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Higher recombination frequencies in female compared to male meisoses in *Brassica oleracea*

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Abstract Linkage maps of the nine chromosomes of *Brassica oleracea*, based on 75 informative molecular markers, have been compared in first and second backcross progeny from a cross between two doubled haploid lines. The second backcross progeny showed greater recombination frequencies for 75% of the pairs of adjacent markers, but there was no obvious indication that this effect was localised to particular regions of the chromosomes. Four chromosomes increased in genetic length more than twofold, while overall, the total map was 66% longer. The possible causes of this discrepancy are analysed. A sex difference in chiasma distribution and/or frequency at meiosis is thought to be the most likely explanation. The implications of this finding for mapping and map-based applications are discussed.

Key words *Brassica oleracea* · Mapping · Meiosis · Molecular markers · Sex differences in recombination

Introduction

Since the 1980s the widespread use of molecular genetic markers for the construction of comprehensive linkage maps has focused attention on the reliability of genetic map data. The basic paradigm of the chromosomal theory of inheritance, developed since the pioneering work of Morgan, Haldane and others early in this century, is that genetic maps are based on recombination frequencies that result from chiasma formation at meiosis. In those species where it is possible to count chiasmata reliably, mean chiasma frequencies can be used to predict the centiMorgan (cM) genetic lengths of the corresponding maps (Nilsson et al. 1993). As more markers are mapped, so the combined map distances between the markers should converge on that predicted by the chiasma frequency. It is important, however, to understand that total map length is not a fixed feature of a given species but may vary due to genotypic, environmental or sex-specific effects (Rees and Thompson 1956). Although differences in recombination have been known to vary considerably with the sex of the parent in sexual organisms (Callan and Perry 1977), data is accumulating that the same is true for male and female meioses in hermaphrodite plants (de Vincente and Tanksley 1991; van Ooijen et al. 1994; Lagercrantz and Lydiate 1995). An understanding of the scale and pattern of variation in recombination frequencies and the resulting map length is of great significance both in terms of evolution and of a wide range of practical applications from plant breeding to map-based gene cloning, all of which may depend greatly for their success on precise knowledge of map distance.

As part of a programme to create a 'substitution library' of chromosomes tracts in *Brassica oleracea* by a combination of backcrossing and selection using molecular markers, we have obtained a data set that allows us to compare recombination in male and female meiosis across the nine linkage groups.

Materials and methods

The parental material for our backcrossing programme consisted of two doubled haploid (DH) microspore culture lines of *Brassica oleracea*. The non-recurrent parent (GD) was derived from the F_1 hybrid calabrese variety 'Green Duke' (*B. oleracea* var 'italica'), while the recurrent parent (RMA12) was obtained by microspore culture of a *B. oleracea* var 'alboglabra' accession. Both lines were chosen for a model programme to map quantitative trait loci (QTL) because they were rapid flowering – the RMA12 line will flower at approximately 2 months from sowing – yet relatively vigorous under field conditions.

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The F_1 between these two lines, RMA12 × GD, was first backcrossed (BC_1), as pollen parent, to the RMA12 DH parent, so that all the subsequent backcross progeny would have the RMA12 cytoplasm – a prerequisite for our QTL mapping programme. A total of 296 random progeny were scored for 126 restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and isozyme markers and, from these, a subset of 75 informative markers were used to select parents for the second backcross generation. Relative map positions were determined using MAPMAKER (Lander et al. 1987), and map distances were calculated in Haldane (1919) centimorgans (cM). All autoradiographs were independently checked to confirm the reliability of interpretation, and any ambiguous genotypes were removed.

A total of 18 of the BC_1 progeny was selected on the basis that each was completely homozygous (as indicated by marker genotypes) for three or more of the nine linkage groups while remaining heterozygous for different short tracts on the others. The total lengths of the heterozygous tracts in the 18 selected lines varied from 11 to 33 markers. These individuals were chosen to allow the introgression of short heterozygous tracts following one or two further backcross generations. Each of the 18 lines was again backcrossed to the RMA12 parent, but this time as seed parent to generate BC_2 families. Thirty BC_2 progeny were genotyped from each of the 18 selected BC_1 parents, but only at those marker loci for which their BC_1 parent was heterozygous, because the remaining loci had already been characterised as homozygous for the RM12 alleles. Comparisons between the number of recombinant and parental genotypes obtained between adjacent pairs of loci in the two backcrosses were made using contingency χ^2 tests.

Results

The 75 loci in the 296 BC_1 progeny could be mapped onto the expected nine linkage groups with no serious ambiguities. The map corresponding to this subset of markers is shown in Fig. 1. The numerical designation of linkage groups, O1 through O9, correspond to the designation of the *B. oleracea* linkage groups in the *B. napus* maps of Parkin et al. (1995). The total inclusive BC_1 map length for the markers used is 1051 cM, with

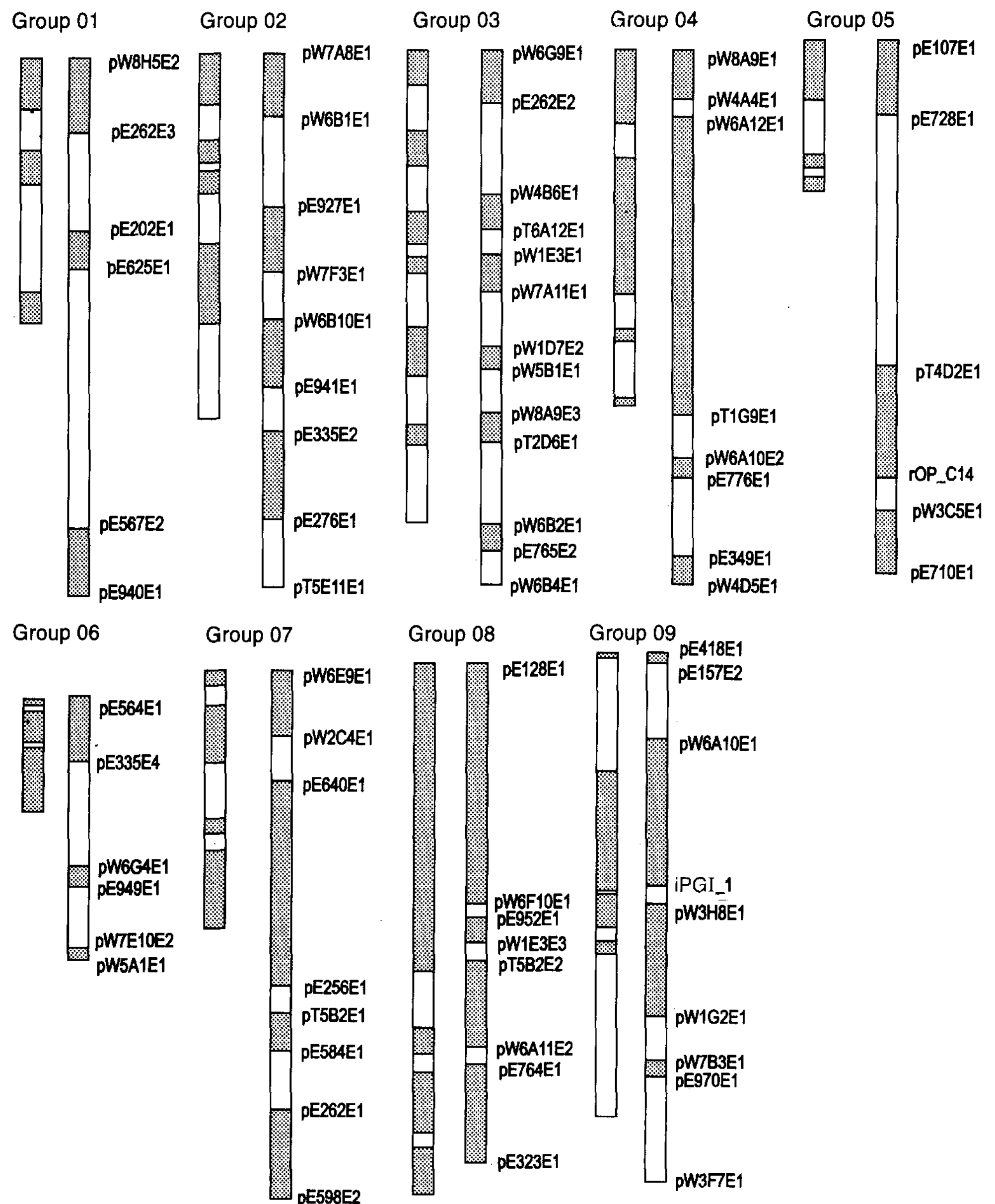

Fig. 1 Comparative ideograms of the nine *Brassica oleracea* linkage groups showing the relative sizes of BC_1 (*left*) and BC_2 (*right*) map intervals. Locus designations are as follows: *pE* Cambridge Laboratory probes, *pW* and *pT* Osborn probes, *iOP* Operon RAPD primer, *iPGI* phosphoglucose isomerase isozyme. *Suffixes E1-E3* represent different *Eco*RI polymorphisms with the same RFLP probe. *Shading* identifies alternate intervals

Table 1 Comparison of recombination frequencies in BC_1 and BC_2 progenies. The contingency χ^2 tests whether the proportion of parental and recombinant gametes is the same in the two backcross generations

Comparison	$BC_1 > BC_2$	$BC_2 > BC_1$	χ^2
Number of recombination frequencies	16	48	16.0
Occasions when contingency χ^2 is significant	1	25	–

Table 2 Relative lengths (cM Haldane) of the nine linkage groups from the two backcross generationss

Chromosome	BC_1	BC_2	BC_2/BC_1
O1	106.3	277.7	2.61
O2	124.5	181.9	1.46
O3	154.0	174.9	1.14
O4	131.3	196.5	1.50
O5	82.0	284.5	3.47
O6	41.1	95.6	2.33
O7	107.4	223.1	2.08
O8	161.3	151.9	0.94
O9	142.9	163.3	1.14
Total	1050.8	1749.4	1.66

the average distance between successive markers being 16.4 cM.

In BC_1, 10 loci showed significant segregation distortion, but at only one of these did the χ^2 have a $P < 0.01$. These 10 loci were randomly distributed over the nine chromosomes but there was clustering of loci showing almost significant distortion in their neighbourhood, which is consistent with viability disturbance rather than mis-classification. In BC_2, however, 12 loci showed distorted segregation, with little apparent correlation with the pattern of distortion in the BC_1 generation. Of these, there was very significant distortion associated with 5 adjacent markers on linkage group O8 in which there was an almost 2:1 excess of heterozygotes.

The data in Table 1 show that in 75% of the 64 pairs of linked loci, the recombination frequency in BC_2 is the larger ($\chi^2_{[1]} = 16, P << 0.001$). For each of these 64 pairs of loci, a 2×2 contingency χ^2 was carried out to compare the frequencies of parental and recombinant gametes in the two backcrosses. The χ^2 was significnat in 26 cases, in 25 of which the recombination frequency in BC_2 was the larger.

The recombination frequencies between adjacent pairs of loci were converted to Haldane (1919) cMs. The total resulting lengths of the nine chromosomes based on this two-point analysis from the BC_1 and BC_2 generations are shown in Table 2, in which it can be seen that the map length of all linkage groups except O8 (the linkage group with segregation distortion) is larger in BC_2 than BC_1. The ratio of the map length of BC_2 to that of BC_1 varies from 3.47 to 0.94 with an average of 1.66. That is, the overall map length obtained from BC_2 is 66% longer; 85% longer if we ignore linkage group O8. Figure 1 illustrates the relative changes in length of the different inter-marker intervals across the nine linkage groups. Inspection of these has failed to indicate any particular pattern to the increase in map length between the generations.

Discussion

The total map length of 1051 cM obtained from our selected markers in the first backcross generation is 23% longer than that presented by Kennard et al. (1994). It is also 42% greater than that expected from the upper limit of published chiasma frequency data for *B. oleracea* of 14.2 (Wills 1966). However, it is consistent with the more comprehensive map of more than 300 loci based on a doubled haploid generation derived from the same parents as in the present study and maps of the *B. oleracea* linkage groups in *B. napus* (Parkin et al. 1995)

The observed increase in overall map length of 66% from BC_1 to BC_2, together with the more than twofold increase in four linkage groups, is a remarkable finding that clearly requires explanation. It appears most likely to have arisen from one of two factors that are confounded in the present design, namely the proportion of loci homozygous in the heterozygous parents and whether recombination occurred during male or female meiosis. Either recombination frequency is higher over the studied intervals in female meiosis or increased homozygosity results in increased recombination in the remaining heterozygous regions. However, an environmental component to the effect cannot be discounted.

The 16 BC_2 families varied in the proportion of homozygous marker loci they contained, which clearly must be closely related to the overall proportion of the genome that was homozygous. To explore whether the increase in recombination was associated with increased homozygosity, as suggested by Tanksley et al. (1992), a linear regression of relative recombination frequency, RF-BC_1/RF-BC_2, onto the number of homozygous marker loci in the BC_1 parent was carried out. This analysis is presented in Table 3 and shows that there is

Table 3 Regression of the ratio RF-BC_2/RF-BC_1 for each backcross against the number of loci homozygous in that backcross

Source	*df*	MS	*F*
Regression	1	18.7185	< 1.0 ns
Residual	285	61.2485	

b = 0.0398

no such significant regression. We are therefore left with the most likely proposition that the recombination frequency is higher in female meiosis in this species or at least that there is a significnat shift in chiasma location within the mapped intervals. It is not uncommon for chiasma distribution to be different in male and female meioses (e.g. Callan and Perry 1977), although comparative studies are relatively rare as it is usually much more difficult to score chiasma frequency in female meioses. In some species, chiasma formation is suppressed altogether in one sex, typically the heterogametic sex, as in male *Drosophila*. Sex differences in recombination frequencies within individual intervals have been known since at least the 1930s (e.g. de Winton and Haldane 1935 in *Primula sinensis*), but the advent of large numbers of polymorphic molecular markers now makes it much easier to obtain accurate data on the pattern of recombination frequencies across the genome in both sexes. However, there is comparatively little published data that allows the examination of sex-specific pattern of recombination across the whole genome of a species. In *Lycopersicon*, de Vicente and Tanksley (1991) used an interspecific hybrid, backcrossed to its parental inbreds both as male and female parent, and found a genome-wide increase in recombination frequency (18%) as measured from female gametes. Similarly, van Ooijen et al. (1994) demonstrated that the female map was 39% longer in *Lycopersicon peruvianum*, while in the pig, Ellegren et al. (1994) showed the same proportional increase in female over male map length. All of these differences favour higher recombination in female meiosis though they are significantly less pronounced than that reported here (66%). The sex of the parent producing the recombinant gametes has also been shown to affect the distribution of crossing over in *Brassica nigra* (Lagercrantz and Lydiate 1995), although the particular pattern of change reported, with more terminal crossing-over in male meioses, is not obviously apparent in the current *B. oleracea* data set.

These findings have important implications for a wide range of applications involving genetical analysis and breeding. First of all, it is clear that considerable caution should be employed in combining maps involving a mixture of information from male and female meioses. Maps derived from F_2 populations are particularly at risk, as are those derived from sib-mating in outbreeding populations, although in the latter case it should be possible, with sufficient marker density, to obtain separate male and female maps and quantify the extent of the heterogeneity.

Marker maps are increasingly being used to study the number, location and effects of polygenes (quantitative trait loci) controlling production traits in crop plants and domestic animals, using segregating F_2 or similar populations. It is hoped that such QTL maps will not only provide data on which to base marker-aided selection schemes but will also eventually lead to the cloning and characterisation of the QTL themselves. However, QTL mapping in such populations generally has very low precision, even if accurate marker maps are available (Darvasi et al. 1993; Hyne et al. 1995), and the increase in heterogeneity caused by the sex differences in the map length of the type reported here will exacerbate this problem. This may mean that precise location of QTL may only be possible using near isogenic lines (NIL) produced from backcrossing.

The reported sex-related heterogeneity in map length is also of considerable relevance to map-based approaches to gene cloning. Together with the reported heterogeneity in the relationships between genetic and physical map distance, which may vary by as much as two orders of magnitude (Wing et al. 1994), sex-specific differences in map distance may make it extremely difficult to predict the required scale of chromosome walking required to cover the interval between two genetic makers. It may also mean that the outcome of many experiments in plant genetics and breeding may depend greatly on the direction of component crosses.

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