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The *Ph1* and *Ph2* loci play different roles in the synaptic behaviour of hexaploid wheat *Triticum aestivum*

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Abstract *Triticum aestivum* is an allohexaploid wheat (AABBDD) that shows diploid-like behaviour at metaphase-I. This behaviour is influenced by the action of several loci, *Ph1* and *Ph2* being the main loci involved. To study the effect of these two loci on chromosome pairing in *T. aestivum* we have analysed the synaptic pattern in fully traced spread nuclei at mid- and late-zygotene, and at pachytene, of three different genotypes of cv Chinese Spring: standard line, *ph1b* and *ph2b* mutants. The analysis of the synaptic progression showed that only a few nuclei accomplish synapsis in the *ph2b* genotype, whereas most nuclei completed synapsis in the standard and *ph1b* genotypes. This result indicates that the *Ph2* locus affects synaptic progression. The number of synaptonemal complex (SC) bivalents and of the different SC multivalent associations were determined in each nucleus. The mean number of lateral elements involved in SC multivalent associations (LEm) at mid-zygotene was relatively high and showed similar values in the three genotypes. These values decreased progressively between mid-zygotene and pachytene in the genotypes with the *Ph1* locus because of the transformation of multivalents into bivalents. In the *ph1b* genotype, this value only decreased between late-zygotene and pachytene. Therefore, multivalent correction was more efficient in the presence than in the absence of the *Ph1* locus. It is concluded that the *Ph1* and *Ph2* loci bring about diploidization of allohexaploid wheat via a different mechanism.

Keywords *Ph1* locus · *Ph2* locus · Hexaploid wheat · Synaptonemal complex · Diploid-like behaviour

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Introduction

Bread wheat, *Triticum aestivum* L., is an allohexaploid species with genomes A, B and D (2n=42, AABBDD), which evolved from a common ancestral genome and, therefore, have partially homologous (homoeologous) chromosomes. Despite these similarities, homoeologous chromosomes do not pair at meiotic metaphase-I. Wheat, therefore, shows strictly disomic inheritance.

On the basis of chromosome pairing at metaphase-I in wheat haploids or hybrids lacking individual chromosomes or chromosome arms, it was demonstrated that the most-important gene (designated *Ph1*) involved in the suppression of homoeologous pairing was located on the long arm of chromosome 5B (Riley and Chapman 1958; Sears and Okamoto 1958). Sears (1977) obtained a mutation (*ph1b*) at this locus that leads to increased homoeologous pairing at metaphase-I. A second locus (*Ph2*) with a lower effect on the suppression of homoeologous pairing was located on the short arm of chromosome 3D (Mello-Sampayo 1968, 1971; Mello-Sampayo and Cannas 1973). Two deletions at this locus were found, *ph2b* (Wall et al. 1971) and *ph2a* (Sears 1982). Additional, less-effective suppressors were discovered on several other chromosomes (Sears 1976; Cuadrado et al. 1991).

Studies of chromosome synapsis at prophase-I in plants with and without the 5B chromosome led Holm and Wang (1988) to suggest that the *Ph1* locus regulates the stringency of synapsis at early zygotene and a suppression of crossing-over between the regions homoeologously synapsed at pachytene. Additional evidence in support of this hypothesis was provided by Gillies (1987), Dubcovsky et al. (1995) and Luo et al. (1996). Feldman (1966), by contrast suggested that the effect of the *Ph1* locus is exerted at premeiotic stages, affecting the alignment of homologous and homoeologous chromosomes. This hypothesis has recently been supported by genomic in situ hybridization studies on the behaviour of homologous chromosomes of barley or rye added to wheat (Aragón-Alcaide et al. 1997; Schwarzacher

1997; Martínez-Pérez et al. 1999). Mikhailova et al. (1998) postulated a *Ph1* locus-effect on both premeiotic alignment and chromosome synapsis, and also on chromatin structure. Using the spreading technique, Martinez et al. (2000) demonstrated that the most-important action of the *Ph1* locus in tetraploid wheats was related to the correction of multivalents throughout prophase-I. Similar studies have not been done with the *Ph2* locus.

In the present study, we analyse the chromosome behaviour at prophase-I by the spreading technique in standard plants (*Ph1Ph1Ph2Ph2*), the *ph1b* mutant (*ph1bph1bPh2Ph2*) and the *ph2b* mutant (*Ph1Ph1ph2-bph2b*), in order to obtain further insights into the effect of the *Ph1* locus on chromosome synapsis and to identify were and how the *Ph2* locus exerts its action on chromosome pairing in hexaploid wheat.

Material and methods

Plants of hexaploid wheat *T. aestivum* cv Chinese Spring (AABBDD, 2n=6*x*=42) from three different genotypes, the standard line (*Ph1Ph1Ph2Ph2*), the *ph1b* mutant (*ph1bph1bPh2Ph2*) and the *ph2b* mutant (*Ph1Ph1ph2bph2b*), were used.

Single anthers of the emerging spikes were squashed in 2% acetic orcein to determine the meiotic stage. Two sister anthers of the same floret at zygotene or pachytene were then prepared for synaptonemal complex (SC) observation, as described by Holm (1986). Surface-spread preparations were silver-stained by Loidl's (1984) method. Nuclei of ten plants of each genotype were observed by electron microscopy for prophase-I analysis. More than 500 nuclei were examined by electron microscopy (EM). A total of 38, 36 and 24 nuclei in the standard, *ph1b* and *ph2b* genotypes, respectively, were photographed (captured nuclei); the remaining nuclei exhibited basically similar features to those selected. A complete reconstruction of some captured nuclei was not possible because they had too many multivalents and/or the SCs were partially destroyed in the synaptic partner exchange regions. Nuclei from different plants did not differ in the initiation and progression of synapsis and, therefore, the nuclei of each genotype were pooled.

For metaphase-I observations, the anthers were fixed in 1:3 acetic-acid ethanol and stored at 4°C. The fixed material was squashed and C-banded according to Giráldez et al. (1979).

Results

Prophase-I observations

Synapsis was initiated in distal regions and progressed with the formation of interstitial synaptic-initiation sites in a similar way in the three genotypes. Nuclei analysed at prophase-I were classified into three substages, mid-zygotene, late-zygotene and pachytene, according to Martínez et al. (1996, 2000). All nuclei at mid-zygotene showed the bouquet structure, and the degree of synapsis was lower than 75%; nuclei at late-zygotene partially maintained the bouquet structure and had a degree of synapsis between 75% and 95%, whereas nuclei at pachytene lost the bouquet arrangement and their synapsis reached nearly 100%. In addition, a decrease in the number of unmatched regions in the SC bivalents and multivalents was observed from mid-zygotene to pachytene. Figure 1 shows some examples of nuclei at different substages. However, important differences were found in the number of nuclei observed at each substage. In genotypes with the *Ph2* locus (standard and *ph1b* mutant lines) most nuclei completed synapsis and reached the pachytene stage. In genotypes lacking the *Ph2* locus, only a few nuclei accomplished synapsis, with the majority of the nuclei showing synaptic features corresponding to the zygotene stage (Fig. 2). Although this refers only to the captured nuclei, the vast majority of the

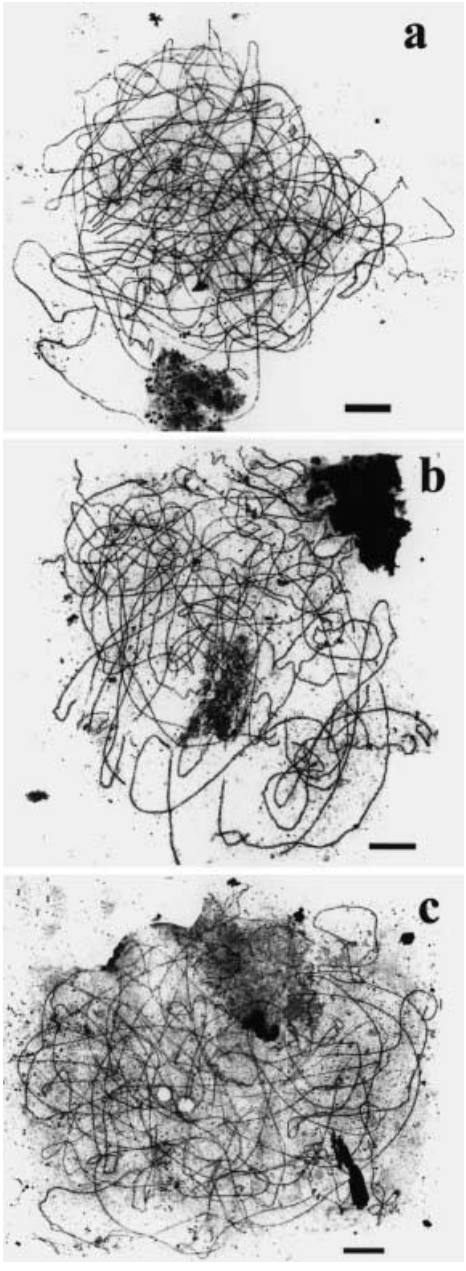

Fig. 1 Electron micrographs of entire nuclei of: **a** *T. aestivum Ph1Ph1Ph2Ph2* at late-zygotene; **b** *T. aestivum ph1bph1bPh2Ph2* at pachytene; **c** *T. aestivum Ph1Ph1ph2bph2b* at late zygotene. Scale bar=5μm

Table 1 Summary of the analysis of the synaptonemal complexes of fully traced nuclei at mid-zygotene (MZ), late-zygotene (LZ) and pachytene (P) of the hexaploid wheat *T. aestivum* standard, and the *ph1b* and *ph2b* mutants. The mean number of the different SC associations per nucleus, the mean number of the lateral elements involved in multivalent associations (LEm) per nucleus, as well as the mean number of interlocks (IL) per nucleus, are indicated

Genotype	Substage	No. of nuclei	Axial element length (μm)	Total synapsis	Bivalent synapsis	II	IV	VI	VIII	LEm	IL
Ph1Ph2	MZ	4	4,721±526	54.2±6.6	57.2±6.8	15.00±1.05	1.75±0.29	0.50±0.33	0.25±0.29	12.00±2.11	4.00±0.82
	LZ	5	4,398±341	82.1±3.2	83.9±2.6	17.80±1.71	1.00±0.55	0.40±0.27		6.40±3.42	2.40±0.51
	P	23	3,789±140	96.3±0.6	97.0±0.7	12.41±0.37	0.48±0.12	0.10±0.08	0.07±0.05	2.70±0.60	1.00±0.24
ph1bPh2	MZ	8	3,757±213	50.8±4.7	51.7±5.5	14.75±0.88	2.37±0.50	0.50±0.19	0.12±0.13	12.50±1.76	3.50±0.46
	LZ	6	3,935±285	80.8±2.4	87.9±2.2	14.83±1.07	2.33±0.34	0.50±0.34	0.17±0.18	12.33±2.15	3.50±0.62
	P[a]	14	3,807±314	94.2±0.8	97.4±0.5	17.14±0.47	1.29±0.16	0.28±0.13	0.07±0.07	7.64±0.91	1.29±0.29
Ph1ph2b	MZ	7	5,536±287	56.7±4.9	57.2±6.8	16.29±0.64	1.29±0.36	0.71±0.18		9.43±1.29	5.57±0.84
	LZ	5	5,053±481	82.9±3.5	83.9±2.6	17.20±1.39	1.00±0.45	0.60±0.24		7.60±2.79	3.00±0.14
	P	4	4,272±271	96.5±0.8	97.0±0.7	20.00±0.58	0.50±0.29			2.00±1.15	1.50±0.96

[a] One nucleus with a trivalent and an univalent

Fig. 2 Histogram showing the percentage of the captured nuclei in each analysed substage at prophase-I of *Ph1Ph2*, *ph1bPh2* and *Ph1ph2b* plants

nuclei analysed by electron microscopy in *ph2b* plants were at zygotene.

General features of SCs in the completely reconstructed nuclei of the three genotypes are summarised in Table 1. Mean values for the total length of the axial elements, the total percentage of synapsis and the percentage of synapsis in SC bivalents, the mean number of bivalents, quadrivalents and other multivalent configurations per nucleus, as well as the number of lateral elements involved in multivalents (LEm) per nucleus, are indicated. Table 1 also includes the frequency of interlocking (IL) per nucleus. No mid-zygotene nucleus showed only bivalents in any of the three genotypes. SC bivalent, quadrivalent and hexavalent configurations were observed in nuclei of the three types of plants at each substage. One octavalent, indicating synapsis between non-homoeologous chromosomes, was observed in a nucleus of the standard genotype and in a nucleus of the *ph1b* genotype. The frequency of multivalents per nucleus, measured as the mean number of LEm, showed similar and relatively high values at mid-zygotene in the standard and the *ph1b* mutant plants (χ^2=0.31; 0.7>*P*>0.5), and in the standard and *ph2b* mutant plants (χ^2=2.15; 0.2>*P*>0.1). These values decreased progressively between zygotene and pachytene in the genotypes with the *Ph1* locus because of the transformation of multivalents into bivalents. In the genotype without the *Ph1* locus, this value barely changed between mid- and late-zygotene, and only decreased between late-zygotene and pachytene, maintaining a higher number of LEm than in the genotypes with the *Ph1* locus at pachytene. Figure 3 includes regions of nuclei showing a higher magnification of synaptic partner exchanges in the standard, *ph1b* and *ph2b* plants. Figure 4 shows the synaptic behaviour of the tetraploid wheat *Triticum turgidum* (Martínez et al. 2000) and the hexaploid wheat *T. aestivum* studied here. The similarities in the correction process between tetraploid and hexaploid wheats are evident.

A total number of 23, 50 and 16 quadrivalents in standard plants, and the *ph1b* and *ph2b* mutants, respectively, showed one synaptic partner exchange (SPE) point.

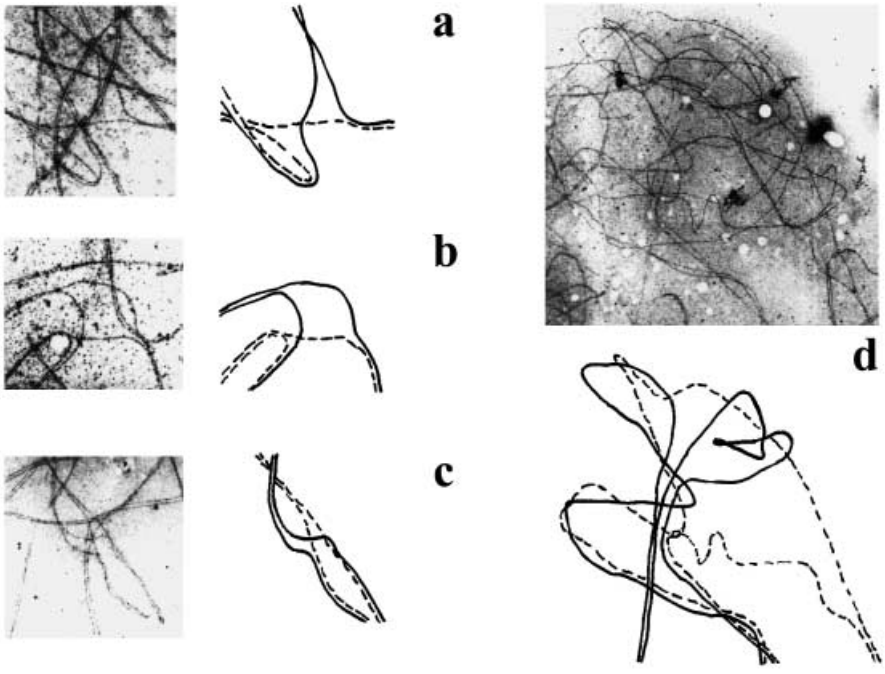

Fig. 3 High-magnification electron micrographs of synaptic partner exchanges in synaptonemal complexes of quadrivalents of *T. aestivum Ph1Ph1Ph2Ph2* (**a**), *T. aestivum ph1bph1bPh2Ph2* (**b** and **c**), and *T. aestivum Ph1Ph1ph2bph2b* (**d**), and their corresponding schematic drawings. **Figures a, b and d** show interstitial synaptic partner exchanges. **Figure c** shows a distal synaptic partner exchange

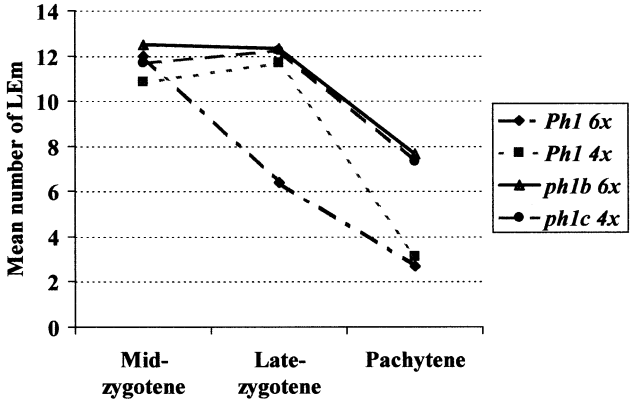

Fig. 4 Representative drawing comparing the evolution of the mean number of multivalent associations (*LEm*) throughout prophase-I in the tetraploid and hexaploid wheat standards and the *ph1c* and *ph1b* mutants. Note the similar mode of multivalent elimination and the small differences at the moment in which the correction mechanism starts

Only one quadrivalent in the *ph1b* genotype had two SPEs. The positional distribution of the SPE points in the quadrivalents with one SPE was analysed according to Santos et al. (1995) and Martínez et al. (1996, 2000). Each chromosome was divided into two halves and each half into five segments of equal relative length. One of these five segments contained the SPE site. The five segments were numbered from 1 to 5 in the direction from the telomere to the chromosome center. The number of the segment containing the SPE site defines its position relative to the chromosome center. Whether a given SPE site was located in the short or in the long arm could not be determined because the technique does not preserve the centromeric region. Therefore, the SPEs located at a similar distance from either chromosome end are combined. The position of the SPE in such quadrivalents is indicated in Fig. 5. Most of the SPEs were centrally located at mid-zygotene in the three genotypes, which is consistent with a synaptic process that starts at the chromosome ends and progresses towards the central region. Progression of synapsis did not alter the centric location of SPEs in genotypes with the *Ph1* locus (standard and *ph2b* lines). In plants lacking the *Ph1* locus, a considerable proportion of the quadrivalents showed the SPE point distally located at pachytene.

Synaptic abnormalities, such as interlockings (ILs), were observed in nuclei of all the genotypes and prophase-I substages analysed (Table 1). Since the 2D analysis does not allow a correct estimation of all interlockings, only interlocks that showed a clear tension between the lateral elements involved were considered. The estimated value of ILs were similar in the three genotypes at mid-zygotene (analysis of variance, F=2.69; P=0.98), decreasing throughout prophase-I.

Metaphase-I observations

Table 2 summarises the chromosome associations observed at metaphase-I. In the standard plants, chromosomes formed mainly ring bivalents, less frequently open bivalents, and rarely pairs of univalents. In plants of the *ph2b* mutant, a higher number of open bivalents and pairs of univalents was found (χ^2=136.33; P<0.001) (Fig. 6b). C-banding differentiated unambiguously between chromosomes from the A/D and B genomes since those of the B genome appear heavily C-banded (Friebe and Gill 1996). No homoeologous association was detected in these two genotypes, which indicates that the

Table 2 Mean number per cell of chromosome associations at metaphase-I in the hexaploid wheat *T. aestivum* standard, and the *ph1b* and *ph2b* mutants

Genotype	Number of cells	Genome	Ring bivalents	Open bivalents	Univalent pairs	Trivalents	Quadrivalents	Un/Cel.
Ph1Ph2	90	A/D	12.94±0.11	1.04±0.11	0.01±0.01			
		B	6.56±0.06	0.44±0.06				
		B-A/D						
		Total	19.50±0.12	1.48±0.12	0.01±0.01			40.49±0.14
ph1bPh2	120	A/D	9.43±0.20	2.97±0.20	0.63±0.10	0.20±0.04	0.36±0.03	
		B	4.62±0.10	1.79±0.10	0.75±0.10			
		B-A/D				0.08±0.02	0.13±0.04[a]	
		Total	14.05±0.15	4.76±0.15	1.38±0.12	0.28±0.04	0.49±0.04	34.22±0.17
Ph1ph2b	120	A/D	11.97±0.05	1.88±0.05	0.14±0.03			
		B	5.81±0.11	1.07±0.08	0.10±0.03			
		B-A/D						
		Total	17.78±0.16	2.95±0.15	0.24±0.04			38.57±0.15

[a] Two nuclei with a pentavalent

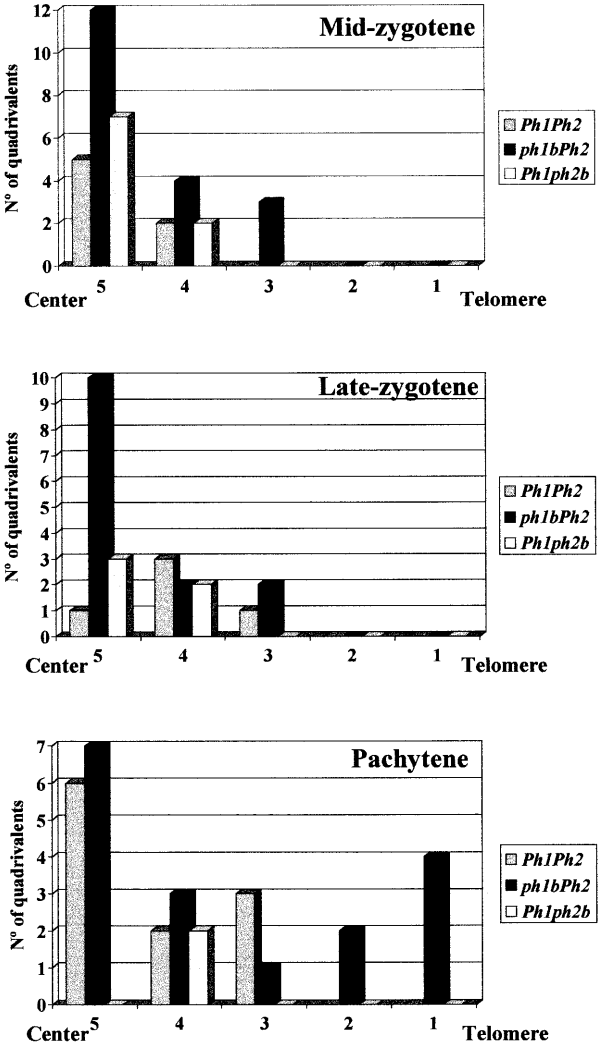

Fig. 5 Location of the synaptic partner exchange point at mid-zygotene, late-zygotene and pachytene

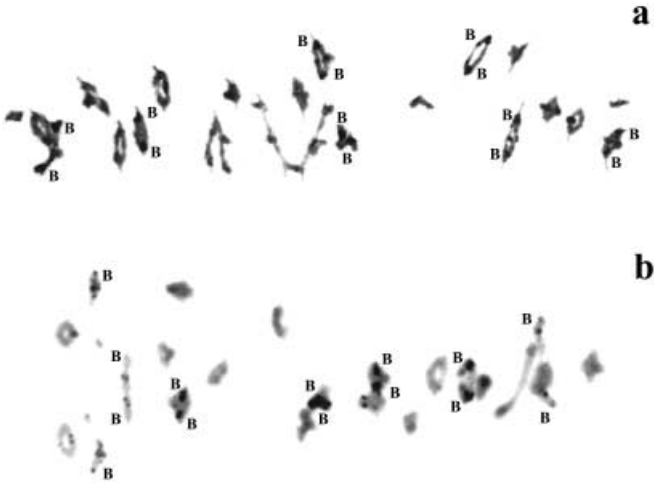

Fig. 6 a Micrograph of a metaphase-I nucleus of *T. aestivum ph1bph1bPh2Ph2* showing five ring bivalents, one open bivalent and two univalents of the B genome, and nine ring bivalents, one trivalent, one quadrivalent and three univalents of the A or D genomes. **b** Micrograph of a metaphase-I nucleus of *T.aestivum Ph1Ph1ph2bph2b* showing four ring bivalents and three open bivalents of the B genome, and 13 ring bivalents and one open bivalent of the A or D genomes

formation of chiasmata presumably occurred only between homologous chromosomes. In the *ph1b* mutant plants some trivalents and quadrivalents involving homoeologous chromosomes were observed (Fig. 6a) indicating the occurrence of homoeologous crossing-over at prophase-I. In addition, a higher frequency of open bivalents and univalent pairs was found in these plants than in plants carrying the *Ph1* locus.

Discussion

Synapsis begins and develops in a similar way in the three genotypes studied. As in many other plant species (Loidl 1990; Cuñado et al. 1996a, b, c) synapsis initiates

at distal regions and continues by the formation of interstitial segments of the SC, which fuse until synapsis is completed at the pachytene stage. However, the number of nuclei that reached this stage was clearly lower in the *ph2b* genotype than in the other two genotypes (Fig. 2), which indicates that the *ph2b* genotype has difficulty in completing synapsis. Holm (1986, 1988a, b, c) analysed synapsis in *T. aestivum* with different doses of the 5B chromosome. Some of these genotypes also displayed difficulties with completing synapsis, which was attributed to the lack of the short arm of the 5B chromosome. The *ph1b* plants did not show problems completing synapsis in the same way as the *ph1c* plants of tetraploid wheats (Martinez at al.2000), which confirms that the *Ph1* locus is not involved in the synaptic progression. Obviously, it is advantageous to use mutants over aneuploid lines.

Synaptic problems could be related to the subsequent metaphase-I behaviour. Partial asynapsis at prophase-I was related to a decrease of chiasmata at metaphase-I in rye inbred lines (Martínez et al. 1995) and *Petunia hybrida* (Abirached-Darmency et al. 1992). In the *ph2b* genotype the number of open bivalents and univalent pairs was significantly higher than in the standard genotype, showing a mean chiasma frequency of 38.5 whereas that of the standard genotype was 40.5. This lower chiasma frequency could be a consequence of the partial asynapsis observed in this genotype. The genotypes used by Holm (1988c) which did not complete synapsis also had a lower chiasma frequency.

Holm and Wang (1988) suggested that the *Ph1* locus had an effect on the stringency of synapsis since the number of zygotene lateral elements involved in multivalent associations in plants with the 5B chromosome was lower than in plants lacking the 5B chromosome. However, in the present study and a previous study of *T. turgidum* (Martínez et al. 2000), the number of lateral elements involved in multivalent associations was similar in plants with and without the *Ph1* locus at mid-zygotene, which suggests an equal synaptic stringency in both genotypes at early prophase. Interestingly, the number of LEm at mid-zygotene was similar in the four genotypes (the *T. aestivum* and *T. turgidum* standard lines, and the *ph1c* and *ph1b* mutants) (Fig. 4), although hexaploid wheat possesses two more genomes than tetraploid wheat, and the A and D genomes are closely related (Naranjo and Maestra 1995). The presence of the D genome could be responsible for a reduction of heterogenetic synapsis in species with this genome. Allotetraploid *Aegilops* species with the D genome show a very high stringency of synapsis involving homologous chromosomes at zygotene (Cuñado et al. 1996c), which would confirm this possibility.

Feldman (1966) argued for a premeiotic effect of the *Ph1* locus on chromosome pairing. Recent studies using FISH and GISH (Aragón-Alcaide et al. 1997; Schwarzacher 1997; Mikhailova et al. 1998; Martínez-Pérez et al. 1999) revealed differences in the arrangement of chromosomes prior to prophase-I between plants with and without the *Ph1* locus. Nevertheless, our data strongly indicate that variation in premeiotic chromosome arrangement is not important and is not causally related to the elimination of heterogenetic chromosome pairing at metaphase-I in allopolyploid wheats, Moreover, similar IL frequencies at mid-zygotene in plants with and without *Ph1* indicate that chromosomes are arranged in a same way at early prophase, at least at the time-point at which our study started.

Multivalent associations are corrected throughout prophase-I. This process is gradual between mid-zygotene and pachytene in genotypes having the *Ph1* locus, but no correction was observed between mid- and late-zygotene in the *ph1b* plants; correction was only apparent between late-zygotene and pachytene. The existence of synaptic partner exchanges located in distal regions at pachytene only in the genotype lacking the *Ph1* locus implies that two different mechanisms are involved in multivalent correction, similar to that observed in allotetraploid wheats (Martínez et al. 1996, 2000). In plants with the *Ph1* locus, a quick process of the opening and closing of the non-strictly homologous branches of the multivalents leads to a rapid dissolution of these associations, with no SPEs observed in distal regions. In plants without the *Ph1* locus, the process of multivalent elimination is slow, with a gradual change of the SPE position from proximal to distal regions. Therefore, *Ph1* activity is directly related to the mechanism of multivalent elimination, arguing against the supposition of Holm and Wang (1988) that multivalent correction was only a consequence of normal synaptic behaviour. These mechanisms seem general in allopolyploid wheats since both allotetraploid (Martínez et al. 1996, 2000) and allohexaploid wheats show a similar multivalent elimination (Fig. 4). Allotetraploid *Aegilops* species do not show multivalent correction between zygotene and pachytene (Cuñado et al. 1996a, b, c). The very low number of multivalents observed in these species at zygotene could make the existence of this kind of mechanism unnecessary.

Multivalent associations are maintained until pachytene in the three genotypes, although multivalent associations are more frequent in the *ph1b* genotype. Recent studies have demonstrated that in some organisms the early steps of recombination precede synapsis (Padmore et al. 1991; Roeder 1997). In tetraploid wheats, Martinez et al. (2000) suggested that some recombination events occur after multivalent correction to explain the very high number of ring bivalents observed at metaphase-I in the standard genotype. The same must be true for hexaploid wheat since it would be otherwise difficult to explain the presence of the SPEs located in proximal regions, the preference for the distal location of chiasmata and the high frequency of ring bivalents at metaphase-I. In the multivalents maintained until crossing-over formation, a restriction of cross-over to strictly homologous regions occurs. This crossing-over restriction was postulated by Gillies (1987) and Holm and Wang (1988) and was strongly supported by the studies of Dubcovsky et al.

(1995) and Luo et al. (1996) on the recombination process in lines with and without the *Ph1* locus. In the *ph1b* genotype, no total restriction of crossing-over to homologous chromosomes takes place, since multivalents involving homoeologous chromosomes were observed at metaphase-I.

No multivalent associations were present in the *ph2b* genotype at metaphase-I. However, the absence of this gene leads to an enhancement of homoeologous pairing in hybrids between wheat and related species at metaphase-I (Mello-Sampayo 1968, 1971). Therefore, it was assumed that *Ph2* has a similar effect on pairing as *Ph1*, only weaker. Results obtained in this study suggest that the *Ph1* and *Ph2* loci exert their effects in different ways: *Ph1* affects correction of synapsis and crossing-over, *Ph2* affects the completion of synapsis. What is not clear is how partial asynapsis leads to homoeologous pairing in hybrids lacking *Ph2* at metaphase-I. The *Ph1* locus completely suppresses homoeologous crossing-over in genotypes with homologous and homoeologous chromosomes, but not in genotypes with only homoeologous chromosomes such as hybrids and haploids (Riley 1960; Kimber 1962; Riley and Law 1965). This different behaviour could be a consequence of the amount of homoeologous synapsis. In genotypes with only homoeologous chromosomes, extensive non-homologous synapsis occurs, whereas in genotypes with homologous and homoeologous chromosomes, synapsis preferentially involves homologous chromosomes. In hybrids and haploids, the *Ph1* locus is not able to correct all of the numerous homoeologous associations, and some crossing-over may take place between homoeologues regions at pachytene. Several nuclei of the *ph2b* genotype at mid- and late-zygotene should be at a stage similar to pachytene. These nuclei had a higher number of multivalent associations than pachytene nuclei. Thus, the delay in the progression of synapsis observed in the *ph2b* genotype leads to an incomplete action of the *Ph1* locus in the process of homoeologous synapsis correction. Consequently, a higher number of homoeologous associations are expected in hybrids lacking *Ph2* than in hybrids with *Ph2*. In hexaploid *ph2b* wheat, the *Ph1* locus would be able to suppress crossing-over between these homoeologous regions. In *ph2b* hybrids, the number of homoeologous associations would be much higher, and the *Ph1* locus would not be able to avoid the formation of several cross-overs between them.

In conclusion, the *Ph1* and *Ph2* loci do not affect the synaptic restriction to bivalents at early prophase. However, the *Ph1* and *Ph2* loci have a different effect on later synaptic behaviour. The *Ph1* locus corrects homoeologous associations throughout meiotic prophase-I and suppresses crossing-over between the remaining homoeologous regions at pachytene. Thus, the *Ph1* locus is an actual homoeologous-pairing (*Ph*) locus, clearly involved in the acquisition of the diploid-like behaviour of allohexaploid wheat. The *Ph2* locus affects the progression of synapsis, probably in a similar way to the diploid species. Therefore, *Ph2* would not be involved in the acquisition of the diploid-like behaviour, and would not be an actual pairing-homoeologous (*Ph*) locus but a synaptic (*Syn*) locus, as Ji and Langridge (1994) suggested analysing the expression of a wheat cDNA clone located on the short arm of chromosome 3D. The effect observed on homoeologous pairing in hybrids lacking *Ph2* would be an indirect effect on the action of the *Ph1* locus.

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