ESTIMATION OF CHROMOSOMAL HOMOLGY BETWEEN
AEGILOPS SPELTOIDES AND THE TETRAPLOID
WHEATS BY USING B-CHROMOSOMES¹⁾

JUN'ICHI SANO AND MASATAKE TANAKA

Plant Germ-plasm Institute, Faculty of Agriculture
Kyoto University, Muko, Kyoto 617

Received July 13, 1979

In order to estimate chromosomal homology between *Aegilops speltoides* (genomic constitution SS) and the tetraploid wheats (AABB or AAGG), effects of the B-chromosomes and genotypes of *Ae. speltoides* on chromosome pairing at MI of meiosis in F₁ hybrids of *Ae. speltoides* × tetraploid wheats were investigated.

In hybrids with B's, chromosome pairing was strikingly affected by the B's and very small amount of pairing was observed. In hybrids without B's, however, extensive pairing was observed, although the amount of pairing varied due to the *speltoides* genotypes involved. In these hybrids with or without B's, although some minor yet distinctive differences in pairing attributable to the kind of genomic constitution of the wheat parent were observed in the multivalent frequency and in the distribution (or terminalization) of chiasmata, both hybrids with different genomic constitution (SAB or SAG) had essentially the same amount of pairing. Since the B-chromosomes used did not cause asynapsis (or desynapsis) of homologous chromosomes in *Ae. speltoides*, the pairing reduced by the presence of B's in the hybrids does not seem to be homologous and is probably homoeologous. Therefore, it can be concluded that there is little chromosomal homology between *Ae. speltoides* and both tetraploid wheats of genomic constitution AABB and AAGG.

INTRODUCTION

There are two kinds of genomic constitutions in the tetraploid wheats, *i. e.*, AABB and AAGG (Lilienfeld and Kihara 1934). The A genome derives from one of the diploid wheats. But the origin of the second genome, B or G, is not known. Many workers suggested that the B genome donor was *Aegilops speltoides* Tausch (2x, genomic constitution SS), for which, Sarkar and Stebbins (1956) provided morphological evidence, Riley *et al.* (1958) showed karyotypic and geographical evidence, and Rees

1) Contribution No. 14 from the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University, Japan.

(1963) gave evidence from the amount of nuclear DNA. It is well known that there is partial homology between the B and G genomes. Examining the wild tetraploid wheats cytogenetically, Tanaka and Ishii (1973) suggested that both genomes had originated in the Zagros Mountains from chromosomal reorganization in the *Ae. speltooides-Triticum boeoticum* amphiploid.

Estimation of the chromosomal homology between *Ae. speltooides* and the tetraploid wheats is difficult, because the former suppresses the activity of the gene *Ph* on wheat chromosome 5BL and thus allows not only homologous but also homoeologous chromosome pairing to occur in hybrids (Riley *et al.* 1961). There may be two ways to overcome this difficulty. One is to find genotypes in *Ae. speltooides* which do not induce homoeologous pairing but permit homologous pairing. Genotypic variation to affect the amount of homoeologous pairing in hybrids with polyploid wheats has been reported in this species (Dvořák 1972; Kimber and Athwal 1972; Shands and Kimber 1973). Several strains were found to cause little or no homoeologous pairing in hybrids. One of these strains examined showed normal homologous pairing at both the diploid and colchicine-induced autotetraploid levels (Larsen and Kimber 1973). The other is to use B-chromosomes of *Ae. speltooides* (Simchen *et al.* 1971). Mcchizuki (1964) found that B-chromosomes of *Ae. mutica* did not affect homologous pairing but reduced homoeologous pairing in hybrids with wheat. Similar effects of B's were reported in interspecific hybrids of *Lolium* (Evans and Macefield 1972) and in *Lolium perenne* × *Festuca arundinacea* hybrids (Bowman and Thomas 1973). Therefore, the B's of *Ae. speltooides* may have a similar activity.

We attempted these two ways mentioned above and report the results here.

MATERIALS AND METHODS

Triticum dicoccoides Körn. ($2n=4x=28$; KU strain no. 110), *T. dicoccum* Schübl. ($2n=4x=28$; 114, 115) and *T. durum* Desf. ($2n=4x=28$; 125) were used as the tetraploid wheats with AABB. *T. araraticum* Jakubz. ($2n=4x=28$; 196-1, 196-2, 8601, 8831) was used as the tetraploid wheat with AAGG. Twelve strains of *Ae. speltooides* without B-chromosomes and two strains with B's were also used. All these *speltooides* strains were originally collected by the Botanical Expedition to the Northern Highland of Mesopotamia, Kyoto University (1970), except one (5715) which was originally collected by the Botanical Mission of the University of Kyoto to the Eastern Mediterranean Countries (1959). All the materials used have been maintained by selfing at the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University.

Each strain of *Ae. speltooides* was crossed with at least one strain of the AABB-wheat and one strain of AAGG-wheat. The hybrids were grown in glasshouse or under field conditions. But some hybrids were placed in a growth cabinet at 22°C with continuous light prior to meiosis. *Ae. speltooides* was grown under field conditions. Cytological observations were made at the first metaphase of meiosis in PMC's fixed in acetic alcohol (1:3) and processed by the aceto-carmine squash technique.

Table 1. Mean chromosome pairing at MI in *Ae. speltoides* × tetraploid wheat hybrids with B-chromosomes

Cross (Genome)	Pl. no.	No. of B's	No. of cells	I			II		III	IV	Xta per cell		Term. coef.
				Rod	Ring	Total					Total	Terminal	
7717×110 (S×AB)	1	1	30	16.17	2.00	0.17	2.17	0.17	0.17	—	2.83	2.03	0.72
7717×114 (")	1	1	120	12.04	3.43	0.13	3.56	0.13	0.56	0.04	5.01	4.86	0.97
7717×125 (")	1	1	30	14.03	2.77	0.27	3.03	0.27	0.30	—	4.07	3.67	0.90
" (")	2	2 ¹⁾	30	18.53	0.90	0.33	1.23	0.33	—	—	1.80	1.43	0.80
7717×196-1 (S×AG)	1 ²⁾	3	30	19.10	0.87	0.03	0.90	0.03	0.03	—	1.13	0.87	0.76
7717×8601 (")	1	0 ³⁾	30	4.53	2.63	0.63	3.27	0.63	1.97	0.90 ⁴⁾	12.33	10.33	0.84
" (")	2	1	50	16.70	1.88	0.08	1.96	0.08	0.10	0.02	2.44	1.58	0.65
" (")	3	2	50	19.52	0.74	—	0.74	—	—	—	0.88	0.54	0.61
7799×114 (S×AB)	1	1	75	9.76	3.64	0.56	4.20	0.56	0.84	0.08	6.81	6.28	0.92
7799×115 (")	1 ²⁾	2	52	18.37	1.19	0.10	1.29	0.10	0.02	—	1.50	1.27	0.85
7799×196-1 (S×AG)	1 ²⁾	3 ⁵⁾	35	19.49	0.63	0.03	0.66	0.03	0.03	0.03	0.86	0.57	0.67
7799×196-2 (")	1	1	75	16.44	1.96	0.13	2.09	0.13	0.11	0.01	2.48	1.47	0.59

1) An isochromosome of the long arm and a telocentric chromosome of the long arm.

2) Placed in a growth cabinet at 22°C with continuous light prior to meiosis.

3) Included in this table for the purpose of comparison.

4) Includes quinquevalents (0.37) and sexivalents (0.03).

5) Includes an isochromosome of the short arm.

Table 2. Mean chromosome pairing at MI in *Ae. speltoides* × tetraploid wheat hybrids without B-chromosomes

Cross (Genome)	Pl. no.	No. of cells	I	II			III	IV-VII	Xta per cell		Term. coef.
				Rod	Ring	Total			Total	Terminal	
5715×114 (S × A B)	1	50	4.80	2.58	1.60	4.18	2.44	0.12	11.30	11.08	0.98
5715×196-1 (S × A G)	1	35	7.23	3.54	0.89	4.43	1.49	0.11	9.00	7.91	0.88
7712×110 (S × A B)	1	30	3.20	1.87	1.53	3.40	3.27	0.27	16.17	12.00	0.74
" (")	2	30	3.23	1.67	1.83	3.50	3.13	0.33	15.40	12.17	0.79
" (")	3	30	2.53	2.00	1.47	3.47	3.43	0.30	15.63	12.47	0.80
	Mean		2.99	1.84	1.61	3.46	3.28	0.30	15.73	12.21	0.78
7712×125 (")	1	35	3.57	1.80	1.86	3.66	3.06	0.20	12.77	12.29	0.96
7712×8831 (S × A G)	1	25	5.48	2.28	2.12	4.40	2.00	0.16	13.28	7.44	0.56
7716×115 (S × A B)	1 ¹⁾	30	6.30	3.37	0.60	3.97	2.17	0.07	9.77	8.37	0.86
7716×125 (")	1	59	8.00	2.95	0.88	3.83	1.73	0.03	8.47	8.31	0.98
7716×196-1 (S × A G)	1 ¹⁾	56	9.02	3.13	0.64	3.77	1.14	0.25	9.23	5.61	0.61
7716×8831 (")	1	35	7.34	3.29	1.00	4.29	1.54	0.11	9.71	8.06	0.83
7719×125 (S × A B)	1	50	6.18	3.10	1.30	4.40	1.90	0.08	10.02	9.50	0.95
7719×196-2 (S × A G)	1	36	7.92	2.64	0.92	3.56	1.50	0.33	9.94	7.50	0.75
7727×114 (S × A B)	1	22	5.45	4.09	1.18	5.27	1.59	0.05	9.86	9.82	1.00
7727×196-2 (S × A G)	1	30	5.03	2.43	1.20	3.63	2.23	0.47	11.23	10.53	0.94
7730×125 (S × A B)	1	46	5.35	2.52	1.37	3.89	2.43	0.13	10.72	10.43	0.97
7730×8831 (S × A G)	1	40	5.93	2.80	1.35	4.15	2.00	0.18	11.30	8.43	0.75
7731×125 (S × A B)	1	34	4.62	2.21	1.44	3.65	2.85	0.12	11.41	11.21	0.98
7731×8831 (S × A G)	1	27	4.41	2.59	0.81	3.41	2.78	0.33	11.96	9.97	0.81
7757 B × 125 (S × A B)	1	40	5.58	2.95	1.35	4.30	2.18	0.08	10.45	10.28	0.98
7757 B × 8831 (S × A G)	1	30	4.50	2.57	1.43	4.00	2.43	0.27	12.47	8.90	0.71
7770×114 (S × A B)	1	50	4.90	3.02	1.40	4.42	2.42	—	10.70	10.46	0.98
7770×196-2 (S × A G)	1	30	3.73	2.03	1.67	3.70	2.27	0.70	13.63	11.10	0.81
7786×125 (S × A B)	1	162	3.87	2.22	1.51	3.73	2.98	0.16	12.16	11.85	0.97
7786×196-2 (S × A G)	1	59	4.69	2.66	1.15	3.81	2.39	0.36	11.90	9.58	0.80
7800×114 (S × A B)	1	25	5.12	2.52	1.76	4.28	2.44	—	11.36	10.92	0.96
7800×196-2 (S × A G)	1	50	6.18	2.96	0.82	3.78	2.06	0.24	9.66	9.20	0.95
7848×110 (S × A B)	1	30	6.40	3.27	1.13	4.40	1.70	0.17	9.83	9.43	0.96
" (")	2	30	5.37	3.00	0.77	3.77	2.33	0.27	10.33	9.77	0.95
" (")	3	30	4.60	2.53	1.57	4.10	2.47	0.20	11.67	11.33	0.97
" (")	4	30	6.03	3.03	1.20	4.23	1.93	0.17	10.33	9.77	0.95
	Mean		5.60	2.96	1.17	4.13	2.11	0.20	10.54	10.08	0.96
7848×114 (")	1	48	4.27	2.33	1.54	3.88	2.85	0.10	12.06	11.65	0.97
7848×125 (")	1	50	6.12	3.48	0.82	4.30	2.04	0.04	9.58	9.26	0.97

(to be continued)

Table 2. (Continued)

Cross (Genome)	Pl. no.	No. of cells	I	II			III	IV-VII	Xta per cell		Term. coef.
				Rod	Ring	Total			Total	Terminal	
7848×8601 (S×AG)	1	30	4.77	2.97	0.53	3.50	2.17	0.60	12.60	9.53	0.76
" (")	2	30	5.40	2.57	0.63	3.20	1.73	0.90	12.03	9.27	0.77
" (")	3	30	5.47	2.47	0.50	2.97	2.10	0.77	11.90	9.00	0.76
" (")	4	30	5.20	2.80	0.57	3.37	1.99	0.74	12.43	9.23	0.74
	Mean		5.21	2.70	0.56	3.26	1.99	0.73	12.24	9.26	0.76
7848×8831 (")	1	30	5.30	2.87	1.20	4.07	2.20	0.20	12.60	8.87	0.70

1) Placed in a growth cabinet at 22°C with continuous light prior to meiosis.

Table 3. Mean chromosome pairing at MI in *Ae. speltoides* with B-chromosomes

Strain no. ¹⁾	No. of B's	No. of cells	I	II			Xta per cell		Term. coef.
				Rod	Ring	Total	Total	Terminal	
7717	5	30	—	0.63	6.37	7.00	15.57	10.63	0.68
7799	1	30	0.07	0.73	6.23	6.97	13.30	12.37	0.93

1) Data were obtained from one plant.

RESULTS AND DISCUSSION

Mean chromosome pairing and chiasma frequency in the hybrids of *Ae. speltoides* × tetraploid wheats are given in Tables 1 and 2.

The hybrids with various numbers of B-chromosomes showed extensive univalent formation (Figs. 1-4) and had high mean univalent frequencies per cell, the range of which was 9.76 (7799×114, plant 1) to 18.53 (7717×125, plant 2) in the hybrids with genomic constitution SAB and 16.44 (7799×196-2, plant 1) to 19.52 (7717×8601, plant 3) in the SAG-hybrids (Table 1). Therefore, when the mean frequency was the highest, only 2.47 (7717×125, plant 2) and 1.48 (7717×8601, plant 3) chromosomes per cell showed pairing on the average in SAB- and SAG-hybrid, respectively. Since the B-chromosomes did not cause asynapsis (or desynapsis) of homologous chromosomes in both *speltoides* strains used (Table 3; Figs. 9, 10), they should not cause asynapsis (or desynapsis) of homologues in hybrids either. If this is true, these data indicate that the average number of chromosomes showing homologous pairing is at most 2.47 per cell in SAB- and 1.48 per cell in SAG-hybrid. This means that there is little chromosomal homology between *Ae. speltoides* and the tetraploid wheats with AABB or AAGG.

In the hybrids without B's involving 13 *speltoides* strains, there was a considerable amount of pairing (Figs. 5-8). The range of mean univalent frequencies of these hybrids was 2.53 (7712×110, plant 3) to 9.02 (7716×196-1, plant 1) per cell (Table 2 and one hybrid in Table 1), which did not overlap with that of the hybrids with B's and significantly differed from it. In these 13 *speltoides* strains including one strain with

Figs. 1-4. Chromosome pairing at MI in *Ae. speltoides* $\times$ tetraploid wheat hybrids with B-chromosomes. ($\times 1250$)

Figs. 1, 2. S $\times$ AB (7717 $\times$ 110); 19_I+1_{II}+1B (1), 17_I+2_{II}+1B (2). Figs. 3, 4. S $\times$ AG (7717 $\times$ 8601); 21_I+2B's (3), 19_I+1_{II}+2B's (4). B-chromosomes are arrowed.

B's (7717), we could not find genotypes which led to such a low level of pairing showing 18 or more univalents per cell as was observed in the hybrids with 2 or 3 B's (Table 1). Therefore, the pairing observed may be non-homologous or homoeologous, as pointed out by Riley *et al.* (1961). The amount of homoeologous pairing observed seems to depend on the *speltoides* genotypes used. The strain 7712, for example, showed a high level of pairing in hybrids, while 7716 showed a low level of pairing

Figs. 5-8. Chromosome pairing at MI in *Ae. speltoides* × tetraploid wheat hybrids without B-chromosomes. ($\times 1250$)

Figs. 5, 6. S×AB (7712×110); $1_I+2_{II}+4_{III}+1_{IV}$ (5), $2_I+2_{II}+5_{III}$ (6). Figs. 7, 8. S×AG (7712×8601); $2_I+2_{II}+1_{IV}+1_V$ (7), $3_I+2_{II}+2_{III}+2_{IV}$ (8).

(Table 2). No apparent difference due to the genomic constitution of the wheat parent was observed. Both the AB and AG genomes showed essentially the same amount of pairing in hybrids with *Ae. speltoides* (Table 2). But the SAG-hybrids showed a slightly higher frequency of multivalents from quadrivalents to septivalents, than the SAB-hybrids. The SAG-hybrids also showed different distribution (or terminalization) of chiasmata from the SAB-hybrids. The SAG-hybrids were observed to have more

Figs. 9-10. Chromosome pairing at MI in *Ae. speltooides* (7717) with 3 B-chromosomes (arrowed). ($\times 1250$)

interstitial-chiasmata than the SAB-hybrids (Table 2). These observations may show to some extent a difference of chromosomal homology or homoeology among the S, A, B, and G genomes, or the AG genomes may contain a weaker suppressor(s) of pairing than the AB genomes.

It is apparent from these data that B-chromosomes of *Ae. speltooides* induce a striking decrease in homoeologous pairing. Dosage effect of the B's seems to exist (Table 1). Genotypes of *Ae. speltooides* also have some effect on homoeologous pairing. Shands and Kimber (1973) reported a "low-pairing" genotype of *Ae. speltooides*, which led to a mean pairing of $20.20_I + 0.40_{II}$ in a hybrid of *T. durum* (AABB) $\times$ *Ae. speltooides* and $7.20_I + 4.87_{II} + 1.55_{III} + 0.17_{IV}$ in a hybrid of *T. timopheevi* (AAGG) $\times$ *Ae. speltooides*. They thought that these results showed a lack of homology between the B and S genomes and a high degree of homology between the G and S. The former conclusion agrees with the present results but the latter does not.

Concerning the effect of B-chromosomes, Dover and Riley (1972b) observed that the presence of B's resulted in a marked reduction in the amount of homoeologous pairing in 5B deficient hybrids of *T. aestivum* $\times$ *Ae. speltooides*. But when 5B was present, the presence of B's did not always result in a striking decrease in homoeologous pairing. By the analogy from the results obtained by Dover and Riley (1972a) in hybrids without B's of *T. aestivum* $\times$ *Ae. mutica*, Vardi and Dover (1972) believed that the B's did not introduce factors altering the level of pairing other than that due to the genotypes of *Ae. speltooides*. It is very difficult, however, to explain the striking reduction of pairing in the present experiment by assuming such genotypes. The B-chromosomes must have a similar effect to the pairing suppressor *Ph* on 5B which inhibits homoeologous pairing but permits homologous pairing. There is a similar pairing suppressor on 3D (Mello-Sampayo 1971). These pairing suppressors may obscure the effect of B's

in *T. aestivum* (AABBDD) × *Ae. speltoides* hybrids. In fact, Dover and Riley (1972b) recognized the effect of B's in 5B deficient hybrids of *T. aestivum* × *Ae. speltoides*. The effect was also recognized in *Ae. speltoides* × tetraploid wheat hybrids which, of course, lack 3D, in the present study. Furthermore, Riley *et al.* (1973) recognized the effect in *Ae. speltoides* × *T. boeoticum* (AA) hybrids which, naturally, lack both 5B and 3D. These pairing suppressors and the activity of the B-chromosomes may counteract the *Ae. speltoides* genotype inducing homoeologous pairing.

LITERATURE CITED

- Bowman, J. G., and H. Thomas, 1973 B chromosomes and chromosome pairing in *Lolium perenne* × *Festuca arundinacea* hybrid. *Nature New Biol.* **245**: 80-81.
- Dover, G. A., and R. Riley, 1972a Variation at two loci affecting homoeologous meiotic chromosome pairing in *Triticum aestivum* × *Aegilops mutica* hybrids. *Nature New Biol.* **235**: 61-62.
- Dover, G. A., and R. Riley, 1972b Prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. *Nature* **240**: 159-161.
- Dvořák, J., 1972 Genetic variability in *Aegilops speltoides* affecting homoeologous pairing in wheat. *Can. J. Genet. Cytol.* **14**: 371-380.
- Evans, G. M., and A. J. Macefield, 1972 Suppression of homoeologous pairing by B chromosomes in a *Lolium* species hybrid. *Nature New Biol.* **236**: 110-111.
- Kimber, G., and R. S. Athwal, 1972 A reassessment of the course of evolution of wheat. *Proc. Natl. Acad. Sci. USA* **69**: 912-915.
- Larsen, J., and G. Kimber, 1973 The effect of the genotype of *Triticum speltoides* on the pairing of homologous chromosomes. *Can. J. Genet. Cytol.* **15**: 233-236.
- Lilienfeld, F., and H. Kihara, 1934 Genomanalyse bei *Triticum* und *Aegilops*. V. *Triticum Timopheevi* Zhuk. *Cytologia* **6**: 87-122.
- Mello-Sampayo, T., 1971 Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. *Nature New Biol.* **230**: 22-23.
- Mochizuki, A., 1964 Further studies on the effect of accessory chromosomes on chromosome pairing. *Japan. J. Genetics* **39**: 356. (Abstr. in Japan.)
- Rees, H., 1963 Deoxyribonucleic acid and the ancestry of wheat. *Nature* **198**: 108-109.
- Riley, R., V. Chapman, and T. E. Miller, 1973 The determination of meiotic chromosome pairing. *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, Missouri, USA pp. 731-738.
- Riley, R., G. Kimber, and V. Chapman, 1961 Origin of genetic control of diploid-like behavior of polyploid wheat. *J. Hered.* **52**: 22-25.
- Riley, R., J. Unrau, and V. Chapman, 1958 Evidence on the origin of the B genome of wheat. *J. Hered.* **49**: 91-98.
- Sarkar, P., and G. L. Stebbins, 1956 Morphological evidence concerning the origin of the B genome in wheat. *Amer. J. Bot.* **43**: 297-304.
- Shands, H., and G. Kimber, 1973 Reallocation of the genomes of *Triticum timopheevii* Zhuk. *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, Missouri, USA pp. 101-108.
- Simchen, G., Y. Zarchi, and J. Hillel, 1971 Supernumerary chromosomes in the second outbreeding species of the wheat group. *Chromosoma* **33**: 63-69.
- Tanaka, M., and H. Ishii, 1973 Cytogenetical evidence on the speciation of wild tetraploid wheats collected in Iraq, Turkey, and Iran. *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, Missouri, USA pp. 115-121.
- Vardi, A., and G. A. Dover, 1972 The effect of B chromosomes on meiotic and pre-meiotic spindles and chromosome pairing in *Triticum/Aegilops* hybrids. *Chromosoma* **38**: 367-385.