First, a break must have occurred at or near the centromere; and second, the telomeric end of the chromosome was joined at the centromeric break point. This scenario raises several questions: First, how did the broken end of the inverted chromosome gain a new telomere necessary for chromosome stability? The answer to this question may be found in recent molecular studies demonstrating that the *de novo* addition of new telomeric repeats can "heal" broken chromosomes, conferring chromosomal stability (YU and BLACKBURN 1991). The inversions that differentiate tomato and potato could therefore have involved only a single chromosomal break that led to an inversion in which the broken end of the chromosome formed a new telomere *de novo*. An alternative explanation is that the inversions were the consequence of homologous recombination between telomeric and centromeric repeats. Studies of tomato telomeres have shown that a few copies of telomeric repeats can be found at interstitial sites and some of these correspond to centromeric areas (GANAL, LAPITAN and TANKSLEY 1991; P. BROUN, unpublished data).

A second, related question is: What became of the telomeric sequences originally at the end of the inverted chromosomes that presumably would have been translocated near to the centromeric break point? One possibility is that they were lost in the breakage/inversion/ligation process. If they were not lost, one would expect to find telomeric sequences at or near the centromeres in the affected chromosomes. This might again explain the occurrence of telomeric repeats at interstitial sites.

Finally, despite the fact that tomato and potato differ for five chromosomal inversions, their pachy-

tene karyotypes are very similar (BARTON 1950; YEH and PELOQUIN 1965). Both have heterochromatin concentrated near the centromeres with the remainder of the chromosomes being largely euchromatic. The fact that their karyotypes are so similar suggests that the breakpoints leading to the translocations either occurred in the euchromatin adjacent to centromeric heterochromatin; or, if the breakpoints occurred in the heterochromatin, the heterochromatin distal to the breakpoint was lost or converted to euchromatin upon transposition to the telomeric end of the chromosomes. Extensive studies of radiation-induced chromosome breakage in tomato have shown that breakages in the centromeric heterochromatin are much more common than those in euchromatin, a finding that supports the notion that the breakpoints occurred in the heterochromatin (KHUSH and RICK 1963).

Applications of high density maps: The map developed for tomato has markers, on *average*, every 1.2 cM–the highest density map yet reported for any crop species. This density of markers makes it very likely that any gene of interest will be within a few map units of at least one molecular marker. Genes of economic importance (*e.g.*, those for disease resistance, growth habit, male sterility, etc.) can thus be readily associated with tightly linked molecular markers that can serve in marker-assisted selection for those genes in breeding programs. Already, genes for more than 15 important traits have been shown to be tightly associated with markers on the map (Figure 1, Table 1). In the near future it is likely that most major genes of economic importance in tomato breeding will have been associated with tightly linked molecular markers.

Although not all of the 1030 molecular markers mapped in tomato have been located in potato, a sufficient number have been mapped to allow one to deduce the position of the remaining markers based on the highly conserved linkage order of the two species. Moreover, nearly all of the RFLP probes mapped in tomato cross-hybridize with potato DNA and can be used for potato mapping (BONIERBALE, PLAISTED and TANKSLEY 1988). Since the potato genome has fewer map units than tomato, the effective density of markers in potato is actually higher than tomato (*ca.* 1 marker every 0.7 cM). In addition, approximately 300 additional RFLP markers have been mapped on potato independently of the work reported in this paper (GEBHARDT *et al.* 1989, 1991). This brings the total number of markers available for potato (and tomato) genetics to more than 1400, making these two species among the most thoroughly mapped of any plant or animal species.

The large number of markers mapped in tomato/potato should facilitate studies on the genetic basis of quantitative traits. In order to have a chance of detecting all of the quantitative trait loci (QTL) affecting a character in a particular cross, it is necessary to have molecular markers evenly distributed throughout the genome. The maps presented here should allow selection of evenly spaced polymorphic markers. In the past, one was never certain whether the true ends of chromosomes extended beyond the last markers on the available linkage map. Recent genetic mapping of several telomeres in tomato indicates that the genetic map does not extend much beyond the end markers of the molecular map reported here (GANAL, BROUN and TANKSLEY 1992). QTL, even at the distal parts of the chromosomes, should therefore be readily detected.

Finally, the availability of high density maps for tomato and potato should facilitate chromosome walking to genes of economic importance. Plants have several advantages for chromosome walking compared with animals, including the ability to generate large segregating populations for high resolution mapping and repeatable methods for the transformation of foreign DNA into their genome. This last attribute is especially important in verifying the identity (via phenotype) of a gene that has putatively been cloned by a chromosome walk. These attributes, combined with the high density molecular maps reported here, should provide the necessary tools for isolating genes whose gene products are unknown, but whose phenotypes are economically important and/or biologically interesting.

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