Theor. Appl. Genet. 56, 193-198 (1980)

The Effect of B-Chromosomes of Rye on the Chromosome Association in F_1 Hybrids *Triticum aestivum* x *Secale cereale* in the Absence of Chromosomes 5B or 5D*

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Summary. *T. aestivum* var. 'Chinese Spring' (monosomic 5B and 5D, respectively) was crossed with *S. cereale* (with and without B-chromosomes). The resulting nullisomic 5B hybrids exhibited a high degree of chromosome association both at 20°C and 10°C. The presence of B-chromosomes reduced association slightly whether 5B was present or not.

In nullisomic 5D hybrids B-chromosomes of rye raise chromosome association at 20°C when compared to hybrids with 5D, with as well as without, B's. At 10°C, due to the absence of the *Ltp* gene on 5D, chromosome association in nullisomic 5D hybrids is low, and no effects of rye B-chromosomes is detectable.

The hypothesis that B-chromosomes of rye carry (an) asynaptic gene(s) decreasing effective pairing, and (an) independent post-synaptic gene(s) increasing chiasma frequency on effective pairing sites, is presented.

Key words: B-Chromosomes – Chromosome associations – *Triticum aestivum* – *Secale cereale* hybrids

1 Introduction

In common wheat *Triticum aestivum* var. 'Chinese Spring' ($2n = 6x = 42$) meiotic chromosome association is controlled by a number of suppressor and promotor genes which are located in several chromosomes of the complement.

In normal plants, the overall effect of this regulatory system is to create a balanced situation in which pairing occurs only between homologous chromosomes, and at a sufficient level. Pairing between homoeologous chromosomes, i.e., those of different related genomes, is supressed mainly by a strong 'dominant' suppressor gene, *Ph*, in the long arm of chromosome 5B (Okamoto 1957; Sears and Okamoto 1958; Riley and Chapman 1958; Wall et al. 1971). Minor homoeologous and general pairing suppressors were also found in chromosomes 3D^{L}, 3A^{S} and 4D (Mello-Sampayo and Canas 1973; Driscoll 1973). Dominant alleles of promoters of homoeologous pairing have been referred to in chromosomes 5D^{L}, 5A^{L} and 5B^{S} (Feldman 1966; Riley et al. 1966; Feldman and Mello-Sampayo 1967; Riley and Chapman 1967) and in chromosomes 5D^{S} (Feldman 1968) and 5A^{S} (Dvorak 1976).

Environmental factors influence the quantitative expression of the genetic component of chromosome pairing (Elliott 1955; Jain 1957; Dowrick 1957). Riley (1966) was able to demonstrate a significant decrease in chromosome association in wheat at low temperature in the absence of chromosome 5D. A low temperature gene (*Ltp*) on the long arm of chromosome 5D of *T. aestivum* var. 'Chinese Spring' was found to stabilise meiotic pairing under low temperatures (Hayter 1969). An equivalent to the *Ltp* locus was presumed to exist on chromosome 5A of tetraploid wheats (Riley and Hayter 1967).

Two diploid species of Triticum, often considered to belong to the related genus Aegilops, *Triticum speltoides* (= *Aegilops speltoides*) and *Triticum tripsacoides* (= *Aegilops mutica*) have accessory chromosomes which have a suppressive effect on homoeologous pairing in hybrids with *Triticum aestivum*. This effect was found to be very similar to that of *Ph* on chromosome 5B (Dover and Riley 1972). Dover (1973) observed however, that B-chromosomes of *T. tripsacoides* induced asynapsis in F_1 hybrids *T. aestivum* x *T. tripsacoides* at low temperatures. It is known that the presence of B-chromosomes in rye increases the variability of the frequency of chiasmata (Jones and Rees 1967). They do not compensate for the absence of *Ph* on 5B of wheat (Roothaan and Sybenga 1976).

The present paper deals with the study of hybrids between *T. aestivum* (normal and monosomic for chromosome 5B or 5D) and inbred lines of *S. cereale* with and without B-chromosomes, at two temperatures.

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* The work was supported by a fellowship of the Gulbenkian Foundation and partly carried out while the author was at the Department of Genetics, Agricultural University, Wageningen, the Netherlands

0040-5752/80/0056/0193/$ 1.20

2 Material and Methods

All wheat genotypes used were derived from stocks originally obtained by E.R. Sears (University of Missouri) of *Triticum aestivum* var. 'Chinese Spring' (2n = 6x = 42). They were either monosomic 5D, monosomic 5B or nullisomic 5B-tetrasomic 5A.

Secale cereale plants with standard B-chromosomes were from an original Transbaikal accession obtained from A. Müntzing at the University of Lund. These B-chromosomes had been transferred earlier to rye material from the Genetics Department, University of Wageningen. This was done by crossing inbred lines of rye without B-chromosomes with the original Transbaikal accessions carrying B-chromosomes. Plants from subsequent generations were crossed and backcrossed six times with different inbred lines.

Hybrids were obtained by crossing *Triticum aestivum* (monosomic 5D, monosomic 5B or nullisomic 5B-tetrasomic 5A), as the female parent, and *Secale cereale* (with or without B-chromosomes).

The hybrids were cytologically selected from the segregating F1 generation. Chromosome numbers in all the tested plants were checked in the root-tips of germinating seedlings after pre-treatment with 1-bromonaphathalene and fixation in acetocarmine. The hybrids studied were either normal or devoid of chromosomes 5B or 5D; or disomic for 5A, with or without B-chromosomes of rye.

The plants were grown over winter in Portugal in greenhouses (normal temperature 20°C ± 2°C) until they were one month old, well before the differentiation of meiotic cells. They were then sectioned in two halves. One of these was kept in the greenhouse and the other transferred to a growth chamber at 10°C temperature and continuous light.

To estimate the chiasma frequency, anthers at first metaphase of meiosis were fixed in acetic-alcohol (1:3) and acetocarmin squash preparations were made. For each hybrid samples of 50-100 pollen mother cells (PMC's) were studied in three to five plants of each type. In the statistical analysis t-tests were used throughout.

At first metaphase of meiosis, the observation of chiasmata implies that pairing occurred and that it was followed by crossing-over between synapsed regions of chromosomes. Metaphase association of chromosome arms looked convincingly like chiamata and were considered as such. Since the presence of more than one chiasma per association was very rare, for convenience all associations were considered equivalent to one chiasma.

3 Results

Chromosome association at 20°C and 10°C in F_1 hybrids *Triticum aestivum* (normal and monosomic 5D) x *Secale cereale* (with and without B-chromosomes) is summarised in Table 1 (10°C) and Figure 1 (20°C). It was not con-

Table 1. Mean chromosome association at low temperature (10°C ± 2) in metaphase of the first division of meiosis in F_1 hybrids *Triticum aestivum* ♀ (normal and monosomic 5D) x *Secale cereale* ♂ (with and without B-chromosomes). (50 cells per plant)

			A-chromosomes					B-chromosomes				
Plant No.	Chromosome No.	Chromosome 5D	Univalents	Bivalents			Chiasmata p/cell	Univalents	Bivalents			Chiasmata p/cell
				Rods	Rings	Total			Rods	Rings	Total	
73	28	present	27.08	0.44	0.02	0.46	0.48					
90	28	present	26.84	0.58	–	0.58	0.58					
93	28	present	27.20	0.40	–	0.40	0.40					
197	28	present	26.20	0.90	–	0.90	0.90					
75	28+2B	present	26.88	0.56	–	0.56	0.56	0.48	0.76	–	0.76	0.76
87	28+2B	present	26.60	0.70	–	0.70	0.70	0.20	0.82	0.08	0.90	0.98
198	28+2B	present	27.28	0.34	0.02	0.36	0.38	0.16	0.88	0.04	0.92	0.96
89	27	absent	26.00	0.48	0.02	0.50	0.52					
90	27	absent	26.32	0.34	–	0.34	0.34					
91	27	absent	25.36	0.82	–	0.82	0.82					
100	27	absent	26.40	0.28	–	0.28	0.28					
101	27	absent	25.24	0.88	–	0.88	0.88					
86	27+2B	absent	25.52	0.74	–	0.74	0.74	0.12	0.80	0.14	0.94	1.08
152	27+2B	absent	25.16	0.94	–	0.94	0.94	0.32	0.84	–	0.84	0.84
155	27+2B	absent	25.12	0.88	0.08	0.96	1.04	0.24	0.78	0.10	0.88	0.98
156	27+2B	absent	26.20	0.40	–	0.40	0.40	0.44	0.70	0.08	0.78	0.86
161	27+2B	absent	25.20	0.90	–	0.90	0.90	0.48	0.76	–	0.76	0.76
154	27+3B	absent	25.24	0.88	–	0.88	0.88	1.28	0.86	–	0.86	0.86
157	27+3B	absent	25.20	0.88	0.04	0.82	0.96	1.20	0.86	0.04	0.90	0.94
158	27+3B	absent	25.36	0.80	0.02	0.92	0.84	1.36	1.36	–	1.36	1.36
96	27+1B+isoB	absent	25.72	0.64	–	0.64	0.64	2.00	–	–	–	0.00
99	27+1B+isoB	absent	25.48	0.76	0.02	0.78	0.80	2.00	–	–	–	0.00

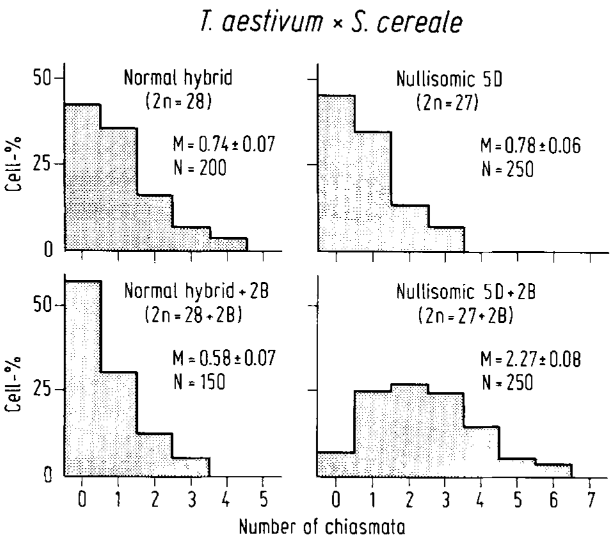

Fig. 1. Distribution of chiasma frequencies per cell in hybrids *T. aestivum* × *S. cereale* at 20°C in the presence and absence of chromosome 5D of wheat and B-chromosomes of rye

sidered necessary to present tables and distributions (figures) for both temperatures.

In plants grown at 10°C no significant differences in chromosome association, which was notably low, were detected. In the absence of the B-chromosomes of rye no difference could be observed between plants with or without chromosome 5D at either temperature. A significant discrepancy became apparent at 20°C when chromosome association of plants carrying rye B-chromosomes was compared. A rise in pairing was found in the absence of chromosome 5D compared to that found in the presence of this chromosome. The increase in chiasma frequency observed was from 0.58 in 28 + 2B plants to 2.28 in 27 + 2B, which is statistically significant at P = 0.001. Three B-chromosomes, or one B-chromosome with a long-arm iso-chromosome, had a very similar effect. The low chiasma frequency of 0.80 at 10°C (in the presence of rye B-chromosomes) in the absence of 5D must be concluded to be due to the destabilising effect of an absence of *Ltp* at low temperature. The difference, with 2.28 at 20°C, is significant (P = 0.001).

Low means of 0.74 and 0.56 chiasmata per cell were observed at first metaphase in F_1 hybrids of normal *T. aestivum* × *Secale cereale* respectively under 20°C and 10°C (Table 1). At this level an effect of an absence of 5D cannot be expressed.

Hybrids deficient for chromosome 5B showed higher pairing at both temperatures (Table 2, Fig. 2). At 20°C, it was observed that the presence of two or four B-chromosomes of rye had a slight but not significant suppressive effect on association, whether chromosome 5B is present or not (P = 0.09).

Hybrids nullisomic for 5B showed no effect of rye B-chromosomes (Cf. Roothaan and Sybenga 1976), but, as expected, homoeologous association was greatly increased. Addition of an extra chromosome 5A (disomic in the hybrid) resulted in a further rise in number of chiasmata per cell, both at 10°C and at 20°C. At 20°C the average chiasma frequency in nulli 5B, disomic 5A was 8.59, in nulli 5B 6.71, a difference which is statistically significant (P = 0.05) even when the extra bivalent due to disomy of 5A is taken into account. B-chromosomes of rye reduced chiasma frequencies slightly and not significantly; they did, however, increase the variability.

4 Discussion

Hybrids between *Triticum aestivum* and either *T. speltoides* (= *Aegilops speltoides*) or *T. tripsacoides* (= *Aegilops mutica*) show that a digenic system existing in the last two diploid species is epistatic to the supressor gene, *Ph*, carried by chromosome 5B of *T. aestivum* (Riley et al. 1961; Riley and Law 1965). On the other hand, hybrids deficient for chromosome 5B have shown low homoeologous pairing, if accessory chromosomes from *T. speltoides* or *T. tripsacoides* are present (Dover and Riley 1972).

The genome of *Secale cereale* has no apparent effect in raising homoeologous pairing in normal hybrids with *T. aestivum*. In the absence of chromosome 5B, the hybrids, as expected, display higher chromosome association at 10°C and 20°C (Fig. 2). An increased dosage of chromosome 5A significantly raises chromosome association in nullisomic 5B hybrids even further. Therefore, the effect of the pairing promoter carried by chromosome 5A (Feldman 1966) is additive to the effect of the absence of 5B. The presence of accessory chromosomes of rye in F_1 hybrids *T. aestivum* × *S. cereale* seems to have a slight supressive effect on chromosome association whether chromosome 5B is present or not, at 20°C (Fig. 2).

In these hybrids chromosome association is usually very low and the variability is always very high, both between PMC's and between plants of the same genotype. Sometimes chromosome associations are observed in rye as a consequence of chromosome stickiness and the existence of large heterochromatic segments at the end of the chromosomes (Lima-de-Faria 1952; Sarma and Natarajan 1973). This non-homologous association is a source of error in the interpretation of meiotic configurations (John and Lewis 1965). These problems make it sometimes difficult to detect differences in chromosome association induced, for instance, by the B-chromosomes of rye. This may be the reason why the differences in chromosome pairing were not statistically significant (9% level of confidence).

No differences in chromosome association were de-

Table 2. Mean chromosome association at low temperature (10°C ± 2) in metaphase of the first division of meiosis in F_1 hybrids *Triticum aestivum* ♀ (normal, monosomic 5B and nullisomic 5B – tetrasomic 5A) × *Secale cereale* ♂ (with and without B-chromosomes). (50 cells per plant)

			A-chromosomes						B-chromosomes				
Plant No.	Chromosome No.	Chromosome 5B	Univalents	Bivalents			Trivalents	Chiasmata p/cell	Univalents	Bivalents			Chiasmata p/cell
				Rods	Rings	Total				Rods	Rings	Total	
82	28	present	26.56	0.52	–	0.52	–	0.52					
83	28	present	26.76	0.62	–	0.62	–	0.62					
196	28	present	26.62	0.54	–	0.54	–	0.54					
84	28+2B	present	26.48	0.56	–	0.56	–	0.56	0.12	0.86	0.08	0.94	1.02
85	28+2B	present	26.92	0.54	–	0.54	–	0.54	0.12	0.80	0.14	0.94	1.08
172	28+2B	present	26.56	0.52	–	0.52	–	0.52	0.08	0.90	0.06	0.96	1.02
199	28+2B	present	26.48	0.76	–	0.76	–	0.76	0.40	0.80	–	0.80	0.80
81	27	absent	17.52	4.12	0.38	4.50	0.14	5.16					
213	27	absent	17.88	4.52	0.34	4.56	–	4.92					
214	27	absent	18.94	3.02	0.80	3.82	0.14	4.90					
168	27+2B	absent	18.42	3.76	0.50	4.26	0.02	4.80	0.28	0.74	0.12	0.86	0.98
170	27+2B	absent	17.80	4.38	0.22	4.60	–	4.82	0.04	0.82	0.16	0.98	1.14
171	27+2B	absent	19.52	3.74	–	3.74	–	3.74	0.12	0.94	–	0.94	0.94
212	27+2B	absent	15.28	6.28	0.56	6.84	–	7.40	–	–	–	–	–
215	27+2B	absent	18.68	4.14	0.02	4.16	–	4.18	0.28	0.28	0.04	0.86	0.90
169	27+4B	absent	17.56	4.30	0.42	4.72	–	5.14	1.21	1.40	–	1.40	1.40
216	27+4B	absent	17.20	4.02	0.58	4.60	0.20	5.58	0.12	1.74	0.20	1.94	2.14
162	26+di5A	absent	12.54	5.94	1.44	7.38	0.22	9.26					
165	26+di5A+2B	absent	13.16	5.68	1.68	7.36	0.04	9.12	0.04	0.96	0.02	0.98	1.00
166	26+di5A+2B	absent	14.38	4.96	1.58	6.54	0.20	8.52	0.12	0.82	0.12	0.94	1.06
190	26+di5A+2B	absent	12.34	6.48	1.00	7.48	0.20	8.88	0.24	0.78	0.10	0.88	0.98

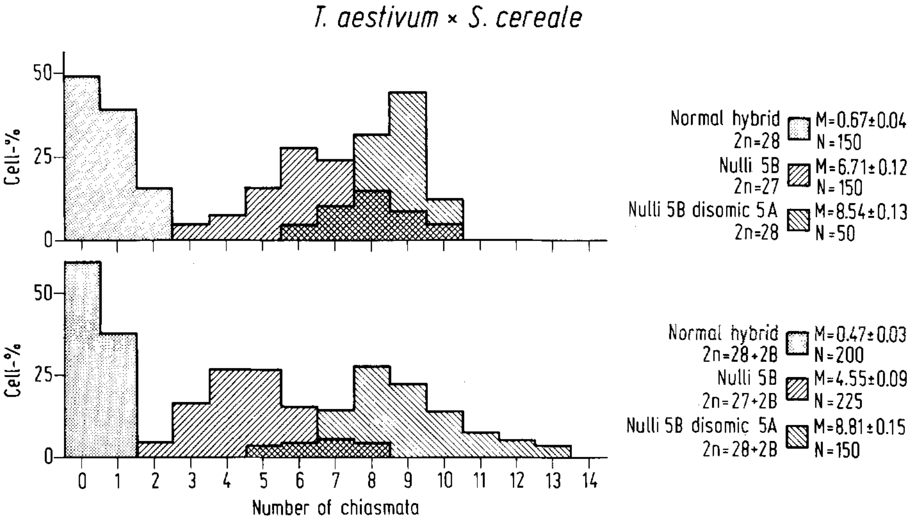

Fig. 2. Distribution of chiasma frequencies per cell in F_1 hybrids *T. aestivum* × *S. cereale* at 20°C in the presence and absence of chromosome 5B of wheat and B-chromosomes of rye, with chromosome 5A of wheat in a monosomic and disomic condition respectively

tected in hybrids with or without chromosome 5D, either at low or normal temperatures. Chromosome 5D is referred to as a carrier of a temperature stabiliser (*Ltp*) gene of chromosome pairing (Riley 1966). It was expected that the absence of this chromosome in the hybrids would produce a slight decrease in association at low temperature. That this difference was not detected in the present experiments is probably a consequence of the low level of association in *T. aestivum* × *S. cereale* hybrids.

Plants deficient for chromosome 5D and simultaneously carrying two or three B-chromosomes show at 20°C a significant rise in chromosome pairing in relation to the hybrids carrying the same dosage of B-chromosomes but not deficient for chromosome 5D (Fig. 1).

Jones and Rees (1967) have found that B-chromosomes of rye increased variability in chiasma frequencies among PMC's and an intra-bivalent asymmetry in chromosome arm association and chismata distribution. Taking into consideration the overall constancy of mean number of chiasmata, which they found in all combinations (with or without B-chromosomes), it may be concluded that B-chromosomes increase the density of chiasma in each pairing chromosome arm (segment). In our hybrids the increase in chiasma frequency might be similarly explained by an effect of B-chromosomes on chiasma density, in the absence of chromosome 5D.

Homoeologous chromosome association between wheat and rye is always low. Besides the presynaptic effect of genes affecting the proper alignment of chromosomes (Feldman 1966) there may exist in those regions where homoeologous chromosomes associate at pachynema, a discontinuous succession of homologous segments for legitimate synapsis. Those segments are potential crossover regions. Increased local density of chiasmata would correspond to a higher probability of crossing-over per unit length of paired segments. This would result in a higher probability of crossing-over at metaphase I per paired homologous segment.

If rye B-chromosomes have an effect on chromosome behaviour in the absence of 5D, irrespective of temperature (although not detectable at low temperatures), which they do not have in the presence of 5D, necessarily 5D must have meiotic effects in addition to *Ltp*. Also, rye B-chromosomes must have several effects. Perhaps, like some genotypes of *Ae. speltoides* but unlike their B-chromosomes, rye B's might slightly overrule *Ph* in 5B and permit some homoeologous pairing unless counteracted by a general pairing restricting gene in 5D. Thus, two effects should be attributed to 5D (1. *Ltp*; 2. slight asynapsis) and three to rye B-chromosomes (1. homoeologous pairing in the presence of 5B; 2. general pairing restriction; 3. increased chiasma formation on effective pairing sites). Under normal conditions, effect 3 balances 2, together causing increased variation. In the absence of 5D, effect 1 is sufficiently strong in combination with 3, to counteract 2, but at low temperatures there simply is not enough pairing for 1 and 3 to have a detectable effect. In the presence of 5D, the combined pairing restricting effects of 5D and effect 2 are strong enough to balance 1 and 3.

This explanation, of course, is speculative. It is not clear for instance why increased chiasma formation at effective pairing sites does not greatly increase chiasma frequency in combinations of rye B-chromosomes and a double dose of 5A, which carries a pairing promotor.

5 Acknowledgement

I am very grateful to Dr. T. Mello-Sampayo and Dr. J. Sybenga for their encouraging discussions and helpful comments during the realization of the work and for reading the manuscript before publication. The technical assistance of Ms. Zaida Cunha and Ms. Anabela Silva is gratefully acknowledged.

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Received July 3, 1979
Communicated by R. Riley

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