pairwise comparisons from the second study, only 11% showed $|D'| > 0.5$. This suggests that admixture probably did not generate the strong signal of LD at long physical distances seen in Utah.

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The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association

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The correct pairing and segregation of chromosomes during meiosis is essential for genetic stability and subsequent fertility. This is more difficult to achieve in polyploid species, such as wheat, because they possess more than one diploid set of similar chromosomes. In wheat, the *Ph1* locus ensures correct homologue pairing and recombination[1]. Although clustering of telomeres into a bouquet early in meiosis has been suggested to facilitate homologue pairing[2,3], centromeres associate in pairs in polyploid cereals early during floral development[4]. We can now extend this observation to root development. Here we show that the *Ph1* locus acts both meiotically and somatically by reducing non-homologous centromere associations. This has the effect of promoting true homologous association when centromeres are induced to associate. In fact, non-homologously associated centromeres separate at the beginning of meiosis in the presence, but not the absence, of *Ph1*. This permits the correction of homologue association during the telomere-bouquet stage in meiosis. We conclude that the *Ph1* locus is not responsible for the induction of centromere association, but rather for its specificity.

We previously showed that centromeres associate in pairs before meiosis in polyploid cereals, but not until the beginning of meiosis in their diploid progenitors[4]. Using fluorescence *in situ* hybridization on intact root sections, we now report that centromeres also associate in pairs in developing xylem vessel cells of bread wheat (AABBDD, $2n = 6x = 42$) but not in those of its diploid progenitors (Fig. 1b, e, and Table 1). Moreover, we show that during this developmental process the chromosomes endoreplicate, becoming polytene. This is indicated by the substantial increase in size of the interphase chromosomes (and nucleus), as compared with the surrounding tissues (Fig. 1a, d, f, g).

The level of centromere association in xylem vessel cells of wheat is unaffected by the presence of *Ph1*, as in floral development[5] (Fig. 1e, h). Thus, neither endoreplication nor the *Ph1* locus can induce centromere association. Although centromeres associate in the xylem vessel cells in the presence and absence of *Ph1*, they are not associated in other root tissues (Fig. 1c). Polyploidy is therefore necessary but not sufficient to induce centromere association—a specific developmental context is also required, as in meiosis, floral development or xylem vessel development.

We have assessed homologue association in these vessel cells by labelling specific pairs of rye chromosomes in wheat–rye addition lines. These rye homologues associate at a high level through their centromeres during vessel development in the presence of *Ph1* (25/25

Table 1 Statistics of the number of centromeres

		Ph1+	*Ph1–*	*t*-test
Wheat–rye	Premeiosis	19.5 (2.7)	16.3 (2.9)	$P < 0.001$
	Telomere bouquet	23.5 (1.5)	13.7 (2.1)	$P < 0.001$
	Xylem vessel	20 (1.7)	16.3 (1.8)	$P < 0.001$
	Non-polytene root	24.9 (1.2)	25.2 (1.3)	$P = 0.6$
Wheat	Xylem vessel	21.7 (2.1)	21.5 (1.8)	$P = 0.798$
T. monococcum	Xylem vessel	12.56 (0.8)		

The s.d. is given in parentheses. Student's *t*-test was used to test the null hypothesis that the two means in the presence and absence of *Ph1* are the same. The null hypothesis can be discounted in all comparisons except the wheat xylem and the wheat–rye non-polytene root. All centromere sites were counted on the original three-dimensional confocal stacks.

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cells at the last stage of development examined) but not in its absence (22/25 cells scored did not show association) (Fig. 1f, g, i, j). In both the presence and absence of *Ph1*, as we have shown above, the number of centromere sites seen indicates centromere pairing (Fig. 1e, h). This suggests that in the presence of *Ph1* most centromeres are homologously associated. In other root cells, neither the centromeres nor the homologues are associated (Fig. 1c, d). In xylem cells in which the homologues are associated at their centromeres, associations also occur at interstital sites in 67% of the cells. But no interstitial associations are observed unless the homologue centromeres are also associated, indicating that centromere association may precede interstitial association.

Homologous chromosomes also associate somatically in many different *Drosophila* tissues[6,7], and numerous studies have shown that this association induces an extra level of epigenetic regulation through trans-sensing or transvection[6–8]. In common with *Drosophila*, wheat chromosomes are organized in a Rabl configuration with the centromeres grouped at one pole of the nucleus and the telomeres at the opposite pole[9,10]. In this configuration, association of homologous centromeres will facilitate the interaction of other chromosomal regions in the large wheat nucleus. Thus, the presence of *Ph1* in wheat has the potential to induce epigenetic control, in the same manner as in *Drosophila*. This level of epigenetic regulation is not available to diploid cereals, and may be a factor in the widespread occurrence of polyploidy in the plant kingdom.

The effect of *Ph1* in the xylem vessel cells is analogous to the premeiotic association observed during floral development[11]. Thus, in those somatic tissues in which the centromeres associate, *Ph1* would promote the specificity of the association to true homologues, and this specificity could be achieved by reducing non-homologous centromere associations. To test this hypothesis, we determined the centromere behaviour during meiosis, floral development and xylem vessel development in hybrid lines possessing only non-homologous chromosomes, in both the presence and

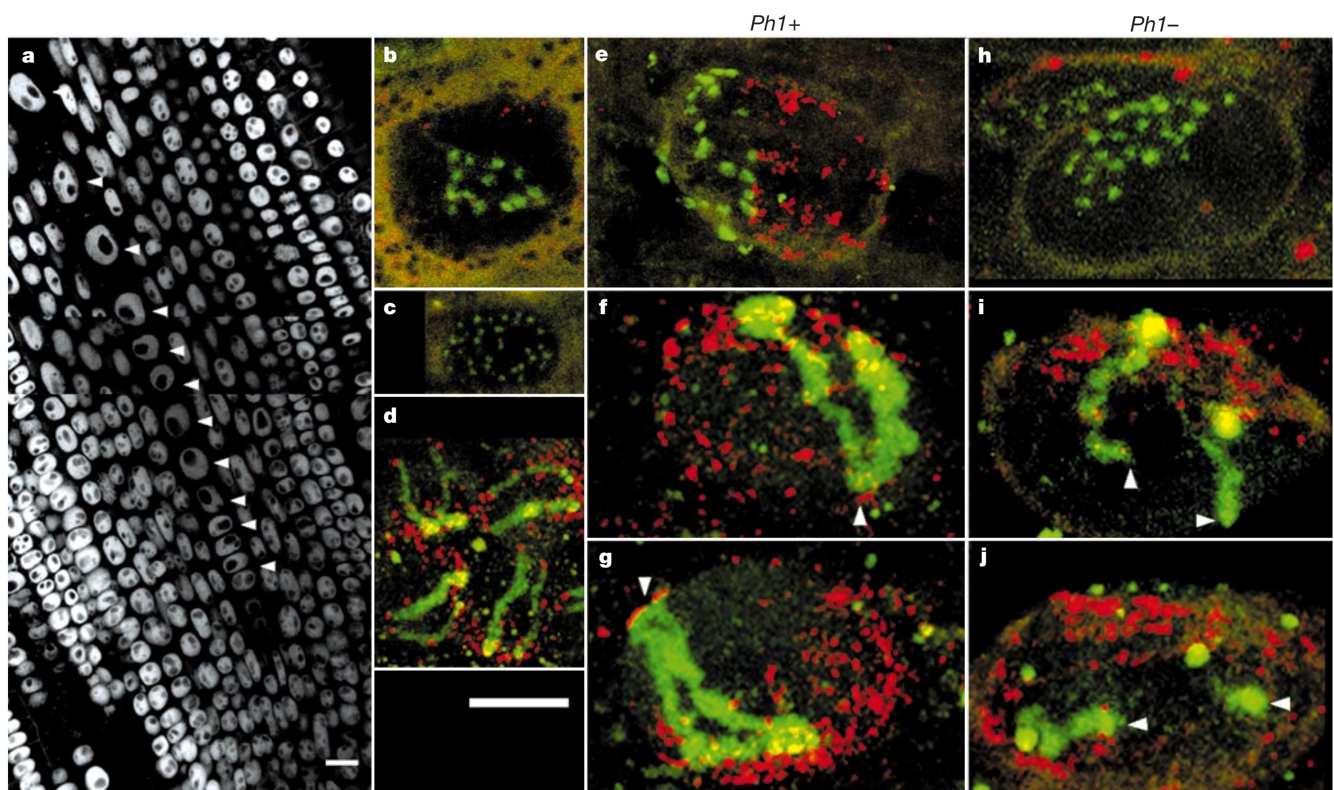

Figure 1 Centromere and homologue behaviour during root development. **a**, Root section labelled with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride). Arrowheads indicate the column of developing xylem vessel cells. **b**, Polytene vessel nucleus in *T. monococcum*, $2n = 14$, showing 13 centromeric sites (green). **c**, Non-polytene root nucleus in hexaploid wheat, $2n = 42$, showing 35 centromeric sites (green). **d**, Non-polytene root nuclei in hexaploid wheat, showing the homologues unassociated (green). **e**, **h**, Polytene vessel nuclei in hexaploid wheat, showing about 21 centromere sites (green) in both the presence (**e**) and absence (**h**) of *Ph1*. **f**, **g**, Polytene vessel nuclei in hexaploid wheat, showing homologues (green) associated through their centromeres (arrowheads). **i**, **j**, Polytene vessel nuclei (*Ph1* mutant), showing homologues (green) unassociated; centromeres are indicated by arrowheads. Telomeres are red in **b**–**j**, which are all at same magnification. Scale bars, 10 μm.

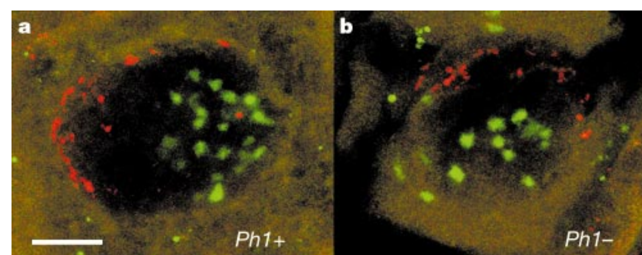

Figure 2 Centromeres (green) and telomeres (red) in root xylem vessel cells of wheat–rye hybrids. **a**, Presence of *Ph1*, showing 20 centromere sites. **b**, Absence of *Ph1*, showing 14 centromere sites. Scale bar, 10 μm.

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absence of the *Ph1* locus. These lines are wheat–rye hybrids, and contain a haploid set of 21 wheat chromosomes and a haploid set of 7 rye chromosomes, making 28 chromosomes in total.

In most root tissues, centromeres are not associated (Table 1). During xylem vessel development in the absence of the *Ph1* locus, however, the centromeres associate, reducing to a mean of 16 centromere sites, whereas in the presence of the *Ph1* locus, a mean of 20 sites is seen (Table 1, and Fig. 2a, b). Similarly, during floral development, a mean of 16 paired centromere sites is formed in the absence of the *Ph1* locus and 19 in its presence (Table 1, and Fig. 3). Thus, in these somatic cells, *Ph1* can reduce but not eliminate non-homologous association. The seven rye chromosomes are visualized as up to seven domains in both the presence and absence of *Ph1*, showing that there is no physical separation of the genomes and that wheat and rye centromeres associate (Fig. 3c, h).

The telomere bouquet is formed in these hybrids at the onset of meiosis in both the presence and absence of *Ph1* (Fig. 3k, l). At the telomere-bouquet stage, in the absence of the *Ph1* locus the centromeres reduce to a mean of 14 sites, showing complete but non-homologous pairing. In the presence of *Ph1* the mean number of sites increases to 24 (Table 1, and Fig. 3l). Previous studies have indicated that in these lines and in other hybrids in which only non-homologous chromosomes are present, the telomere and interstitial regions can synapse to the same degree in both the presence and absence of *Ph1* (refs 12, 13).

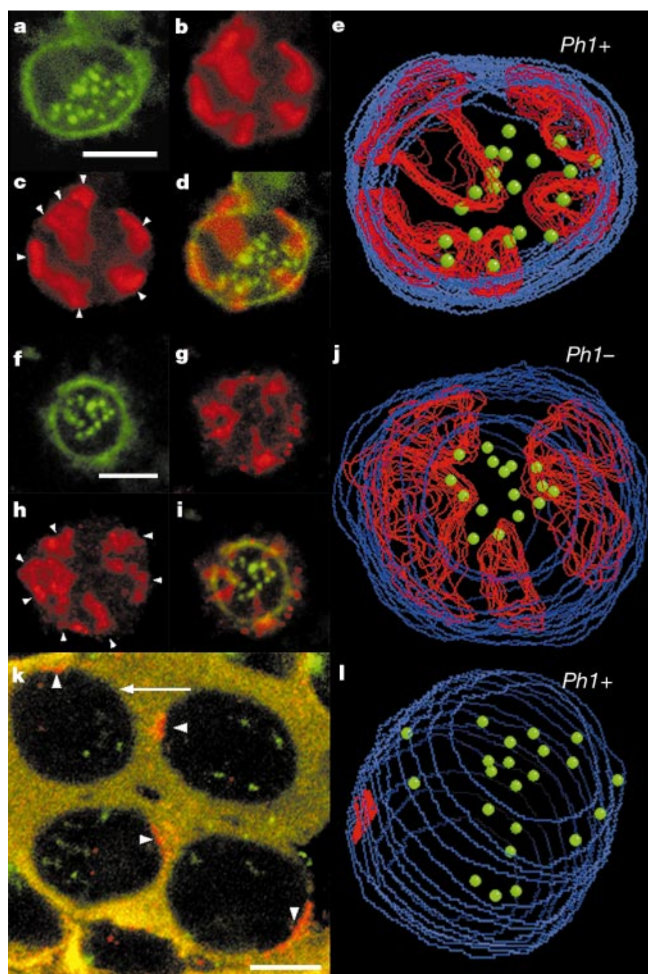

Figure 3 Premeiotic and meiotic behaviour of centromeres in wheat–rye hybrids. **a**–**e**, Premeiotic pollen mother cell (*Ph1* present). **a**, Single confocal section showing 20 centromere sites. **b**, equivalent section to **a** showing five rye chromosome domains. **c**, Confocal section showing seven rye chromosomal domains (arrowheads). **d**, Overlay of **a** and **b**. **e**, Three-dimensional reconstruction of the nucleus shown in **a**–**d**. **f**–**j**, Premeiotic pollen mother cell (*Ph1* absent). **f**, Single confocal section showing 15 centromere sites out of the 16 present in the entire three-dimensional stack. **g**, Equivalent section to **f**, showing four rye chromosome domains. **h**, Confocal section showing seven rye chromosome domains (arrowheads). **i**, Overlay of **f** and **g**. **j**, Three-dimensional model of the nucleus shown in **f**–**i**. **k**, Confocal section showing four meiocytes possessing telomere bouquets (red, arrowheads) in the presence of *Ph1*, centromeres shown in green. **l**, Three-dimensional model of the nucleus arrowed in **k**, showing 22 centromere sites. Scale bars, 10 μm.

This confirms that, at the time of synapsis, the *Ph1* locus increases the specificity of interaction between true homologues not at these sites, but rather at the centromeres, as we have shown. Non-homologous centromere associations are mostly eliminated at the telomere-bouquet stage of meiosis in wheat–rye hybrids in the presence of *Ph1*, but not in its absence. Because higher levels of recombination occur in the absence of *Ph1* than in its presence[14,15], we suggest that the maintenance of centromere association is important for recombination to occur at all in these hybrids.

We can now explain the previous studies on chromosome synapsis in the presence and absence of *Ph1* (refs 16–18). In tetraploid and hexaploid wheat, the centromeres associate during floral development whether *Ph1* is present or not; however, a higher proportion of paired sites will be homologous in the presence of *Ph1*. This premeiotic association of centromeres provides an initial sorting of the chromosomes. The sites remain paired through floral development to the telomere-bouquet stage of meiosis. At the telomere-bouquet stage, any non-homologous centromere associations separate in the presence of *Ph1*, enabling a further sorting of these chromosomes.

Studies of synapsis in the presence of *Ph1* indicate that most chromosomes synapse as bivalents, although some multivalents are formed early on (involving on average a total of six chromosomes)[16] . As the synapsis of chromosomes progresses the number of multivalents declines, suggesting a correction of pairing, in agreement with our observations. In the absence of *Ph1*, non-homologous associations made before meiosis are maintained. Thus, telomere-led synapsis results in a high level of multivalents (involving a total of up to 17 chromosomes), which remain uncorrected[17,18]. As a result, at least 14 chromosomes are incorrectly paired at metaphase I (ref. 19).

Deletion of the *Ph1* locus has two major phenotypic effects: first, chromosome pairing is disrupted, which leads to the synapsis of non-homologous chromosomes; second, non-homologous recombination is induced[20]. The effect on chromosome pairing has been proposed to occur either premeiotically[21] or during synapsis[17,18]. Our data suggest that the effect of *Ph1* on premeiotic alignment and correction during synapsis can be explained by a mechanism involving centromere association. At meiosis, any pre-existing incorrect associations will disrupt chromosome pairing unless separated and re-paired correctly.

This might be achieved either by the separation of all centromere associations or by a more targeted separation solely of incorrect associations. Our studies suggest that the latter occurs. Linking two non-homologous chromosome arms through a single centromere is not sufficient to induce recombination between them in the presence of *Ph1*, showing that the *Ph1* recombination phenotype cannot be simply explained by centromere association[22]. This phenotypic effect may be the indirect consequence of globally disrupting chromosome pairing, which results in the induction of non-homologous recombination[23]. As yet, however, we cannot exclude the possibility that the *Ph1* locus contains more than one gene with individual phenotypic effects.

The mechanism that we have shown for the effect of *Ph1* on homologue pairing depends on the presence of chromosome-specific centromeres. It has been previously shown in cereals that centromeres can evolve rapidly leading to chromosome-specific organization[24,25]. Our results reveal a function that exploits the specificity of these macromolecular structures. □

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Methods

Plant material

The roots and anthers used in this study came from hexaploid wheat (*Triticum aestivum*, cv. Chinese spring); a Chinese spring mutant (*ph1b*) lacking the *Ph1* locus; a *Ph1* mutant line carrying 1RS chromosome arm substitution for 1BS wheat chromosome arm; a hexaploid wheat addition line carrying an extra pair of 1RL (*Secale cereale*) telocentric chromosomes; Chinese spring/*Secale cereale* cv. Petkus F_1 hybrids with and without the *Ph1* locus; and the diploid wheat progenitor, *Triticum monococcum*. All the lines lacking *Ph1* used here carried the *ph1b* deficiency.

Sectioning and fluorescence *in situ* hybridization

The centromere (CCS1) and telomere (TTTAGGG repeat) probes used, tissue sectioning and specimen preparation, *in situ* hybridization and probe preparation, and the labelling of the two homologous rye arms (see Fig. 1f, g, i, j) have all been described[4,5].

Fluorescence microscopy and image processing

We collected confocal optical section stacks using a Leica TCS SP or SP2 confocal microscope as described[5]. Confocal images were processed by Adobe Photoshop and the public domain program NIH Image (W. Rasband). The models were created using Object-image 1.62n7 (a modified version of NIH Image; N. O. Visscher) and Rotater 3.5.

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Phagocytosis and clearance of apoptotic cells is mediated by MER

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Apoptosis is fundamental to the development and maintenance of animal tissues and the immune system[1]. Rapid clearance of apoptotic cells by macrophages is important to inhibit inflammation and autoimmune responses against intracellular antigens[2–4]. Here we report a new function for Mer, a member of the Axl/Mer/Tyro3 receptor tyrosine kinase family. *mer*^{kd} mice with a cytoplasmic truncation of Mer had macrophages deficient in the clearance of apoptotic thymocytes. This was corrected in chimaeric mice reconstituted with bone marrow from wild-type animals. Primary macrophages isolated from *mer*^{kd} mice showed that the phagocytic deficiency was restricted to apoptotic cells and was independent of Fc receptor-mediated phagocytosis or ingestion of other particles. The inability to clear apoptotic cells adequately may be linked to an increased number of nuclear autoantibodies in *mer*^{kd} mice. Thus, the Mer receptor tyrosine kinase seems to be critical for the engulfment and efficient clearance of apoptotic cells. This has implications for inflammation and autoimmune diseases such as systemic lupus erythematosus.

Several receptors have been identified that participate in the recognition and phagocytosis of apoptotic cells. These include the scavenger receptor class A, CD36 and the phosphatidylserine receptor, which bind phosphatidylserine on the surface of apoptotic cells; the vitronectin receptor ($\alpha v\beta 3$ integrin) in cooperation with CD36, which use thrombospondin as a molecular bridge to recognize apoptotic cells; and CD14, the lipopolysaccharide (LPS) receptor, and complement, which recognize an unknown ligand on apoptotic cells[5–12]. The family of Axl/Mer/Tyro3 receptor tyrosine kinases have several functions in different tissues[13–17]. Previously, we showed that a functional knockout mouse with a truncated cytoplasmic tail of the Mer receptor tyrosine kinase (*mer*^{kd}) was hypersensitive to high doses of LPS, and suggested that Mer may

Table 1 Flow cytometry analysis of the percentage of dead cells present in the thymus of dexamethasone-treated mice

Group	Mean (% dead cells)	Fold increase compared with WT
Wild type	4.40	1
mer^{kd}	32.38	7.4
Saline controls	1.05	–
Bone marrow chimaeras*		
WT into WT	1.10	1
mer^{kd} into *mer*^{kd}	51.50	46.8
WT into *mer*^{kd}	3.15	2.9
mer^{kd} into WT	0.98	1
Saline controls	1.15	–

Each experimental group included up to six mice per analysis. WT, wild type.
* The data is representative of two independent experiments.

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