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Chromosome pairing relationships among the A, B, and D genomes of bread wheat [+,*]

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Summary. Chromosome pairing and chiasma frequency were studied in bread wheat euhaploids (2n=3x=21; ABD genomes) with and without the major pairing regulator *Ph1*. This constitutes the first report of chromosome pairing relationships among the A, B, and D genomes of wheat without the influence of an alien genome. All *Ph1* euhaploids had very little pairing, with 0.62–1.05 rod bivalents per cell; ring bivalents were virtually absent and mean arm-binding frequency (*c*) values ranged from 0.050 to 0.086. In contrast, the *ph1b* euhaploids had extensive homoeologous pairing, with chiasma frequency 7.5–11.6 times higher than that in the *Ph1* euhaploids. They had 0.53–1.16 trivalents, 1.53–1.74 ring bivalents, and 2.90–3.57 rod bivalents, with *c* from 0.580 to 0.629. N-banding of meiotic chromosomes showed strongly preferential pairing between chromosomes of the A and D genomes; 80% of the pairing was between these genomes, especially in the presence of the *ph1b* allele. The application of mathematical models to unmarked chromosomes also supported a 2:1 genomic structure of the *ph1b* euhaploids. Numerical modeling suggested that about 80% of the metaphase I association was between the two most related genomes in the presence of *ph1b*, but that pairing under *Ph1* was considerably more random. The data demonstrate that the A and D genomes are much more closely related to each other than either is to B. These results may have phylogenetic significance and hence breeding implications.

Key words: Homoeologous chromosome pairing – *Ph1* pairing regulator – Genome relationships – Euhaploid – Giemsa N-banding

Introduction

Common bread wheat (*Triticum aestivum* L. em. Thell.) is the most important single food source for man, with over 500 million tons of grain produced annually. It is an allohexaploid (2n=6x=42) whose three related genomes (A, B, and D) came from three distinct diploid species. The degree of relationship among the three genomes remains a central question in wheat cytogenetics today.

The donors of the A and D genomes are two wild grasses, *Triticum monococcum* L. and *Aegilops squarrosa* L., respectively (Morris and Sears 1967). Since it has not been possible to unequivocally assign the B genome to an existing diploid species (Morris and Sears 1967; Kimber and Feldman 1987), it is likely that the original B-genome donor is extinct. Alternatively, the B-genome donor might have been substantially modified through introgressive hybridization with related taxa (Gill and Chen 1987). Thus, the chromosome pairing relationships among the A, B, and D genomes cannot be assessed accurately for two main reasons. First, not all diploid progenitors of wheat are currently known; and second, pairing in diploid hybrids between known progenitors is not sound evidence of genomic relationship (Kimber and Feldman 1987; Jauhar 1988, 1990a).

Homoeologous relationships among the A, B, and D genomes have been fully established by nullisomic-tetrasomic compensation (Sears 1954, 1966) and by isozyme analysis (Hart 1987). The synteny relationships among the enzyme loci studied are almost entirely conserved

+ This paper is dedicated to the memory of the late Ernest R. Sears

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(Hart 1987). Genetic homoeologies detected by nulli-tetra compensation tests have also been demonstrated cytologically (Riley and Kempanna 1963; Riley and Chapman 1966; Feldman and Avivi 1984). These homoeologies confirm the overall conservatism of genomic organization in hexaploid wheat, but do not specify the exact relationship among the genomes or the extent of their recombinational and substitutional modification. Furthermore, the affinity between equivalent chromosomes of these genomes in disomic wheat and normal haploids is suppressed largely by the genetic activity of the "pairing homoeologous" gene, *Ph1*, on the long arm of chromosome *5B* (Okamoto 1957; Riley and Chapman 1958; Sears and Okamoto 1958; Sears 1976). Therefore, the chromosome pairing relationships among the A, B, and D genomes can be assessed in euhaploids ($2n=3x=21$) having the recessive mutant allele *ph1b* and in nullihaploids ($2n=3x-1=20$) that lack chromosome *5B*. These haploids offer two advantages over diploid hybrids: they have known genomic constitution, and they provide an opportunity for preferential pairing among three competing chromosomes in each homoeologous group. Moreover, increased pairing in the *ph1b* polyhaploids offers a robust basis for assessing affinities among the A, B, and D genomes and for studying the effect of the *Ph1* locus on chromosome pairing by N-banding and by the application of mathematical models.

In this paper, chromosome pairing relationships have been investigated in ABD euhaploids with and without *Ph1*. Pairing was studied directly for a set of cells with N-banded meiotic genomes, and further evaluated by means of numerical meiotic models for a larger set of cells with unbanded chromosomes. Chromosome pairing among A, B, and D genomes has been studied previously in wheat × *Aegilops* hybrids (ABDS) (Alonso and Kimber 1983), and in wheat × rye hybrids (ABDR) (Naranjo et al. 1987, 1988). The present study, however, is the first report of relative chromosome pairing affinities among A, B, and D genomes in *ph1b*-ABD euhaploids without the genetic or competitive influence of an alien genome.

Materials and methods

Seven euhaploids ($2n=3x=21$; ABD genomes) were extracted from two highly crossable spring wheat cultivars, 'Fukuhokomugi' (Fukuho) and *ph1b ph1b* mutant 'Chinese Spring' (CS), upon pollination with winter barley (*Hordeum vulgare* L.) cultivars 'Luther' and 'Boyer.' The *ph1b ph1b* mutant of CS, produced by Sears (1977), has a small deficiency by which the *Ph1* locus (the major pairing regulator) is deleted, but the rest of the chromosome complement is apparently intact. However, for all practical purposes it is designated by the recessive allele *ph1b*. The euhaploids are interchangeably referred to as polyhaploids or simply as haploids in this paper.

The genetic identity of the *ph1b ph1b* mutant and normal Chinese Spring was verified by electrophoretic characterization of their seed gliadins (prolamins). Native seed gliadins (i.e., alcohol-soluble prolamins derived from the seed gluten) were extracted from the two genotypes by the method of Konarev et al. (1981). Twenty-five microliter aliquots of the extracts were loaded into the sample wells of 180 mm × 120 mm × 1.5 mm vertical polyacrylamide slab gels (buffer pH = 3.2) and separated for 5.5 h at 30 mA per gel in an LKB 2001 Vertical Electrophoresis System. The gels were then simultaneously stained and fixed in 0.07% Coomassie Blue G250, 7% ethanol, and 12% trichloroacetic acid. Electrophoretograms of the two genotypes were compared.

The Fukuho polyhaploids had *Ph1*, whereas the CS polyhaploids (designated *ph1b* euhaploids) lacked it. The wheat polyhaploids were obtained when barley chromosomes were completely eliminated in early stages of wheat × barley hybrid embryo development. The hybridizations were performed as a part of a breeding program designed to transfer dwarf-bunt resistance from barley into wheat. The wheat cultivars were manually emasculated and pollinated with barley pollen in the field, in the greenhouse, or on detached spikes in liquid medium in a growth chamber. The haploids varied in their origin, although all were embryo-rescued on Orchid Agar (Difco) medium. 'Boyer' was the pollen parent only for haploid no. 72; the others had 'Luther.' Pollinations were performed in the field for haploids no. 54, 57, 156, and 191, in the greenhouse for haploid no. 72, and on detached spikes for haploids no. 134 and 137. In the field and greenhouse, the emasculated florets were treated with 2,4-D ($25\ mg\ l^{-1}$ 24 h before pollination) and GA_3 ($75\ mg\ l^{-1}$ at 6 h and 30 h after pollination). Detached spikes were cultured using the technique of Singh and Jenner (1983), except that we applied growth regulators in the liquid medium (Riera-Lizarazu 1990). Immediately following emasculation, i.e., 48 h prior to pollination, the detached spikes were cultured in test tubes.

For somatic chromosome counts, the method of staining with aceto-orcein (Jauhar 1990b) was followed, and it allowed us to study the details of primary and secondary constrictions. For N-banding of meiotic chromosomes, spikes were fixed in 99% ethanol:glacial acetic acid (3:1). Anthers at metaphase I (MI) were squashed in 45% acetic acid and the slides were processed and stained according to Gill (1987).

The frequencies of A-D bivalents were scored in those cells with any pairing. The B-genome chromosomes were easily recognized by their heavy banding, the A- and D-genome chromosomes being lightly banded. Thus, associations involving A- and D-genome chromosomes were readily scorable and they accounted for most of the homoeologous pairing. However, the distinction between A-B and B-D associations was not always possible, but these constituted only a small proportion of the total associations. The frequencies of A-D, A-B, and B-D associations were recorded within this observational limitation, and the percentages of paired arms in these associations were calculated.

For meiotic analysis of unbanded chromosomes, spikes were fixed in Carnoy's fluid (6:3:1, 95% ethanol:chloroform: glacial acetic acid) containing a few drops of saturated aqueous solution of ferric chloride (Jauhar 1975, 1990b). Anthers were squashed in 1.5% acetocarmine. Ring and rod configurations were separately scored at MI, and the mean arm-binding frequency (c) was calculated. Because c is different from chiasma frequency, the latter value was also calculated. In this study, the terms "chromosome pairing" and "chromosome association" have been used interchangeably, although there are subtle differences between them.

The pattern of affinities among the A, B, and D genomes was estimated for meiotic data from unbanded chromosomes with the partitioning model of Alonso and Kimber (1981), the

general and restricted preferential pairing models of Sybenga (1988), and the probability-estimation model of Crane and Sleper (1989). The Alonso-Kimber model (1981) gives a single solution (i.e., a single value for each variable), while the latter models generally give a range of solutions that account for exactly the same configuration frequencies. All these models rest upon a set of assumptions: the homoeologous groups behave identically, chiasma interference affects pachytene bivalents and multivalents equally, and the arms of each chromosome pair independently. While these assumptions are generally implicit in genome analysis of hybrids, their violation is plausible and can affect the optimized pattern of genomic affinity. Although the models do not identify which genomes are the closest or most remote (the information from N-banded chromosomes is helpful for that distinction), they do permit calculation of ranked, expected proportions of MI association between closest (s_1), intermediate (s_2), and most remote (s_3) genomes.

The models helped to ascertain the relative affinities of the A, B, and D genomes under conditions when the major pairing regulator is active (*Ph1* euhaploids) or disabled (*ph1b* euhaploids). For ease of comparison, the model results are presented in terms of the proportions of MI association for each pair of genomes, signified in descending order by s_1, s_2, and s_3. Following Alonso and Kimber (1981) and Crane and Sleper (1989), the pattern of affinity was designated 3:0 if all genomes were nearly the same (s_1, s_2, and s_3 all near ⅓ with high c), 1:1:1 if the genomes were far apart (s_1, s_2, and s_3 all near ⅓ with low c), normal 2:1 if two genomes were close (s_1 near 1) and inverse 2:1 if one genome was intermediate between the other two ($s_1 = s_2 = ½$).

Crane and Sleper (1989) gave equations for calculating s_1, s_2, and s_3 for all three models. In terms of the Alonso-Kimber (1981) variables x and y, $s_1 = x/(x+2y)$ and $s_2 = s_3 = y/(x+2y)$, where x and y are relative affinities as defined by Alonso and Kimber (1981). In Sybenga's (1988) general preferential pairing model, $s_1 = (1/3) + p_2$, $s_2 = (1/3) + p_3$, and $s_3 = (1/3) + p_1$; for his restricted preferential pairing model, $s_1 = (1/3) + p$ and $s_2 = s_3 = (1/3) - (p/2)$. In the Crane-Sleper (1989) model, $s_1 = r_1 a_1 / T$, $s_2 = r_2 a_2 / T$, and $s_3 = r_3 a_3 / T$, where $T = r_1 a_1 + r_2 a_2 + r_3 a_3$ and the ratio $b_i / a_i = h$ is uniform over all pairs of genomes.

Results

Table 1 presents the chromosome pairing data for the *Ph1* and *ph1b* euhaploids (2n = 3x = 21; ABD) analyzed in this study and for three others from the literature (Riley and Chapman 1958; Kimber and Riley 1963). One of the three haploids was a nullihaploid that lacked chromosome *5B* and hence the *Ph1* locus. Because of size differences among chromosomes of the three genomes, intergenomic (homoeologous) pairing resulted in heteromorphic configurations (Figs. 3a, b, d).

Pairing in Ph1 euhaploids

All the *Ph1* euhaploids had very low pairing (Figs. 1a–d). Only 6.5–11.0% of the complement paired homoeologously with 0.7–1.2 chiasmata per cell, and c values from 0.05 to 0.11. Ring bivalents were virtually absent, as evidenced by chiasma frequency of about one per bivalent (Table 1). Many of the rod bivalents were heteromorphic (Figs. 1c, d).

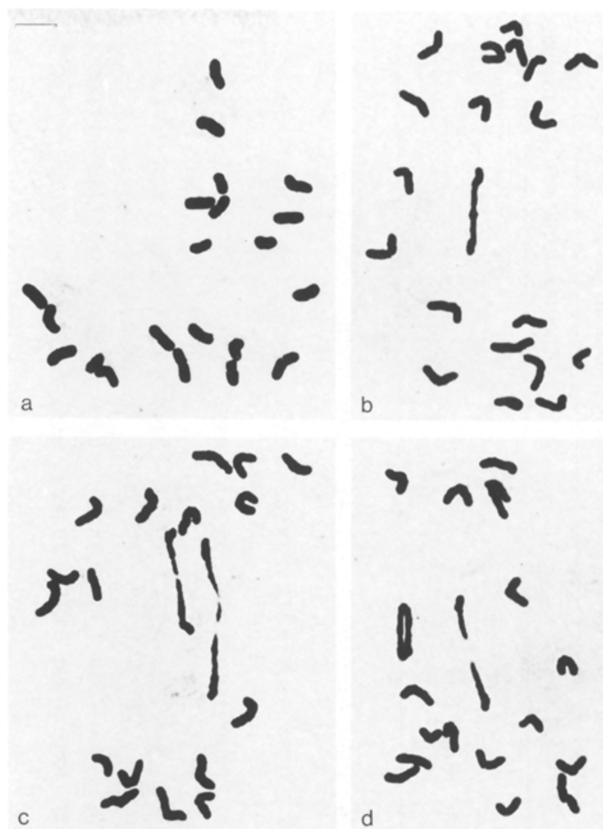

Fig. 1a–d. Meiotic stages showing chromosome pairing in normal bread wheat euhaploids (2n = 3x = 21; ABD) with *Ph1* intact. Note very little homoeologous pairing. **a** Late metaphase I with 21 condensed univalents; note the size variation among the univalents, the B-genome univalents being the largest and the D-genome univalents the smallest. **b** Late metaphase I – anaphase I with 1 bivalent (II) and 19 univalents (I); note relatively less condensed univalents moving to the two poles. **c** Late metaphase I – anaphase I with 2 II + 17 I, the univalents moving to poles at random. At least one of the bivalents is distinctly heteromorphic. **d** Metaphase I with 2 II + 17 I; note a rod and a ring bivalent, a rare occurrence in *Ph1* euhaploids. Both bivalents are heteromorphic and are probably between A- and D-genome chromosomes. (The bar represents 10 μm)

Pairing in the ph1b ph1b disomic and ph1b euhaploids

The *ph1b ph1b* disomic mutant of 'Chinese Spring' (CS) is very similar to its parental genotype in morphology and gliadin phenotype; both have nearly identical seed gliadin banding patterns (Fig. 2). It is believed that electrophoretic similarity of proteins provides a direct measure of gene homology (Metakovsky et al. 1989). Therefore, the mutant stock probably has a small deletion involving the locus *Ph1* (Sears 1977) and appears not to be contaminated with other wheat germplasm. Nevertheless, the mutant was less crossable to barley than was the normal CS. The mutation perhaps also involves deletion or altered expression of one or more crossability determinants closely linked to the *Ph1* locus. The small

Table 1. Chromosome pairing in bread wheat (*Triticum aestivum*) euhaploids (ABD; 2n=3x=21) with and without *Ph1* and in nulli-*5B* haploids (ABD; 2n=3x−1=20)

Haploid and genomic constitution	Chrom. number 2n	No. of cells scored	Mean and range of chromosome configurations at metaphase I										I	*c*	Chiasma frequency			Percentage of complement paired
			V	IV			III			II					Per cell	Per paired chrom.	Per II	
				Ring	Chain	Total	Frying pan	Chain	Total	Ring	Rod	Total						
Euhaploids with *Ph1*																		
Euhaploid no. 54	21	138	–	–	–	–	–	0.036 (0–1)	0.036 (0–1)	0.007 (0–1)	0.616 (0–3)	0.623 (0–3)	19.645 (12–21)	0.050	0.70 (0–5)	0.52	1.01 (1–2)	6.45
Euhaploid no. 72	21	55	–	–	–	–	–	0.091 (0–1)	0.091 (0–1)	0.018 (0–1)	0.855 (0–3)	0.873 (0–3)	18.982 (15–21)	0.077	1.07 (0–5)	0.53	1.04 (1–2)	9.61
Euhaploid no. 134	21	100	–	–	–	–	–	0.040 (0–2)	0.040 (0–2)	0.040 (0–1)	1.050 (0–4)	1.090 (0–4)	18.700 (13–21)	0.086	1.21 (0–6)	0.53	1.04 (1–2)	10.95
Euhaploid no 137	21	30	–	–	–	–	–	–	–	–	0.933 (0–4)	0.933 (0–4)	19.133 (13–21)	0.067	0.93 (0–4)	0.50	1.00	8.89
Euhaploid-Riley[a]	21	100	–	–	–	–	–	0.070	0.070	–	1.380	1.380	18.050	0.109	1.52	0.51	1.00	14.05
Euhaploid-Kimber[b]	21	400	–	–	–	–	–	0.008	0.008	0.005	0.890	0.895	19.180	0.065	0.92	0.50	1.01 (1–2)	8.67
Euhaploids without *Ph1*																		
Euhaploid no. 57	21	58	–	–	–	–	0.017 (0–1)	0.517 (0–3)	0.534 (0–3)	1.741 (0–5)	3.569 (1–7)	5.310 (1–8)	8.776 (4–13)	0.580	8.10 (4–12)	0.66	1.33 (1–2)	58.21
Euhaploid no. 156	21	40	–	–	0.025 (0–1)	0.025 (0–1)	0.150 (0–1)	0.975 (0–3)	1.125 (0–3)	1.525 (0–5)	2.900 (1–6)	4.425 (1–7)	8.675 (5–14)	0.591	8.55 (4–14)	0.69	1.36 (1–3)	58.69
Euhaploid no. 191	21	50	0.020 (0–1)	0.040 (0–1)	0.080 (0–1)	0.120 (0–1)	0.220 (0–2)	0.940 (0–4)	1.160 (0–5)	1.540 (0–5)	2.920 (0–6)	4.460 (1–7)	8.040 (2–13)	0.629	9.08 (5–15)	0.70	1.35 (1–3)	61.75
Nulli-*5B* haploids																		
Nullihaploid-Riley[a]	20	75				0.020			0.860	0.960	3.200	4.160	9.020	0.493	6.90	0.63	1.23	54.90

[a] Riley and Chapman (1958)
[b] Kimber and Riley (1963)

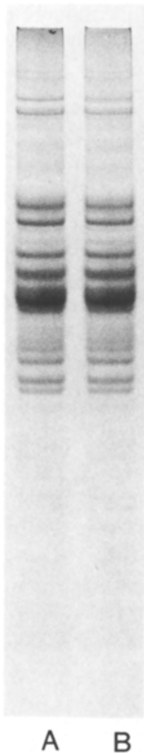

Fig. 2. Electrophoretogram comparing profiles of native gliadin proteins extracted from seeds of Chinese Spring wheat (*A*) and its *ph1b ph1b* mutant (*B*). Note the identical banding patterns in the two profiles, indicating the close similarity of the two genotypes

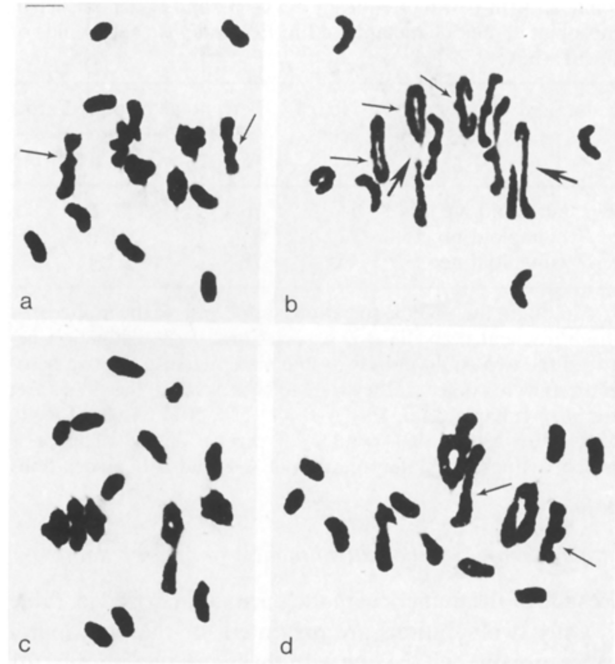

Fig. 3a–d. Meiotic metaphase I (MI) in *ph1b* euhaploids derived from *ph1b ph1b* mutant of 'Chinese Spring.' Note extensive homoeologous pairing. **a** Metaphase I with 6 II + 9 I; note 3 ring and 3 rod bivalents. As revealed by N-banding, the bivalents are formed by corresponding chromosomes of the A and D genomes, and the large B-genome chromosomes remain unpaired. Heteromorphy of the two rod bivalents is clearly noticeable (*thin arrows*). **b** MI with 2 III (one V-shaped and one frying pan, marked by *thick arrows*) + 5 II (two rings and 3 rods) + 5 I. Note heteromorphy of ring bivalents and the ring portion of the frying pan trivalent (*thin arrows*). **c** MI with 1 III (frying-pan) + 4 ring II + 10 I. **d** MI with 3 III + 2 rod II + 8 I; heteromorphy of the two rod IIs is evident (*thin arrows*). (The bar represents 10 μm)

deletion might also reduce the viability or longevity of embryo sacs. The mutant shows very little homoeologous pairing in its disomic *ph1b ph1b* condition, even though it lacks the homoeologous pairing suppressor. The mean pairing was 0.07 V + 0.67 IV (0.13 ring + 0.54 chain) + 0.27 III + 18.33 II (11.73 ring + 6.60 rod) + 1.53 I, with 33.13 chiasmata per cell. Normal CS formed 21 bivalents with 40.52 chiasmata per cell. Thus, pairing in the *ph1b ph1b* mutant was primarily restricted to homologous partners.

The *ph1b* euhaploids showed substantial homoeologous pairing (Figs. 3a–d); 58.2–61.8% of the complement paired, with 8.1–9.1 chiasmata per cell (Table 1). The frequencies of multivalents and ring bivalents increased appreciably, with *c* from 0.58 to 0.63. Again, many of the rod bivalents were heteromorphic (Figs. 3a, d) as were the less condensed rings (Fig. 3b). Most of the univalents (Figs. 3a, c) were larger than their chiasmatically bound homoeologues, implying that these univalents belonged to the B genome, the largest of the wheat genomes (Gill 1987). In microsporocytes with bivalents and univalents, the B-genome univalents could be easily recognized by their large size and heavy banding. The *ph1b* euhaploids exceeded the nulli-*5B* haploid in chiasma frequency (Table 1).

Giemsa N-banding of meiotic chromosomes

Meiotic N-banding showed that about 80% of the associations were between the lightly banded A and D genomes (Table 2). Heteromorphic A-D bivalents (both rod and ring) were frequent. The large B-genome univalents were heavily banded and were thus easily recognized. The trivalents consisted of an A- and a D-genome chromosome almost always associated with each other, with a heavily banded B-genome chromosome either at one end of the linear or V-shaped figure (thus forming BAD- or ADB-chromosome trivalents) or as the "handle" of a frying pan. Trivalents were frequently heteromorphic as well. Although the A-B and B-D associations could not be distinguished as reliably as the A-D ones, such a distinction was attempted for haploids 156 (81.5% A-D, 11.5% A-B, and 7.0% B-D) and 191 (78.6% A-D, 13.0% A-B, 8.4% B-D). In both cases, the B genome appeared to be closer to A than to D, although the pattern of affinity was only moderately asymmetric.

Table 2. Arm-pairing frequency of A-, B-, and D-genome chromosomes at meiotic metaphase I in *Ph1* and *ph1b* euhaploids of bread wheat

Euhaploid	No. of cells scored	Percentage of paired arms	
		A-D	A-B+B-D[a]
Ph1 euhaploid no. 134	15	70.4	29.6
ph1b euhaploid no. 156	32	81.5	18.5
ph1b euhaploid no. 191	45	78.6	21.4

[a] Although the A-B associations appeared to be noticeably more frequent than the B-D associations, clear distinction between the two could not always be made. Hence, the two associations were pooled. If they were to be separated, the values are: for *ph1b* euhaploid no. 156: A-B=11.5%, B-D=7.0%; for no. 191: A-B=13.0%, B-D=8.4%. A certain degree of error is probable due to misinterpretation of A-B and B-D associations

Application of mathematical models to meiotic data

Results of the numerical models are summarized in Table 3. Only two solutions are presented for the probability estimation model: the one with the highest s_3:s_2 ratio, for comparison with the restricted preferential pairing model, and the one with the lowest s_3:s_2 ratio, for comparison with the general preferential model's most inverse 2:1 solution. Model results varied widely for the *Ph1* polyhaploids. The value of *c* was simply too low for reliable conclusions from unmarked chromosomes. For example, haploid 137 lacked ring bivalents and trivalents, such that any pattern could and did fit (Table 3). However, in general the *Ph1* polyhaploids conformed to a 1:1:1, nonpreferential genomic structure.

Model solutions for the *ph1b* haploids conformed more closely to the results of N-banding, with s_1 values near 0.8. However, these solutions did not specify the secondary A-B and B-D relationships; thus, s_2 and s_3 varied widely within the permitted ranking, where $s_2 \geq s_3$. This is a general problem in genome analysis from unmarked chromosomes; secondary relationships can vary widely, but the primary relationship (here A-D) generally does not. Haploids 156 and 191 had fewer rod bivalents than expected and therefore gave bad fits with all the models. Because the optimized arm ratio was 1.00 in all the models, this deficiency might indicate a slight variation in *c* among homoeologous groups of the predominantly pairing A and D genomes. The optimum short-to-long arm ratio for haploid 57 was lower (0.69) because it had fewer trivalents and more rods, but since all three *ph1b* polyhaploids came from the same inbred Chinese Spring stock, the difference apparently was not biologically significant. In general, the pairing with *ph1b* was more preferential than with *Ph1* and conformed to a moderately normal 2:1 pattern.

The solutions in Table 3 also permit comparison of the models and their possible biases. For haploids 54, 72,

Table 3. Results of application of numerical meiotic models to metaphase I pairing data in *Ph1* euhaploids and *ph1b* euhaploids of bread wheat

Haploid		A-K	Res. Syb.	Gen. Syb.	C-S, norm	C-S, inv.
Ph1 euhaploids						
No. 54	s_1	0.560	0.333	0.333	0.414	0.457
	s_2	0.220	0.333	0.333	0.293	0.429
	s_3	0.220	0.333	0.333	0.293	0.114
	SSD	0.0040	0.0040	0.0040	0.0019	0.0020
No. 72	s_1	0.603	0.333	0.333	0.334	0.381
	s_2	0.199	0.333	0.333	0.333	0.381
	s_3	0.199	0.333	0.333	0.333	0.238
	SSD	0.0277	0.0278	0.0278	0.0134	0.0135
No. 134	s_1	0.870	0.667	0.500	0.560	0.506
	s_2	0.065	0.167	0.500	0.220	0.494
	s_3	0.065	0.167	0.000	0.220	0.000
	SSD	0.0046	0.0052	0.0052	0.0015	0.0016
No. 137	s_1	0.333	0.333	0.333	0.999	0.567
	s_2	0.333	0.333	0.333	0.0005	0.433
	s_3	0.333	0.333	0.333	0.0005	0.000
	SSD	0.0061	0.0000	0.0000	0.0000	0.0000
Riley	s_1	0.333	0.333	0.333	0.351	0.378
	s_2	0.333	0.333	0.333	0.324	0.378
	s_3	0.333	0.333	0.333	0.324	0.245
	SSD	0.0032	0.0016	0.0016	0.0010	0.0011
Kimber	s_1	0.333	0.544	0.439	0.544	0.701
	s_2	0.333	0.228	0.439	0.228	0.299
	s_3	0.333	0.228	0.123	0.228	0.000
	SSD	0.0019	0.0000	0.0000	0.0000	0.0000
ph1b euhaploids						
No. 57	s_1	0.854	0.870	0.864	0.870	0.864
	s_2	0.073	0.065	0.136	0.065	0.136
	s_3	0.073	0.065	0.000	0.065	0.000
	SSD	0.0378	0.0000	0.0000	0.0000	0.0000
No. 156	s_1	0.780	0.735	0.694	0.768	0.742
	s_2	0.110	0.132	0.306	0.116	0.258
	s_3	0.110	0.132	0.000	0.116	0.000
	SSD	0.3405	0.3969	0.3969	0.3272	0.3272
No. 191	s_1	0.760	0.731	0.688	0.755	0.723
	s_2	0.120	0.135	0.312	0.123	0.277
	s_3	0.120	0.135	0.000	0.123	0.000
	SSD	0.1622	0.1886	0.1886	0.1590	0.1590

A-K = Alonso-Kimber model (1981)
Res. Syb. = Restricted preferential pairing model of Sybenga (1988)
Gen. Syb. = General preferential pairing model of Sybenga (1988)
C-S, norm. = Crane-Sleper model (1989) (most normal 2:1 solution found)
C-S, inv. = Crane-Sleper model (1989) (most inverse 2:1 solution found)
s_1, s_2, s_3 = Proportions of metaphase I associations due to each pair-wise combination of the A, B, D genomes. On the basis of banding of meiotic chromosomes, s_1 represents A-D association, s_2 = A-B association, and s_3 = B-D association
SSD = Sum of squared differences between observed and expected frequencies

and 134, the Alonso-Kimber (1981) model gave a more preferentially normal 2:1 solution than the most normal 2:1 solution to the Sybenga (1988) or Crane-Sleper (1989) models.

Discussion

The *Ph1* euhaploids (2n = 3x = 21; ABD) with the major homoeologous pairing suppressor showed very little pairing, with virtually no ring bivalents. Clearly, in the presence of *Ph1* the A, B, and D genomes maintained their meiotic integrity. This observation is consistent with previous reports (e.g., Riley and Chapman 1958; Kimber and Riley 1963), but N-banding and the large univalent size demonstrated that the limited pairing that occurred was mostly between A and D genomes.

The *ph1b ph1b* mutant of 'Chinese Spring' wheat has opened up new possibilities for studying intergenomic pairing among the three wheat genomes. Although in this recessive high-pairing mutation (in disomic condition) pairing was predominantly restricted to homologous partners, the *ph1b* euhaploids 57, 156, and 191 (2n = 3x = 21; ABD) showed extensive homoeologous pairing, up to nine times that in the *Ph1* euhaploids (Table 1). The chiasma frequency in the euhaploids without *Ph1* was 7.5–11.6 times that with *Ph1*. That *Ph1* is the major suppressor of homoeologous pairing (Sears 1976) is further demonstrated by the present study.

The increased pairing in the *ph1b* haploids could arise from inactivated pairing regulation, both directly and indirectly. The direct effect would be an increased ability to form chiasmata even when a given level of genomic dissimilarity exists in the haploid complement. The indirect effect would result from the formation of translocation heterozygotes through homoeologous recombination in previous generations of the parental mutant *ph1b ph1b*. Adjacent disjunction from such a translocation multivalent would have resulted in duplication of a chromosome segment and deficiency for its homoeologue in a derived haploid. However, the effect on overall genetic balance and viability would generally be small, because less than a whole chromosome would be involved.

The parental *ph1b ph1b* disomic had a mean of 1.01 multivalents per cell, and two of the three possible two-by-two disjunctions of a quadrivalent are adjacent. Therefore, if all the multivalents reflected recombinational translocation heterozygosity and disjunctions were randomly two-by-two, about two-thirds of the haploids would be expected to contain such a duplicated segment. Thus, the expected contribution of homoeologous substitution to mean bivalent frequency in a sample of *ph1b* haploids would be about 0.7, out of a total frequency of 4.4–5.3 (Table 1). An increased ring bivalent frequency would result only in the unlikely event that the duplicated segment included substantial parts of both arms. The frequency of 0.7 is several-fold too low to account for pairing in *ph1b* haploids, and therefore the *ph1b* allele must affect pairing more directly.

The amount of pairing and chiasma frequency were higher in our *ph1b* euhaploids than in the nulli-*5B* haploid (Table 1), although in both cases *Ph1* is missing. The excessive pairing observed in these euhaploids was probably due to the presence of a pairing promoter (Sears 1976) on the short arm of chromosome *5B*. Nevertheless, some nulli-*5B* haploids are reported to have 60.4% homoeologous pairing (Kimber and Riley 1963), which is comparable to that in the *ph1b* euhaploids.

Homoeologous pairing led to the formation of heteromorphic bivalents (Figs. 3a, b) because of the size differences of the chromosomes of the three genomes, the B genome being the largest and D the smallest (Gill 1987). The most heteromorphic bivalents probably involved B and D homoeologues. N-banding clearly showed that most of the pairing in the *ph1b*-ABD euhaploids involved chromosomes of A and D genomes (Table 2). The B genome also appeared to be more closely related to A than to D, although it was able to pair with both. This would seem consistent with the intermediate size of the A-genome chromosomes.

A certain degree of preferential pairing between the A- and D-genome chromosomes has been reported previously. Okamoto and Sears (1962) studied pairing in the progeny of haploids and identified the chromosomes involved in translocations. They reported that most homoeologous recombinants were between A- and D-genome chromosomes. However, Riley and Kempanna (1963) studied chromosome pairing relationships in the absence of chromosome *5B* and found no evidence of preferential pairing between the chromosomes of particular genomes. Preferential A-D association has been reported in interspecific wheat hybrids also. Using double-double telocentric wheat, in which two homoeologous chromosomes were marked by their two separate telocentric arms, Alonso and Kimber (1983) observed preferential association of A with D, and B with S in ABDS hybrids. The pairing frequencies of the B and S genomes were similar to those of the A and D. These observations showed that the B genome of wheat had higher affinity with the S genome (of *Aegilops speltoides* or *Ae. longissima*) than with either A or D. (The genome in *Aegilops speltoides* is probably the B genome or very close to the B genome of hexaploid bread wheat.) A telocentric chromosome is readily recognizable in both somatic and meiotic plates and thus provides a useful cytological marker to study the pairing behavior of particular chromosomes. However, because a telocentric lacks one arm, the pairing potential of the entire chromosome will seldom be realized (Okamoto and Sears 1962). Sallee and Kimber (1978) concluded that the pairing of the whole

chromosome is not more than would be predicted from the pairing of the two telos. However, the fact that recombination is strongly reduced near the centromere of a telo casts some doubt on this conclusion.

Meiotic C-banding of a Chinese Spring aneuhaploid (*3AL*) and two nulli-tetra (nulli *3A*-tetra *3B*, and nulli *5B*-tetra *5D*) Chinese Spring hybrids with rye suggested preferential pairing between chromosomes of unbanded genomes, i.e., A and D (Hutchinson et al. 1983), as did a series of wheat (normal, *ph1b, 5B*-deficient, and *3D*-deficient) × rye hybrids (Naranjo et al. 1987, 1988). On the other hand, in an earlier study of hybrids between group-5 telocentric lines of Chinese Spring wheat and *Ae. speltoides,* Riley and Chapman (1966) observed that the association of telosomes of *5B* and *5D* was much more frequent than other possible homoeologous associations. However, it is now known that arm *5AL* is involved in a cyclical translocation with chromosome *4A* (new designation) and *7B* (Naranjo et al. 1987, 1988). This may explain the lack of pairing of *5AL* with *5DL* in Riley and Chapman's (1966) study. Since Giemsa-marked meiotic associations were available, the genomic structure of the *ph1b*-ABD euhaploids could be objectively evaluated in the present study. The A- and D-genome chromosomes are most closely related, at least in terms of their pairing relationships (Table 2). The application of the three mathematical models to the pairing data also led to the same conclusions. The values of s_1 (proportion of MI associations between two most closely related genomes A and D) near 0.80, s_2 (between the next most closely related genomes, probably A and B) near 0.115, and s_3 (between the least related genomes, probably B and D) near 0.07 were obtained from some solutions to the Sybenga (1988) and the Crane-Sleper (1989) models.

Variation in model results for the *Ph1* euhaploids is expected because of their low frequencies of trivalents and ring bivalents, the two associations that contain all the information on pairing preferentiality (Sybenga 1988). The low *c* values permitted solutions to fit a variety of genomic structures. However, except for haploid 137 (which lacked sufficient information to assign any genomic structure), all the solutions with *Ph1* were substantially less preferential than with *ph1b*.

With the *ph1b* allele, all models gave at least some normal 2:1 solutions, and there were no 1:1:1 or nearly 1:1:1 solutions. It can be inferred, on the basis of the banding patterns reported above, that chromosomes of the A and D genomes paired preferentially. According to Kimber (1984), *Ph1* affects only *c* and not the relative affinity of the genomes (*x*). However, it appears from the present data that *Ph1* had an effect on apparent genomic structure apart from the major effect on *c*. Kimber's (1984) hypothesis is consistent neither with model results (admittedly unreliable individually but perhaps more robust collectively) in the *Ph1* euhaploids, nor with the observed correction of synaptonemal complexes (SC) at zygotene (Hobolth 1981; Holm 1986). Meiotic data from the *ph1b ph1b* hexaploid also show that chiasma frequency is lowered to 33.13 per cell (compared to 40.52 in the parental normal Chinese Spring) and multivalent frequency increases (0.07 V + 0.67 IV + 0.27 III + 18.33 II + 1.53 I per cell). This double effect would be a logical consequence of a gene that influences the rate, duration, and timing of SC correction relative to formation of recombination nodules. Nevertheless, our banding data support only a limited effect, at most in the "wrong" direction, such that the *Ph1* haploid is actually less preferential. A larger sample size and more genome-specific banding would help resolve the direction and statistical significance of *Ph1*'s effect.

Conclusion

It is remarkable that a single gene mutation can drastically alter the pairing pattern in the polyhaploids of wheat. Such a mutation can be very useful in promoting pairing between alien chromosomes and wheat chromosomes in wheat hybrids, thus facilitating interspecific gene transfer. Analysis of chromosome pairing in wheat *ph1b* euhaploids constitutes an excellent means of elucidating relationships among the A, B, and D genomes because their individual chromosomes can be identified by their diagnostic banding patterns. The present study demonstrates that the A- and D-genome chromosomes pair preferentially, while the B-genome chromosomes remain largely unpaired. About 80% of the metaphase I associations were between chromosomes of A and D. This shows that the A and D genomes are much more closely related to each other than either one is to the B genome. These observations may shed light on the phylogeny of hexaploid wheat and may have important breeding implications.

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