Chromosoma (2001) 110:371–377
DOI 10.1007/s004120100156

ORIGINAL ARTICLE

E. Sánchez-Morán · E. Benavente · J. Orellana

Analysis of karyotypic stability of homoeologous-pairing (*ph*) mutants in allopolyploid wheats

Received: 6 March 2001 / In revised form: 11 May 2001 / Accepted: 11 May 2001 / Published online: 20 July 2001

Abstract Karyotypic analysis of wheat lines with different genotypes for the homoeologous-pairing loci *Ph1* and *Ph2* was carried out by means of a genomic in situ hybridization method that allowed unequivocal identification of the A, B and D genomes. Chromosomal rearrangements mainly affecting the A and D genomes were found in all plants of allohexaploid wheat (AABBDD) lacking *Ph1* activity. The frequency of intergenomic exchanges per plant in *ph1b* mutant and nulli-5B lines was 4.31 and 3.40, respectively. In addition, an unbalanced genomic constitution was found in a few plants, some even showing a euploid chromosomal number. By contrast, rearranged karyotypes were detected neither in the *ph1* mutant line (*ph1c*) of allotetraploid wheat (AABB) nor in the allohexaploid wheat lines lacking *Ph2* activity, namely *ph2b* mutant and nulli-3D lines. These results were compared with the chromosomal pairing behaviour displayed by mutant lines *ph1c*, *ph1b* and *ph2b* at first meiotic metaphase. Despite the finding of standard, non-rearranged karyotypes in the *ph1c* tetraploid mutant, the frequency of A-B homoeologous metaphase I association was similar to that observed in the *ph1b* hexaploid mutant. The results presented clearly demonstrate that inactivity of the *Ph1* locus induces karyotypic instability in wheat. Intergenomic exchanges have probably been accumulating since the original *ph1* mutant and aneuploid lines were obtained, which should be taken into account when it is planned to use these lines for basic research on *Ph1* function or in applied wheat breeding programmes.

Edited by: D. Schweizer

E. Sánchez-Morán (✉) · E. Benavente · J. Orellana
Unidad de Genética, Departamento de Biotecnología,
E.T.S.I. Agrónomos, Universidad Politécnica de Madrid,
28040 Madrid, Spain

E. Sánchez-Morán
Present address: Departamento de Genética, Facultad de Biología,
Universidad Complutense de Madrid, 28040 Madrid, Spain,
e-mail: uge@eucmos.sim.ucm.es

Introduction

Allopolyploids are polyploids obtained after chromosomal doubling of interspecific hybrids between closely related species. Despite the fact that allopolyploid species contain two or more different genomes with a high level of sequence homology, diploidization mechanisms have evolved that confine crossing over to homologous chromosomes, thus ensuring balanced gamete production, genome stability and fertility. The diploid-like meiotic behaviour of wild allopolyploids is achieved by different strategies (for a review see Cuñado and Santos 1999) that are facilitated by two complementary systems: the structural differentiation of homoeologous chromosomes and different genetic systems that promote homologous pairing.

The *Ph1* gene, located on the short arm of chromosome 5B (Riley and Chapman 1958), is the main one responsible for the suppression of homoeologous pairing in both *Triticum turgidum* (genome composition AABB) and *T. aestivum* (genome composition AABBDD). Lack of activity of *Ph1*, whether resulting from nullisomy or induced mutations at this locus, namely *ph1c* in the allotetraploid durum wheat (Giorgi 1978) and *ph1b* in the allohexaploid bread wheat (Sears 1977), promotes homoeologous pairing in wheat-alien hybrid combinations and has been extensively used for genetic introgression into cultivated wheats. In allohexaploid wheat, the *Ph2* gene located on the short arm of chromosome 3D is also involved in the control of homologous pairing. However, it is known that nullisomy for 3DS or mutation at this locus, namely *ph2b* (Mello-Sampayo 1971), has only an intermediate promoting effect on homoeologous pairing in the hybrids (e.g. Maestra and Naranjo 1998).

To explain the effect of the *Ph1* locus, two main hypotheses have been proposed: (1) the presynaptic hypothesis of Feldman (1966) suggests that the *Ph1* locus controls premeiotic alignment of homologous and homoeologous chromosomes, leading to exclusively homologous pairing at zygotene. Recent fluorescence in situ hybridization (FISH) experiments carried out in dif-

ferent wheat-alien translocation and addition lines have indicated that the *Ph1* locus can modify the spatial organization of chromosomes at premeiotic interphase by controlling, in some cases, the subcellular elements involved in chromosomal positioning, i.e. microtubules and centromeres (Aragón-Alcaide et al. 1997a, b; Mikhailova et al. 1998; Martínez-Pérez et al. 1999). These studies have also revealed some differences between *ph1* mutant and normal plants in centromere structure at premeiotic interphase. However, the metaphase I pairing analysis reported by Dvorak and Lukaszewski (2000) is not consistent with the idea that centromere behaviour prior to meiosis mediates the effect of *Ph1* on homoeologous pairing. (2) The synaptic hypothesis proposes that the *Ph1* locus affects the stringency of both synapsis and crossing over, which is confined to homologous chromosomes at meiotic prophase (Hobolth 1981; Jenkins 1983; Holm and Wang 1988). Dubcovsky et al. (1995) and Luo et al. (1996) used molecular markers to distinguish between the homoeologous genomes and concluded that *Ph1* is involved in the detection of sequence homology along the entire length of synapsed chromosomes, suppressing recombination between homoeologous segments. The possibility that *Ph1* is a regulatory locus that can show pleiotropic effects, thus explaining contradictory inferences as to its mode of action, has even been considered (Dvorak and Lukaszewski 2000).

The consequences of mutation at the *Ph2* locus have been studied in interspecific hybrid combinations; such mutations are postulated to have meiotic effects only quantitatively different from those of mutation at the *Ph1* locus (e.g. Maestra and Naranjo 1998). Although the mode of action of *Ph2* seems far from being elucidated, a recent synaptonemal complex analysis of *ph1b* and *ph2b* mutant lines has shown that they have a different effect on later synaptic behaviour (Martínez et al. 2001).

Despite the extensive use of mutant and aneuploid lines lacking *Ph* activity to obtain wheat-alien hybrid combinations and derivatives for both basic research on *Ph* loci meiotic functions and breeding programmes, the consequences of such a disruption of the homoeologous pairing control system on intergenomic recombination in wheat itself have never been reported. With that in mind, we analysed the karyotypic structure of *ph1* and *ph2* mutant wheat as well as nulli-5B and nulli-3D tetrasomic lines of hexaploid wheat. The metaphase I meiotic behaviour of some of the mutant lines was also examined. We have used an improved method of genomic in situ hybridisation (GISH) that allows simultaneous discrimination of the A, B and D wheat genomes (Sánchez-Morán et al. 1999).

Materials and methods

The materials examined for this study included different genotypes of *T. turgidum* L. (2*n*=4*x*=28; genome constitution AABB) and *T. aestivum* L. (2*n*=6*x*=42; genome constitution AABBDD). The tetraploid wheats used were: cvs Langdon and Cappelli and the cv. Cappelli *ph1c* mutant line. Hexaploid wheats examined were: cv. Chinese Spring; cv. Chinese Spring *ph2b* mutant line; 41 plants from four different stocks of the cv. Chinese Spring *ph1b* mutant line; and five plants of each of the nulli-tetrasomic lines N5BT5A, N5BT5D, N3DT3A and N3DT3B. For all materials except those noted, ten plants per genotype were examined.

The root tips were immersed in ice-cold water for 24 h to accumulate mitotic metaphases. They were then fixed in ethanol:acetic acid (3:1), squashed in a drop of 45% acetic acid and the cover slips removed by freezing. The slides were air dried and stored at 4°C until in situ hybridization experiments were performed. For meiotic chromosomal preparations, fixed anthers at the metaphase I stage were subjected to the same procedure.

Total genomic DNA isolated from leaves of *T. monococcum* and *Aegilops squarrosa* was mechanically sheared to 10–12 kb pieces and then labelled with digoxigenin-11-dUTP and biotin-16-dUTP, respectively, by nick translation (Roche) according to the manufacturer's instructions. Total genomic DNA isolated from *Aegilops* species of the Sitopsis section was autoclaved for 5 min to allow fragmentation into pieces of 100–200 bp in size and employed as blocking DNA. The procedure for GISH analysis of hexaploid wheat lines was as described in Sánchez-Morán et al. (1999) with a ratio of 1:2:300 for A genome probe, D genome probe and blocking DNA in the hybridization mixture. In tetraploid wheat lines, digoxigenin-labelled A genome probe and blocking DNA from *Aegilops* species of the Sitopsis section were used in a 1:150 (probe:blocking DNA) ratio. To detect probe hybridization sites, we used 5 ng/µl of goat anti-digoxigenin antibody conjugated with fluorescein isothiocyanate (Roche) for digoxigenin-labelled probes (A genomic DNA), and 5 ng/µl of avidin conjugated with Cy3 cyanine dye (Amersham) for biotinylated probes (D genomic DNA). The slides were screened with a Zeiss Axiophot epifluorescence microscope equipped with different sets of filters. Photographs were taken on Kodak Ektachrome film ASA/ISO 400. The slide films were scanned and the images processed using Adobe PhotoShop software.

Fig. 1a–m Fluorescence micrographs after genomic in situ hybridization (GISH) of *ph1c*, *ph2b* and *ph1b* mutant lines. **a, b** Identification of A (*green*) and B (*brown*) genomes at mitotic metaphase (**a**) and meiotic metaphase I (**b**) in *ph1c* mutant wheat (2*n*=4*x*=28, genome constitution AABB). **c, d** Simultaneous discrimination of A (*yellow-green*), B (*brown*) and D (*red*) genomes at mitotic metaphase (**c**) and meiotic metaphase I (**d**) in *ph2b* mutant wheat (2*n*=6*x*=42, genome constitution AABBDD). **e–m** Discrimination of A, B and D genomes in *ph1b* mutant wheat (2*n*=6*x*=42, genome constitution AABBDD). Genome identification is as in **c, d**. **e** Somatic metaphase of a *ph1b* aneuploid plant with 11, 14 and 17 chromosomes belonging to the A, B and D genomes, respectively. *Arrows* indicate chromosomes with evidence of intergenomic exchanges between A and D genomes. **f–i** Details of the translocations detected in **e**. The notation is according to the PAINT system (see Fig. 2). **f, g** t(Ad), chromosome of the A genome with a terminal segment from the D genome in the long arm. **h, i** t(Da), chromosome of the D genome with a terminal segment from the A genome in the long arm. **j** t-ins(dAda), chromosome of the A genome with a terminal translocation in the short arm and an insertion (interstitial translocation) in the long arm, both belonging to the D genome. **k** ins(Bdb), chromosome of the B genome with an insertion from D genome in the long arm. **l** t(aD), chromosome of the D genome with a large segment from the A genome in the short arm. **m** Meiotic metaphase I of a *ph1b* aneuploid plant showing: three ring bivalents, one rod bivalent and one univalent belonging to the A genome; five ring bivalents, one rod bivalent and two univalents belonging to the B genome; five ring bivalents and one rod bivalent belonging to the D genome. Homoeologous associations between A and D genomes (*asterisk*) can be observed as a rod bivalent and a complex hexavalent configuration. *Arrowheads* in **a–e** indicate the 4AL/7BS intergenomic translocation shared by tetraploid and hexaploid wheats. *Bars* represent 10 µm except in **j**, **k** and **l** where they represent 20 µm

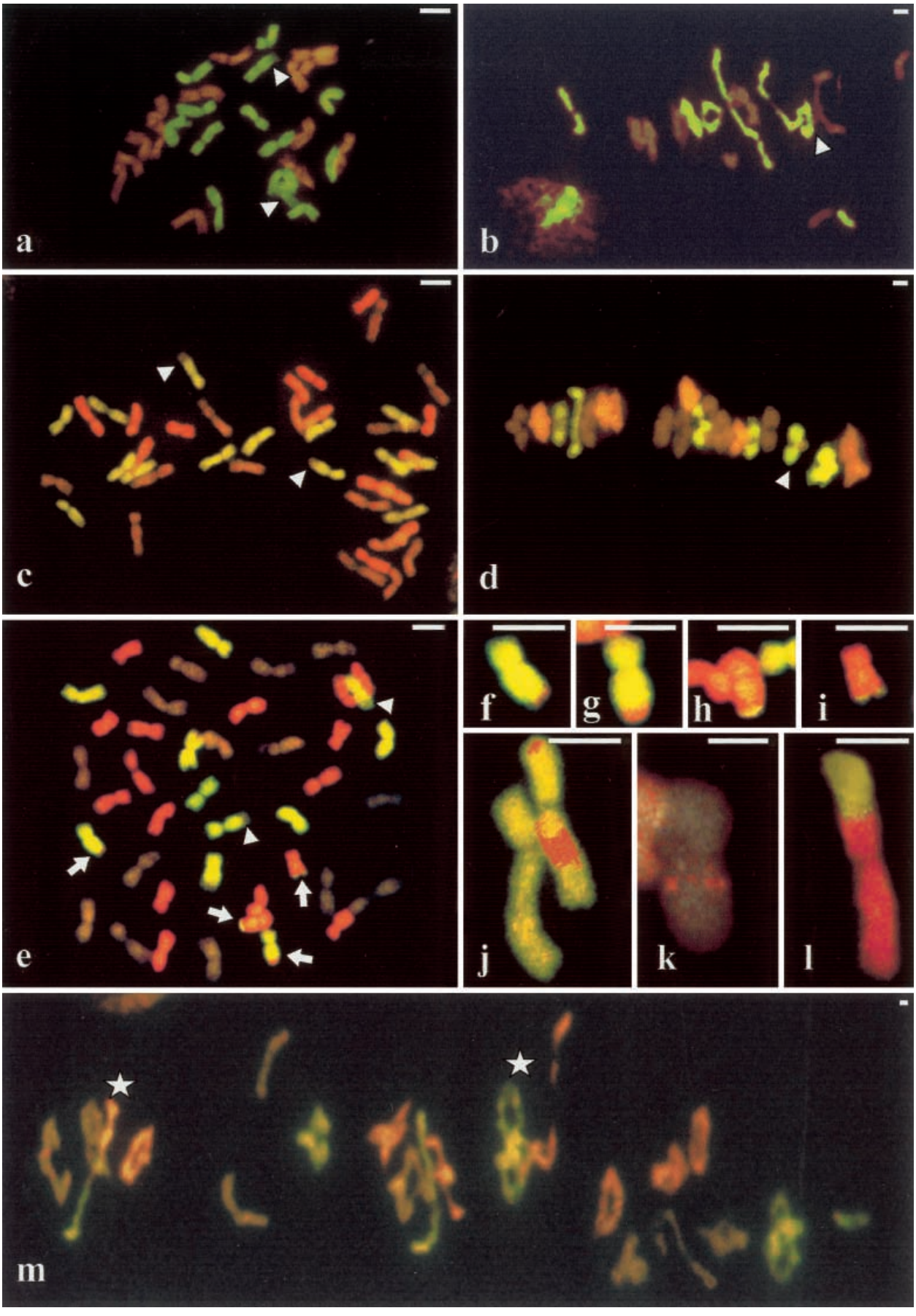
a
b
c
d
e
f
g
h
i
j
k
l
m

Table 1 Chromosomal rearrangements observed in *ph1b* mutant (four different stocks) and nulli-5B allohexaploid wheat lines classified by the PAINT system. See Fig. 2 for graphical representation of the rearranged patterns. [t, terminal translocation; ins, insertion (interstitial translocation)]

Line	No. of plants	A genome							B genome			D genome						Total
		t		ins			t-ins A-D	Total	t Ba	ins Bdb	Total	t		ins		t-ins daDa	Total	
		dA	Ad	dAd	adA	Ada						aD	Da	daD	Dad			
Stock 1	15	13	24	1	–	3	1	42	–	1	1	2	10	3	4	–	19	62
Stock 2	5	3	13	1	–	–	–	17	2	–	2	5	7	–	3	–	15	34
Stock 3	6	3	9	–	–	–	–	12	–	–	–	1	15	–	1	1	18	30
Stock 4	15	9	25	–	1	–	–	35	–	–	–	1	14	–	1	–	16	51
ph1b total	41	28	71	2	1	3	1	106	2	1	3	9	46	3	9	1	68	177
N5BT5A	5	4	7	–	–	–	–	11	–	–	–	3	5	–	–	–	8	19
N5BT5D	5	3	4	–	–	1	–	8	–	–	–	1	4	–	2	–	7	15
N5B total	10	7	11	–	–	1	–	19	–	–	–	4	9	–	2	–	15	34

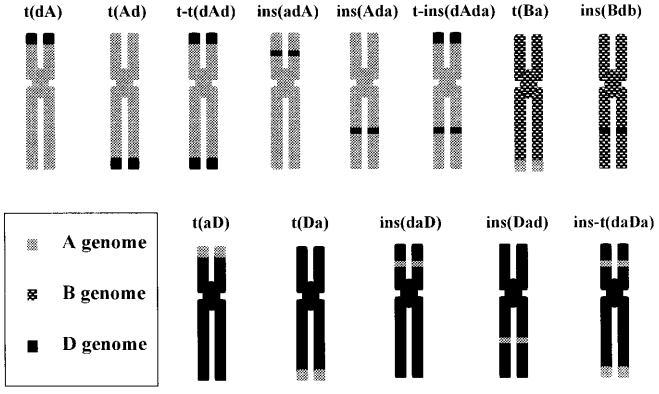

Fig. 2 Graphic representation of the chromosomal rearrangements (*t* terminal translocation, *ins* insertion, interstitial translocation) observed in *ph1b* mutant and nulli-5B plants whose frequencies are shown in Table 1. Rearranged patterns are named according to the PAINT system. Microphotographic examples of some of them appear in Fig. 1f–l

Results

Genomic in situ hybridization using digoxigenin-labelled DNA from *T. monococcum* (putative donor of the A genome of allopolyploid wheats) and biotinylated DNA from *Ae. squarrosa* (putative donor of the D genome of hexaploid wheat) as probes, and blocking with genomic DNA from *Aegilops* species of the Sitopsis section (putative donors of the B genome of allopolyploid wheats) was carried out to identify unequivocally the wheat genomes in mitotic and meiotic cells (Fig. 1). Chromosomes of the A genome were visualized as green and yellow-green fluorescence in durum (AABB) and bread (AABBDD) wheats, respectively; chromosomes of the D genome were revealed by red fluorescence, whereas B genome chromosomes were brown.

In normal and *ph1c* mutant tetraploid wheat, as well as in normal, *ph2b* mutant and nulli-3D hexaploid wheat, fluorescence hybridization signals were homogeneously distributed along the chromosomes of the different genomes and only the 4AL/7BS intergenomic translocation shared by all allopolyploid wheats could be distinguished (Fig. 1a–d). By contrast, the plants of *T. aestivum* either homozygous for the *ph1b* allele or nullisomic for chromosome 5B showed a great number of karyotypic alterations such as structural rearrangements (terminal and interstitial translocations) and aneuploidies (Fig. 1e–m). In all, 211 translocations were observed, 177 in *ph1b* and the remaining 34 in nulli-5B plants (Table 1). The PAINT system – Protocol for Aberration Identification and Nomenclature Terminology (Tucker et al. 1995) – was followed to classify the different types of chromosomal aberrations found (Fig. 2).

Each *ph1b* plant showed different numbers and types of chromosomal rearrangements, which almost exclusively involved the A and D genomes (98.3%). Most exchanges were located at terminal regions, but a number of insertions (11.8%) were observed. Similarly, only translocations between the A and D genomes were observed in the ten nulli-5B plants studied, and they were also preferentially located at terminal regions (91.1%) (Table 1). There were no remarkable differences between *ph1b* and nulli-5B plants either in the frequency and genomic composition of rearranged chromosomes or in the location of exchanges. Two *ph1b* plants, with euploid chromosomal number (2*n*=42), showed an unbalanced genome constitution consisting of 11, 14 and 17 chromosomes belonging to the A, B and D genomes, respectively, as well as several chromosomal translocations (Fig. 1e–i). When, in a given plant, two rearranged chromosomes have a similar pattern of fluorescence and the exchanged segment is the same size and in the same position, they can be considered as homozygous. However, about 65% of the rearrangements found were heterozygous because only one chromosome with a specific pattern was observed.

The karyotypic constitution of eight distinct progenies, obtained by selfing of single *ph1b* plants showing different karyotypes, was also examined. In all of them, we detected not only those translocations present in the parent plant but also new chromosomal rearrangements. A mean of 0.9 de novo rearrangements per plant was found (Table 2). This demonstrates that intergenomic exchanges are produced in every generation.

Table 2 Observed chromosomes in eight different *ph1b* progenies. Mean values per plant appear in brackets. The parental karyotype and the number of offspring are indicated. [t, terminal translocation; ins, interstitial translocation (insertion)]

	No. of plants	A genome		B genome		D genome		Total	De novo rearrangements
		t	ins	t	ins	t	ins		
ph1b-1		3	–	–	–	1	–	4	
offspring	11	29 (2.64)	3 (0.27)	–	–	13 (1.18)	3 (0.27)	48 (4.36)	8 (0.73)
ph1b-2		1	–	–	–	1	–	2	
offspring	3	4 (1.33)	–	–	–	4 (1.33)	–	8 (2.67)	2 (0.67)
ph1b-3		3	–	–	–	2	–	5	
offspring	5	16 (3.2)	1 (0.2)	2 (0.4)	–	12 (2.4)	–	31 (6.2)	6 (1.2)
ph1b-4		2	–	–	–	2	–	4	
offspring	6	12 (2.0)	–	–	–	16 (2.67)	2 (0.33)	30 (5.0)	6 (1.0)
ph1b-5		–	–	–	–	1	–	1	
offspring	2	1 (0.5)	–	–	–	3 (1.5)	–	4 (2.0)	2 (1.0)
ph1b-6		1	–	–	–	1	–	2	
offspring	2	4 (2.0)	–	–	–	3 (1.5)	–	7 (3.5)	3 (1.5)
ph1b-7		2	–	–	–	1	–	3	
offspring	2	5 (2.5)	–	–	–	2 (1.0)	–	7 (3.5)	1 (0.5)
ph1b-8		–	–	–	–	1	–	1	
offspring	2	1 (0.5)	–	–	–	3 (1.5)	–	4 (2.0)	2 (1.0)

Table 3 Mean number of meiotic configurations and homoeologous associations per cell observed at metaphase I in wheat *ph* mutant lines. (I, univalents; IIrod, rod bivalents; IIring, ring bivalents; Biv, bivalents; Multi, multivalents)

Line	A genome			B genome			D genome			Homoeologous Biv			Multi	Homoeologous associations		A-D
	I	IIrod	IIring	I	IIrod	IIring	I	IIrod	IIring	A-B	B-D	A-D		A-B	B-D	
ph1c	0.52	1.91	4.75	0.69	2.69	3.91	–	–	–	–	–	–	0.13	0.11	–	–
ph1b	3.8	1	1.33	1.33	1.83	4.33	1.5	1.16	4.16	–	–	0.16	1.16	0.16	–	1.16
ph2b	0.05	0.8	6.15	–	1.15	5.85	–	1.05	5.95	–	–	–	–	–	–	–

The results obtained in somatic cells were compared with those observed in 50 pollen mother cells at metaphase I of each mutant genotype (Table 3). In the hexaploid *ph2* mutant (*ph2b*) most of the chromosomes formed homologous bivalents and no homoeologous association was observed (Fig. 1d). However, associations between homoeologous chromosomes were observed in both *ph1* mutant lines. In the allotetraploid mutant (*ph1c*), there were a small number of univalents and some multivalent associations were also observed (Fig. 1b). In the allohexaploid mutant (*ph1b*), a drastic decrease in the number of ring bivalents was paralleled with an increase in the number of univalents. In addition, the frequency and complexity of multivalents were much higher than in *ph1c* plants (Fig. 1m). Most of the homoeologous associations (93%) occurred between the A and D genomes. The frequency of A-B metaphase I association was similar to that found in the allotetraploid mutant. As expected from karyotypic observations, chromosomal associations occurred mostly at distal regions.

Discussion

It is usually assumed that *ph1* mutant lines, either in allotetraploid (*ph1c*) or allohexaploid (*ph1b*) wheat, as well as nulli-5B plants have the same karyotypic structure as *Ph1* normal plants. However, very little effort has been made to test this premise despite the reduction in viability displayed by the *ph1b* mutant (Sears 1977), and the singular meiotic behaviour reported for some *ph1b* wheat-rye hybrids (Koebner and Shepherd 1985; Naranjo et al. 1988; Cuadrado et al. 1997) could be interpreted as indirect evidence of the existence of intergenomic rearrangements in these lines. The methodologies employed in those former cytogenetic studies, C-banding and FISH with multiple repeated DNA probes, were unable to detect any karyotypic instability in *ph1b* mutant wheat. However, the results presented here clearly demonstrate that the absence of the *Ph1* locus induces chromosomal rearrangements in allohexaploid wheat (Table 1).

Most intergenomic exchanges observed involved the A and D genomes. Because of their preferential association at metaphase I in interspecific hybrid combinations, it is generally assumed that these wheat genomes are very closely related (e.g. Fernández-Calvín and Orellana 1994; Maestra and Naranjo 1998). In accord with this, we have found that A-D homoeologous metaphase I associations are the most frequent (88%) in the *ph1b* mutant line (Table 3). On the other hand, it is well established that in cereal materials chiasmata tend to be formed at distal chro-

mosomal regions (e.g. Lukaszewski 1995), which concurs quite well with the predominant distal position of exchange points reported here (Fig. 1f–i, l). All evidence thus supports the idea that the intergenomic rearrangements found in *ph1b* mutant and nulli-5B hexaploid wheat plants originated by recombination between homoeologous partners promoted by disruption of the meiotic control activity of the *Ph1* gene. The appearance of new chromosomal exchanges in the offspring of *ph1b* mutants (Table 2) shows that rearrangements can appear generation after generation and have probably been accumulating since the original lines were produced.

The finding of standard, non-rearranged karyotypes in all *ph1c* mutant plants examined (Fig. 1a) could be interpreted as a distinct effect of inactivity of *Ph1* when in the tetraploid wheat genetic background. However, it has to be kept in mind that only the A and B genomes are present in allotetraploid wheats and that fewer than 0.1 rearrangements per plant involving these genomes were detected in the *ph1b* mutant line (Table 1). On the other hand, the frequency of A-B homoeologous metaphase I association was very similar in both *ph1* mutant lines (0.11 and 0.16 associations per cell in tetraploid and hexaploid wheat, respectively; see Table 3). It is thus likely that the lack of chromosomal rearrangements reported here in the *ph1c* mutant line does not reflect differing activity of the *Ph1* gene depending on the presence or absence of the D genome but is just a consequence of the low degree of relatedness between its two constituent genomes. Even the chance of appearance and further accumulation of A-B rearrangements in the *ph1b* line could be higher just because of the much more frequent use of this line compared with the tetraploid since the original mutant plants were obtained.

Most hypotheses about how the *Ph1* locus prevents homoeologous pairing assume that the presence of multivalent pairing configurations is exclusively due to the existence of homoeologous pairing. We have detected a mean number of 1.98 chromosomal exchanges per plant that could be considered in heterozygous condition. It is even likely that these data are actually an underestimation of the real number of translocations produced in these genotypes since there is a technical limit to the ability of GISH procedures to detect small exchanged segments. Therefore, a proportion of metaphase I multivalents observed in *ph1b* mutants and nulli-5B plants have certainly originated not from homoeologous pairing and recombination mainly involving chromosomes of A and D genomes, but from homologous pairing in heterozygotes for reciprocal translocations.

Meiotic analyses of interspecific wheat-alien hybrids using the *ph2b* mutant wheat line have shown that mutation at the *Ph2* locus has an intermediate effect on the frequency of homoeologous metaphase I association compared with that reported for mutation at *Ph1* (e.g. Benavente et al. 1998; Maestra and Naranjo 1998). It would then be expected that the karyotypic stability and meiotic behaviour of the *ph2b* mutant and nulli-3D lines were only quantitatively different from those observed in the *ph1b* mutant and nulli-5B lines. However, the wheat plants lacking *Ph2* activity examined here showed neither chromosomal rearrangements (Fig. 1c) nor homoeologous metaphase I association (Table 3). A recent analysis of synaptonemal complex formation in normal, *ph1b* and *ph2b* mutant allohexaploid wheat has shown that the *Ph1* and *Ph2* loci differ in late synaptic behaviour (Martínez et al. 2001). The metaphase I association pattern reported by these authors in the mutant genotypes agrees with the data for the *ph1b* and *ph2b* lines in Table 3. From a comparison of prophase I and metaphase I observations, Martínez et al. (2001) have proposed that the *Ph1* locus is involved in the restriction of both synapsis and crossing over to homologous chromosomes, whereas the *Ph2* locus affects the progression and completion of synapsis, and have further suggested that only *Ph1* represents a real '*Ph*' locus, actually involved in the diploid-like meiotic behaviour of allopolyploid wheats. Our results seem to support this hypothesis.

The findings reported here are of great importance in the interpretation of much cytogenetic data in the literature. The analysis of *ph1b* mutant or nulli-5B plants as well as their use as wheat parental lines without previous checking for karyotype stability may lead to erroneous conclusions on either the control of the diploidization system in allopolyploid wheats and the evolutionary relationships between wheat and wild relatives. In our opinion, experiments involving *ph1c* and *ph2b* mutants would be more suitable for these purposes because they show standard, non-rearranged karyotypes.

Acknowledgements This work has been supported by grant No. AGF96-0443 from the Comisión Interministerial de Ciencia y Tecnología of Spain.

References

Aragón-Alcaide L, Reader S, Miller T, Moore G (1997a) Centromeric behaviour in wheat with high and low homoeologous chromosomal pairing. Chromosoma 106:327–333

Aragón-Alcaide L, Reader S, Beven A, Shaw P, Miller T, Moore G (1997b) Association of homologous chromosomes during floral development. Curr Biol 7:905–908

Benavente E, Orellana J, Fernández-Calvín B (1998) Comparative analysis of the meiotic effects of *ph1b* and *ph2b* mutations in wheat×rye hybrids. Theor Appl Genet 96:1200–1204

Cuadrado A, Vitellozzi F, Jouve N, Ceoloni C (1997) Fluorescence in situ hybridization with multiple repeated DNA probes applied to the analysis of wheat-rye chromosome pairing. Theor Appl Genet 94:347–355

Cuñado N, Santos JL (1999) On the diploidization mechanism of the genus *Aegilops*: meiotic behaviour of interspecific hybrids. Theor Appl Genet 99:1080–1086

Dubcovsky J, Luo MC, Dvorák J (1995) Differentiation between homoeologous chromosomes 1A of wheat and $1A^m$ of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc Natl Acad Sci USA 92:6645–6649

Dvorak J, Lukaszewski AJ (2000) Centromere association is an unlikely mechanism by which the wheat *Ph1* locus regulates metaphase I chromosome pairing between homoeologous chromosomes. Chromosoma 109:410–414

Feldman M (1966) The effect of chromosomes 5B, 5D and 5A on chromosomal pairing in *Triticum aestivum*. Proc Natl Acad Sci USA 55:1447–1453

Fernández-Calvin B, Orellana J (1994) Metaphase I-bound arms frequency and genome analysis in wheat-*Aegilops* hybrids. 3. Similar relationships between the B genome of wheat and S or Sl genomes of *Ae. speltoides*, *Ae. longissima* and *Ae. sharonensis*. Theor Appl Genet 88:1043–1049

Giorgi B (1978) A homoeologous pairing mutant isolated in *Triticum durum* cv. Cappelli. Mutant Breed Newslett 11:4–5

Hobolth P (1981) Chromosome pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. Carlsberg Res Commun 46:129–173

Holm PB, Wang Z (1988) The effect of chromosome 5B on synapsis and chiasma formation in wheat, *Triticum aestivum* cv. 'Chinese Spring'. Carlsberg Res Commun 53:191–208

Jenkins G (1983) Chromosome pairing in a *Triticum aestivum* cv. Chinese Spring. Carlsberg Res Commun 48:255–283

Koebner RMD, Shepherd KW (1985) Induction of recombination between rye chromosome 1RL and wheat chromosomes. Theor Appl Genet 71:208–215

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and Triticale. Theor Appl Genet 90:714–719

Luo M-C, Dubcovsky J, Dvorák J (1996) Recognition of homoeology by the wheat *Ph1* locus. Genetics 144:1195–1203

Maestra B, Naranjo T (1998) Homoeologous relationships of *Aegilops speltoides* to bread wheat. Theor Appl Genet 97:181–186

Martínez M, Cuñado N, Carcelén N, Romero C (2001) The *Ph1* and *Ph2* loci play different roles in the synaptic behaviour of hexaploid wheat *Triticum aestivum*. Theor Appl Genet (in press)

Martínez-Pérez E, Shaw P, Reader S, Aragón-Alcaide L, Miller T, Moore G (1999) Homologous chromosome pairing in wheat. J Cell Sci 112:1761–1769

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

Mikhailova EI, Naranjo T, Shepherd K, Wennekes-van Eden J, Heyting C, de Jong JH (1998) The effect of the wheat *Ph1* locus on chromatin organisation and meiotic chromosome pairing analysed by genome painting. Chromosoma 107:339–350

Naranjo T, Roca A, Goicoechea PG, Giráldez R (1988) Chromosome pairing in hybrids of *ph1b* mutant wheat with rye. Genome 30:639–646

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–714

Sánchez-Morán E, Benavente E, Orellana J (1999) Simultaneous identification of A, B, D and R genomes by GISH in wheat-rye derivatives. Heredity 83:249–252

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

Tucker JD, Morgan WF, Awa AA, Bauchinger M, Blakey D, Cornforth MN, Littlefield LG, Natarajan AT, Shasserre C (1995) PAINT: a proposed nomenclature for structural aberrations detected by whole chromosome painting. Mutat Res 347:21–24