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ORIGINAL PAPER

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Control of conformation changes associated with homologue recognition during meiosis

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Abstract During early meiosis, chromosomes pair via their telomeres and centromeres. This pairing induces a conformational change which propagates from these regions along each chromosome, making the chromatin of the partners accessible for intimate pairing. In the present study, we show by exploiting wheat–rye hybrids that the signal is initiated in both the presence and absence of either the *Ph1* or *Ph2* locus. However, the chromatin change only continues to propagate through rye telomeric heterochromatin when *Ph1* is absent. This failure to propagate the chromatin change through the rye heterochromatin in the absence of *Ph2* correlates with a subsequent lack of wheat–rye chromosome association at metaphase I.

Introduction

Meiosis is a specialised type of cell division in which two rounds of chromosome segregation follow a single round of DNA replication. Integral parts of the process include the recognition, pairing and synapsis of homologues, which in most of the eukaryotes are pre-requisites for genetic recombination and balanced segregation of half-bivalents at anaphase I. The first division of the meiosis is in fact a unique chromosome-segregation event in which homologues segregate from each other.

Many of the components of the meiotic recombination machinery are known, especially from yeast, as well as some structural components of the synaptonemal complex. However, little is known about how homologues recognise each other in the first place. It is clear that during early meiosis chromosomes must become competent to pair, in contrast to their behaviour during interphase. The telomeric and centromere regions of chromosomes are commonly associated with the nuclear envelope, having critical roles in meiotic chromosome pairing (Prieto et al. 2004). They are involved in sorting the chromosomes into pairs. This is achieved early in meiosis through the clustering of the telomeres into a 'bouquet' and the centromeres into seven groups (Martinez-Perez et al. 2003; Prieto et al. 2004). Studies exploiting maize and *Caenorhabditis elegans* have indicated that the onset of pairing at meiosis is associated with conformational changes in the chromosomes (Dawe et al. 1994; MacQueen and Villeneuve 2001). In maize, it is observed that this is not a generalised conformational change of the whole chromosome, but is a localised effect. Thus, these conformational changes are distinct from the generalised condensation of chromosomes which is initiated at the onset of meiosis and end with condensed chromosomes at metaphase I. As two homologues pair or 'zip up', the chromatin immediately preceeding the 'pairing fork' undergoes a conformational change, becoming elongated, after which it intimately pairs. However, as yet no mutants have been identified in either *C. elegans* or maize which affect this conformational change. Recently this 'localised' conformational change has also been observed in hexaploid wheat chromosomes which are pairing at meiosis and interestingly, the behaviour of the conformational change is affected by the *Ph1* locus. In the presence of *Ph1*, euchromatin, but not some telomeric heterochromatin, can undergo the conformational change, whereas in its absence, both euchromatin and the telomeric heterochromatin undergo the change. Thus, in hexaploid

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wheat, when two chromosomes recognise each other by their telomere (or centromere regions), the chromatin immediately adjacent to these regions undergoes a conformational change. The conformational change is confined to these interacting chromosomes. Thus, as the chromosome regions pair along the chromosome, the chromatin immediately adjacent then undergoes a conformational change which is then followed by its intimate pairing (Dawe et al. 1994; Prieto et al. 2004).

Hexaploid wheat (*Triticum aestivum*) contains three closely related genomes A, B and D. Chromosome pairing during meiosis in this allohexaploid is restricted to true homologues, despite the fact of the presence of related (homoeologous) chromosomes. This means that hexaploid wheat has a diploid-like behaviour which results in 21 bivalents during metaphase I in meiosis. There are several pairing homologous (*Ph*) genes controlling chromosome pairing in wheat (Sears 1976). The strongest effect is associated with the *Ph1* locus, which is located on the long arm on chromosome 5B. This suppresses homoeologous chromosome pairing in wheat (Riley and Chapman 1958; Sears and Okamoto 1958). Other loci have been described as suppressors of homoeologous pairing and include *Ph2* located on the short arm of chromosome 3D (Upadhya and Swaminathan 1967; Mello-Sampayo and Lorente 1968; Mello-Sampayo 1971). We have shown recently that the *Ph1* affects the conformational change which makes the chromosomes competent to intimately pair at meiosis. In hybrids in the presence of the *Ph1* locus, interactions between the homoeologues fail to generate this conformational change in the rye telomeric heterochromatin, whereas in the absence of *Ph1*, the interactions between the homoeologues do induce the change in the rye telomeric heterochromatin.

In the present study, we have investigated wheat–rye hybrids via in situ hybridisation of their intact meiocytes to determine whether the lack of the conformational change when homoeologues interact in the presence of *Ph1* is because the signal to change is not initiated, or because it is initiated but not propagated. We have also investigated whether the *Ph2* locus affects the conformational change in early meiosis and its subsequent effect on pairing at metaphase I.

Material and methods

Plant material

The anthers and the roots used in this study came from *Secale cereale* cv. Petkus (diploid rye), *T. aestivum* cv. Chinese Spring (CS)/*S. cereale* cv. Petkus F_1 hybrids, with and without the *Ph1* locus (carrying the *ph1b* deficiency), and *T. aestivum* cv. CS/ *S. cereale* cv. Petkus F_1 hybrids, with and without the *Ph2* locus. All lines lacking *Ph2* used here carried the *ph2b* deficiency.

Meiotic spreads and fluorescence in situ hybridisation

The determination of intergenomic pairing at meiotic metaphase I was performed upon meiotic spreads of pollen mother cells and utilised genomic in situ hybridisation (GISH) according to Reader et al. (1996). The probe was total genomic *S. cereale* cv. Petkus DNA labelled with fluorescein-12-dCTP (NEN Life Sciences), and the incubation and stringency washes were performed at 60°C.

Slides were analysed using a conventional epifluorescence microscope (Nikon Microphot) with a photomicrographic attachment.

Sectioning and fluorescence in situ hybridisation

The probes used in this paper were total genomic *S. cereale* cv. Petkus DNA, the sequence pSc250-amplified by PCR using total rye genomic DNA as template to label rye heterochromatin knob DNA (Vershinin et al. 1995) and the telomeric probe amplified by PCR using the oligomer primers $(5'\text{-TTTAGGG-}3')_5$ and $(5'\text{-CCCTAAA-}3')_5$ in the absence of template DNA (Cox et al. 1993).

Preparation of the meiotic chromosome spreads for premeiosis analysis, their labelling by in situ hybridisation and subsequent scoring has all been described previously (Aragon-Alcaide et al. 1996, 1997).

Biotin-labelled and digoxigenin-labelled probes were detected with streptavidin-Cy3 conjugate (Sigma, St. Louis, Mo., USA) and anti-digoxigenin-FITC (Roche, Postfach, Basel, Switzerland), respectively. Chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindole) and mounted in Vectashield.

Confocal fluorescence microscopy and image processing

We collected confocal optical stacks using a Leica TCS SP as described previously (Martinez-Perez et al. 1999). Confocal images were processed by the public domain program ImageJ, written by Wayne Rasband (wayne@codon.nih.gov) at the Research Services Branch, National Institute of Mental Health, Bethesda, Md., USA. All the images of single meiocytes are taken from whole anther sections which are two layers thick. The meiocytes were analysed from 3D confocal data stacks. Projections were made for the images shown in this paper.

Images were captured with a CCD camera using the appropriate Metamorph software (Universal Imaging, Molecular Devices, Downingtown, UK).

Final figures were processed with PhotoShop, version 4.0, software (Adobe Systems, San Jose, Calif., USA). Images were printed on a Hewlett Packard Deskjet HP 950C Color Printer.

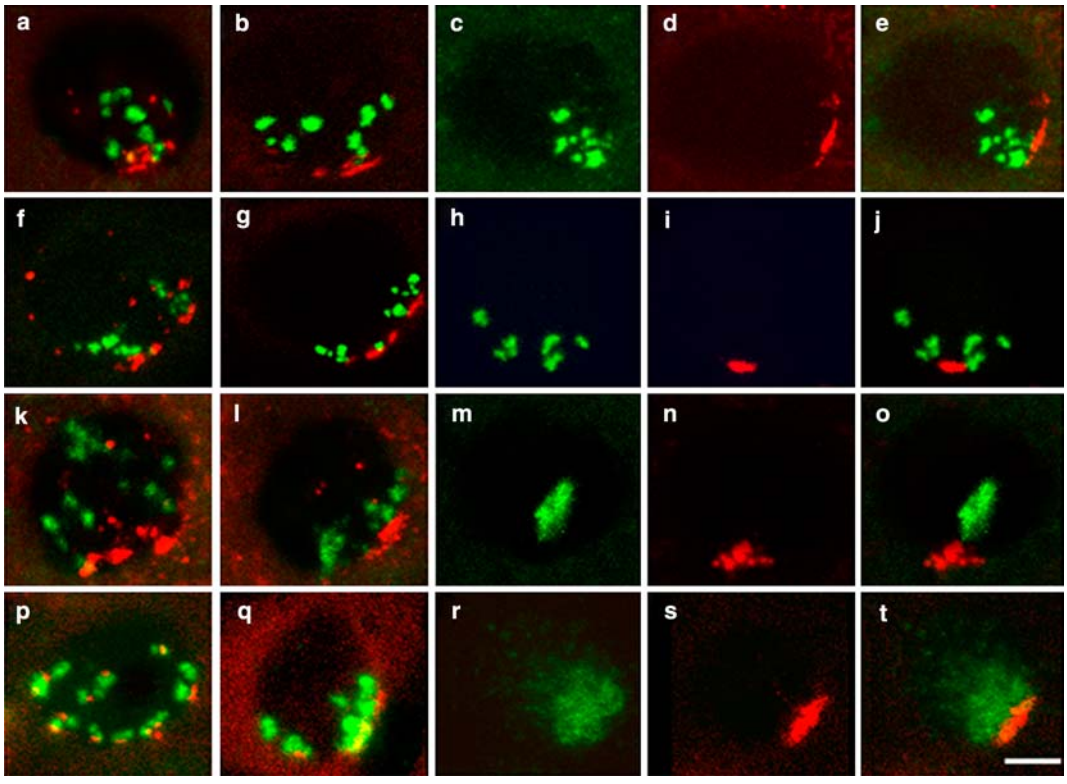

Fig. 1 Projection of confocal sections from meiocytes during meiosis in the wheat–rye hybrids and in the diploid rye showing telomere (*red*) and rye heterochromatin behaviour (*green*). **a** Early wheat–rye meiotic nucleus, *Ph1*+ and *Ph2*+. **b** Later stage than **a**, *Ph1*+ and *Ph2*+. **c**–**e** Later meiotic nucleus at the telomere bouquet stage, *Ph1*+ and *Ph2*+ (rye heterochromatin knobs, telomeres and overlay, respectively). **f** Early meiotic nucleus, *Ph1*+ and *Ph2*−. **g** Later stage than **f**, *Ph1*+ and *Ph2*−. **h**–**j** Later meiotic nucleus at the telomere bouquet stage, *Ph1*+ and *Ph2*− (rye heterochromatin knobs, telomeres and overlay, respectively). **k** Early meiotic nucleus, *Ph1*− and *Ph2*+. **l** Later stage than **k**, *Ph1*− and *Ph2*+. **m**–**o** Later meiotic nucleus at the telomere bouquet stage, *Ph1*− and *Ph2*+ (rye heterochromatin knobs, telomeres and overlay, respectively). **p** Early meiotic nucleus in the diploid rye. **q** Later stage than **p** in the diploid rye. **r**–**s** Later meiotic nucleus at the telomere bouquet stage in the diploid rye (rye heterochromatin knobs, telomeres and overlay, respectively). *Scale bar* = 10 μm

Results and discussion

Chromosome organisation at early meiotic stages in wheat–rye hybrids

Wheat–rye hybrids contain a haploid set of 21 wheat chromosomes and a haploid set of 7 rye chromosomes, making 28 homoeologues and no homologues. We have visualised by in situ hybridisation the behaviour of the rye heterochromatin knobs and the telomeres in meiocytes from these hybrids in the presence and in the absence of *Ph2* (with *Ph1* present in both situations) either just prior to or during early stages of meiosis. The anthers used in this study came from more than 50 F_1 hybrids either in the presence or in the absence of *Ph2* with nearly 250 meiocytes being analysed.

In the presence of *Ph2*, the heterochromatin knobs on each rye chromosome remain as tight foci, showing that the chromatin conformation in these chromosome regions do not change either before or during the telomere bouquet in the meiocytes examined (Fig. 1a–e). However, the rye heterochromatin knobs are no longer immediately adjacent to the telomeres in the telomere bouquet, in contrast to their location prior to meiosis. Thus, the DNA regions between the knobs and the telomeres have undergone an extensive elongation. It implies that the conformational change is initiated, but that the chromatin change does not propagate through the heterochromatin knobs, as they remain tightly focussed. No differences are observed in the heterochromatin of hybrids with or without *Ph2* (but with *Ph1* present). Rye heterochromatin knobs are tight in appearance in the absence of *Ph2* either before or during the telomere cluster stage (Fig. 1f–j). In contrast, in meiocytes examined from the hybrid lacking *Ph1* (but with *Ph2* present), the knobs are observed as groups of elongated structures as the telomeres cluster to form the bouquet (Fig. 1k–o). At the telomere bouquet stage all the heterochromatin knobs are found as a single elongated structure, implying interactions with one another (Fig. 1m–o). Thus, the conformational change was not limited to the regions close to the telomeres, but also extended through the rye heterochromatin knobs in hybrids in the absence of *Ph1*. At present, it is unclear in the presence of *Ph1* whether the failure of the rye telomeric heterochromatin to undergo conformational changes stops subsequent changes in conformation in regions proximal to the heterochromatin. The results are

summarised diagrammatically in Fig. 2. The effect on the telomeric heterochromatin knobs could help to explain the basis for homoeologous pairing in the absence of *Ph1*. In the presence of *Ph1*, regions which are highly homologous (such as heterochromatin) do not undergo the conformational change and may therefore be excluded from the pairing process. In contrast, in the absence of *Ph1*, the chromatin changes do occur in these highly homologous regions, and they can engage in multiple associations between homologous, homoeologous and non-homologous chromosomes. Moreover, the whole chromosome may be slightly more condensed in the presence of *Ph1* when pairing than in its absence, which might further exclude highly homologous repeats from the pairing process. It has also been observed in the absence of *Ph1* that the elongation of chromatin associated with pairing can occur in meiocytes which have not fully formed the telomere bouquet (Prieto et al. 2004). In contrast, the conformational changes associated with pairing are only observed at the telomere bouquet formation in the presence of *Ph1* (Prieto et al. 2004). This implies that pairing is being initiated earlier in the absence of the *Ph1* as the telomeres are beginning to cluster. One explanation for this is that the stringency at which interactions between telomere regions can trigger the pairing process is reduced in the absence of *Ph1*. Thus, there will be a higher chance of an interaction occurring between telomere regions as they cluster in the absence of *Ph1*, which triggers the pairing process. An earlier initiation of pairing in the absence of *Ph1* implies that the chromosomes will pair in slightly different overall condensation states in the presence and absence of *Ph1*. In the absence of *Ph1*, the chromosomes will be less condensed than in the presence of *Ph1*. This is consistent with the proposal by Maestra et al. (2002) that although there is no apparent difference in the overall structure of chromosomes in the presence and absence of *Ph1* prior to meiosis, the chromosomes may be less condensed when pairing during early meiosis in the absence of *Ph1* than its presence. The more 'open chromatin' (less condensed) of the entire chromosome at the time of pairing combined with the ability of highly homologous heterochromatin to extensively elongate as the chromosomes pair may explain the basis of marked increase in homoeologous and non-homologous interactions in the absence of *Ph1*. However, it is difficult to provide clear-cut data for this proposal at the telomere bouquet, as visualising the behaviour of whole chromosome additions is difficult to interpret at this stage while reducing the complexity by visualising single arm additions (telosomes) has additional complications. The two telomeres of the telosome join the telomere bouquet, bringing centromere of the telosome into the bouquet. Thus, the telosome is looped back at this stage and in

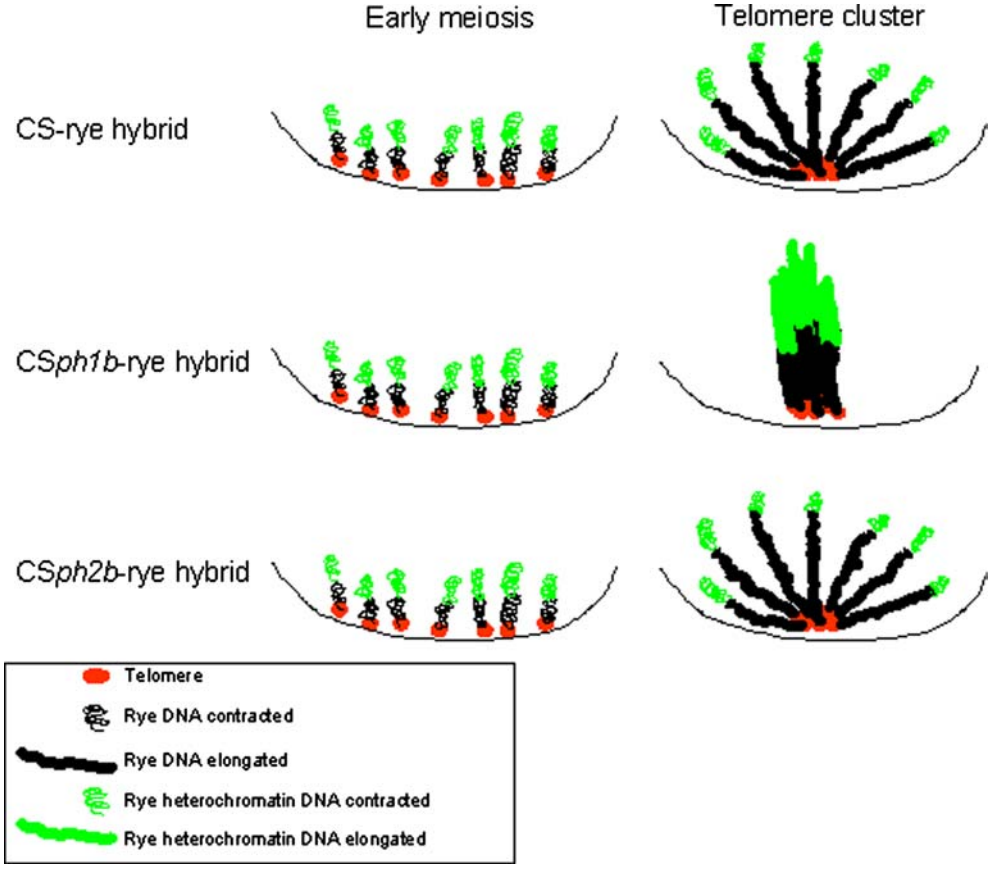

Fig. 2 Diagram of the chromosome associations at the telomere end at early meiosis and during the telomere bouquet in wheat–rye hybrids with *Ph1* and *Ph2* loci, *Ph1* mutants and *Ph2* mutants, respectively. Wheat chromosomes are not shown. At the telomere bouquet the signal to condense the DNA is triggered from the telomeres along the chromosome, but it is only propagated to the rye heterochromatin knobs in the hybrids lacking *Ph1* but not *Ph2*

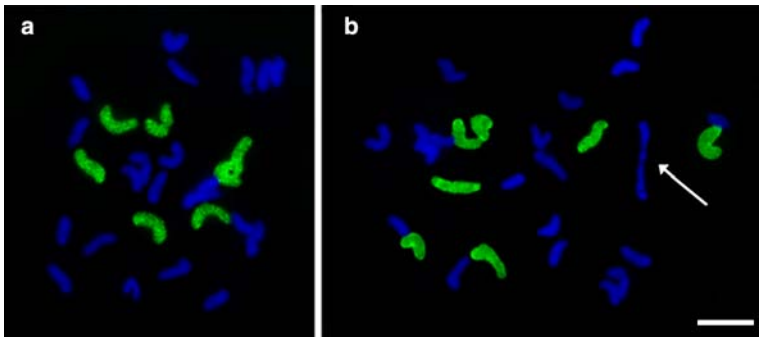

Fig. 3 In situ hybridisation in chromosome spreads in meiotic metaphase I. Rye chromosomes are shown in *green* and wheat chromosomes are in *blue*. **a** Euploid wheat–rye hybrid. **b** Wheat–rye hybrid in the absence of *Ph2*. *Scale bar* = 10 μm

Table 1 Analysis of the chromosome association in wheat–rye hybrids in the presence and in the absence of *Ph1* and *Ph2* at metaphase I

	Genotype		
	CS[a] euploid × rye	CS *ph2b* × rye	CS *ph1b* × rye
Number of meiocytes analysed	500	92	100
Chromosome number	28	28	28
Wheat-wheat			
Arm association	242	155	714
Arm association/cell	0.48	1.68	7.14
Wheat-rye			
Arm association	39	7	59
Arm association/cell	0.08	0.08	0.59
Rye-rye			
Arm association	8	4	5
Arm association/cell	0.02	0.04	0.05
Total			
Arm association	547	166	778
Arm association/cell	0.58	1.80	7.78

[a]*CS* Cv. Chinese Spring

some cases can stretch right around the nucleus (Martinez-Perez et al. 1999; Carlton and Cande 2002; Maestra et al. 2002).

If the rye heterochromatin knobs interact with each other in the absence of *Ph1* in the wheat–rye hybrid, it raises the question of whether they also interact with each other during meiosis in diploid rye which does not apparently carry a *Ph1* locus. Visualising by in situ hybridisation, the rye heterochromatin knobs and the telomeres in 75 diploid rye meiocytes from 15 plants, we observe that the rye heterochromatin knobs are tight in appearance in premeiosis and during meiosis before the telomere bouquet in the meiocytes examined (Fig. 1p, q). When the meiosis reaches the telomere bouquet stage, the rye heterochromatin knobs are seen as diffuse elongated structures (Fig. 1r–t). Rye heterochromatin knobs do not associate in a single structure at the telomere bouquet stage in diploid rye, in contrast to the behaviour of the rye chromosomes in the wheat–rye hybrid in the absence of *Ph1* but in the presence of *Ph2*. This would suggest that there is/are factor(s) which reduce inter chromosome pairing of heterochromatin in rye, which are titrated out in the wheat–rye hybrid in the absence of *Ph1*. However, *Ph1* compensates for this factor or factors in the hybrid.

Chromosome associations in the hybrids at metaphase I

Chromosome associations in wheat–rye hybrids have already been described in the absence of the *Ph1* locus (Naranjo et al. 1996, 1988; Wang and Holm 1988). The lack of conformational change in the rye heterochromatin in the absence of *Ph2* (but in the presence of *Ph1*) implies that there could be little wheat–rye chromosome pairing observed later in metaphase I. To assess this, we have distinguished rye chromosomes from wheat chromosomes in wheat–rye hybrids by GISH using total rye genomic DNA as probe on metaphase I spreads (Fig. 3). Using GISH, we are able to study the relative frequency of wheat–wheat and wheat–rye homoeologous pairs in these wheat–rye hybrids in the presence and in the absence of *Ph1* and *Ph2*. In the presence of both *Ph2* and *Ph1* we commonly see all the 28 chromosomes as univalents in meiocytes in metaphase I (Fig. 3a). In the hybrids lacking *Ph2* (but with *Ph1* present), a small increase in the chromosome association is observed (from 0.58 to 1.8 arm association/cell, Table 1). Chromosome associations are usually rod bivalents (Fig. 3b). No multivalent associations are detected at metaphase I in this hybrid. The frequency of the chromosome association and the genome involved in these chromosome associations is shown in Table 1. In the absence of *Ph2* (but presence of *Ph1*), there are a higher number of chromosome associations between wheat chromosomes (1.68 arm association/cell) than between wheat–rye and rye–rye chromosomes which are practically zero (0.08 and 0.04 arm association/cell, respectively).

Chromosome associations in the wheat–rye hybrids lacking *Ph2* (but with *Ph1* present) have also been compared with chromosome associations in wheat–rye hybrids lacking *Ph1* (but with *Ph2* present). The number of chromosome associations is lower in the absence of *Ph2* (1.80 arm association in total/cell) than observed in hybrids lacking *Ph1* (7.78 arm association in total/cell). A higher number of wheat–wheat chromosome associations is observed in the absence of *Ph1* (7.14 arm association/cell), and it is higher than the chromosome

association between wheat–rye chromosomes (0.59 arm association/cell, Table 1). Rye–rye associations are almost zero in the absence of *Ph1* (0.05 arm association/cell). These results suggest that the *Ph2* locus, as well as *Ph1*, affects pairing of chromosomes at metaphase I. However, the *Ph2* effect is not as dramatic as *Ph1*. Although in the wheat–rye hybrid lacking *Ph2*, there are some chromosome associations between wheat chromosomes, and it is very unusual to find chromosome pairing between wheat–rye and rye–rye chromosomes (Table 1). This result implies that the *Ph2* locus is not involved directly in homologue recognition, and it supports the results reported by other authors suggesting a later function for *Ph2* during meiosis, e.g., affecting synapsis or synaptic progression (Ji and Langridge 1994; Martinez et al. 2001). Although there is some level of chromosome pairing in the absence of *Ph2* involving wheat chromosomes, the lack of the propagation in the chromatin change through the rye heterochromatin regions in the absence of *Ph2* correlates with a lack of wheat chromosomes pairing with those of rye, and similarly, rye chromosomes with rye at metaphase I.

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