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Sex influence on recombination frequency in *Secale cereale* L.

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Abstract The variation in recombination frequency (rf) is important to plant breeders since their major objective is to obtain favorable recombinants of linked genes. One source of variation in rf is sex. Sex differences for recombination frequencies were studied in four of the seven chromosomes of *Secale cereale* L. cv 'Ailés' using isozyme and storage protein loci and were determined on the basis of reciprocal crosses between heterozygous plants of cv. 'Ailés' and homozygous plants of the inbred line 'Riodeva'. The differences were found to be strongly segment-specific. In some cases the level of crossing-over in male and female meiosis was about the same (between *Pgm1* and *Ndh1* loci on chromosome arm *4RS*). However, for most of the chromosome segments in *1R*, *3RL* and *6RL* the male rf was significantly higher than the female rf. Different hypotheses about the mechanisms of plant sex differences for recombination are discussed.

Key words Sex influence · Recombination frequency · Linkage maps · *Secale cereale*

Introduction

Genetic linkage maps are based on recombination frequency, which depends on the number of crossing-overs during meiosis. A number of different factors can affect the crossing-over frequency, such as the individual genotype, sex, centromere, changes in chromosome structure (inversions and translocations), changes in chromosome number and, during meiosis, the total lack of pairing (asynapsis) or chiasmata (desynapsis). Temperature, maternal age, nutritive factors (such as ion concentrations), antibiotics (mytomycine C and actinomycine D), X-rays and cytoplasmic effects are the most common environmental factors that may affect the crossing-over frequency.

Sex is one of most important genetic factors affecting recombination frequency. There are three major ways it may function:

1) The two sexes have normal chiasmatic meiosis, but they show quantitative differences in the number and the chromosomal location of the crossovers. This is the case in humans (Donis-Keller et al. 1987), horse (Anderson and Sandberg 1984), salmonid fishes (Johnson et al. 1987) and *Xenopus laevis* (Graf 1989) where males normally show less meiotic crossing-over than females. However, female grasshoppers normally show fewer crossovers (Hewitt, 1976; Fletcher and Hewitt 1980; Cano et al. 1987; Cano and Santos 1990)

2) One sex has non-chiasmatic meiosis (the crossing-over is blocked); thus the male (XY) of *Drosophila* and the female (WZ) of *Bomyx mori* have non-chiasmatic meiosis. In plants, there is also an example of non-chiasmatic meiosis, in the embryo-sac mother cells in species of the genus *Fritillaria* (Noda 1968).

3) In haplo-diploid species like the bee, the haploid sex (always the male) lacks both crossing-over and the independent assortment of non-homologous chromosomes (White 1973).

Haldane (1922) presented the first general treatment of the problem, advancing the empirical claim that recombination tends to be reduced in the heterogametic sex. Huxley (1928) similarly suggested that whenever a marked sex difference in recombination occurred, it was always the heterogametic sex that had the lower value. Trivers (1988) suggests that both reduced recombination and heterogamety are the consequence of a more intense selection in one sex (usually the male) than in the other (Burt et al. 1991). However, there are some important exceptions to the Haldane-Huxley rule.

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1) The heterogametic sex (male or female) shows a higher recombination in some amphibian, marsupial and grasshopper species (John and Lewis 1965; Morescalchi 1973; Rahn and Martínez 1983; Bennett et al. 1986; Hayman et al. 1988; Cano and Santos 1990; Van Oorschot et al. 1992).

2) The proposed explanation cannot account for the observed differences in hermaphrodites as the two sexes share a common genotype. In four species of *Allium*, in *Arabidopsis*, *Pinus*, pearl millet and maize the chiasmata frequency is significantly higher in the pollen mother cells (Gohil and Kaul 1980; Vizir and Korol 1990; Busso et al. 1995; Groover et al. 1995; Zhuchenko and Korol 1985). In contrast, female recombination frequency is higher in some species of the genus *Tulbaghia* (Vosa 1972) and also in an interspecific hybrid in tomato (Vicente and Tanksley 1991). Finally, in wheat (Wang et al. 1995) and *Brassica* (Lagercrantz and Lydiate 1995), both increased and reduced male recombination frequencies have been observed for different chromosome regions.

One of the limitations of previous studies has been an inability to compare recombination rates over the entire genome of male and female gametes derived from the same plant. The objective of this study was to compare the male and female recombination frequencies in ten backcrossed populations derived from five rye plants using isozyme and storage protein loci located on chromosomes *1R, 3R, 4R* and *6R*. If differences in recombination exist between sexes, the question remains as to whether this is a genome-wide phenomenon or is it restricted to certain regions or specific-chromosomes. Additionally, is this a phenomenon observed in only one individual genotype or is it more general and occurring in several plants?

Materials and methods

The materials used in this work were ten backcross families derived from five hybrids obtained from crossing five different plants *of Secale cereale* L. cv 'Ailés' (A1–A5) with plants of the inbred line 'Riodeva' (30 generations of selfing). Each hybrid ('Ailés' × 'Riodeva') plant was crossed both as male and female with the inbred line 'Riodeva', and two types of backcross populations were obtained, depending on the segregant parent (BC-M and BC-F, respectively; Table 1).

Table 1 Materials used in this work

Plants of cv Ailés	Backcross populations (Ailés×Riodeva)×Riodeva	Number of plants analyzed
Ailés-1	BC_1-Female (BC_1-F)	100
Ailés-1	BC_1-Male (BC_1-M)	55
Ailés-2	BC_2-Female (BC_2-F)	77
Ailés-2	BC_2-Male (BC_2-M)	61
Ailés-3	BC_3-Female (BC_3-F)	174
Ailés-3	BC_3-Male (BC_3-M)	66
Ailés-4	BC_4-Female (BC_4-F)	169
Ailés-4	BC_4-Male (BC_4-M)	145
Ailés-5	BC_5-Female (BC_5-F)	126
Ailés-5	BC_5-Male (BC_5-M)	126

Table 2 Chromosomal location of the loci studied in this work. For references see the review of Schlegel et al. (1986)

Isozyme and storage protein loci studied	Chromosomal location
Posphoglucose isomerase-1 (*Pgi1*)	1RS
Chloroform-methanol proteins (*Cm1, Cm2, Cm3, Cm4*)	1RS
6-Phosphogluconate dehydrogenase-2 (*Pgd2*)	1RL
Malate dehydrogenase-1 (*Mdh1*)	1RL
Secalin-3 (*Sec3*)	1RL
Glutamate oxaloacetate transaminase-4 (*Got4*)	3RL
Malate dehydrogenase-2 (*Mdh2*)	3RL
Phosphoglucose mutase-1 (*Pgm1*)	4RS
NADH dehydrogenase-1 (*Ndh1*)	4RS
Leucin aminopeptidase-1 (*Lap1*)	6RS
Aconitase-1 (*Aco-1*)	6RL
NADH dehydrogenase-3 (*Ndh3*)	6RL
Esterase-6 (*Est6*), Esterase-8 (*Est8*), Esterase-10 (*Est10*)	6RL

All crosses were made at approximately the same time in a greenhouse. In general, we obtained a lower number of seeds in the BC-M populations, most probably due to the fact that the mother plant belonged to an inbred line. The five hybrid plants ('Ailés' × 'Riodeva') used to obtain the ten backcross families showed seven bivalents in Metaphase I.

The isozyme and storage proteins loci analyzed are listed in Table 2.

Results

A total of 18 isozyme and storage protein markers previously located in rye (Table 2) were used to construct the linkage maps for the five BC-F and the five BC-M populations. The order of the markers deduced from both populations was the same and consistent with the information available about their position on rye chromosomes *1R, 3R, 4R* and *6R* (Figueiras et al. 1985; Benito et al. 1990; 1991a, b; Figueiras et al. 1991). However, the male and female recombination frequencies (mrf and frf, respectively) were different and the total map length estimated from male and female gametogenesis for the chromosome segments studied also differed significantly.

The male recombination frequency is higher than the female rf

Since each pair of BC-F and BC-M backcross populations was generated using the same F_1 hybrid plant ('Ailés' × 'Riodeva') as female or male parent, the recombination detected in these backcross populations reflects the crossing-over that ocurred in female or male gametogenesis. Therefore, the differences detected in each pair of backcross populations can not be attributed to differences in the individual genotype. Since the recurrent parent ('Riodeva') in either case was a homozygous inbred line of rye (more than 30 generations of selfing), any difference in recombination

Table 3 Comparisons of female and male genetic length among different rye chromosomes

Plant	Chromosome	Sum of genetic distances between pairs of successive loci	Genetic length	χ^2 P
1	*1R* Female	2.00+27.00+22.00+9.00	60.00	8.62**
1	*1R* Male	0.00+49.09+34.55+14.55	98.10	$P<0.01$
1	*6R* Female	10.00	10.00	0.272
1	*6R* Male	12.73	12.73	$P>0.05$
2	*4RS* Female	14.30	14.30	0.708
2	*4RS* Male	19.70	19.70	$P>0.05$
2	*6R* Female	41.45+28.57	70.02	1.05
2	*6R* Male	50.00+33.33	88.33	$P>0.05$
3	*1R* Female	0.57+5.33+5.48+35.62+13.01	60.01	9.39**
3	*1R* Male	3.03+6.06+13.64+46.97+21.21	90.91	$P<0.01$
3	*4R* Female	20.55	20.55	0.013
3	*4R* Male	21.21	21.21	$P>0.05$
3	*6RL* Female	10.30+10.27+15 75	36.32	12.85***
3	*6RL* Male	28.79+27.27+13.64	69.70	$P<0.001$
4	*3RL* Female	4.14	4.14	11.58***
4	*3RL* Male	21.38	21.38	$P<0.001$
5	*1RL* Female	1.58	1.58	2.06
5	*1RL* Male	4.76	4.76	$P>0.05$

frequency between each pair of backcross populations (BC_1-F and BC_1-M, for example) could potentially be attributed to specific sex-differences in crossing-over rates. The effect of the individual genotypes on recombination frequency can be analyzed by comparing the results obtained from the different pairs of backcross populations (BC_1 and BC_2, for example).

The map from the male gametogenesis gave a total length of 258.09 cM (maximum) versus 156.21 cM (maximum) for the female gametogenesis (2.3 times higher). This difference was determined to be significant (contingency χ^2 =67.29, $P<0.01$) based on a comparison of total crossovers per gamete for male versus female gametes.

A chromosome-by-chromosome comparison of map length indicated that in all cases the map lengths obtained from male gametogenesis were higher than those from the female side (Table 3, Figure 1). Three of the four chromosomes studied – *1R*, *3R* and *6R* – showed significant differences between mrf and frf; no significant effects of sex could be detected for the segment of chromosome *4R*. In any case, the direction of the difference was always towards an higher recombination on the male side.

Interval comparisons: the differences between mfr and frf are strongly segment-specific

The clear reduction in frf does not exclude the possibility that certain regions of the chromosomes are unaffected or even experienced greater frf. To test this possibility, we calculated the recombination frequencies within individual intervals (defined as regions between pairs of adjacent linked markers) by counting the total number of recombinants occurring within each interval from each data set (BC-F and BC-M) (Figure 1). Two-point genetic distances for the same interval were statistically compared by a two-way χ^2 contingency test.

From the 44 intervals analyzed, 36 (82%) were found to have a greater mrf, 7 intervals (16%) showed greater frf and 1 (2%) gave the same frequency of recombination in both male and female gametes. The χ^2 test revealed that 12 of those intervals were significantly different ($P<0.05$) and always with mrf exceeding frf, suggesting that the higher recombination frequency in the male meiosis is a general property of the whole genome.

Despite this consistent trend, it is important to note that while the interval *Pgm1-Ndh1* on the chromsome arm *4RS* was similar in the two different plants analyzed, *Mdh2-Got4* on *3RL* showed a highly significant sex-difference in the Ailés-4 plant (Fig. 1, Table 3). In contrast, the intervals studied in chromosome *6R* did not differ neither in the Ailés-1 nor Ailés-2, but were significant in the Ailés-3 plant; in this last case the two succesive intervals of the 6RL chromosome arm (*Aco1-Ndh3* and *Ndh3-Est6*) showed parallel effects. A similar situation is observed for chromosome *1R*, where only three intervals showed significant differences: *Pgi1-Cm3* in plant Ailés-3, and *Sec3-Cm1* and *Mdh1-Cm1* in plant Ailés-1 (these two later segments include the centromere). Therefore, not only may the mrf and frf vary among plants, but they may also vary among different segments of the same plant; that is, the significant differences between mrf and frf are strongly segment specific.

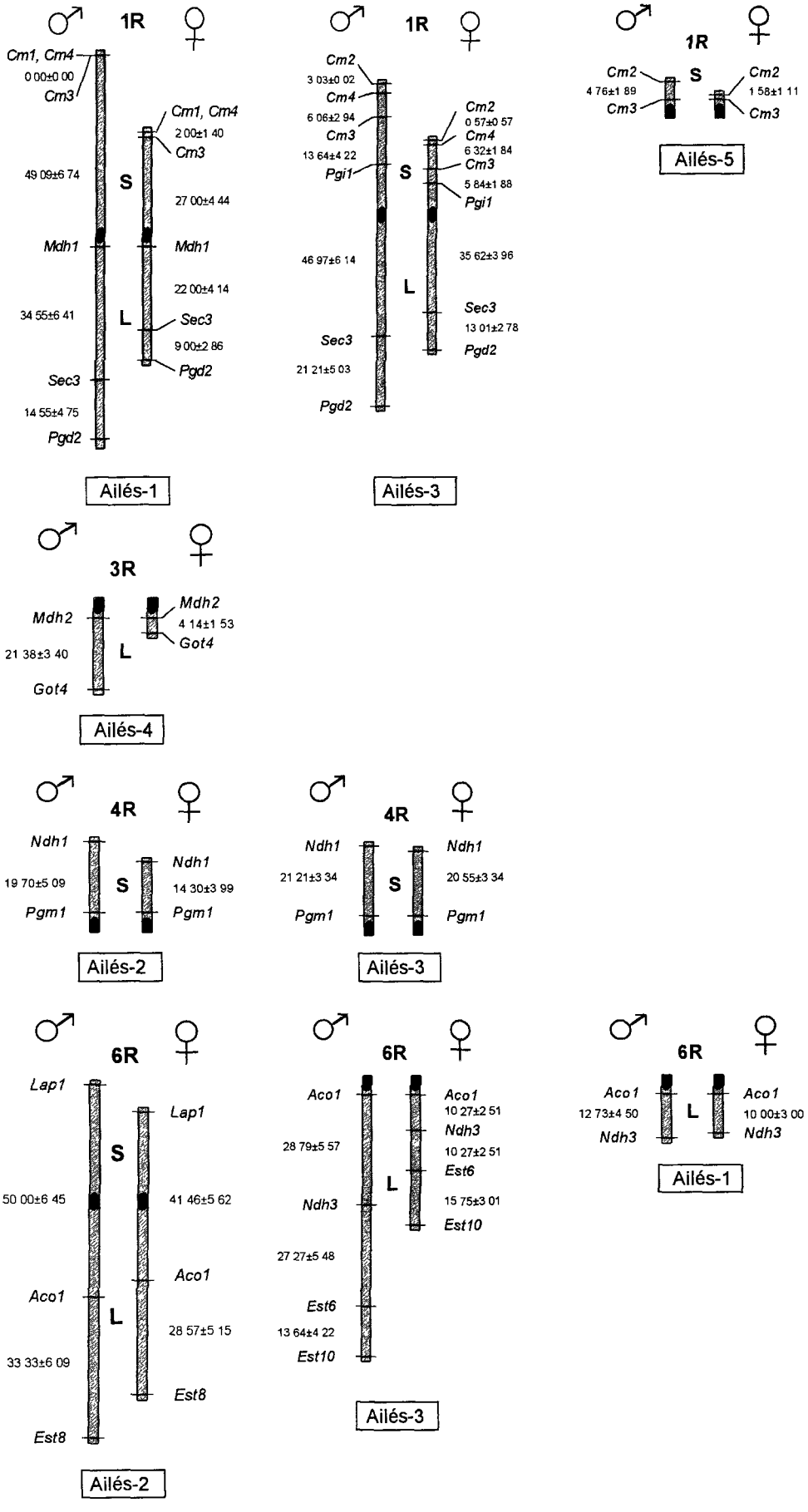

Fig. 1 Chromosome-by-chromosome comparison of linkage map lenghts as estimated from male and female gametogenesis

Discussion

Our results suggest a general reduction in female recombination frequency and support the notion that crossing-over was reduced during female gametogenesis compared to male gametogenesis. However, the heterogeneity of the results shows that there are several points of caution that should be taken into account when considering the general problem of sex differences in recombination frequency, since the comparisons can be established for different chromosomes or chromosome-segments from a same plant or vice-versa.

1) First, considering the consistent trend towards higher recombination frequency in male gametogenesis in rye, it should be stated that only some segments (intervals) of chromosomes *1R, 3R, 4R* and *6R* showed this trend. This phenomenon must be assesed in the future using markers located on chromosomes *2R, 5R* and *7R*.

2) The behaviour of the same chromosome may differ among plants. For example, the male map length of chromosome *6R* in the three plants analyzed is higher than the female length. However, the differences were significant only in one plant (Table 3).

3) The behaviour of each chromosome in the same plant may also be different. The lengths of chromosomes *1R* and *6R* were significantly higher on the male than female side in Ailés-3, whereas the length of chromosome *4R* remained constant (Table 3). Chromosome *1R* presented a significantly higher mrf whereas chromosomes *4R* and *6R* showed higher, but non-significant, mrf (Ailés-1 plant). Parallel examples have been reported in other cases: the chromosome 9 of the interspecific hybrid of tomato analyzed by Vicente and Tanksley (1991) showed a higher mrf while the remaining chromosomes had higher frf: Wang et al (1995) observed that on chromosomes *7B* and *7D* of wheat the mfr was larger than frf, while on *7A* the reverse was true.

4) The same interval can present a different behaviour in different plants. The *Sec3-Cm4* interval showed a significant difference in Ailés-1 but not in Ailés-3. Therefore, different background genotypes could be related but with a different distribution and rates of recombination. In *Brassica nigra* enhanced male recombination frequencies were associated with proterminal regions, while in regions adjacent to putative centromeres enhanced female recombination frequencies were detected (Lagercrantz and Lydiate 1995); therefore, it is not possible to attribute the effect to sex because the estimation of male and female rf are based on two different genotypes.

Our data suggest that the higher mrf generally observed in rye is due to strongly segment specific differences and that these differences also depend on the genotype.

The nature of sex influence in rf

The mechanism of the sex difference in crossing-over frequency in hermaphrodite plants remains unknown. It is not possible to suggest a general mechanism because some species show a reduced crossing-over frequency in male gametogenesis and others the opposite. In some cases the data are even contradictory: for example, cytogenetic analyses suggest that chiasma frequency is higher in human male gametogenesis (Lange et al 1975; Jagiello et al 1976), but genetic data indicate that males have shorter map lengths (Donis-Keller et al. 1987). Therefore, it is speculative to suggest that reduced crossing-over in males might be a general rule for eukaryotes (Vicente and Tanskley 1991) since in many animal and plant species a reduced crossing-over in females seems to be common.

One possible mechanism of a sex difference in recombination related with differential duration of meiotic Prophase I was suggested by Fogwill (1958) to explain the higher chiasma frequency in female meiosis in *Fritillaria* and *Lilium*: a longer Prophase I would be related to a higher opportunity to crossover. In our material, *Secale cereale,* male meiosis takes more than twice as long as female meiosis (Bennet et al. 1973).

Sex differences in recombination might be useful in genetic mapping. Low values of recombination frequency increase the number of pairs of linked loci. In this work, we have observed several cases in which the same pair of loci behave as linked when the female gametes were analyzed but are independent when male gametes were studied. This was the case for *Sec3-Cm1, Cm3-Cm4, Mdh1-Cm1, Cm3-Cm4, Pgd2-Cm1, Cm3-Cm4, Aco1-Lap1* and *Pgi1-Sec3*.

Differences between mrf and frf can potentially be exploited for practical purposes. Backcross breeding is a normal method used to introduce genes for desirable traits from one variety or species to another. One problem of this technique is the "linkage drag" or the simultaneous introduction of undesirable genes linked to the traits being introduced (Zeven et al. 1983, Young and Tanksley 1989). If recombination rates are higher in males, then exercising backcross breeding using the recurrent parent as the female should minimize linkage drag.

Our results in *Secale cereale* and the results previously obtained in other species underscore the important influence of sex in recombination frequency. In the future, when genetic maps obtained in the same species by different authors are compared it will be very important to specify whether these maps are based on the frequency of crossing-over in male or female gametogenesis.

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