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Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers

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Abstract Sex as a factor affecting recovered recombination in plant gametes was investigated in pearl millet, *Pennisetum glaucum*, by using reciprocal three-way crosses [(A × B) × C v C × (A × B)]. The two populations were mapped at 42 loci pre-selected to cover the majority of the genome. No differences in recombination distances were observed at the whole-genome level and only a few individual linkage intervals were found to differ, all in favour of increased recombination through the male. Distorted segregations found in the three-way crosses provide evidence of post-gametic selection for particular gene(s) or chromosome regions. The significance of these results for the design of pearl millet breeding programmes and inheritance experiments, as well as for other experimental strategies, is discussed.

Key words *Pennisetum glaucum* · RFLP analysis
Recombination rate · Segregation distortion
Genetic mechanisms

Introduction

Recombination at meiosis and the factors that influence recombination rates have been studied extensively. One such factor is the relative recombination rates recovered in male and female gametes. In animals such as *Drosophila* (Baker et al. 1976) and humans (Donis-Keller et al. 1987) the evidence shows that recombination is much higher in the female. However, analysis of recombination frequencies in mouse (Reeves 1990) and silk worm (Maeda 1939) demonstrated higher values in male gametes. In plants, where a clear effect of sex on recombination could be exploited in breeding programmes, the evidence is also equivocal. A number of studies, e.g., in *Zea mays* (Robertson 1984) and *Arabidopsis thaliana* (Zhuchenko et al. 1989), have shown a tendency towards increased recombination in the male. However, these results do not exclude the possibility that the differences are restricted to particular chromosome regions since all the studies involved a limited number of recombination intervals using available morphological or protein marker loci.

With the development of DNA markers and the construction of extensive genetic maps, analyses of recombination frequencies can be extended to an entire genome. In a comparison of male versus female recombination in two backcross populations of tomato using RFLP markers, De Vicente and Tanksley (1991) reported a significantly higher recombination rate in female meiosis. This result was associated with a skewed allelic segregation, as has been observed in many of the molecular mapping projects in plant crop species, e.g., in barley (Heun et al. 1991) and in pearl millet (Liu et al. 1994). These reports indicated that the skewed segregation could not explain the differences in recombination rates. It is clearly important to establish whether the direction and extent of the sex differences in recombination rates in tomato reflect a general situation in plants. From a practical standpoint both differential recombination in male and female meiosis and distorted segregation, if predictable, can be exploited to the plant breeders' advantage.

In this paper we report the results of experiments in pearl millet, *Pennisetum glaucum*, designed to examine the effects of sex on recombination and segregation distortion with a view to possible exploitation in breeding strategies.

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Materials and methods

Genetic material

Three genotypes, each of which had been inbred for more than nine generations, were used in three-way crosses: 81B (a downy mildew-

resistant selection from gamma-irradiated Tift 23DB), ICMP 451 (derived from LCSN 72-1-2-1-1, a selection made in Upper Volta from the ICRISAT Center Late Composite) and BKM 1163 (an inbred derived from crosses between Tift 23DB and a Ghanaian landrace).

F_1 plants of 81B×ICMP 451 were obtained by protogyny-facilitated hand pollination without employing emasculation. A single typical F_1 plant was then crossed as both male and female with the third parent, BKM 1163. All pollinations were carried out at 35 °C in a glasshouse with natural daylength at the ICRISAT Center, and were repeated on 3 consecutive days to reduce the probability of selfing.

The parents were morphologically distinct: 81B is dwarf and has hairy leaf blades; ICMP 451 is tall, with bristled panicles and smooth leaf blades; and BKM 1163 is dwarf, with smooth leaf blades and amber seed colour. F_1 plants of 81B × ICMP 451 were distinguished from selfs by the dominant characteristics of the male parent (smooth leaf blades, bristled panicles, and tall plants). F_1 seed of the three-way cross having BKM 1163 as the male parent, TWC♂, [(81B × ICMP 451) × BKM 1163] were distinguished from selfed seed by xenia for amber coloured endosperm colour, whereas selfed and F_1 seed in TWC♀ [BKM 1163 × (81B × ICMP 451)] were indistinguishable.

From each three-way cross, TWC♀ and TWC♂, 112 plants were grown to the five-leaf stage and then harvested directly into liquid nitrogen before freeze drying. This material, together with leaf samples from the parental and F_1 genotypes, was further characterized at the Cambridge Laboratory.

RFLP analysis

Sixty-eight low-copy PSM DNA clones were chosen to provide coverage of the current pearl millet map (Liu et al. 1994). Of these 41 were found to be informative and were used for mapping the three-way crosses. Analyses with the restriction enzymes *Eco*RI, *Eco*RV, *Dra*I and *Hin*dIII were carried out as described by Liu et al. (1994). The segregation data were analyzed using Mapmaker v1.9, Whitehead Institute, Mass., USA (Lander et al. 1987). The maps are presented in cM, using the Haldane mapping function.

Results

The use of three-way crosses allowed off-type progenies, in particular those produced by selfing, to be identified unequivocally (Fig. 1). After elimination of such progenies, the segregation data for the 112 TWC♀ plants and the remaining 107 TWC♂ plants were analyzed to produce two genetic maps (Fig. 2). In all cases the marker order and linkage group assignment, of the 42 loci that could be mapped using the 41 probes, was consistent between the reciprocal crosses and the map constructed in an independent F_2 (LGD-1-B-10 × ICMP 85410) population by Liu et al. (1994).

Recombination in male and female gametes

The total map distances estimated in the two populations did not differ significantly, being 234 cM in TWC♀ and 267 cM in TWC♂. These values were also similar to the 255.3 cM map distance spanned by the same markers in the (LGD-1-B-10 × ICMP 85410) F_2 map.

Analysis of the seven linkage groups showed that in only one, the short linkage group 3, was there a difference ($P< 0.05$) between the length of the TWC♂ map (22 cM) and the length of the TWC♀ map (9 cM). However, observation of the remaining six groups shows clearly that the sex difference does not reflect a general trend. The analysis of the 35 linkage intervals for which reciprocal data were available showed that only three intervals were significantly different at $P< 0.05$, and of these only two were significantly different at $P< 0.01$. These were *Xpsm223-Xpsm607* in linkage group 1, $\chi^2_{(1)}=7.4$, and *Xpsm451B-Xpsm248* in linkage group 3, $\chi^2_{(1)}=7.1$, with the TWC♂ map having the larger value in each case.

Segregation distortion

The three-way cross design allowed transmission of the alternative 81B and ICMP 451 alleles through pollen and egg cells to be scored with precision. Severe distortion from the expected 1:1 ratio was observed, only in TWC♂, for alleles at loci in linkage groups 1, 4 and 7. The 12 loci sig-

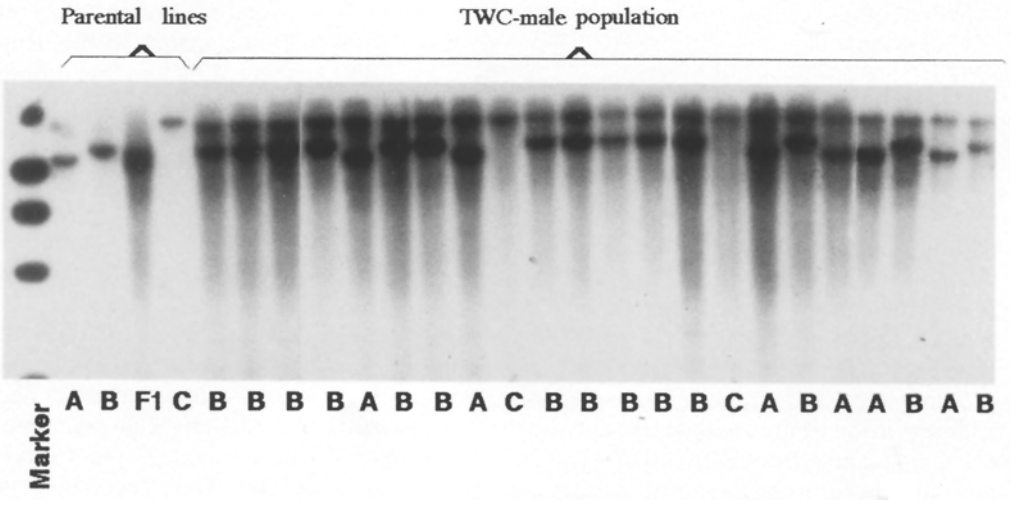

Fig. 1 Segregation of *Xpsm466* in parents (*A,B*), the F_1 plant and a sample of the TWC♂ [C × (A × B)] population digested with *Dra*I. Note, the individual progenies are classified by parental genotype (*A* or *B*), always recovered with the female *C* allele, and off-type (homozygous *C*) in the case of two selfed progeny

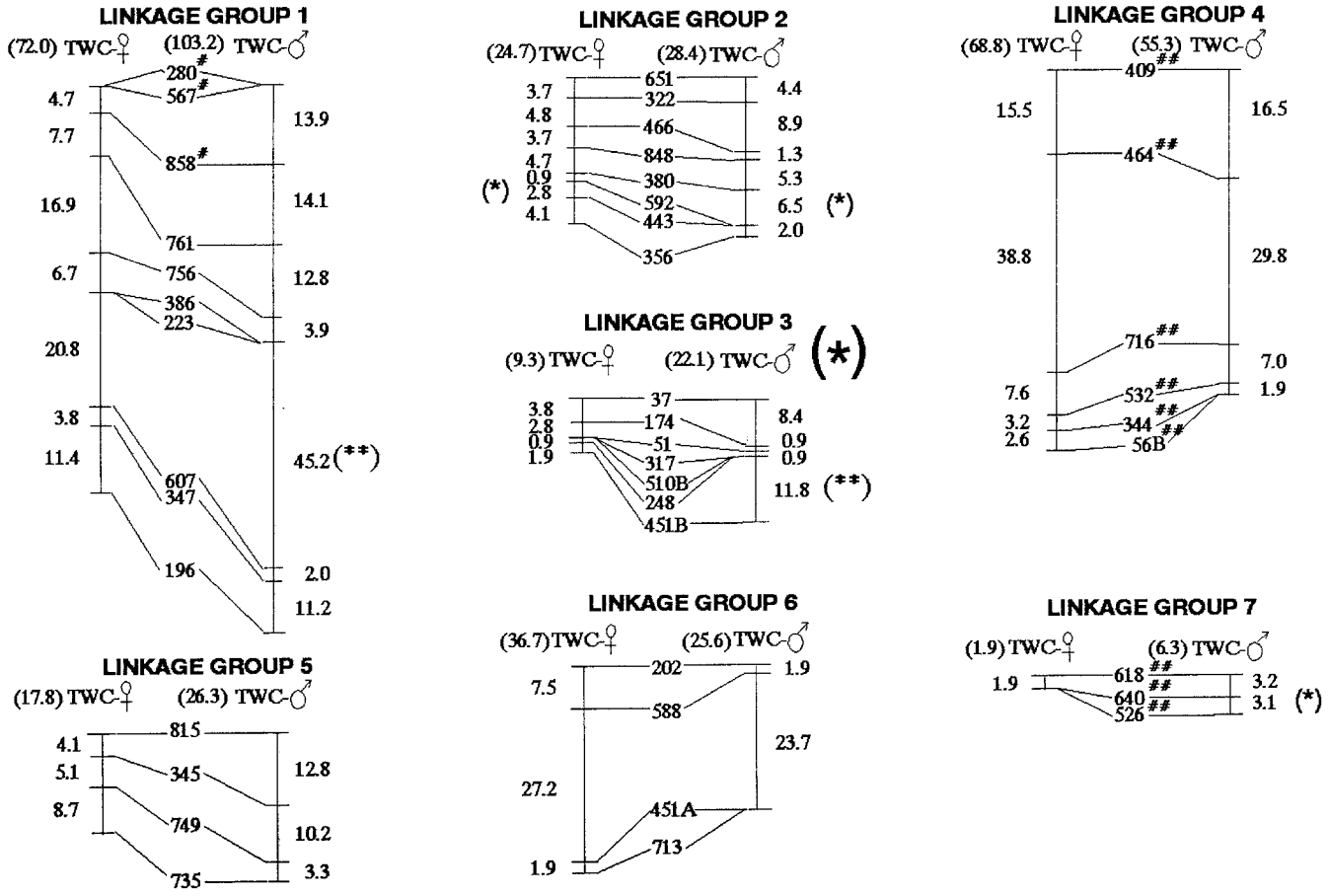

Fig. 2 Male and female genetic maps. Note, linkage groups or intervals showing differences between the TWC♂ and TWC♀ map are shown by $*P< 0.5$ and $**P< 0.01$ in the direction of greater recombination. Loci showing distorted segregation, found only in the TWC♂ population, are shown by $\#P< 0.05$ and $\#\#P< 0.01$

nificantly affected in TWC♂ all showed segregation in favour of the ICMP 451 alleles (Fig. 3) and smaller non-significant biases occurred in TWC♀ along part of linkage group 1 and most of linkage group 4 (Fig. 3).

Discussion

Analysis of the 81B × ICMP 451 three-way crosses confirms the findings of Liu et al (1994) in that the markers mapped in the expected linear arrangement on the same chromosomes, separated by linkage distances similar to those previously observed. Thus the analyses also confirmed the extremely short genetic map described by Liu et al. using 181 random markers. Although the map has not been capped by mapped telomeres it is unreasonable to expect that, with an average of 26 markers per chromosome, a large portion of the genome lies outside the distal markers. Genetic lengths of between 100 and 200 cM per chromosome appear to be the norm in other cereals, e.g., barley (Kleinhofs et al. 1993) and wheat (Devos et al. 1993). It is probable therefore that the low level of recombination per chromosome will limit the rate of progress achieved by pearl millet breeders. Thus any means of maximising recombination will be of value. For example, more generations of random mating can be made, before selection commences, in the creation of composite populations. Also, in population improvement by recurrent selection, mass selection and full-sib progeny selection, which allow frequent recombination generations, can be used rather than methods which predominantly use selfing generations. However, an advantage of the genetically short chromosomes is that they provide an excellent test bed for experiments, such as the one reported here, to measure the effects of factors that influence recombination. Moreover, marker-assisted recovery of non-target recurrent parent chromosomes in backcross breeding programmes will be easy, and relatively inexpensive, in this species.

Differences in male and female recombination

The experiments described here were designed to eliminate other factors that could affect recombination rates in plants. Temperature and photoperiod at meiosis were controlled. Individual genotype effects were eliminated by the use of a single F_1 plant as both male and female in the backcrosses. The effects of non-collinearity, as might be found

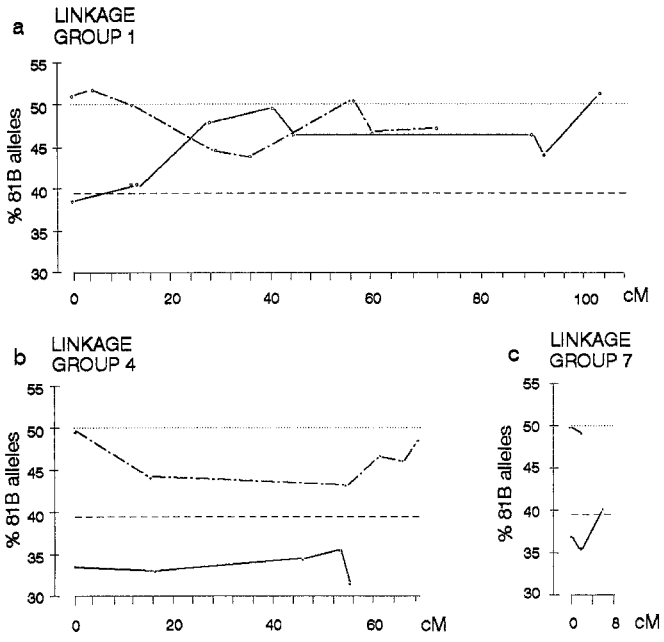

Fig. 3 Disturbance in the ratio of alleles recovered in TWC♂. Note, for the three regions of the genome affected TWC♀ is shown as (–·–·–) and TWC♂ as (———) against the expected 50% allelic recovery rate (.....) and the 95% confidence limit (------)

in interspecific crosses, were minimised by using adapted *P. glaucum* inbreds, and this was eventually confirmed by the maps themselves. Any cytoplasmic effect or cytoplasmic-nuclear interaction effect was avoided since both females in the three-way crosses had Tift 23DB cytoplasm. Moreover, the use of three-way crosses allowed the unequivocal identification of off-type progeny (Fig. 1), particularly those that might arise by selfing, as is quite possible with the hand pollination methods used to produce large amounts of hybrid seed per panicle in pearl millet. However, one effect could not be controlled. Strong selection for or against specific alleles in various regions of the genome, through either the male or female gametes, could decrease measured recombination values. This possibility is discussed further below.

The TWC♂ and TWC♀ maps indicate clearly that the differences in recovered male and female recombination rates are minimal. Results recently obtained in this laboratory (Liu and Gale, unpublished) indicate that the lack of large differences in male and female recombination rates extends to interspecific crosses in *Pennisetum*.

Distorted segregations

Selection for coupling linkages would result in reduction in recombination. Such selection would be reflected in segregation of markers distorted from the expected 1:1 ratios. Such distortion was found, but only in TWC♂. Three regions of the genome were affected and in each case the selection favoured transmission of alleles from ICMP 451 [parent B in C × (A × B)]. There is no evidence, however, that selection is for coupling linkages. The distortion in linkage group 1 was most severe at the top of the group and waned for markers progressively farther down the chromosome (Fig. 3 a) This suggests that the effect is caused by a single gene or gene cluster. Certainly recombination values in TWC♂ are not decreased in that region. The other two linkage groups affected, 4 and 7 (Fig. 3 b,c), are too short to distinguish the alternatives of selection at multiple loci from selection at a single locus associated with linkage drag. The net effects of these disturbances in segregation in the male population would not argue against the findings from the TWC♂/TWC♀ comparisons. Indeed they would, if anything, result in an underestimate of recombination in the TWC♂.

Interestingly, two out of three regions of the genome identified in this study as sources of distorted segregation corresponded with those observed by Liu et al. (1994) in a different mapping population (LGD-1-B-10 × ICMP 85410). However, it cannot be determined whether the distortions on linkage groups 4 and 7 observed by Liu et al. (1994) were due to selection of alleles transmitted through the male, since these experiments employed a F_2 population produced by selfing.

The occurrence of disturbed segregation ratios in specific regions of the genome in two independent crosses warrants further study to determine the most likely among several possible available explanations. Gametophytic competition has been observed for several characters in this crop (Sarr and Pernes 1988; Sarr et al. 1988; Robert et al. 1989, 1991), but sporophytic selection could also have contributed to the distortions observed in the population mapped by Liu et al. (1994).

Should gametophytic selection be found to be a widespread and common phenomenon in pearl millet, knowledge of the mechanism involved will facilitate its possible application. Breeders may be able to exploit the genes responsible for gametic selection once genotypes of pearl millet at these loci are known. This information can be obtained by assaying inbred lines for genotype at selected RFLP loci, but will be more costly to obtain for populations under recurrent selection, where large numbers of plants will have to be assayed with very tightly linked markers to obtain reliable estimates of allelic frequencies. Options available, once performance of a genotype can be predicted, range from being able to maximise transmission (e.g., in the return to a recurrent parent genotype), or to preferentially exclude deleterious alleles known to be carried in these regions of the genome.

Conclusions

A major significance of these results is that there is likely to be little difference in recombination rates recovered from either male or female gametes for the pearl millet breeder to exploit. Of course this conclusion has been obtained from a comparison of only two three-way crosses. However, since the mapping results are similar to those

found possible in an independent F_2 population, there is no reason to believe that they are in any way exceptional. These equivalent recombination rates suggest that backcrossing programmes can be carried out in the most convenient manner, rather than using the F_1 as female as would be suggested from the tomato results (De Vicente and Tanksley 1991). However, breeders wishing to avoid surprises due to segregation distortion should use the segregating genome as the female in crosses. Other applications, such as the construction of inbred substitution lines by marker-assisted selection for entire linkage groups of a donor inbred, will not be able to take advantage of reduced recombination in one gamete or the other to hold linkage groups together. Fortunately, given the low rates of recombination observed in *P .glaucum*, such aides are probably not necessary.

Our results indicate that segregation will be more predictable among female gametes than among male gametes in pearl millet. If breeders are seeking Mendelian segregation ratios in this species, they should examine test-cross progenies where the F_1 has been used as the female parent (contrary to standard practice where it is more often used as the male parent), rather than F_2 populations, or test-crosses where the F_1 is used as the pollen parent. This has recently been demonstrated to hold true where segregation distortion due to gametophytic selection was found in progenies that are predicted to segregate 3:1 or 1:1 for the yellow seedling character of ICMB 88004 (K. N. Rai, personal communication). Further, in a backcrossing program to introduce one or more genes of interest into an elite background, pearl millet breeders should use the recurrent parent as the male, which can conveniently be done by using stored pollen (Hanna 1990) if this facility is available. The male recurrent parent will permit the use of standard formulae (e.g., Sedcole 1977) to calculate the number of plants required to ensure transmission of the donor gene(s) of interest each generation, facilitating their timely and efficient backcross transfer.

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