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An RFLP linkage map of *Lycopersicon peruvianum*

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Abstract In order to map genes determining resistance to bacterial canker in tomato, backcrosses were made between a resistant and a susceptible *Lycopersicon peruvianum* accession. The linkage study with RFLP markers yielded a genetic map of *L. peruvianum*. This map was compared to that derived from a *L. esculentum* × *L. pennellii* F_2 population, based on 70 shared RFLP markers. The maps showed a good resemblance in both the order of markers and the length of the chromosomes, with the exception of just one relocated marker on chromosome 9. Because backcrosses were made with the F_1, either as the pollen parent or as the pistil parent, linkage maps from male and female meioses could be estimated. It was concluded that recombination at male meiosis was reduced, and that gametophytic selection for parental genotypes at more than one locus per chromosome might be partly responsible for the reduction of the estimated male map length.

Key words Molecular markers
Sex differences in recombination

Introduction

The mapping of genes determining important agricultural traits using molecular markers is at present one of the major issues in the field of plant breeding. In tomato many traits have now been mapped (e.g., Klein-Lankhorst et al. 1991; Paterson et al. 1991; Van der Beek et al. 1992; and references therein), and the results of these mapping studies are currently being implemented in practical plant-breeding programmes. Many of these traits originate from wild relative species of tomato. *L. peruvianum* is one of the wild relatives which harbours many genes of importance for tomato breeding. In a search for resistance genes for bacterial canker in tomato (*Lycopersicon esculentum*), caused by *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davies et al. (referred to as *Cm*), Lindhout and Purimahua (1987) detected a high level of resistance to bacterial canker in five accessions of the wild relative of tomato, *L. peruvianum*. These accessions are useful for introducing resistance into commercial tomato cultivars by marker-aided introgression.

The study described in this paper was undertaken to map genes coding for *Cm* resistance and to generate a linkage map of *L. peruvianum* with molecular markers, which can also be used for mapping other traits present in *L. peruvianum*. For this purpose a set of genomic tomato DNA clones was used, which had been mapped in an F_2 population of *L. esculentum* × *L. pennellii* (Tanksley and Mutschler 1990; Tanksley et al. 1992). Generally, the *Lycopersicon* species are expected to have congruent linkage maps, since Bonierbale et al. (1988) demonstrated the resemblance between the tomato and the potato linkage maps, both species being members of the *Solanaceae*. In this study we compared the linkage map of *L. peruvianum* with the map of the same DNA markers determined in the *L. esculentum* × *L. pennellii* F_2 population.

Because it was concluded from a previous study (Lindhout and Purimahua 1989) that the resistance was of a recessive nature, a backcross to the resistant parent was used as a mapping population. In order to study possible maternal effects, the backcross was made in two ways, first with the F_1 as pollinator, and second with the F_1 as pistil parent. This also offered the opportunity to calculate and compare the map lengths from female and male meioses.

In the present paper, the results are described regarding the level of polymorphism between the parental *L. peruvi-*

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anum accessions, the RFLP linkage map, and the differences between the male and the female map. Another paper will focus on the mapping of the *Cm* resistance genes (Sandbrink et al., manuscript in preparation).

Materials and methods

Plant material

Reciprocal F_1 crosses were made between two *L. peruvianum* accessions, the *Cm* resistant LA 2157 (referred to as R) and the *Cm* susceptible LA 2172 (referred to as S). Dr. C. Rick collected LA 2157 at Túnel Chotano and LA 2172 at Cuvita in Peru (Rick 1986). Both accessions are maintained at the Centre of Genetic Resources (CPRO-DLO), Wageningen, The Netherlands. Three reciprocal backcross populations were made between the F_1 and the resistant parent, **A**: (R×S)×R (280 plants), **B**: R×(R×S) (199 plants), and **C**: R×(S×R) (287 plants). Pollination was performed by mixing pollen from five male parent plants and applying it to the pistils of five female parents.

Prior to *Cm* inoculation (Sandbrink et al., manuscript in preparation) a few leaves were harvested for RFLP analysis from all plants, and after completion of the *Cm* disease test redundant leaf material was harvested from resistant plants only.

DNA isolation

DNA was isolated from leaf tissue according to a method developed by the group of Dr. S.D. Tanksley (Cornell University, Ithaca, N.Y., USA) as described by Van der Beek et al. (1992).

Restriction enzyme digestion, electrophoresis and blotting

Total DNA samples (5 µg) were digested with the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, or *Xba*I (Amersham) according to the manufacturer's recommendations. The restriction fragments were separated overnight on 1% agarose gels in TAE (Sambrook et al. 1989) (1.2 V/cm). DNA was denatured in 0.4 M NaOH, 0.6 M NaCl for 30 min., neutralized in 1.5 *M* NaCl, 0.5 M Tris-HCl, pH 7.5 for 30 min., transferred to Genescreen-Plus (Dupont Nemours) nylon membranes according to the manufacturer's instructions and fixed by baking for 2 h at 80 °C.

Southern hybridization

A set of 124 tomato genomic (TG) DNA clones was obtained from Dr. S. D. Tanksley (Cornell University, Ithaca, N.Y., USA). The inserts of the clones were amplified by PCR using M13 sequence primers and radiolabeled by the random primer extension method according to Feinberg and Vogelstein (1983). DNA-DNA hybridization was carried out overnight in a hybridization oven (Hyb-Aid) at 65 °C in 1 M NaCl, 1% SDS , 10% dextran sulphate and 0.1 mg/ml salmon sperm DNA. After hybridization the filters were washed twice for 1 h at 65 °C with 0.5×SSC, 1% SDS. The filters were autoradiographed on KODAK XAR-5 films for 6 h to 1 week. The hybridized probes were removed by incubating the filters at 95 °C in 0.1×SSC, 1% SDS for 30 min. Blots could be used routinely at least ten, and in several cases up to 25, times.

Detection and level of polymorphism between LA 2157 and LA 2172

The TG clones and the restriction enzymes *Eco*RI and *Eco*RV were utilized in an initial search for sufficient polymorphic loci distributed evenly over the genome. In regions where linkage with *Cm* resistance was suspected, additional clones were tested for polymorphisms, extending the number of restriction enzymes to six with *Dra*I, *Hae*III, *Hin*dIII, *Xba*I. The polymorphisms were screened for homozygosity with sets of ten individual plants from both parental accessions, because (1) pollen mixtures derived from several plants had been used for cross-pollination and (2) no DNA or plant material of the actual parental plants used to produce the segregating populations was available. Clones were selected for linkage analysis of the backcross progeny when they showed polymorphism between, but not within, the two accessions.

Linkage analysis

Initially, the experiment was designed to determine marker genotypes in the Cm-resistant plants only (see Sandbrink et al., manuscript in preparation). As a consequence of distorted segregation ratios (see Sandbrink et al., manuscript in preparation), a group of susceptible plants was genotyped in addition to the resistant plants. Hence, the marker genotypes were determined for non-random subgroups of the backcross populations. When there is selection for a certain genotype of a single locus, such as a resistance locus, the estimator of the recombination frequency in a backcross remains unbiased, independent of the level of interference. For instance, when there is selection for only the genotype xX at the X-locus (Table 1), the frequencies f_{xyz}, f_{xyZ}, f_{xYZ}, and f_{xYz} are zero. The expected values of the recombination frequency estimators are:

$$E(\hat{r}_{xy})=r_{xy}-Cr_{xy}r_{yz}+Cr_{xy}r_{yz}+0+0=r_{xy},$$
$$E(\hat{r}_{yz})=r_{yz}-Cr_{xy}r_{yz}+Cr_{xy}r_{yz}+0+0=r_{yz},$$
$$E(\hat{r}_{xz})=r_{yz}-Cr_{xy}r_{yz}+r_{xy}-Cr_{xy}r_{yz}+0+0=r_{xy}+r_{yz}-2Cr_{xy}r_{yz}=r_{xz}.$$

This also holds when the selected locus lies in between two loci. For instance, when there is selection for only the genotype yY at the Y-locus (Table 1), the frequencies f_{Xyz}, f_{XyZ}, f_{xyz}, and f_{xyZ} are zero. The expected values of the estimators are:

$$E(\hat{r}_{xy})=0+0+r_{xy}-Cr_{xy}r_{yz}+Cr_{xy}r_{yz}=r_{xy},$$
$$E(\hat{r}_{yz})=r_{yz}-Cr_{xy}r_{yz}+0+0+Cr_{xy}r_{yz}=r_{yz},$$
$$E(\hat{r}_{xz})=r_{yz}-Cr_{xy}r_{yz}+0+0+r_{xy}-Cr_{xy}r_{yz}=r_{xy}+r_{yz}-2Cr_{xy}r_{yz}=r_{xz}.$$

With the pooled data of backcross populations A, B and C, a linkage map for the species *L. peruvianum* was constructed with the computer software JoinMap (Stam 1993). This map (referred to as the *peruvianum* map) was compared to the map of these markers which has been determined with an F_2 population from a *L. esculentum* × *L. pennellii* cross (Tanksley et al. 1992) (referred to as the *esculent-*

Table 1 Estimation of recombination frequencies in a progeny of a cross XYZ/xyz × xyz/xyz (haplotypes separated by the slash). r_{xy}, r_{yz}, and r_{xz} are the recombination frequencies between loci X and Y, Y and Z, and X and Z, respectively, C, coefficient of coincidence (=1−interference), N_t, total number of individuals

Genotype	Frequency	Expected relative frequency, multiplied by two
xyz/XYZ	f_{XYZ}	$1-r_{xy}-r_{yz}+Cr_{xy}r_{yz}$
xyz/XYz	f_{XYz}	$r_{yz}-Cr_{xy}r_{yz}$
xyz/Xyz	f_{Xyz}	$r_{xy}-Cr_{xy}r_{yz}$
xyz/XyZ	f_{XyZ}	$Cr_{xy}r_{yz}$
xyz/xyz	f_{xyz}	$1-r_{xy}-r_{yz}+Cr_{xy}r_{yz}$
xyz/xyZ	f_{xyZ}	$r_{yz}-Cr_{xy}r_{yz}$
xyz/xYZ	f_{xYZ}	$r_{xy}-Cr_{xy}r_{yz}$
xyz/xYz	f_{xYz}	$Cr_{xy}r_{yz}$

Unbiased estimators of r_{xy}, r_{yz}, and r_{xz}

$$\hat{r}_{xy}=(f_{Xyz}+f_{XyZ}+f_{xYZ}+f_{xYz})/N_t$$
$$\hat{r}_{yz}=(f_{XYz}+f_{XyZ}+f_{xyZ}+f_{xYz})/N_t$$
$$\hat{r}_{xz}=(f_{XYz}+f_{Xyz}+f_{xyZ}+f_{xYZ})/N_t$$

um × *pennellii* map). In order to compare the lengths of the two maps, the *peruvianum* map was recalculated with MAPMAKER (Lander et al. 1987), the program used to construct the *esculentum* × *pennellii* map.

Because the F_1 was used both as pistil parent (population A), and as pollen parent (populations B and C), the possible difference in recombination between the male and the female meiosis could be studied. Accordingly, a female map was constructed with population A, and a male map with the pooled data of populations B and C, using JoinMap.

The linkage maps were drawn with the computer program DrawMap (Van Ooijen 1994).

Results

Detection and level of polymorphism between LA 2157 and LA 2172

In the present study 124 TG clones were used to screen for RFLPs between the *L. peruvianum* accessions LA 2157 and LA 2172. For 86 clones (69%) a polymorphism was detected with one or more enzymes. Of these, 80 provided polymorphisms only between, and not within, the accessions with at least one of the restriction enzymes used. The degree of polymorphism thus encountered, and the distribution over the enzymes used, is presented in Table 2. In the end, 72 clones, yielding 73 polymorphisms, were selected for the linkage study.

Data on polymorphisms detected with *Eco*RI and *Eco*RV were used to calculate the genetic distance between LA 2157 and LA 2172 according to formula 6b of Upholt (1977). The data obtained with the other enzymes were not included for this purpose, because these enzymes were only used when no polymorphism was found with *Eco*RI or *Eco*RV. The incorporation of these data would underestimate the genetic distance. Using the set of 124 clones a total of 613 *Eco*RI and *Eco*RV fragments were detected stemming from either LA 2157 or LA 2172 (thus, non-polymorphic fragments were counted as two fragments). The fraction of shared fragments was 0.708, equalling a genetic distance of 1.96 nucleotide substitutions per 100 basepairs.

Comparison of the *peruvianum* map to the *esculentum* × *pennellii* map

After the resistance screening (see Sandbrink et al., manuscript in preparation) 152 plants, classified as resistant, were genotyped with 73 markers. An additional group of 116 susceptible plants was genotyped using 21 markers, possibly linked to a resistance locus (markers indicated with an asterisk in Table 3 of Sandbrink et al., manuscript in preparation). A *peruvianum* linkage map was calculated from these pooled data of the three backcross populations (Fig. 1). Comparison with the *esculentum* × *pennellii* map published by Tanksley et al. (1992) reveals that both maps are congruent, except for the following differences. TG325 and TG190 are not present on the *esculentum* × *pennellii* map of Tanksley et al. (1992). With TG166 two independently segregating bands (indicated with an appended A and B in Fig. 1) were detected in the *L. peruvianum* backcross populations. TG258 resides on the *peruvianum* map on chromosome 6, but in the *esculentum* × *pennellii* map on chromosome 1. Since this clone hybridized to multiple fragments in the *L. peruvianum* backcross population, the segregating band is presumed to be different from the band segregating in the *L. esculentum* × *L. pennellii* F_2 population employed by Tanksley et al. (1992).

With respect to the order of the loci on the map only some minor changes were observed (see the shaded areas on chromosomes 1, 4, and 12 in Fig. 1), which are considered to be within the range of statistical variation associated with map estimation. On chromosome 9, however, there were several rearrangements in the order of markers. TG9 is closely linked to TG79 and TG35 on the *peruvianum* map, but more than 35 cM apart from these loci on the *esculentum* × *pennellii* map. The map with the segment from TG144 to TG223A (Fig. 1) inverted towards TG328, however, was the second best order of chromosome 9, as calculated by MAPMAKER with a log-likelihood only 0.42 more negative. Except for the position of TG9, this map has a good resemblance to the *esculentum* × *pennellii* map.

Table 2 Degree of DNA polymorphism between LA 2157 (R) and LA 2172 (S). A, no polymorphism detected between R and S; B, polymorphic between R and S, but not within R or S; C, polymorphic within R; D, polymorphic within S; Percent useful clones: percentage of clones that are useful for the present linkage study (=B/total)

Restriction enzyme	Number of clones					Percent useful clones
	A	B	C	D	Total	
*Eco*RI	72	41	2	9	124	33
*Eco*RV	71	38	0	15	124	31
*Dra*I	39	14	0	6	59	24
*Hin*dIII	43	10	0	6	59	17
*Xba*I	29	6	0	4	39	15
*Hae*III	26	9	0	4	39	23

Table 3 Genetic map length of the chromosomes (Chrom) in the *esculentum* × *pennellii* map (*e* × *p*), as determined by Tanksley et al. (1992), and in the *peruvianum* map (*per*), determined in the present study, covered by the most distal shared markers. Both maps are calculated with MAPMAKER and given in centiMorgans with the Kosambi mapping function. Nr, number of markers in between, and including, the most distal shared markers in the *peruvianum* map

Chrom	Nr	*e*×*p*	*per*
1	10	118	128
2	6	95	78
3	6	71	90
4	6	54	55
5	3	44	38
6	8	93	83
7	8	71	91
8	6	70	77
9	7	89	115
10	4	90	109
11	2	27	44
12	7	91	165
Total	73	913	1073

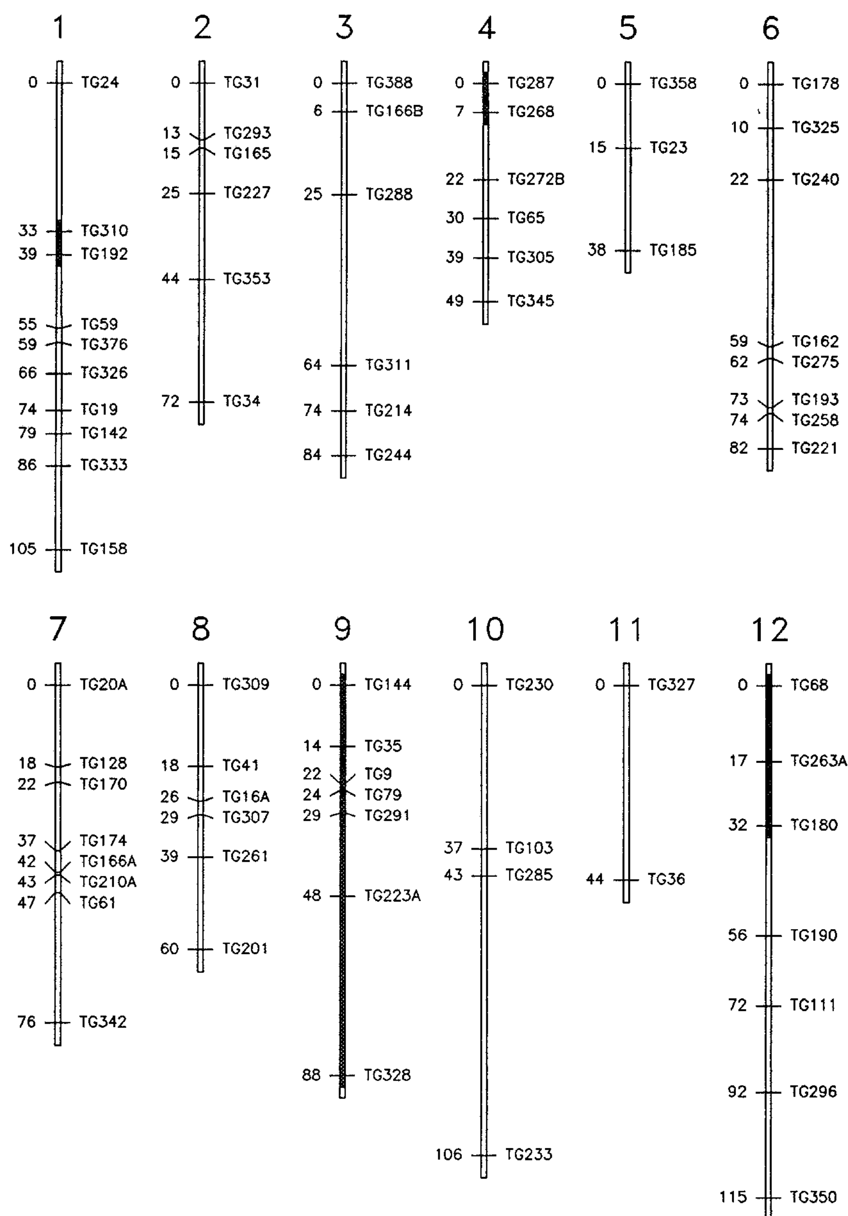

Fig. 1 Genetic linkage map of 73 RFLP markers, determined with backcross populations A, B, and C, within the species *L. peruvianum*. The markers were determined on 152 resistant plants; 21 markers were determined additionally on 116 susceptible plants. *Shaded areas* indicate modified orders compared to the *esculentum* × *pennellii* map. Calculated with JoinMap; in centiMorgan units with the Kosambi mapping function

The genetic length of the *peruvianum* and *esculentum* × *pennellii* maps covered by the most distal shared markers differed by 160 cM (Table 3). This difference was mainly due to chromosome 12, which contributed 74 cM. Chromosome 9 contributed 26 cM to the difference, which may be due to the rearrangements on this chromosome.

Comparison of the male and female maps

A linkage map from female meiosis was calculated using the data of backcross population A, and a map from male meiosis using the pooled data of populations B and C (Fig. 2). In the female map the chromosomes are longer than in the male map, except for chromosomes 6 and 11. Averaged over all chromosomes, the female map was 39% longer than the male map. A comparison of the length of the segments between adjacent markers revealed that 43 out of 61 segments were longer in the female map.

Discussion

RFLP variation within *L. peruvianum*

The genetic distance between the *L. peruvianum* accessions LA 2157 and LA 2172, as calculated from our data, is only 2.0% (proportion of nucleotide substitution). This is relatively low compared to the distances (3.3–3.9%) between *L. peruvianum* accessions reported by Miller and Tanksley (1990). Rick (1986) showed that crossability among *L. peruvianum* populations is generally lower than that found with LA 2157 and LA 2172. Although the ac-

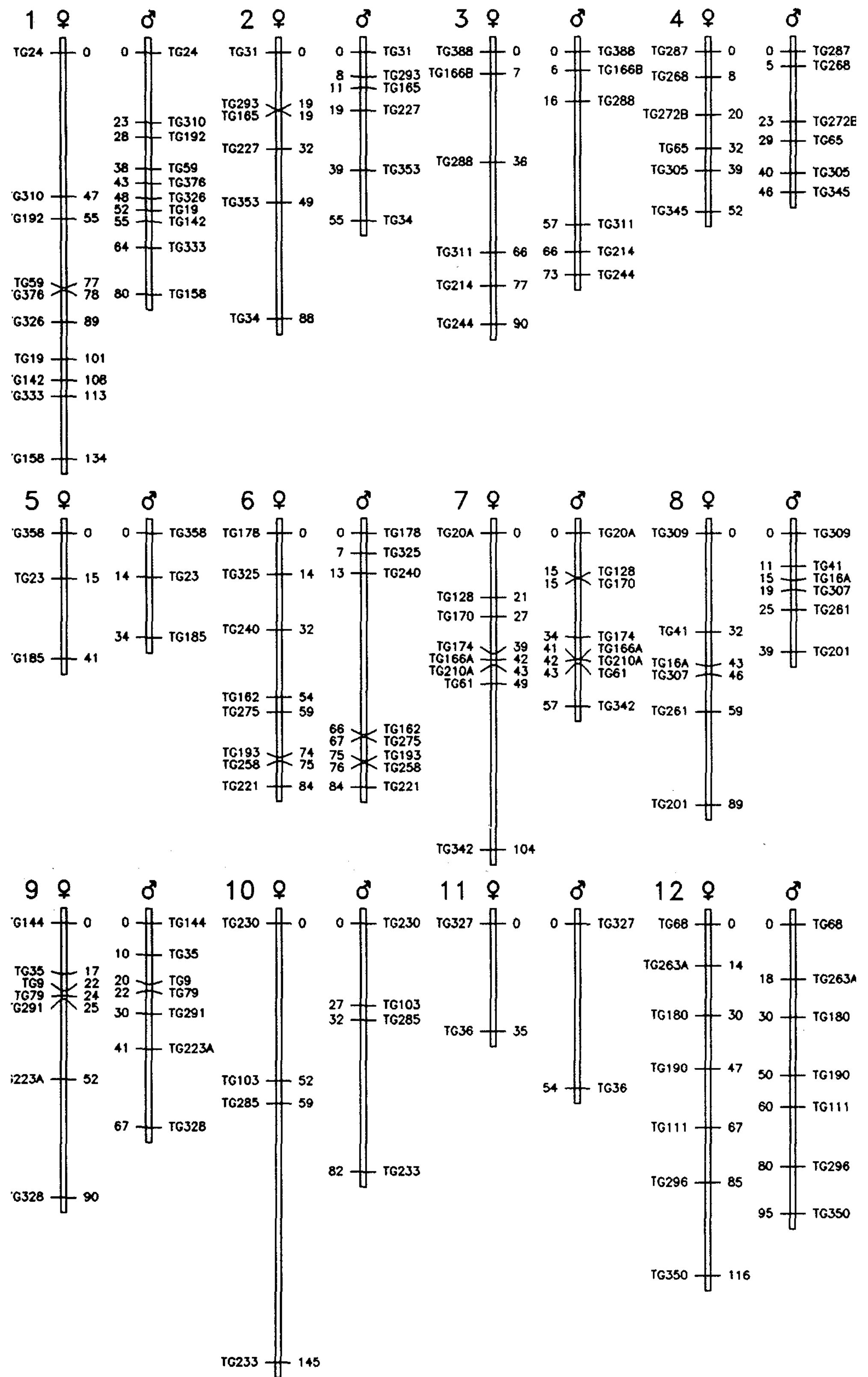

Fig. 2 Genetic linkage maps from male and female meioses determined with the backcross populations within *L. peruvianum*. Calculated with JoinMap; in centiMorgan units with the Kosambi mapping function

cessions used by Miller and Tanksley (1990) were not included in the crossability study of Rick (1986) (except for LA 462), both the observed genetic distance and the crossability indicate that LA 2157 and LA 2172 are phylogenetically more closely related than those accessions.

Comparison of the *peruvianum* map to the *esculentum* × *pennellii* map

In this study a linkage map of the species *L. peruvianum* was calculated for a set of 73 molecular markers, of which

70 had also been used in a mapping study involving an F_2 of *L. esculentum* and *L. pennellii* (Tanksley et al. 1992). When comparing these two maps, only one marker (TG258) was found to map on another chromosome, presumably because the corresponding DNA clone is not a single-copy clone. The order of markers on the maps was identical, with the exception of chromosome 9, which showed a significantly different map position of TG9.

For the comparison of the lengths of the *esculentum* × *pennellii* and *peruvianum* maps MAPMAKER had to be used. The major difference was observed for chromosome 12. However, when comparing the *peruvianum* map calculated with MAPMAKER to the map calculated with JoinMap (compare Table 3 with Fig. 1), the major difference (43%) was also found for chromosome 12. The other chromosomes were on average only 10% (from 0% to 31%) longer when calculated with MAPMAKER. Hence, the difference in length of chromosome 12 between the *esculentum* × *pennellii* and the *peruvianum* map may be a consequence of the different mapping algorithm of MAPMAKER.

Comparison of the male and female maps

In this study the recombination at male meiosis was shown to be less frequent than at female meiosis. The genome-wide difference was twice as large as the difference found in an F_1 between *L. esculentum* and *L. pennellii*, as described by De Vicente and Tanksley (1991). They found the female linkage map, covering all chromosomes with 85 markers, to be 18% longer than the male map. Rick (1969) found a similar effect in the recombination between three neighbouring pairs of markers in a cross between *L. esculentum* and *L. pennellii*. Possibly, this is a more general phenomenon within the *Solanaceae*, but up to now such a relationship between sex and recombination for all species within a taxon has only been described for the *Liliaceae* (Burt et al. 1991). Hence more species need to be studied to validate this generalization.

Rick (1969) suggested that selection against recombinant genotypes of male gametes or zygotes, in favour of parental genotypes, might play a role in the difference of the estimated recombination between sexes. If gametophytic selection in favour of parental genotypes is based on just one locus per chromosome, the estimation of recombination frequency is unbiased (see *Linkage analysis* paragraph). Hence, even under extreme segregation distortion caused by selection at one locus, the estimated recombination frequency is an unbiased estimate of the frequency of the recombination that took place in the meiosis. However, if gametophytic selection in favour of parental genotypes acts on two (or more) loci on one chromosome, this in effect is selection against recombinant genotypes, as suggested by Rick (1969). The result will be, that the recombination frequency of any pair of loci sharing a chromosomal segment, with the two (or more) loci under gametophytic selection, will be underestimated. Although larger differences in map length were found for chromosomes that showed larger differences in allele frequency between cross A on the one hand and crosses B and C on the other (chromosomes 1, 2, 7, and 8), the chromosomes without any real differences in allele frequency (chromosomes 3, 4, 5, 10, and 12) also had larger female than male map lengths [see Sandbrink et al. (manuscript in preparation) for the allele frequencies]. Therefore, it is concluded that recombination is reduced at male meiosis, though gametophytic selection for parental genotypes at more than one locus per chromosome may be partly responsible for the reduction of the estimated male map length.

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