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Metaphase-I bound-arm frequency and genome analysis in wheat-*Aegilops* hybrids. 2. Cytogenetical evidence for excluding *Ae. sharonensis* as the donor of the B genome of polyploid wheats

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Summary. Genome affinities were analyzed at meiosis in C-banded metaphase-I cells of wheat × *Ae. sharonensis* hybrid plants. The results showed that the most frequent type of pairing occurred between chromosomes of the A and D genomes in all plants, as well as in cells with different numbers of associations. These findings clearly indicated that *Ae. sharonensis* can be excluded as the donor of the B genome of wheat.

Key words: Genome analysis – Wheat × *Ae. sharonensis* hybrids – Phylogenetic relationships – B genome C-banding

Introduction

The origin of the B genome of wheat is still unknown and remains a central question in wheat cytogenetics.

Attempts to obtain information about the genome relationships in polyploids are essential for understanding evolutionary processes and have consequences for planning practical experiments involving the introduction of alien variation. Although many techniques have been employed to determine the donor of the B genome (see Fernández-Calvín and Orellana 1990 for a review), analysis of meiotic pairing behavior in interspecific hybrids (Kihara 1929, 1930) and intergeneric amphiploids (Miller 1981) appears to be the most reliable method for establishing genome affinities. One problem of the meiotic pairing studies is the difficulty of identifying the chromosomes of the different genomes during meiosis. In recent years, however, chromosome banding techniques have opened the way for the characterization of chromosomes and could provide new information on the phylogenetic relationships of wheat and its relatives.

Previous meiotic pairing analyses suggested that the B genome is derived from species of the *Sitopsis* section of *Aegilops;* however, these analyses have not permitted an unequivocal identification of the precise donor of the B genome (see Kerby and Kuspira 1987).

There is little information on the evolutionary affinity of *Ae. sharonenis* with tetraploid and hexaploid wheat compared with that of *Ae. longissima, Ae. bicornis* and *Ae. speltoides* (see Kerby and Kuspira 1987). The only results available are contradictory, probably due to being obtained by traditional staining methods (Riley et al. 1958; Kushnir and Halloran 1981).

The aim of the present work is to determine the degree of affinity between the genomes that are in competition for pairing in wheat × *Ae. sharonensis* hybrids by using the C-banding procedure at meiosis.

Materials and methods

Five hybrids obtained from a cross between hexaploid wheat *Triticum aestivum* cv Chinese Spring (genome constitution AABBDD), as female, and *Ae. sharonensis* accession 4 (genome constitution S^1S^1), as male, formed the material for this study. The accession of *Ae. sharonensis* was kindly supplied by Dr. S. Ohta, Plant Germplasm Institute, Kyoto University, Japan.

Seeds were germinated on moist wet filter paper in Petri dishes at 20 °C. Primary roots 1 cm long were excised and immersed in tap water at 0 °C for 24–30 h to accumulate metaphase cells and shorten the chromosomes. Subsequently the tips were fixed in acetic-ethanol 1:3 and stored at 0–4 °C for several months. In order to obtain meiotic cells, anthers of hybrids were fixed in acetic:ethanol 1:3, and stored for 1–4 months at 0–4 °C. The fixed material was squashed and stained following the Giemsa C-banding technique described previously (Giraldez et al. 1979).

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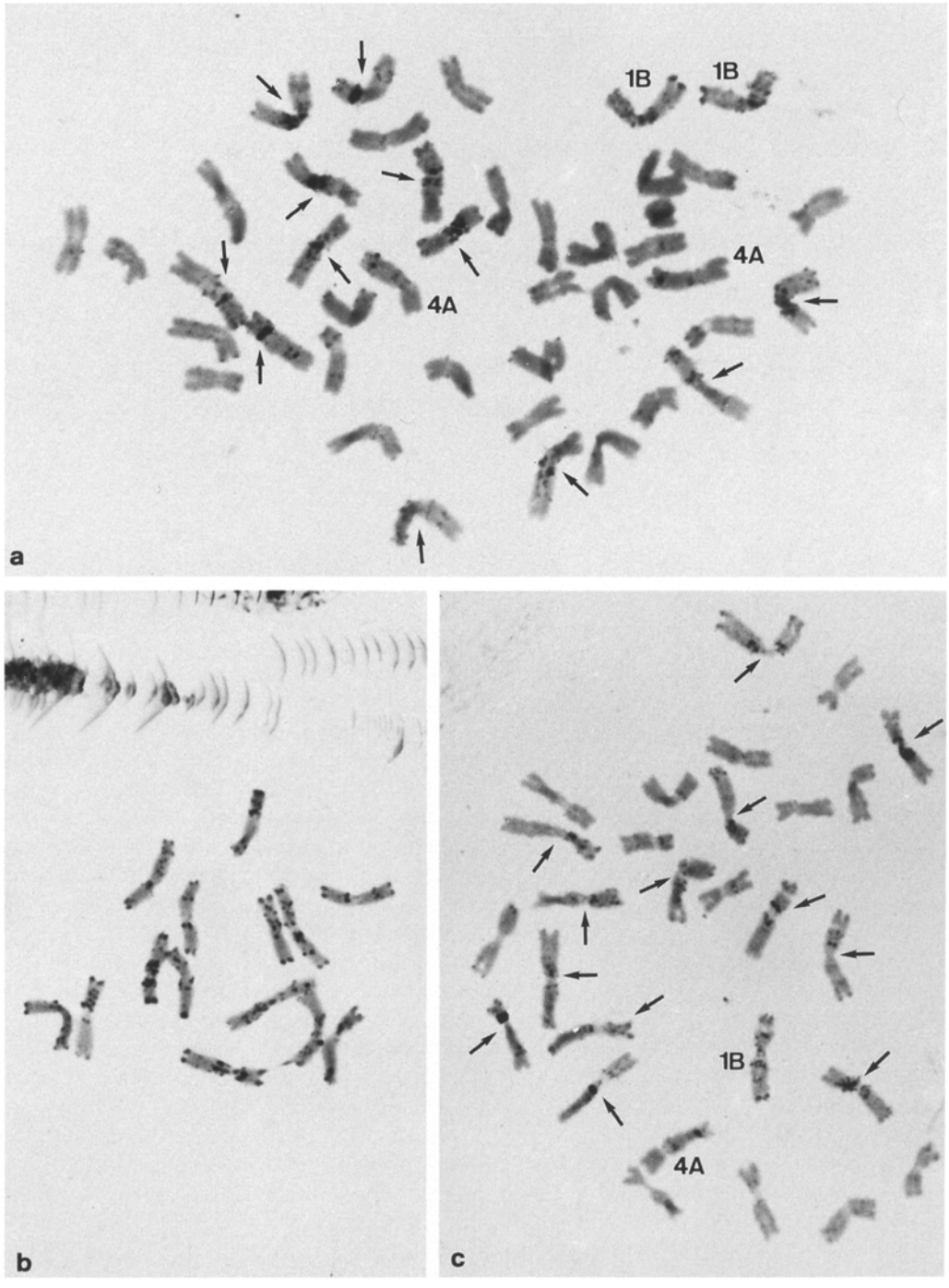

Fig. 1. **a** Somatic metaphase cell of *Triticum aestivum* cv Chinese Spring. Chromosomes of the B genome, other than the 1Bs, are indicated with *arrows*. **b** Somatic metaphase cell of *Aegilops sharonensis* accession 4. **c** Somatic metaphase cell of Chinese Spring × *Ae. sharonensis* hybrid. Chromosomes of the B and S^1 genomes are indicated with *arrows*

Results

The use of the C-banding procedure allowed us to identify the chromosomes of the genomes involved in different meiotic configurations in the hybrids between Chinese Spring and *Ae. sharonensis*. The degree of C-banding differentiation among the genomes is higher in mitotic than in meiotic cells (Fig. 1). Chromosomes of the A and D genomes are indistinguishable from each other because all of them showed a C-banding pattern charac-

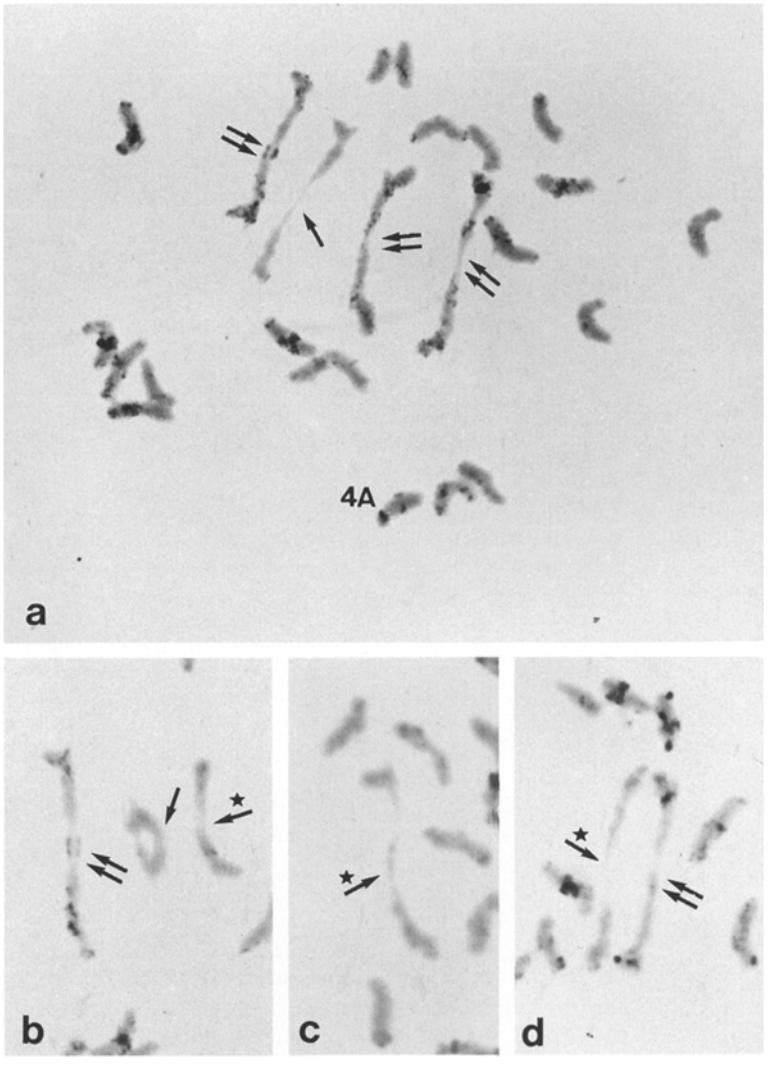

Fig. 2. **a** Metaphase-I cell of a Chinese Spring × *Ae. sharonensis* hybrid. **b–d** Different meiotic configurations; A-D associations are indicated with *single arrows*, B-S associations are indicated with *double arrows*, A-D heteromorphic bivalents are indicated with a *star*

terized by an absence of prominent C-bands, being almost euchromatic except for chromosome 4A which possesses a fine and weak interstitial band located sub-distally on the long arm (see Gill et al. 1991). By contrast, chromosomes of the B genome were much more heterochromatic showing prominent pericentromeric C-blocks as well as some dispersed and intercalary heterochromatin. In this genome telomeric C-heterochromatin is observed infrequently except at the telomere of the long arm in chromosome 1B (Fig. 1 a). However, telomeric C-heterochromatin is very common in almost all chromosomes of the S^1 genome of *Ae. sharonenis*. These chromosomes also demonstrated intercalary and dispersed C-heterochromatin as did chromosomes of the B genome (Fig. 1 b).

These different C-banding patterns allow one to distinguish three different groups of chromosomes (AD, B and S^1) in somatic metaphase cells of the hybrids (Fig. 1 c). In these cells it was even possible to identify specific chromosomes of the B and S^1 genomes. However, in meiotic cells the difficulties of identifying not only specific chromosomes but even genomes are much greater (Fig. 2). For instance the condensation of the chromosomes at first meiotic metaphase makes difficult to detect telomeric C-bands in many cells and consequently chromosomes of the B and S^1 genomes cannot be identified at this stage. In exceptional meiotic metaphase-I cells four (A-D, AD-B, AD-S^1 and B-S^1) out of the six (A-B, A-D, A-S^1, B-D, B-S^1 and D-S^1) possible types of associations could be distinguished but this is not a general feature and probably the use of these few cells could produce a biased sample. For this reason AD-B and AD-S^1 types of associations were pooled and so only three kinds of pairing could be unequivocally ascertained, A-D, AD-BS^1 and B-S^1. The presence of heteromorphic bivalents has been taken as a criterion for

Table 1. Number of different meiotic configurations observed at metaphase-I in Chinese Spring × *Ae. sharonensis* hybrids

Plant	Bivalents[a]						Univalents		III	No. cells
	A-D		AD-BS		B-S		AD	BS		
	R	O	R	O	R	O				
CS × *Ae. shar* 4-1	5	74	1	21	1	55	1,215	1,262	3	100
CS × *Ae. shar* 4-2	5	88	–	27	1	60	1,181	1,248	3	100
CS × *Ae. shar* 4-3	3	58	–	19	1	40	1,259	1,299	–	100
CS × *Ae. shar* 4-4	2	73	–	17	–	62	1,228	1,255	3	100
CS × *Ae. shar* 4-5	1	51	–	20	1	34	1,276	1,310	–	100
Total	16	344	1	104	4	251	6,159	6,374	9	500

[a] R, ring bivalents; O, open bivalents; III, trivalents

Table 2. Mean number of associations per cell observed at metaphase-I for each type of pairing identified. Comparisons between types of pairing are also given

Plant	Type of pairing			Total
	A-D	AD-BS	B-S	
CS × *Ae. shar* 4-1	0.86	0.26	0.58	1.70
CS × *Ae. shar* 4-2	1.01	0.29	0.63	1.93
CS × *Ae. shar* 4-3	0.64	0.19	0.42	1.25
CS × *Ae. shar* 4-4	0.79	0.20	0.63	1.62
CS × *Ae. shar* 4-5	0.53	0.20	0.36	1.09
Total	0.77	0.23	0.52	1.52

Comparisons	t-value	*df*
A-D/AD-BS	7.990**	4
A-D/B-S	5.968**	4
AD-BS/B-S	6.360**	4

** $P<0.01$

Table 3. Total associations observed at metaphase-I for each type of pairing in cells with different numbers of bound arms

No. bonds/cell	Number of associations		
	A-D	AD-BS	B-S
1	81	25	67
2	126	37	93
3	83	30	58
4	63	16	29
5	14	2	9
6	12	2	4
7	–	–	–
8	4	2	2

detecting pairing between the A or D genome chromosomes with those of the B genome chromosomes in wheat haploids (Jauhar et al. 1991), but unfortunately in our case this could lead to erroneous data since many heteromorphic bivalents were formed by chromosomes of the A and D genomes exclusively (see Fig. 2b–d).

Table 1 shows the numbers of meiotic configurations scored in all plants analyzed. As expected in low homoeologous pairing hybrids the larger configurations were less frequent and a maximum of two arms were involved in each association. No quadrivalents were found and all the trivalents analyzed were V-shaped. From the different meiotic configurations it was possible to estimate the mean number of associations per cell for each category of distinguishable pairing (Table 2).

If there is no preferential pairing between the genomes that are in competition in the hybrids then meiotic associations should be distributed at random between all homoeologous chromosomes. In this event the mean number of A-D associations per cell would not differ from the B-S^1 type, since in both the same number of homoeologous chromosomes is involved. In the same way, we would also expect AD-BS^1 pairing to be the most frequent since four genomes are involved. However, the highest mean number of associations per cell corresponded to the A-D type followed by the B-S^1 type, with AD-BS^1 being the least frequent. These differences were significant when paired t-tests were performed (see Table 2). The possibility of identifying every type of pairing at metaphase-I allowed us to analyze whether A-D associations were more frequent than B-S associations in cells with different numbers of bound arms. Table 3 shows the number of associations observed for the three identifiable types (A-D, AD-BS^1 and B-S^1) in cells with different numbers of bound arms. As expected, in all cell types the most frequent pairing was that involving the A and D genomes.

Discussion

The results of this work clearly indicated that pairing was not at random among all genomes that are in competition in Chinese Spring × *Ae. sharonensis* hybrids. Prefren-

tial pairing affinities between the chromosomes of the A and D genomes and between the chromosomes of the B and S^1 genomes have been detected.

There is unequivocal evidence that A and D genomes derive from *Triticum monococcum* and *Aegilops squarrosa*, respectively. Additionally, all previous data strongly suggest that the B genome of wheat has originated from species of the section *Sitopsis* of *Aegilops*, which includes *Ae. bicornis, Ae. longissima, Ae. searsii, Ae. sharonensis* and *Ae. speltoides* (see Kerby and Kuspira 1987).

If meiotic pairing frequency reflects, in some way, the phylogenetic relationships between the genomes that are in competition it would be expected that the B and the S^1 genomes would pair more frequently than the A and D genomes in $ABDS^1$ hybrids. However comparisons of the mean number of bound arms per cell between A-D and B-S^1 types clearly indicated that the A-D type of pairing was significantly the most frequent (Table 2).

Kushnir and Halloran (1981) studying Chinese Spring (*ph1b* mutant) × *Ae. sharonensis* hybrids with high homoeologous pairing concluded that the bivalents found at metaphase-I were mainly due to pairing of the B genome chromosomes of *T. aestivum* with those of *Ae. sharonensis* and therefore proposed that *Ae. sharonensis* could be the donor of the B genome of wheats. However Riley et al. (1958), in a previous publication showed that chromosome pairing in the intergeneric hybrid between *T. aestivum* and *Ae. sharonensis* was very low and concluded that *Ae. sharonensis* should be excluded as a donor of the B genome of wheat.

These contradictory conclusions were arrived at for several reasons. In both cases the metaphase-I data were obtained using traditional staining methods which did not allow the identification of specific types of pairing. Moreover, the two studies were carried out in different types of hybrids; Riley et al. (1958) analyzed low homoeologous pairing hybrids whereas Kushnir and Halloran (1981) used high homoeologous pairing hybrids. Obviously in the latter case the results could have been misinterpreted because most of the pairing was ascribed to the B-S^1 type. Kushnir and Halloran did not take into account the fact that A and D genomes could pair with an appreciable frequency, although a certain level of preferential pairing between both genomes had been reported previously. Okamoto and Sears (1962) studied the progeny of wheat polyhaploids and observed that most homoeologous recombinants were between chromosomes of the A and D genomes (Riley and Kempanna 1963). These findings seem indirectly, to indicate, the existence of such a type of preferential pairing. This was recently confirmed by direct observations in C-banded metaphase-I cells of haploid wheat (Jauhar et al. 1991), wheat-rye hybrids (Hutchinson et al. 1983; Naranjo et al. 1987, 1988) and pentaploid wheat-*Aegilops* hybrids (Fernández-Calvín and Orellana 1991, 1992).

It is surprising that the less related genomes (A and D) showed similar or higher pairing than the hypothetically more close genomes (B and S^1). Chromosomes of the B genome always showed lower homologous pairing than the A genome in tetraploid wheat, in spite of being larger than the A chromosomes (Orellana et al. 1989). This minor meiotic pairing between homologues could be a reflection of an intrinsic characteristic of the B genome and so might be the rule between homoeologues too. This possibility, although speculative, might explain the excess of the A-D type of pairing found. It is also possible that another species of the *Sitopsis* section may be the donor of the B genome of wheat, but this seems less probable in the light of the results of Alonso and Kimber (1983) in wheat × *Ae. longissima* and wheat × *Ae. speltoides* hybrids. These authors used telocentric chromosomes as cytogenetic markers and found that the pairing frequencies of the B and S genomes were very similar to those of the A and D genomes.

Since it has not been possible to unequivocally assign the B genome to an existing diploid species, it may be that the original B genome donor is extinct (Morris and Sears 1967). Alternatively the B genome donor might have been substantially modified through introgressive hybridization with related taxa, so making its identification difficult (Gill and Chen 1987).

It has also been suggested that tetraploid wheat could have originated through hybridization of several amphidiploids having one wheat genome in common and differing by the second genome (Sarkar and Stebbins 1956; Zohary and Feldman 1962). Hybridization of several different amphidiploids might produce new chromosome combinations affecting only the second genome, thus differentiating it from the common genome. In this way, several diploids of the section *Sitopsis* could have contributed to the formation of the B genome.

From this point of view, if any chromosome group of the B genome was very similar to that of *Ae. sharonensis* we would expect that these chromosomes would almost always be paired and, consequently, differences of pairing preferences between the distinguishable types should be observed in cells with different numbers of associations. However, in all types of cells analyzed by us, the most frequent type of pairing was that formed between A and D genomes (Table 3). This result again indicates that there is sufficient evidence for excluding *Ae. sharonensis* as the donor of the B genome of polyploid wheats.

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