Theor Appl Genet (1994) 88:97–101

P. D. Chen · H. Tsujimoto · B. S. Gill

Transfer of *Ph*^{I} genes promoting homoeologous pairing from *Triticum speltoides* to common wheat

Received: 10 May 1993 / Accepted: 23 July 1993

Abstract Diploid-like chromosome pairing in polyploid wheat is controlled by several *Ph* (pairing homoeologous) genes with major and minor effects. Homoeologous pairing occurs in either the absence of these genes or their inhibition by genes from other species (*Ph*^{I} genes). We transferred *Ph*^{I} genes from *Triticum speltoides* (syn *Aegilops speltoides*) to *T. aestivum*, and on the basis of further analysis it appears that two duplicate and independent *Ph*^{I} genes were transferred. Since *Ph*^{I} genes are epistatic to the *Ph* genes of wheat, homoeologous pairing between the wheat and alien chromosomes occurs in the F_1 hybrids. Using the *Ph*^{I} gene stock, we could demonstrate homoeologous pairing between the wheat and *Haynaldia villosa* chromosomes. Since homoeologous pairing occurs in F_1 hybrids and no cytogenetic manipulation is needed, the *Ph*^{I} gene stock may be a versatile tool for effecting rapid and efficient alien genetic transfers to wheat.

Key words *Triticum aestivum* · *T. speltoides* · Meiotic chromosome pairing · Alien transfer

Introduction

Common wheat (*Triticum aestivum* L.) is an allohexaploid (2n = 6x = 42) species with the genomic constitution AABBDD. Although F_1 hybrids between different diploid (2n = 14) progenitor species show considerable pairing, diploid-like strictly homologous chromosome pairing is observed in hexaploid wheat. The suppression of meiotic pairing between the genetically related A-, B-, and D-genome chromosomes (termed homoeologous chromosomes) is controlled by a major gene, *Ph1* (pairing homoeologous), located on chromosome 5B (Riley and Chapman 1958; Sears and Okamoto 1958). Another gene, *Ph2* on 3D, has a moderate suppression effect on homoeologous pairing (Mello-Sampayo 1971). Minor suppressors of homoeologous pairing have been located on 3AS and 4D, and promotors, on group 5 chromosomes (see review by Sears 1976). Thus, diploid-like pairing in hexaploid wheat is controlled by one major gene, *Ph1*, on 5BL and one intermediate-pairing gene, *Ph2*, on 3DS and a number of minor genes that either suppress or promote homoeologous pairing.

When all of the genetic elements of the *Ph* system are functioning and balanced, homoeologous pairing rarely occurs. During the evolution of polyploid wheat, *Ph* genes ensured genome stability and fertility. Furthermore, the *Ph* system of wheat prevents meiotic pairing and recombination between wheat and alien chromosomes in F_1 hybrids. However, in the absence of *Ph* genes (nullisomic or mutant), alien chromosomes do pair and recombine with wheat chromosomes, and a number of agronomically important genes have been transferred to wheat by *ph*-induced chromosome pairing (Sears 1977, 1981, 1982).

Riley et al. (1961) reported a genetic sytem in *T. speltoides* that is also able to effect chromosome pairing in hexaploid wheat even in the presence of the 5B system. In hybrids between *T. speltoides* and *T. aestivum*, the normal 5B system in *T. aestivum* was apparently suppressed by genes from *T. speltoides*, thereby allowing pairing between homoeologous chromosomes. Later, Dvořák (1972) and Kimler and Athwal (1972) found that *T. speltoides* is polymorphic for the promotion of homoeologous pairing, and high- intermediate-, and low-pairing type strains have been identified. Chen and Dvořák (1984) suggested that probably two genes in *T. speltoides* were involved in the promotion of homoeologous pairing, with one system being composed of two duplicate gene loci segregating independently of each other and the other system

Contribution no. 93-435-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA

Communicated by K. Tsunewaki

P. D. Chen[1] · Tsujimoto[2] · B. S. Gill (✉)
Wheat Genetics Resource Center, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506-5502, USA

Present addresses:
[1] Department of Agronomy, Nanjing Agriculture University, Nanjing, China
[2] Kihara Institute for Biological Research, Minami-ku, Yokohama 232, Japan

being composed of several minor genes modifying the effects of major genes.

Since genes in *T. speltoides* are epistatic over the *Ph* gene of wheat, we designated these genes Ph^I. Ph^I genes have been used to transfer genes from alien species into wheat (Riley et al. 1968). Because Ph^I genes are dominant and cause homoeologous pairing in F_1 hybrids, they allow an easier transfer of alien genetic material to wheat than the recessive *ph1* mutant or nullisomic-5B induced introgression. However, other deleterious genes of *T. speltoides* are introduced into wheat at the same time. In this paper, we report on the genetics, selection, and identification of a high-pairing line of common wheat carrying Ph^I genes transferred from *T. speltoides*. We will further demonstrate the effectiveness of the Ph^I genes to promote homoeologous pairing.

Materials and methods

T. speltoides (Tausch) Gren ex. Richter (TA 1786) was collected from Turkey by Dr. B. L. Johnson (original accession no. G1936). *T. variabilis* (TA 1904) is of unknown origin and was obtained from the late Dr. E. R. Sears. (his accession no. P83-9.2-1). The *T. durum-Haynaldia villosa* (syn. *Dasypyrum villosum*) amphiploid was produced at Nanjing Agricultural University, China. Mutants *ph1b*, *ph2a*, and *ph2b* were obtained from E. R. Sears. These materials are maintained by the Wheat Genetics Resource Center at Kansas State University.

T. speltoides TA 1786 as male parent was crossed with 'Chinese Spring' (CS), and the hybrid was backcrossed to CS two times. The plants with multivalents at metaphase I (MI) of meiosis in the BC_1 and BC_2 generations were selected and backcrossed to CS. The BC_2F_2 plants were testcrossed to *T. variabilis*, and MI pairing was analyzed in the F_1 hybrids to identify low-, intermediate-, or high-pairing lines. The BC_2F_3 plants with high-pairing genes were further testcrossed to the *T. durum-Haynaldia villosa* amphiploid to determine homoeologous pairing between wheat and *H. villosa* chromosomes. CS and mutants *ph1b*, *ph2a*, and *ph2b* were used as controls in testcrosses.

Root tips were pretreated for 20–24 h at 4 °C and fixed 95% ethanol-glacial acetic acid (3:1). Anthers with pollen mother cells (PMCs) at the MI stage of meiosis were selected and fixed in 95% ethanol-glacial acetic acid (3:1). Mitotic and meiotic preparations were made by squashing in 45% acetic acid with or without staining with 1% aceto-carmine. C-banding analysis was according to the method described by Gill et al. (1991). The method of in situ hybridization using biotinylated total genomic DNA of *H. villosa* as the probe was that of Mukai and Gill (1991). The ratio of biotinylated DNA to cold wheat DNA was about 1:200 ~ 300.

Results

Demonstration of Ph^I genes in *T. speltoides*

The F_1 hybrids of *T. speltoides* (TA 1786) with CS at MI of meiosis showed 5.78 I + 7.70 II + 1.56 III + 0.46 IV and 15.5 chiasma per PMC, indicating that TA 1786 is a high-pairing type. The F_1 hybrid was highly male sterile and partly female fertile. Some BC_1 plants were obtained by backcrossing the F_1 to CS. After a second backcrossing to CS, a number of BC_2 plants showed a chromosome number approaching 42 and partial fertility. Multivalents were observed more frequently at MI of meiosis in some BC_2 plants, which indicated that these plants were carrying Ph^I genes that inhibit the *Ph* gene system of *T. aestivum*. These plants were selected and self-pollinated to produce BC_2F_2 plants.

Testcrosses with T. variabilis

Some of the BC_2F_2 plants showed high frequencies of PMCs with multivalents at MI suggesting that the Ph^I gene(s) had

Table 1 MI chromosome pairing in testcrosses of BC_2F_2 plants with *T. variabilis*. Only data on bivalent or multivalent associations are shown

Line	Cells scored	II			III	IV	V	Chiasma	Pairing[a] type
		(Rod)	(Ring)	Total					
640-1	65	5.74	0.57	6.31	0.78	0.03		8.53	HP
-2	65	2.20	0.08	2.28	0.90	0.02		4.22	LP
-4	38	3.82	0.18	4.00	0.84			5.86	IP
641-2	100	1.40	0.11	1.51	0.57			2.76	LP
-3	59	5.90	0.56	6.46	0.86	0.07		8.95	HP
-4	50	4.72	0.14	4.86	0.68			6.36	IP
-5	78	3.67	0.66	4.33	0.59	0.04		6.30	IP
642-1	50	2.66	0.04	2.70	0.14			3.02	LP
-3	83	5.77	0.37	6.14	1.12	0.10		9.05	HP
-4	101	3.65	0.08	3.37	0.29			4.39	LP
-5	61	4.20	0.21	4.41	0.26	0.05		5.29	IP
-7	53	3.28	0.25	3.53	1.00	0.04		5.90	IP
-8	50	1.58	0.00	1.58	0.04			1.66	LP
-9	70	2.80	0.07	2.87	0.23			3.40	LP
643-1	72	2.14	0.00	2.14	0.06			2.26	LP
-2	78	6.59	0.29	6.88	0.97	0.08	0.01	9.39	HP
-4	63	2.40	0.03	2.43	0.17			2.80	LP
-5	99	3.80	0.28	4.08	0.43	0.02		5.28	IP
-6	70	4.24	0.19	4.43	0.44			5.50	IP
-7	52	2.31	0.15	2.46	0.10			2.81	LP
-8	52	1.94	0.12	2.06	0.85			3.88	LP
-9	80	6.43	0.46	6.89	0.93	0.16	0.01	9.73	HP
-10	102	2.45	0.06	2.51	0.25			3.07	LP

[a] *HP*, High pairing, *IP*, intermediate pairing, *LP*, low pairing

been transferred from *T. speltoides*. In order to verify the presence of Ph^1 genes transferred from *T. speltoides*, we test-crossed BC_2F_2 plants with *T. variabilis*. The F_1 hybrid between normal *T. aestivum* and *T. variabilis* shows low homoeologous pairing at MI. Analysis of the chromosome pairing at MI of meiosis in 23 testcross plants of four BC_2F_2 lines with *T. variabilis* is shown in Table 1. Of 23 testcross plants, 5 showed 6.14–6.89 II + 0.78–1.12 III + 0.03–0.16 IV and 8.53–9.73 chiasma per PMC at MI (Fig. 1a). This level of pairing is classified as high pairing. Seven testcross plants showed 2.51–5.30 II + 0.08–1.00 III + 0.00–0.05 IV and 5.28–6.36 chiasma per PMC (Fig. 1b); this is classified as an intermediate level of homoeologous pairing. Eleven testcross plants showed 1.51–2.46 II + 0.04–0.90 III and 1.66–4.39 chiasma per PMC (Fig. 1c) and were taken as an example of low pairing. This latter level is similar to or slightly higher than the level observed in control CS × *T. variabilis* F_1 hybrids (2.41 II + 0.07 III and 2.66 chiasma).

Testcrosses of BC_2F_3 plants from the above four BC_2F_2 lines were successively made with *T. variabilis*. In the progenies from testcrosses between high-pairing lines and *T. variabilis*, 32 of 39 (from 640-1) and 21 of 27 (from 641-3) plants analyzed still showed high pairing (> 7.0 chiasma per PMC) in two families. Only 7 of the 39 and 6 of the 27 plants analyzed showed intermediate pairing (4.5–7.0 chiasma per PMC). No low-pairing plants were recovered from the two high-pairing lines. In the progenies from testcrossing between low-pairing lines (640-2 and 641-2) and *T. variabilis*, 9 plants from both families showed low pairing. This suggested that the recessive genes controlling low pairing were homozygous. In the progenies from testcrossing between intermediate-pairing lines and *T. variabilis*, high-(1 plant), intermediate-(1 plant), and low-pairing (5 plants) were observed. This suggested that the genes were still heterozygous.

The comparison of chromosome pairing at MI of PMC in testcrosses of high-pairing lines, 'Chinese Spring', and *Ph* mutants with *T. variabilis* is shown in Table 2. The results indicate that the level of homoeologous pairing of the lines developed in this work is higher than that of the *ph2* mutant and lower than that of the *ph1b* mutant.

Testcrosses with the *T. durum-Haynaldia villosa* amphiploid

In order to determine whether the Ph^1 genes promote homoeologous pairing between wheat chromosomes and alien chromosomes, some high-pairing BC_2F_3 plants were crossed with a *T. durum-Haynaldia villosa* amphiploid (AABBVV). The V genome chromosomes contain large telomeric C-bands and thus can be distinguished from the A-, B-, or D-genome chromosomes of wheat (Fig. 2). Chromosome pairing in PMCs of testcross progenies was analyzed.

The F_1 hybrids of high-pairing lines with *T. durum-H. villosa* amphiploid had 10–26% PMCs involved in homoeologous pairing between the V genome chromosomes and the A-, B-, or D-genome chromosomes. This frequency is higher than observed in the control CS × *T. durum-H. villosa* amphiploid (3%) and those of low-pairing lines × the *T. durum-H. villosa* amphiploid (6–9%). This result, which is parallel to the situation in testcrosses with *T. variabilis*, indicates that the Ph^1 genes of *T. speltoides* transferred into wheat

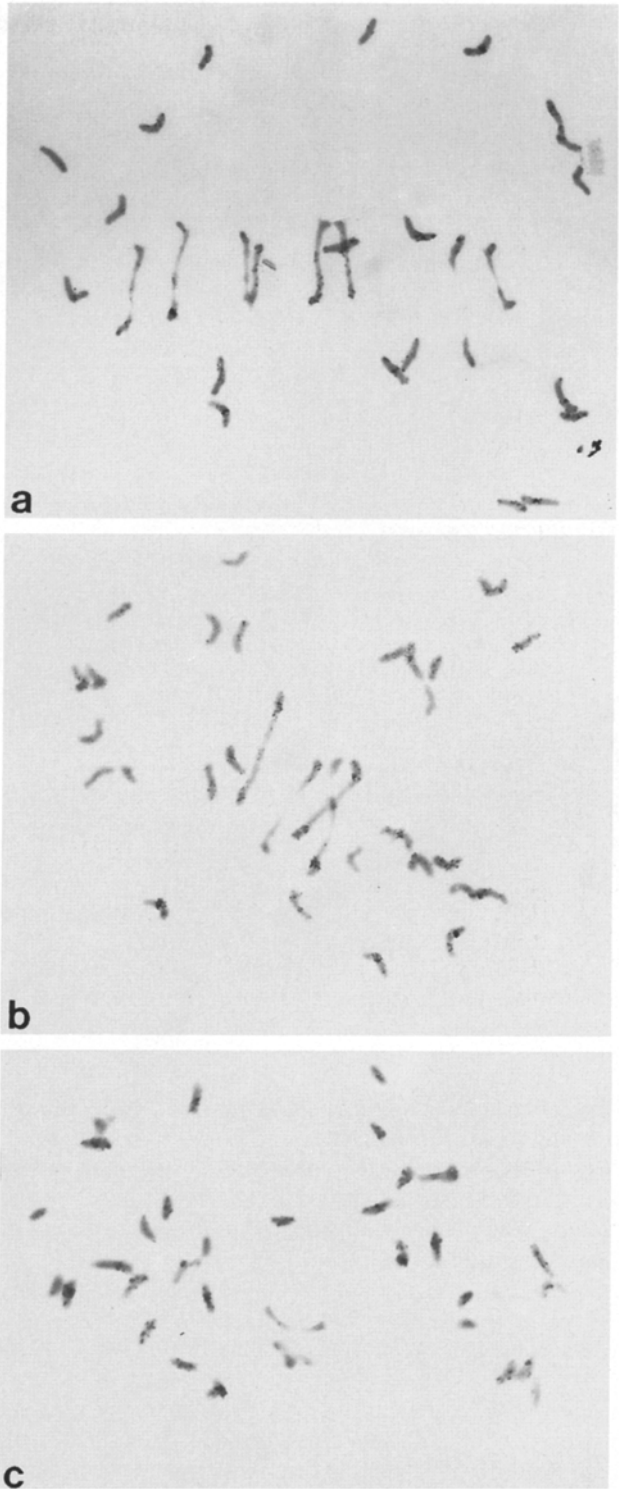

Fig. 1a–c Chromosome pairing at MI in high-, intermediate-, and low-pairing 'Chinese Spring' wheat lines in testcrosses with *T. variabilis*. **a** High-pairing line, **b** intermediate-pairing line, **c** low pairing line

Table 2 The comparison of chromosome pairing at MI in testcrosses of high-pairing line 640-1, 'Chinese Spring' (CS) and mutants *ph1b*, *ph2a* and *ph2b* with *T. variabilis*. Only data on bivalent or multivalent associations are shown

Line	Number of cells	II			III	IV	V	Chiasma
		II(rod)	II(rung)	Total				
CS	73	2.30	0.11	2.41	0.07			2.66
ph2b(3DS)	150	4.05	0.04	4.09	0.28	0.01		4.72
ph2a(3DS)	200	4.96	0.22	5.18	0.51	0.03	0.01	6.55
HP line (640-1)	65	5.74	0.57	6.31	0.78	0.03		8.53
ph1b(5BL)	150	6.53	1.29	7.82	2.06	0.33	0.02	14.30

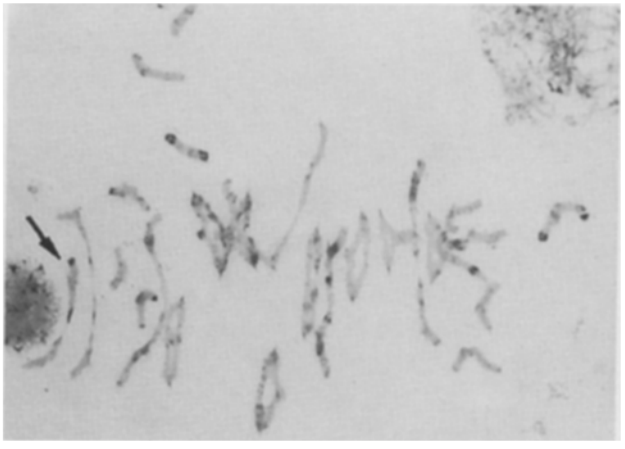

Fig. 2 A rod bivalent (*arrowed*) involving homoeologous pairing between a wheat chromosome and a *H. villosa* chromosome at MI in the F_1 hybrid of a high-pairing wheat line × *T. dururm-H. villosa* amphiploid (AABBDV)

do promote homoeologous pairing between alien chromosomes and wheat chromosomes.

The PMCs were further analyzed by genomic in situ hybridization. A darkly-labelled *H. villosa* chromosome can be clearly seen to pair with wheat chromosomes in a multivalent association (Fig. 3).

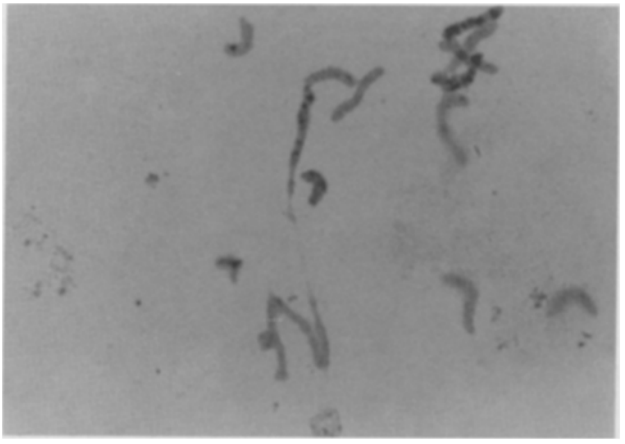

Fig. 3 Genomic in situ hybridization using labeled total genomic DNA of *H. villosa* as probe showed homoeologous pairing between wheat (*light*) and *H. villosa* chromosomes (*dark*) in progenies of crosses of high-pairing Ph^1 line with the *T. durum* × *H. villosa* amphiploid

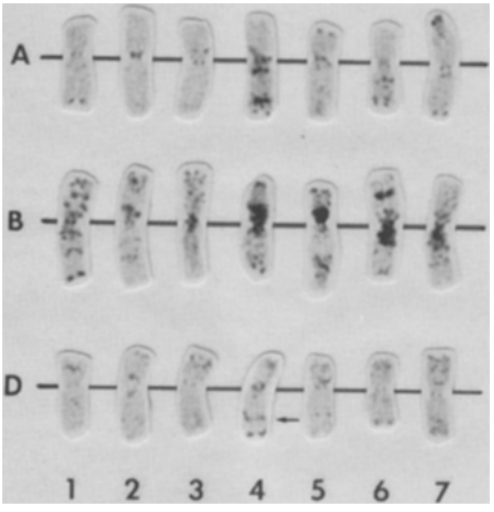

Fig. 4 C-banded karyotype of a high-pairing line derived from *T. speltoides* having a translocated chromosome T4D (*arrow*)

The chromosome constitution of high-pairing lines

The chromosome constitution of high-pairing lines was analyzed by C-banding. A modified chromosome 4D was observed in all five high-pairing lines, with the long arm of this modified 4D showing an extra telomeric band (Fig. 4). A high-pairing line (2n = 42) with this chromosome pair substituting for a pair of normal 4D was recovered, and a quadrivalent consiting of two 4B and two modified 4D chromosomes was observed in C-banded PMCs of this line. The long arm of the modified 4D paired with the short arm of 4B, suggesting that the modified 4D is involved in a translocation between 4DL and 4BS of wheat or 4SS of *T. speltoides* (henceforth designated T4D). Another line with a pair of T4D substituting for a pair of 4B was also recovered. This line showed some difference in plant height and spike morphology and was partially fertile even though missing the 4B pair. One plant of this line produced more than 100 seeds in the greenhouse.

Discussion

The results indicate that Ph^1 genes from *T. speltoides* that inhibit the *Ph* system of wheat and promote homoeologous chromosome pairing have been transferred to common wheat. The level of pairing observed in high-pairing lines was about 50% of the pairing observed in the original F_1 hybrid between CS and the high-pairing *T. speltoides* (TA1786). High-, inter-

mediate-, and low-pairing lines of wheat were observed. The high-pairing lines were homozygous and produced mostly high-pairing plants and only a few intermediate-pairing plants. Low-pairing lines were homozygous for genes for low pairing. The intermediate-pairing lines produced high-, intermediate-, and low-pairing plants. The distribution of pairing levels was consistent in successive generations.

The results support the hypothesis that high chromosome pairing in *T. speltoides* is controlled by two major genes that show different effectiveness and are additive and also by some minor genes (Chen and Dvořák 1984). As a result, there is a gradual increase in homoeologous pairing from low to high pairing depending upon the number of genes. The plants homozygous for two dominant and independent Ph^I genes are true breeders for high pairing. The various intermediate pairing are determined by dosages of the two Ph^I genes that can vary from 1 to 3. This also explains why intermediate-pairing types segregate to produce high-, intermediate-, and low-pairing types.

Since the translocated chromosome T4D is observed in all five high-pairing lines, the Ph^I genes may be located on the segment transferred from *T. speltoides*. The effectiveness of the Ph^I gene on T4D might be stronger than that of the Ph^I gene located on another as yet unidentified chromosome. However, further research is needed to confirm these observations.

Two types of plants containing the T4D were observed. In one, the T4D chromosome substituted for the 4D pair. In another, the 4B pair was substituted by a T4D pair. In the latter case, the plants were fertile, although plants nullisomic for 4B are always sterile. There are two possible explanations for the observed results: (1) the transferred segment on 4D is derived from 4S and contains both high-pairing and fertility genes; (2) the transferred segment on 4D is of wheat origin and the high-pairing gene is located elsewhere.

Many desirable genes are present in wild relatives of common wheat. However, a lack of recombination between wheat and alien chromosomes hinders the exploitation of wild relatives for wheat improvement. Several approaches have been proposed to overcome the barrier. Spontaneous translocations between alien and wheat chromosomes can occasionally occur in wide crosses. Various types of radiation, including X-rays, gamma rays, and neutrons, have been used to induce translocations (reviewed in Mukai et al. 1993). The genetically induced homoeologous pairing methods either using high-pairing *T. speltoides* (Riley et al. 1968) or the *Ph* system (Sears 1976) provide the opportunity for targeted transfer of small chromosome segments. The Ph^I stock combines the advantages of both of the above homoeologous transfer methods. It has the advantage of the *T. speltoides* method as homoeologous pairing can be observed in F_1 hybrids but without the introduction of any other deleterious *T. speltoides* chromosomes. It also has the advantage over the *ph1* mutant method because it eliminates the need for aneuploids such as nullisomic-5B stock. Although the pairing level is not high, it is effective as shown by induced homoeologous pairing between *H. villosa* and wheat chromosomes. Further work with a variety of alien genetic sources will reveal the extent of the usefulness of this novel genetic engineering stock.

Acknowledgements This research was completed when Dr. P. D. Chen and Dr. H. Tsujimoto were visiting scientists with the Wheat Genetics Resource Center. The research was supported by a USDA grant to the Wheat Genetics Resource Center.

References

Chen KC, Dvořák J (1984) The inheritance of genetic variation in *Triticum speltoides* affecting heterogenetic chromosome pairing in hybrids with *Triticum aestivum*. Can J Genet Cytol 26:279–287

Dvořák J (1972) Genetic variability in *Aegilops speltoides* affecting homoeologous pairing in wheat. Can J Genet Cytol 14:371–380

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Kimber G, Athwal RS (1972) A reassessment of the course of evolution of wheat. Proc Natl Acad Sci USA 69:912–915

Mello-Sampayo T (1971) Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. Nature New Biol 230:22–23

Mukai Y, Gill BS (1991) Detection of barley chromatin added to wheat by genomic in situ hybridization. Genome 34:448–452

Mukai Y, Friebe B, Hatchett JH, Yamamoto M, Gill BS (1993) Molecular cytogenetic analysis of radiation-induced wheat-rye terminal and intercalary chromosomal translocations and the detection of rye chromatin specifying resistance to Hessian fly. Chromosoma 102:88–95

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behavior of hexaploid wheat. Nature 182:713–715

Riley R, Kimber G, Chapman V (1961) Origin of genetic control of diploid-like behavior of polyploid wheat. J Hered 52:22–25

Riley R, Chapman V, Johnson R (1968) The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. Genet Res 12:199–219

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet 10:31–51

Sears ER (1977) An induced mutant with homoeologous pairing in wheat. Can J Genet Cytol 19:585–593

Sears ER (1981) Transfer of alien genetic material to wheat. In: Evans IT, Peacock WI (eds) Wheat science-today and tomorrow. Cambridge University Press, UK, pp 75–89

Sears ER (1982) A wheat mutation conditioning an intermediate level of homoeologous chromosome pairing. Can J Genet Cytol 24:715–719

Sears ER, Okamoto M (1958) Intergenomic chromosome relationships in hexaploid wheat. Proc Tenth Int Cong Genet 2:258–259