Theor Appl Genet (1995) 91: 744–746

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A comparison of male and female recombination frequency in wheat using RFLP maps of homoeologous group 6 and 7 chromosomes

Received: 20 March 1995 / Accepted: 24 March 1995

Abstract A novel approach was used to compare male and female recombination rates in wheat. Doubled haploid lines were developed from an F_1 using two distinct approaches: the anther-culture technique and the *Hordeum bulbosum* system, from which sets of lines were developed from "male" and "female" meioses, respectively. The genotype of the lines was established at RFLP and isozyme markers polymorphic on chromosomes of homoeologous groups 6 and 7, and "male" and "female" linkage maps were calculated using this information. The markers in one segment of chromosome 6B exhibited disturbed segregation frequencies in the anther-culture population. The "male" and "female" maps differed significantly in recombination frequency between some markers on two chromosomes, and these were consistent in direction within chromosomes and inconsistent in direction between chromosomes. In two of the four chromosomes studied the "male" map was much longer than the "female" map. These results suggest that significant differences may exist in male and female recombination frequencies in bread wheat which are specific to certain chromosomal segments but are inconsistent in direction between chromosomes. Other factors, such as environmental influences, may also be important in creating differences.

Key words Wheat · Anther culture · Doubled haploid lines · Recombination frequency · Sex difference in recombination

Communicated by G. Wenzel

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Introduction

Genetic advance in a breeding programme depends on the generation and selection of new recombinant products from intervarietal or interspecific crosses. In this respect the amount of genetic variation released in the segregating generations of a particular cross depends on the random reassortment of chromosomes and the amount of recombination within chromosomes. The latter can vary in crop species and can be influenced by both environment and genotype. Environmental effects include temperature and stress conditions, and these have been used, for example, to increase chiasma frequency with the objective of increasing recombination frequency. Genetic variation can be demonstrated for factors affecting recombination, such as chiasma frequency, and, in some species, e.g. *Primula sinensis* (de Winton and Haldane 1935), *Arabidopsis* (Vizir and Korol 1990), *Brassica oleracea* (M. J. Kearsey, personal communication) and *Brassica nigra* (Lagercrantz and Lydiate 1995), differences exist between male and female gametes in the amount of recombination. In crops such as wheat, sex differences in recombination could have important consequences for genetical studies and breeding strategies but no published information on any comparison is yet available. With the advent of RFLP-based maps of wheat (Chao et al. 1989; Devos and Gale 1993) it is now possible to carry out such studies with relative ease.

A novel approach to evaluating the relative rates of male and female recombination frequency in wheat is to use doubled haploid populations, rather than the usual reciprocal backcross generations. Male gametes can be fixed in the homozygous state using anther-culture techniques and female gametes can be fixed by developing lines from interspecific crosses where chromosome elimination takes place, such as with *Hordeum bulbosum* (Barclay 1975) or *Zea mays* (Laurie and Bennett 1986). In the present work comparative maps for the chromosomes of homoeologous groups 6 and 7 were produced using RFLP and isozyme markers on separate populations of doubled haploid lines

derived from the same cross and developed using these alternative systems.

Materials and methods

Development of mapping populations

Genotypes derived from the European spring wheat varieties Highbury and Sicco were employed for the investigation. As a first step, both varieties had been subject to cytogenetic manipulation to introduce chromosome 5B from the variety Chinese Spring (CS), and to create the single chromosome substitution lines Highbury (CS 5B) and Sicco (CS 5B). CS 5B carries the gene *kr1* which confers interspecific compatibility and therefore renders both substitution lines cross-compatible with *H. bulbosum*.

The substitution lines were intercrossed to form the F_1 hybrid. The F_1 was then used to derive two populations of DH lines, the first using the *H. bulbosum* system (Snape et al. 1979), which produced a female gamete population of which 45 lines were used here (HB population), and the second using anther culture to produce a population from the male gametes, of which 69 were used (AC population) (Snape et al. 1986).

Mapping procedures

RFLP and isozyme markers

Chromosomes of homoeologous groups 6 and 7 were chosen as the target chromosomes for this investigation as they had served as the basis of earlier studies (Chao et al. 1989; Kleinhofs et al. 1988). Initially, the parents were assessed for polymorphism for the available probes using nine restriction enzymes. From the result of this screen chromosomes 6B, 7A, 7B and 7D were chosen for detailed analysis of the recombinant doubled haploid populations.

The maps were developed using the informative probe/enzyme combinations which consisted of both known-function clones, 2437 [locus *Xpsr8(Cxp3)*], 501 [locus *Xpsr2(a-amy-1)*], PSR470 [locus *Xpsr470(Wx)*], bNRp10 [locus *Xak466(Nra2)*], and pcwx27 [locus *Xhhu2(Ppcl)*], (origins described in Kleinhofs et al. 1988; Chao et al. 1989); as well as the anonymous cDNA probes, PSR103, PSR117, PSR119, PSR141, PSR150, PSR167, PSR160, and PSR687. In addition, two isozyme markers, Peroxidase-4 (Liu et al. 1990) and Aminopeptidase-3 (Koebner and Martin 1989), were used.

Isolation of genomic DNA, Southern hybridization and isozyme analysis

The techniques employed for DNA extraction, enzyme digestion, electrophoresis, Southern hybridization, probe labelling and hybridization were essentially as described by Sharp et al. (1988).

Linkage analysis

In each population the lines were expected to segregate 1:1 for the alternative alleles at each marker locus and the observed ratio was tested for significance by chi-square analysis. MAPMAKER, Version 2.0, (Lander et al. 1987) was used with each set of data to establish linkage groups and the Kosambi mapping function was selected to convert recombination frequency to centimorgans (cM). For two-point analysis a LOD score of 2.0 and a recombination frequency of 0.45 were the thresholds for linkage, and for three-point analysis a LOD score of 3.0 was applied. Where map orders differed, the male and female maps were ordered according to established maps (Devos and Gale 1993; Gale and Devos, unpublished) and map distances re-computed.

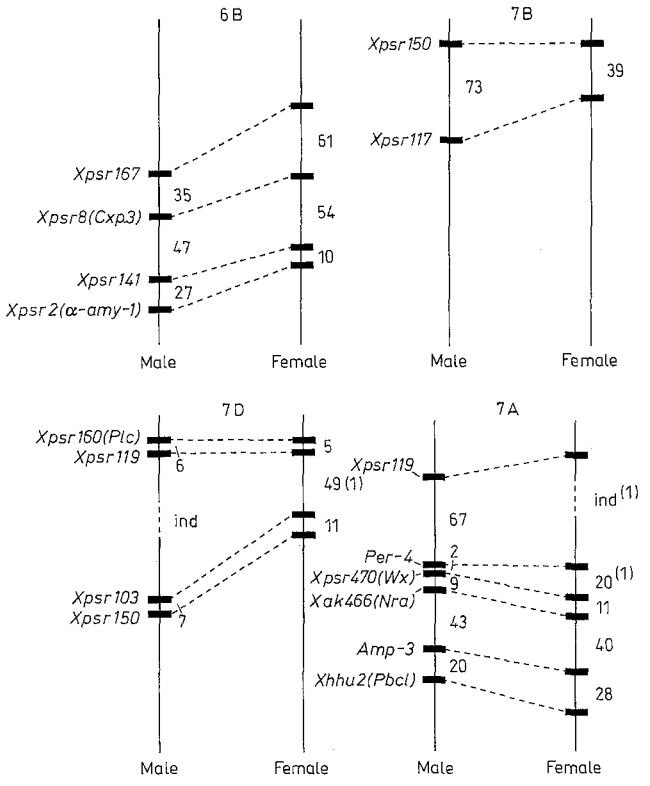

Fig. 1 A comparison of "male" (left) and "female" (right) genetic maps of chromosomes 6B, 7A, 7B and 7D of wheat. Distances in cM (Kosambi); ((1))=maps differ significantly; ind=independent segregation)

To compare 'male' and 'female' recombination frequencies, for each pair of adjacent marker loci in a linkage group, the observed proportions of parental and recombinant lines in each of the two populations were compared by 2×2 contingency chi-square tests.

Results

Construction of "male" and "female" maps

In the HB population, one of the 16 loci exhibited aberrant segregation patterns, significant at the 5% level. This was *Xhhu2(Ppcl)* on chromosome 7A, for which the lines were segregating in an approximate allelic ratio of 2 Highbury:1 Sicco. An excess of Sicco alleles was present in the AC lines at three adjacent loci in the centromeric region of chromosome 6B causing a significantly disturbed segregation frequency on this segment. These were *Xpsr8(Cxp3)*, *Xpsr141* and *Xpsr2(a-Amy-1)*. This distortion remained when these 69 lines were combined with the 45 HB lines, whilst that for *Xhhu2(Ppcl)* disappeared. This provides evidence that, in the development of the AC population, differential segregation or gametic selection exists for the centromeric region flanked by the 6B markers.

In each analysis the 16 loci formed four linkage groups (Fig. 1). The most likely gene orders in the two populations were not identical, but, on the basis of the standard

Table 1 Total map lengths (cM) for the four chromosomes as obtained from female and male meiosis

Ratio	Chromosome							
	6B		7A		7B		7D	
	♀	♂	♀	♂	♀	♂	♀	♂
	125	107	99	74	39	73	65	124
♀/♂	1.17		1.338		0.543		0.524	

errors of the estimates of their recombination fraction, they were indistinguishable from each other and from established maps (Devos and Gale 1993). The four loci on chromosome 6B mapped to a single linkage group which straddles the centromere, *Xpsr167*:*Xpsr8*(*Cxp3*) located on the short arm and *Xpsr141*:*Xpsr2*(*a-Amy-1*) on the long arm. Chromosome 7A comprised the marker group *Xpsr119*:*Per-4*:*Xpsr835*(*Wx*):*Xak466*(*Nra2*):*Amp-3* on the short arm linked to *Xhhu2*(*Ppcl*) on the long arm via *Amp-3*. The four loci, *Xpsr160*, *Xpsr119*, *Xpsr103* and *Xpsr150*, all on the short arm of 7D, fell into two groups of closely-linked loci, *Xpsr160*:*Xpsr119* and *Xpsr103*:*Xpsr150*, the latter being proximal to the centromere.

Comparison of male and female recombination frequencies

To compare male and female recombination frequencies, 12 contingency chi-square tests were performed, of which three were significant, more than would be expected by chance. The intervals were *Xpsr119*:*Per-4*:*Xpsr835*(*Wx*) on 7A, and *Xpsr119*:*Xpsr103* on 7D. *Xpsr119*:*Xpsr103* in the "male" map and *Xpsr119*:*Per-4* in the "female" map were segregating independently. On 7D the "male" recombination frequency was larger than that of the "female" while on 7A the reverse was true. Map distances (cM) for each chromosome are given in Table 1 together with the female/male ratio of lengths, excluding the section *Xpsr119*:*Per-4* of chromosome 7A.

Discussion

These results indicate that significant differences in recombination frequency may exist between male and female gametes of wheat. These differences are specific to certain chromosomal segments, are consistent in direction within chromosomes, and are inconsistent in direction between chromosomes. In two of the four chromosomes the maps derived from female gametes were shorter by approximately one half.

Early reports of a sex difference in recombination were made more than 50 years ago for *Primula sinensis* (de Winton and Haldane 1935) and more recently for *Arabidopsis* (Vizir and Korol 1990). Recent investigations into crop plants have revealed the same phenomenon. In *Brassica oleracea* (M. J. Kearsey personal communication) the "female" maps were larger overall than the "male" maps, being consistent in direction and distributed over the whole genome, while in *Brassica nigra* (Lagercrantz and Lydiate 1995) the recombination frequencies were consistently larger. Genome-wide differences have also been revealed in *Lycopersicon* (De Vincente and Tanksley 1991).

These results, although not covering the whole genome, offer evidence in favour of the existence of sex differences in recombination in wheat. However, the source of the differences is not clear. Environmental or genotype×environmental factors must also be considered. The methods of producing the DH lines were inherently different but they were also developed in different places and at different times: the *H. bulbosum* population in Cambridge in 1980 and the anther-culture population in France in 1984. However, given the small population sizes used and the consequent low power of the test, it can be concluded that sex differences in recombination frequency may well exist in bread wheat.

References

Barclay IR (1975) High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410–411

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495–504

De Vincente MC, Tanksley SD (1991) Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato. Theor Appl Genet 83:173–178

Devos KM, Gale MD (1993) The genetic maps of wheat and their potential in plant breeding. Outlook Agric 22:93–99

Koebner RMD, Martin PK (1989) Chromosome control of aminopeptidase of wheat and its close relatives. Theor Appl Genet 78:657–664

Kleinhofs A, Chao S, Sharp PJ (1988) Mapping of nitrate reductase genes in barley and wheat. In: TE Miller, RMD Koebner (eds) Proc 7th Int Wheat Genet Symp. Institute of Plant Science Research, Cambridge, pp 541–546

Lagercrantz U, Lydiate DJ (1995) RFLP mapping in *Brassica nigra* indicates differing recombination rates in male and female meiosis. Genome 38:255–264

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Laurie DA, Bennett MD (1988) The production of haploid wheat plants from wheat maize crosses. Theor Appl Genet 70:100–105

Liu CJ, Chao S, Gale MD (1990) The genetic control of tissue-specific peroxidases *Per-1*, *Per-2*, *Per-3*, *Per-4* and *Per-5* in wheat. Theor Appl Genet 79:305–313

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Snape JW, Chapman V, Moss J, Blanchard CE, Miller TE (1979) The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity 42:291–298

Snape JW, De Buyser J, Henry Y, Simpson E (1986) A comparison of methods of doubled haploid production in a cross of wheat, *Triticum aestivum*. Z Pflanzengzuchtg 96:320–330

Vizir IY, Korol AB (1990) Sex difference in recombination frequency in *Arabidopsis*. Heredity 54:379–383

Winton D de, Haldane JBS (1935) The genetics of *Primula sinensis*III. Linkage in the diploid. J Genet 67–100