Theor Appl Genet (1991) 83:173–178

THEORETICAL AND APPLIED GENETICS

Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato

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Received May 3, 1991; Accepted May 29, 1991
Communicated by G. Wenzel

Summary. We have determined that meiotic recombination differs between male and female gametes derived from the same plant. A single F_1 plant was backcrossed to each of the parents, *Lycopersicon esculentum* and *L pennellii*, as the male (BCE) and female (BCP) parent, respectively. A total of 85 RFLP markers, covering more than 75% of the tomato genome, was used to construct a genetic map for both populations. Since both recurrent parents were homozygous, recombination measured in each population reflects crossing-over rates leading to male (BCE) and female (BCP) gametes. Comparisons were made by interval (genetic distance between two adjacent markers), by chromosome, and for the total length of the genome. Significantly less recombination was observed for male gametes at all levels. No significant relationship was found between areas of reduced recombination and approximate location to the centromere. That selection plays some role could not be eliminated, but no clear evidence was observed for single-locus selection as a major factor in the general reduction of crossing-overs in male gametes.

Key words: Lycopersicon – Sex – Crossing-over – RFLP

Introduction

A number of variables (both environmental and genetic) can affect the amount of crossing-over that occurs during meiosis (reviewed in Stadler 1926; Graf 1989). One such variable is sex. In *Drosophila* (cf. Baker et al. 1976), horses (Andersson and Sandberg 1984), humans (Donis-Keller et al. 1987), salmonid fish (Johnson et al. 1987), and *Xenopus laevis* (Graf 1989), for example, males normally experience less meiotic crossing-over than females. However, other cases in which females show less recombination have been reported (silkworm, Maeda 1939). The reasons for these sex differences are unknown, but it has been suggested that anomalous meiotic pairing of chromosomes occurs in males (Darlington 1934), or that crossing-over occurs at chromosomal sites specific for each sex (White et al. 1986).

Whether or not meiotic recombination is significantly greater in one sex organ than the other has also been a subject of some debate for plant scientists but, unfortunately, the experimental data on this subject is equivocal (Robertson 1984; Carlson 1988; Zhuchenko et al. 1989). Unlike the situation in animals, most plants carry both sex organs in the same individual and these organs differentiate from the same meristematic tissues (Murphy and Thompson 1988).

One of the limitations of previous studies has been the inability to compare recombination rates over the entire genome of male and female gametes derived from the same plant. All previous studies have been restricted to comparing a few linked chromosomal intervals bounded by morphological markers (Rick 1969; Robertson 1984; Zhucheko et al. 1989). To test a hypothesis of a general reduction in recombination in one sex over the other requires the ability to measure crossing-over in all chromosomes throughout the genome simultaneously. In the past this was impractical due to the lack of sufficient numbers of markers. With the development of high-density RFLP maps, such as that available in tomato (Bernatzky and Tanksley 1986b), it is now feasible to conduct such studies. By choosing markers at regular intervals, one can design crosses in which recombination can be measured throughout the genome in male and female gametes from the same plant.

The objective of this study has been to determine whether or not progeny derived from male and female gametes have different levels of recombination and, if so, whether this is a genome-wide phenomenon or is restricted only to certain regions of specific chromosomes. Information from such a study might be valuable not only for what it reveals about sex differences in plants, but also for its practical implications. If differences in recombination exist between the sexes, these might be exploited in crossing schemes to either reduce crossing-over (e.g., in the construction of chromosome substitution/addition lines) or to increase recombination (e.g., in cases where undesirable linkages need to be broken or for the construction of high-resolution RFLP maps around genes targetted for cloning).

Materials and methods

Plant materials and crosses

Lycopersicon esculentum cv Vendor Tm2a and *L. pennellii* (LA716) were hybridized using *L. esculentum* as the female parent, due to unilateral compatibility between the two species (Hardon 1967). A single F_1 plant was backcrossed to each of the parents. For the backcross to *L. pennellii*, the F_1 was used as the female. Hereafter, this population is referred to as the BCP (backcross *pennellii*). For the backcross to *L. esculentum* (hereafter referred to as BCE, backcross *esculentum*) the F_1 was the male parent. All crosses were made at approximately the same time (8:00 a.m.–10:00 a.m.) in a greenhouse with a light regime of 16 hr light 8 h dark.

Seeds from the BCE and BCP were germinated in flats in the greenhouse in the spring of 1988. Germination rates were low for both populations (<50%), however a total of 78 BCE and 115 BCP plants was ultimately obtained.

RFLP analysis

DNA was prepared from each plant from the BCE and BCP populations from fresh frozen tissue as described by Bernatzky and Tanskley (1986a), except that mercaptoethanol was substituted by sodium bisulfite. Total DNA was digested with *Eco*RI, *Eco*RV, *Dra*I, *Hae*III, *Bst*NI, or *Xba*I (depending on which enzyme was needed to detect polymorphism with the clones used), separated on agarose gels, and blotted as described by Bernatzky and Tanksley (1986a).

Clones for RFLP probing were chosen at intervals of approximately every 10–20 map units, based on a previously established tomato RFLP map (Bernatzky and Tanksley 1986b). The selected 85 clones encompass ca. 1,200 cM representing more than 75% of the tomato genome (Fig. 1).

Probes were labelled with ^{32}P-dCTP using the random hexamer method (Feinberg and Vogelstein 1983). Hybridization and autoradiography were as published (Bernatzky and Tanksley 1986b).

Construction of RFLP maps and statistical analyses

Genetic maps were constructed for each of the two populations (BCE, BCP) based on RFLP segregation data and utilizing the MAPMAKER computer program described by Lander et al. (1987). Markers were placed in a linear order on the maps only if the order was preferred by a LOD>3 (Fig. 1).

Interval comparisons were made using Chi-square 2 × 2 contingency tables. Total recombination per chromosome was compared by *t*-tests.

Results

A total of 85 markers was used to construct linkage maps for both the BCE and BCP populations (Fig. 1). The order of markers deduced from both populations was the same and is consistent with previous information regarding the position of these markers on tomato chromosomes (Bernatzky and Tanksley 1986b; M. C. de Vicente and S. D. Tanksley, unpublished results); however, the total map length differed significantly for the two populations (see next section).

Progeny derived from female gametes are more recombinant than those derived from male gametes

Both backcross populations originated from the same hybrid plant; however, since BCE was generated using the F_1 as the male parent, recombination detected in this population reflects crossing-over that occurred in male gametes. On the other hand, BCP was produced using the F_1 as the female parent, and thus recombination in the population reflects crossing-over in females gametes. Since the recurrent parent in either case was homozygous, any difference in recombination between these two populations could potentially be attributed to difference in male versus female crossing-over rates. The map from the BCE population (male gametes) gave a total length of 1,097 cM versus 1,299 cM for the BCP population (female gametes). This difference was determined to be significant ($P<0.01$) based on a comparison of total crossovers per gamete for male versus female gametes (Table 1).

A chromosome-by-chromosome comparison of map length was made in hopes of determining whether the lower value for male gametes was due to a general reduction in recombination throughout the genome or only to specific areas of particular chromosomes. For 11 of the 12 chromosomes, the map length was greater for the BCP population, suggesting that recombination is reduced genome-wide for plants derived from male gametes (Table 1). Only for chromosome 9 did the map length of the BCE population exceed that of the BCP population (Table 1).

Interval comparisons

While the above results suggest that recombination is reduced in most chromosomes for plants derived from male gametes, it does not eliminate the possibility that certain regions of the chromosomes are unaffected or even experience greater recombination. To test this possi-

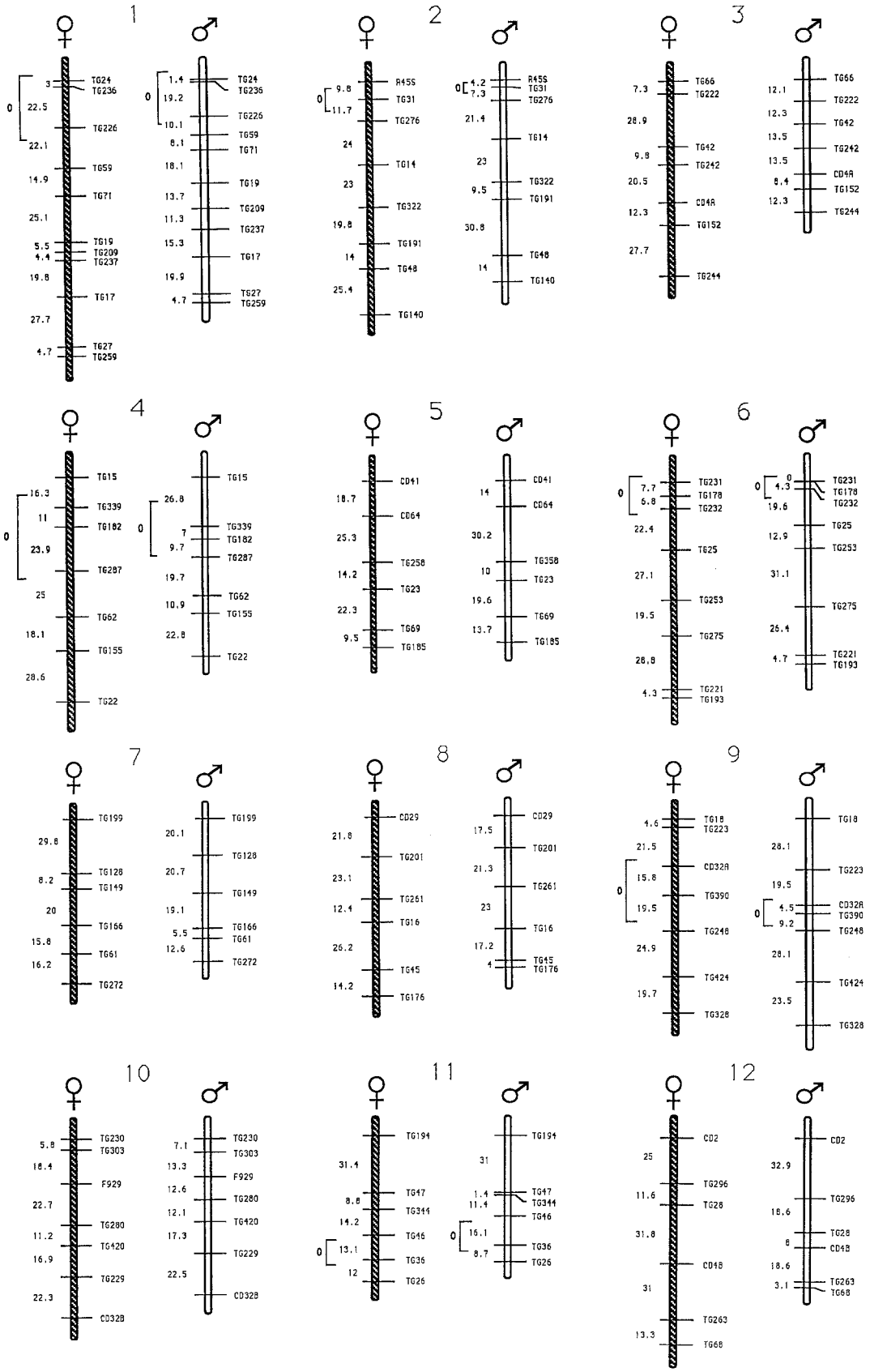

Fig. 1. Genetic maps of the BCP (female) and BCE (male) populations. The approximate location of the centromere (0) is shown in those chromosomes on which it is known

Table 1. Comparisons of recombination percentages (recombinants/total) for each chromosome as well as for the entire genome, based on the BCE and BCP populations. Probability is for the difference between BCE and BCP being attributable to chance (based on *t*-test analysis)

Chromosome	Recombination percentage		Male/female	Probability
	BCE (male)	BCP (female)		
1	122	150	0.81	0.02
2	110	128	0.86	0.10
3	72	107	0.67	0.03
4	97	123	0.79	0.10
5	88	90	0.97	0.68
6	99	117	0.85	0.13
7	78	90	0.87	0.03
8	83	98	0.85	0.79
9	113	106	1.07	0.68
10	85	97	0.87	0.05
11	69	80	0.86	0.12
12	81	113	0.72	0.02
Total	1097	1291	0.84	0.01

Table 2. Comparisons of recombination percentages (recombinant/total) for intervals between linked markers. Only those significant different ($P \leq 0.05$) are shown

Chromosome	Interval	BCE (male)	BCP (female)	Male/female	Probability
1	TG226-TG59	10	22	0.45	0.025
2	TG191-TG48	30	14	2.10	0.025
3	TG222-TG42	12	29	0.41	0.01
3	TG152-TG244	12	28	0.43	0.025
4	TG182-TG287	10	24	0.42	0.05
6	TG231-TG178	0	8	0.00	0.05
7	TG128-TG149	21	8	2.63	0.05
7	TG166-TG61	6	16	0.38	0.05
8	TG45-TG176	4	14	0.29	0.05
9	TG18-TG223	28	5	5.60	0.001
9	CD32A-TG390	5	16	0.31	0.05
11	TG47-TG344	1	9	0.11	0.05
12	TG28-CD4B	8	32	0.25	0.001
12	TG263-TG68	3	13	0.23	0.05

bility, recombination frequencies within individual intervals (intervals are defined as regions between pairs of linked, adjacent markers) were calculated by counting the total number of crossovers occurring within each interval from each data set (BCE and BCP), after removing all missing values.

Of the 73 intervals analyzed, 51 (70%) were found to have more map units in the BCP than in the BCE population (data not shown). A two-way Chi-square contingency test revealed that 11 of these were significantly different ($P < 0.05$) (Table 2). These significant intervals were found on 10 of the 12 chromosomes, which supports the conclusion that recombination is generally suppressed in the population derived from male gametes (Table 2). However, there were three intervals that were significantly greater in the BCE population. One of these intervals (TG18-TG223) is on chromosome 9, which may explain why this chromosome was the only one that did not have significantly more recombination in the female-derived BCP population. Two other intervals (one on chromosome 2 and one on chromosome 7) were also significantly greater in the BCE population. However, overall these two chromosomes still had less recombination in the BCE population, presumably due to other intervals in which recombination was reduced (Table 1).

Areas of reduced recombination are not confined to centromeres

The question arises whether some or all of the reduction in recombination is in some way related to major cytological features. For example, crossing-over may be especially suppressed in centromeric regions (Rick 1969) or enhanced in proterminal regions of chromosomes in male gametes (Johnson et al. 1987). Results form this study, however, do not support these proposals. First of all, the reduction in recombination seems to affect most intervals (70% of those examined) (Fig. 1). If suppression were restricted to centromeric regions, only a few intervals (i.e., those adjacent to centromeres) would show reductions. We found that not all significantly different intervals between both populations were near the centromeric region (Fig. 1). In those chromosomes in which the approximation location of the centromere is known, the intervals that differed most significantly were normally not coincidental with the centromere.

Possible reasons for lower recombination in progeny derived from male gametes

It has been suggested that selection may favor parental genotypes in progeny from interspecific crosses in plants (Rick 1969). We were thus interested in whether selection could account for some or all of the reduction in recombination observed for the male-derived BCE progeny.

One manifestation of selection in an interspecific backcross is the skewing of single-locus segregations from the expected 1:1 ratio (Gadish and Zamir 1986). Such skewing has been observed in interspecific crosses in tomato (Rick 1969, 1972), pepper (Tanksley 1983), and cotton (Stephens 1949) among others. A hypothesis by which reduced recombination is attributed to selection for parental genotypes predicts a skewed segregation of genotypes in favor of the parental alleles. To test this hypothesis, we calculated the homozygote:heterozygote ratio for all loci in both the BCE and BCP populations.

Of 85 loci, 18 (21%) were significantly skewed in the BCE population; however, only 3 of these (17%) were in favor of the homozygote (parental type). The other loci

favored the heterozygous (nonparental) genotype, a result that does not support the selection hypothesis. Moreover, the BCP population also showed skewed segregation for the same number of loci (18 loci); however, this time 8 (44%) were in favor of the homozygous parental type. This result also does not favor the hypothesis that single-locus selection is reponsible for reduced recombination in the male-derived progeny.

Discussion

This is the first published study in which a genome-wide test for male versus female recombination has been done in higher plants. Our results demonstrate a general reduction in the male-derived population, and support the notion that crossing-over was reduced during male gametogenesis compared to female gametogenesis in the hybrid plant used in this study. However, there are several points of caution that should be raised in interpreting these results and in extrapolating the conclusions to other plant species. First, this study was performed on a single hybrid tomato plant and may not reflect the situation in other plant species. Second, this study involved an interspecific cross and there is no guarantee that the same results would be observed in intraspecific crosses. However, recent data from this laboratory support the notion that male recombination might generally be reduced in tomato, where a small section of two chromosomes carrying disease resistance genes has been mapped in intraspecific crosses of the wild tomato species *L. peruvianum*. Results from these studies also reveal significantly lower levels of recombination in progeny derived from male gametes versus female gametes (M. W. Ganal and R. Messeguer, unpublished results).

Finally, it is worth pointing out that our results are consistent with those from at least two unrelated animal species, humans (Donis-Keller et al. 1987) and *Drosophila* (Morgan 1912; Baker et al. 1976), in which crossing-over is suppressed in males. Based on this comparison, it would be tempting to speculate that reduced crossing-over in males might be a general rule for eucaryotes. However, there are major differences in gametophytic generations in plants versus animals that make it more difficult to directly relate reduced recombination with reduced crossing-over. In most animals, the male gametes are short-lived and few genes are expressed after meiosis (Beatty 1975). The male gametophytes of plants, on the other hand, have a more involved and autonomous life cycle. A large percentage of the gene products presents in pollen are transcribed by the haploid genome, and competition among pollen grains during germination and growth through the female stylar tissue for fertilization opens up ample opportunity for selection (Heslop-Harrison 1975). Thus, to attribute reduced recombination in male-gamete-derived progeny to less crossing-over during meiosis requires that one eliminate selection as the causal factor. In the experiments described in this paper, we have attempted to test for the effects of selection, and the results from those tests fail to implicate selection as the primary factor responsible for the reduced recombination observed in the male-derived progeny. However, we acknowledge that selection as a factor affecting recombination cannot be completely eliminated.

Implications of results for genetic and breeding studies in tomato

Differences in male-female recombination can potentially be exploited for practical purposes. For example, backcross breeding is a method commonly used to introduce genes for one or a few desirable traits from one variety or species to another. One of the drawbacks of this technique is the simultaneous introgression of undesirable genes linked to the traits(s) being introduced – a phenomenon that has been referred to as "linkage drag" (Zeven et al. 1983; Young and Tanksley 1989). If recombination rates are higher in females, then exercising backcross breeding using the recurrent parent as the male should minimize linkage drag. By the same reasoning, the recurrent parent could be used as the female in cases where it is desirable to minimize recombination, e.g., in the creation of alien substitution lines (Rick 1969).

Sex differences in recombination might also be exploited for high-resolution genetic mapping that would preface chromosome walking with plants derived from recombinant female gametes versus male gametes. This laboratory has recently taken advantage of this point in high-resolution mapping of disease resistance genes in tomato (M. W. Ganal, personal communication).

Acknowledgements. This study was supported by USDA Competitive Grant 88-37262-3921 and U.S. Israel Binational Agricultural Research and Development Fund Grant US-1388-87 to SDT. CV was supported by a fellowship from the Instituto Nacional de Investigaciones Agrarias, Spain. Thanks to M. Briggs for technical support and to Drs. L. O'Donoughue, M. Ganal, and M. Roeder for reviewing the manuscript.

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