

Chromosomes form into seven groups in hexaploid and tetraploid wheat as a prelude to meiosis

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Summary

Hexaploid wheat possesses 42 chromosomes derived from its three ancestral genomes. The 21 pairs of chromosomes can be further divided into seven groups of six chromosomes (one chromosome pair being derived from each of the three ancestral genomes), based on the similarity of their gene order. Previous studies have revealed that, during anther development, the chromosomes associate in 21 pairs via their centromeres. The present study reveals that, as a prelude to meiosis, these 21 chromosome pairs in hexaploid (and tetraploid) wheat associate via the centromeres into seven groups as the telomeres begin to cluster. This results in the association of multiple chromosomes, which then need to be resolved as meiosis progresses. The formation of the seven chromosome clusters now explains the occasional occurrence of remnants of multiple associations, which have been reported at later stages of meiosis in hexaploid (and tetraploid) wheat. Importantly, the chromosomes have the opportunity to be resorted via these multiple interactions. As meiosis progresses, such interactions are resolved through the action of loci such as *Ph1*, leaving chromosomes as homologous pairs.

Keywords: centromeres, telomeres, *Ph1*, wheat, polyploidy, pairing.

Introduction

At the onset of meiosis in diploid species (e.g. yeast (*Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*), mammals, maize and rye), the telomeres cluster in a group termed the bouquet on the nuclear membrane while the centromeres remain unassociated (Bass *et al.*, 1997; Chikashige *et al.*, 1997; Mikhailova *et al.*, 2001; Niwa *et al.*, 2000; Trelles-Sticken *et al.*, 1999). Homologous (or identical) chromosomes are then intimately paired (synapsed) at sites along their length in a process initiated close to the telomere regions (Gillies, 1985). It has been proposed that the telomere bouquet facilitates the initial sorting of chromosomes into homologous pairs (Rockmill and Roeder, 1998; Trelles-Sticken *et al.*, 1999). However, many species are allopolyploids, including more than 70% of plants and many amphibians. The general effects of polyploidy have been reviewed recently by Pikaard (2001) and Osborn *et al.* (2003). Allopolyploids possess two or more sets of related chromosomes as a result of sexual hybridisation between species containing related, but not completely homologous, genomes. These related chromosomes are termed homoeologues. Thus, homoeologous

chromosomes have a similar linear sequence of genes but a different repetitive content, while homologues have the same linear sequence of genes and repetitive content. Sexual hybridisation between an allopolyploid and a wild relative generally produces an interspecific hybrid containing a haploid set of the allopolyploid and wild relative chromosomes (i.e. only homoeologues). Although chromosomes in such hybrids do not have homologous partners with which to pair, and despite the difference in repetitive and, to some extent, gene content between the homoeologues, there is often a level of pairing and recombination between the allopolyploid and wild relative chromosomes. This then raises the question of how pairing and subsequent recombination between the homoeologues is actually controlled within the allopolyploid itself. This is important because for allopolyploids to be stable and fully fertile, they must behave as diploids at meiosis so that only homologues pair. Clearly, the overall efficiency and accuracy of the mechanisms used to achieve this pairing have a profound effect on the fertility of every allopolyploid.

Our previous studies of chromosome pairing in hexaploid (*Triticum aestivum*) and tetraploid (*T. durum*) wheat (allopolyploids) and their polyploid relatives have shown that chromosomes associate in pairs prior to meiosis via their centromeres during anther development (Maestra *et al.*, 2002; Martinez-Perez *et al.*, 1999, 2000). This contrasts with the observations made on chromosome pairing in some of the diploid progenitors of wheat, where chromosomes first start associating via their telomeres and centromeres during meiosis (Martinez-Perez *et al.*, 2000). Pre-meiotic centromere association can be induced in an artificial polyploid made from two diploids, which do not show pre-meiotic centromere association themselves (Martinez-Perez *et al.*, 2001). However, in the case of wheat, it remains unclear whether the pre-meiotic centromere association was induced on polyploidisation, or already existed in one of its progenitors. The exact progenitor of one of hexaploid and tetraploid wheat genomes is currently not known, so it cannot be ruled out that this diploid progenitor already exhibited pre-meiotic centromere pairing.

Furthermore, by visualising homologous pairs of chromosomes, it has been shown that between 70 and 80% of pre-meiotic centromere associations in hexaploid wheat are initially non-homologous interactions (Aragon-Alcaide *et al.*, 1997; Maestra *et al.*, 2002). In fact, in interspecific hybrids of hexaploid wheat and rye (which carry 28 chromosomes – a haploid set of 21 from wheat and a haploid set of 7 from rye, where there are no homologous chromosomes), the centromeres still associate pre-meiotically, indicating that either homoeologous or non-homologous pairing can take place at this stage (Martinez-Perez *et al.*, 2001). By the start of the telomere bouquet formation during early meiosis (when the centromeres are still polarised with respect to the telomeres), there is a marked increase in the level of homologue association, resulting in 75 and 90% of homologues being associated via their centromeres (Aragon-Alcaide *et al.*, 1997; Schwarzacher, 1997). At later stages, when the telomere bouquet begins to de-cluster, the centromeres are observed as paired (Martinez-Perez *et al.*, 1999). We assumed this implied that centromeres are maintained in pairs throughout the process. However, it is also clear that incorrect centromere associations can be made pre-meiotically (Maestra *et al.*, 2002). Therefore, the centromere associations need to be resorted at some stage. Hexaploid and tetraploid wheat possess a well-characterised gene locus *Ph1*, which controls the specificity of pairing at meiosis, probably by reducing the stability of non-homologous/homoeologous pairs (Riley and Chapman, 1958; reviewed Moore, 2002). We showed that *Ph1* cannot prevent non-homologous/homoeologous chromosomes from associating via their centromeres when homologous chromosomes are absent in the hexaploid wheat-rye hybrid (Martinez-Perez *et al.*, 2001). However, during the late stages of the telomere bouquet formation in this

hybrid, the centromeres are separated in the presence of *Ph1* but are paired in its absence (Martinez-Perez *et al.*, 2001). Thus, this locus does affect the ability to resolve incorrect pairing at centromere sites and at other sites along the chromosome arms during meiosis (Holm, 1988; Martinez *et al.*, 2001; Martinez-Perez *et al.*, 2001). All these studies suggest that there is a degree of incorrect pre-meiotic associations, which must be corrected. It raises the question of how is this achieved in those species in which pre-meiotic centromere association occurs. Do the centromeres simply separate when the telomere bouquet is being formed to enable the chromosome pairs to be corrected or does another mechanism occur? The present study shows that complex multiple associations between centromere pairs occur in wheat as the telomere bouquet is being formed. We suggest that this is part of the correction mechanism for these associations.

Results and discussion

Centromeres reduce to seven clusters

In the present study, three-dimensional confocal data was examined from anther sections in which centromeres and telomeres were visualised by fluorescence *in situ* hybridisation (FISH). From these data sets, we identified those meiocytes where the telomere bouquet was in the process of being formed (Figure 1). This was defined from our previous studies as being when the telomeres had partially associated but not yet formed a single tight cluster. The centromeres in these meiocytes were either located at the opposite nuclear pole with respect to the telomeres or at least still located at the nuclear periphery (Martinez-Perez *et al.*, 1999). Previous studies using squashed meiotic preparations have reported that this stage is difficult to analyse because the centromeres have a diffuse structure (Maestra *et al.*, 2002). We reasoned that use of intact anther preparations may better preserve such structures, allowing their analysis at this stage. Although we have collected very large data sets from anther sections at pre-meiotic and meiotic stages, relatively few meiocytes were identified at the stage of the initiation of telomere clustering. Previous studies on telomere bouquet formation in maize had analysed 9 and 11 meiocytes at this stage (Bass *et al.*, 1997). It is possible, but not yet proven, that the stage from initiating the clustering of the telomeres to its full formation occurs fairly rapidly in cereals.

Surprisingly, our images of these meiocytes show that, just prior to the formation of a tight telomere bouquet, the number of centromere sites reduces to approximately seven diffuse groups, which are located at the nuclear periphery on the pole opposite to the telomeres. This is true for both wild-type hexaploid wheat and the *Ph1* mutant

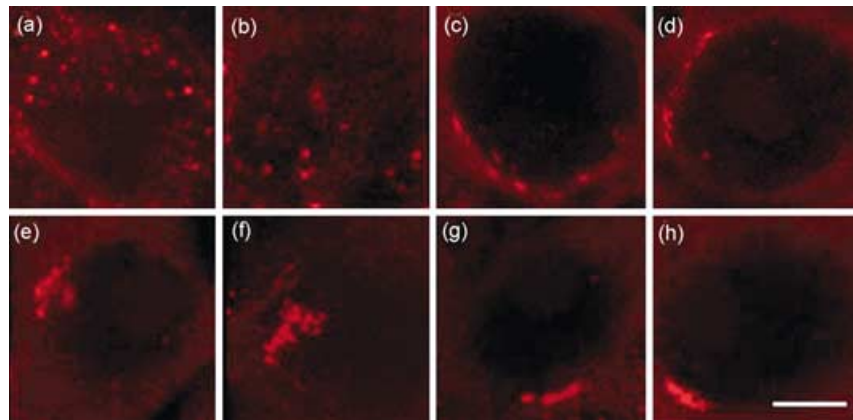


Figure 1. Telomere bouquet formation during meiosis.

All images are projections from confocal sections from hexaploid wheat, in which the telomeres have been labelled by *in situ* hybridisation. The chosen images show meiocytes before, during and after completion of the telomere bouquet formation. The images are sequentially ordered to reflect the stages of telomere bouquet formation.

(a, b) Pre-meiotic meiocytes in which the telomere bouquet has yet to start forming. The telomeres are spread around one pole of the nucleus. The telomeres in (b) are spread around one pole but have started to reduce in number.

(c–f) The telomeres have started to form a bouquet. The telomeres cluster in small groups, and then these groups form large ones. In the present study, meiocytes at this stage were selected for further analysis of their centromere behaviour.

(g, h) Meiocytes exhibiting a fully formed telomere bouquet. Analysis of meiocytes at this stage has been previously reported in Martinez-Perez *et al.* (1999, 2000). The centromeres for meiocytes (d) and (f) are shown in Figures 2(b) and 4, respectively. Bar, 10 μ m.

(Figures 2a–d and 3a; Table 1). The hexaploid wheat genome is derived from three ancestral genomes (A, B and D). Its 21 pairs of chromosomes can be divided into seven homoeologous groups of six chromosomes (two homologues from each of the ancestral genomes). The gene order

along the chromosomes of each homoeologous group is similar. Studies have reported a marked increase in the level of homologue association via the centromeres at this stage, with up to 90% of homologues associating at their centromeres (Aragon-Alcaide *et al.*, 1997; Schwarzacher,

Figure 3. 3D rotations of meiocytes exhibiting centromere clustering.

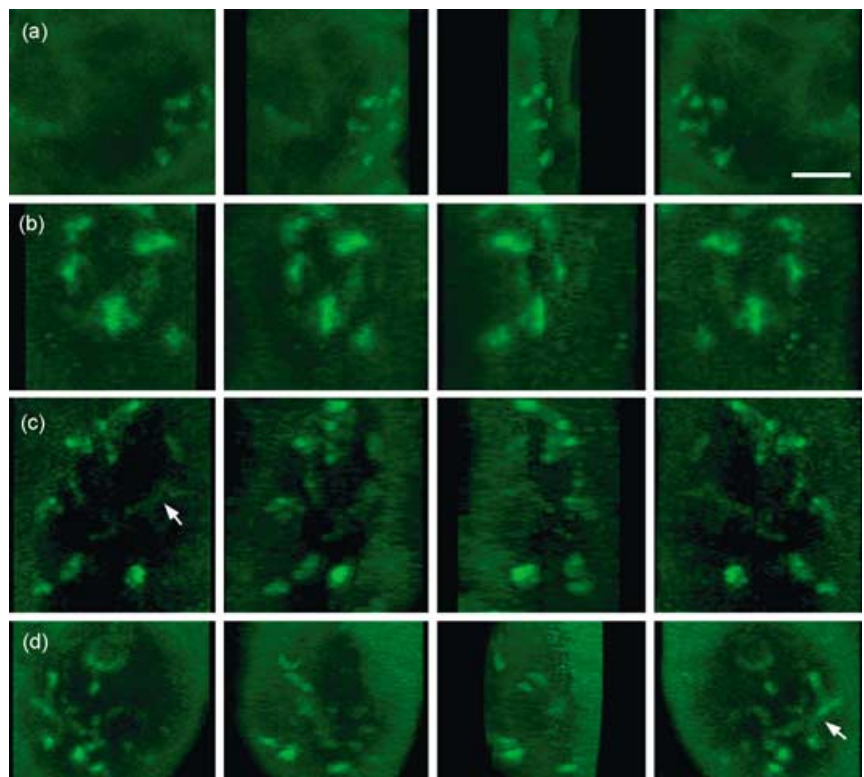
All images are projections of rotations (0° , 50° , 120° and 220°) of 3D confocal data stacks of meiocytes labelled with an *in situ* probe for centromeres.

(a) Rotations of the hexaploid wheat meiocyte in Figure 2(b) reveal seven centromere clusters.

(b) Rotations of the tetraploid meiocyte in Figure 2(f) also show seven centromere clusters.

(c) Rotations of the wheat–rye hybrid meiocyte in Figure 2(j), show seven clusters, with one of them resolving as a higher order structure (white arrow).

(d) Rotations of a hexaploid wheat–rye meiocyte show unpaired centromeres and a quadripartite structure (white arrow). Bar, 10 μ m.



1997). The simplest explanation for the coincidence of the increase in the level of homologue association with the presence of the seven groupings is that the groupings result from the pairing of homoeologous centromeres. If centromere pairs are formed during pre-meiosis either from homologues or homoeologues, then their clustering into seven groups at the initiation of the bouquet stage would lead to an apparent increase in homologue association. However, a definitive proof of this will require the development of probes and *in situ* hybridisation methodo-

logy to visualise sites on each of the six homoeologues belonging to a given group. In tetraploid wheat (lacking the D genome), the 28 chromosomes also reduce to approximately seven clusters at the initiation of the telomere bouquet (Figures 2e,f and 3b; Table 1). Similarly, there are seven clusters at this stage in a hexaploid wheat-rye hybrid and in a *Ph1* mutant-rye hybrid, both containing 28 chromosomes derived from four genomes (the haploid set of chromosomes (21) from hexaploid wheat and the haploid set (7) from rye) (Figure 2g; Table 1). Thus, the

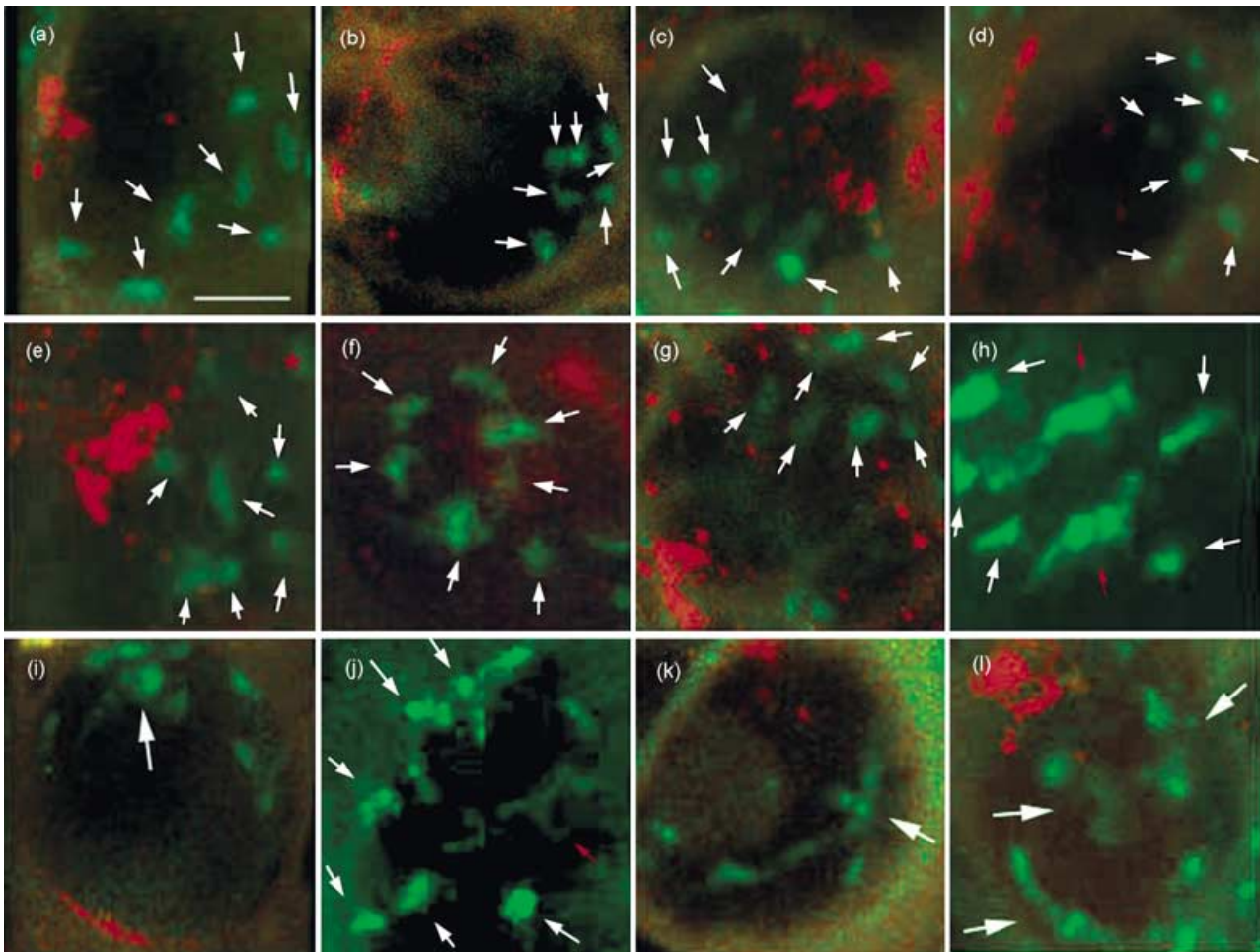


Figure 2. Centromere behaviour on initiation of telomere bouquet formation.

All images are projections of confocal section stacks. All the images of single meiotic nuclei are taken from whole anther sections, which are between two and three cell layers thick. When making projections of single meiotic nuclei from such sections, centromeres and telomere sites from adjacent nuclei will occasionally appear on the image. Such sites, when present, are indicated. The meiotic nuclei reflect the general trend found in the different lines analysed. Images (a–e) show representative meiotic nuclei from the different lines with seven centromere clusters. Images (f–l) are examples of later stages from the same lines in which these clusters elongate forming different structures. The projections show the centromeres (green) in the meiotic nuclei during telomere bouquet formation (red). There are seven centromere clusters (indicated by white arrows) in meiotic nuclei from (a, b) hexaploid wheat, (c, d) *Ph1* mutant line and (e, f) tetraploid wheat; the red asterisk indicates a centromere site from an adjacent nucleus; (g) the hexaploid wheat-rye hybrid. (In (g), the telomere cluster of this meiotic nucleus is at the bottom left hand corner; the other telomeres seen in this projection are from an adjacent nucleus.) (h) The seven centromere clusters in the tetraploid wheat meiotic nucleus have already formed elongated or bipartite structures (red arrows). (i) Most of the seven centromere clusters in this meiotic nucleus of hexaploid wheat have yet to elongate; however, one cluster elongates forming a tripartite structure (white arrow). (j) Most of the seven centromere clusters in this meiotic nucleus of the hexaploid wheat-rye hybrid have started to elongate. One of these clusters is more advanced in the process, revealing a complex elongated structure (red arrow). (k) A partial projection to show a centromere cluster elongating as a quadripartite structure from this meiotic nucleus of the hexaploid wheat-rye hybrid. (l) Three of the seven centromere clusters in this meiotic nucleus from *Ph1* mutant-rye hybrid elongate as bipartite structures (white arrows). Rotated projections of three of these meiotic nuclei (b), (f) and (j) are shown in Figure 3. Bar, 10 μ m.

Table 1 All the meiocytes examined exhibiting incomplete telomere bouquet formation from the different lines had close to seven centromere sites. For hexaploid and tetraploid wheat and the wheat-rye hybrid, 12 of these meiocytes were randomly selected and 3D reconstructions of the meiocytes were made. The number of centromere clusters in each of these 12 meiocytes analysed is given. From the large number of confocal sections of the *Ph1* mutant and *Ph1* mutant-rye hybrid analysed, we could only identify seven meiocytes from each in which the telomere bouquet was in the process of being formed. It was harder to find the meiocytes at this stage because of the lack of synchronisation between the developing meiocytes in the anthers derived from lines lacking *Ph1*, which contrasts to the synchronisation in its presence. The number of clusters in these meiocytes is given

	Number of meiocytes with each type of centromere cluster				
	5 clusters	6 clusters	7 clusters	8 clusters	9 clusters
Hexaploid wheat			9	1	2
Tetraploid wheat	3	1	7	1	
Wheat × rye	1	3	7	1	
<i>Ph1</i> mutant			4	1	2
<i>Ph1</i> mutant × rye	1	1	4	1	

occurrence of seven centromere clusters is not dependent on the presence of homologous chromosomes or on the number of genomes present, and suggests that the process is not simply a random super-clustering of centromere pairs. The centromeres also form seven pairs as the telomere bouquet is initiated in the progenitor diploids of wheat (Martinez-Perez *et al.*, 2000). Moreover, chromosomes of autotetraploid cereals, which possess two chromosome sets of identical chromosomes (i.e. seven groups, each consisting of four homologues), intimately pair (synapse) during meiosis as seven quadrivalents (structures consisting of four paired chromosomes), in contrast to allopolyploids such as hexaploid and tetraploid wheat where synapsis is completed as bivalents (two paired chromosomes) (Gillies *et al.*, 1987; Holm, 1986). It is only early during the synapsis of chromosomes in hexaploid and tetraploid wheat that multiple interactions between chromosomes are observed, but these interactions are then resolved as synapsis progresses (Holm, 1986; Martinez *et al.*, 2001). We have previously reported that the 28 centromeres of an autotetraploid are found as seven clusters when the telomere bouquet is fully formed (Martinez-Perez *et al.*, 2000). As the seven centromere clusters are maintained in the autotetraploid through synapsis in contrast to the allopolyploids described above, its presence is more prominent. During our previous studies, we missed the formation of these clusters in allopolyploids because we scored meiocytes where the telomere bouquet was complete rather than where the telomere bouquet is in the process of being formed.

The formation of centromere pairs into clusters implies that the centromeres are engaged in multiple associations. Association of chromosomes first into pairs and then into seven groups at the beginning of meiosis suggests quite an elaborate mechanism for sorting chromosomes in these polyploids. Thus, we propose that there is an additional sorting mechanism involving centromeres during early meiosis prior to that provided by telomeres in species such

as wheat, which pre-meiotically associate their chromosomes via their centromeres. In fact, it has been shown even in a diploid species that homologous chromosomes pair to wild-type levels, albeit with a 2–3-h delay in the yeast mutants, in which the telomere bouquet is not formed. Thus, although the telomere bouquet facilitates the pairing process, there are also other pairing mechanisms that are telomere bouquet independent (Trelles-Sticken *et al.*, 2000).

It is difficult to envisage the centromere clustering mechanism described above evolving rapidly following polyploidisation. It is more likely that the potential to pair centromeres pre-meiotically exists in the diploid cereals. If the centromeres do form into pairs during pre-meiosis, then there is a potential in the polyploid situation for these pairs to cluster into larger groupings as a prelude to meiosis. The centromeres do form seven pairs in the diploid progenitors of wheat as the telomere bouquet is being initiated (Martinez-Perez *et al.*, 2000). Pre-meiotic centromere association is induced in an artificial polyploid made from two diploid relatives of wheat, which do not themselves exhibit general pre-meiotic centromere association (Martinez-Perez *et al.*, 2000). Thus, polyploidisation would be a factor that induces the expression of this process. The observation that, in the diploid cereal species barley, centromeres can associate in pairs in somatic cells during S phase when the centromeres are being replicated is consistent with this conclusion (Jasencakova *et al.*, 2001). The centromere pairs then separate after replication. We hypothesise that the centromeres fail to separate after replication in wheat and its related polyploids. This may be because the heterochromatin regions are more 'sticky' as a result of changes either in methylation or in the abundance of heterochromatin-associated proteins following polyploidisation. If diploids do have the potential to associate their centromeres, then it would be predicted that the occasional diploid cereal will be identified in the future, which exhibits pre-meiotic centromere association. It also

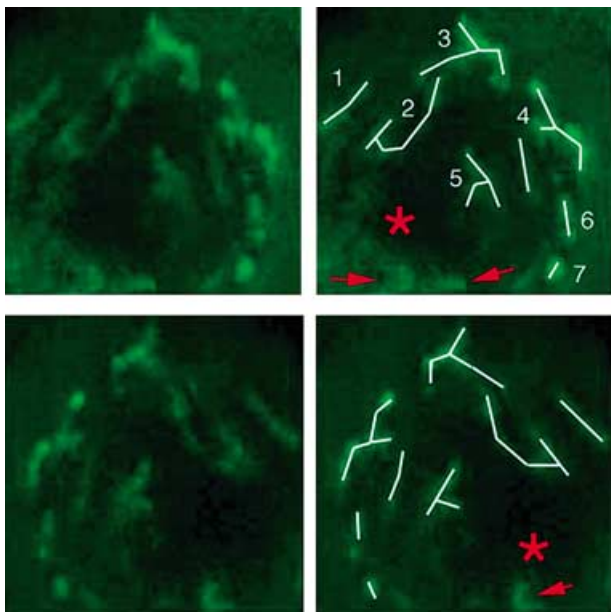


Figure 4. Centromere clusters resolving in hexaploid wheat. Two copies of the images for two rotations (at 0° and 160°) are presented from a confocal section stack of a hexaploid wheat meiocyte. The outlines of the centromere clusters are traced on one copy. These outlines are based only on the structures observed in the 3D reconstructional analysis of the meiocyte where the signal to background noise ratio is higher than that in the projection of the stack of images. Centromere sites from an adjacent nucleus are indicated by the red arrows. The centromeres at different stages of elongation have been interpreted as showing the resolution of seven clusters (numbered 1–7). Some clusters (1, 6 and 7) have yet to initiate resolution. Several clusters (2, 3 and 5) resolve as tripartite structures. One ‘cluster’ (4) is interpreted as having already separated one elongated structure while in the process of separating the remaining structures. The location of the telomere bouquet (shown in Figure 1f) in the nucleus is shown as a red asterisk.

remains to be determined whether the ability to pre-meiotically associate the centromeres either arose in a limited number of cereals, or is present in the rushes and sedges group from which the cereals evolved. These species have an unusual meiosis in that, unlike other species, they fail to resolve pairing between the homologous chromosomes during meiosis I, resulting in their segregation to the same spindle pole (Nordenskiöld, 1962).

The seven centromere clusters resolve as complex structures

The grouping of the centromeres into seven clusters in the polyploids also implies that multiple interactions are occurring between the centromeres. The 42 centromeres of hexaploid wheat are observed as 21 paired sites in late stages of the telomere bouquet in both the presence and the absence of *Ph1*, while in the hybrid at similar stages of the telomere bouquet, the centromeres are only in pairs in the absence of *Ph1* and are separated in its presence (Martinez-Perez *et al.*, 2001). If the diffuse centromere clusters are simply associations of pairs of chromosomes, then their resolution would be immediately back as pairs. However, if the associations are more complex, then the clusters would resolve as complex structures. All the images of meiocytes examined from the different lines at this later stage show a general trend in that the seven centromere clusters now form elongated structures (Figure 2h–l). The diffuse nature of these structures means that the signal to noise ratio is lower than that at the previous stages, and this is particularly evident when projections are made of the collected image stacks. In the case of the images of the centromere clusters in hexaploid wheat, elongated tripartite structures are revealed (Figure 4). We interpret

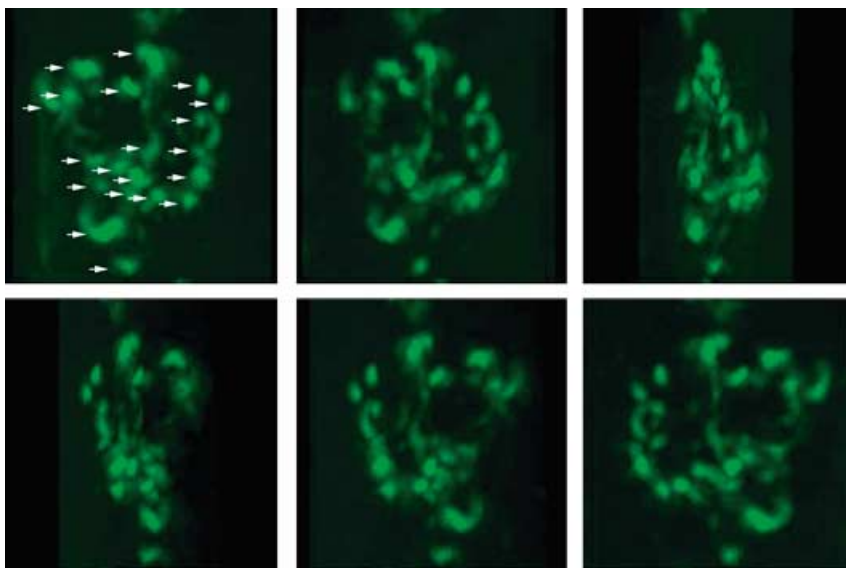


Figure 5. Centromere clusters resolve into elongated structures in hexaploid wheat. Six different rotations (0°, 20°, 50°, 160°, 180° and 230°) of a 3D data set of a hexaploid wheat meiocyte showing the resolution of the centromere clusters into 21 centromere elongated sites (white arrows).

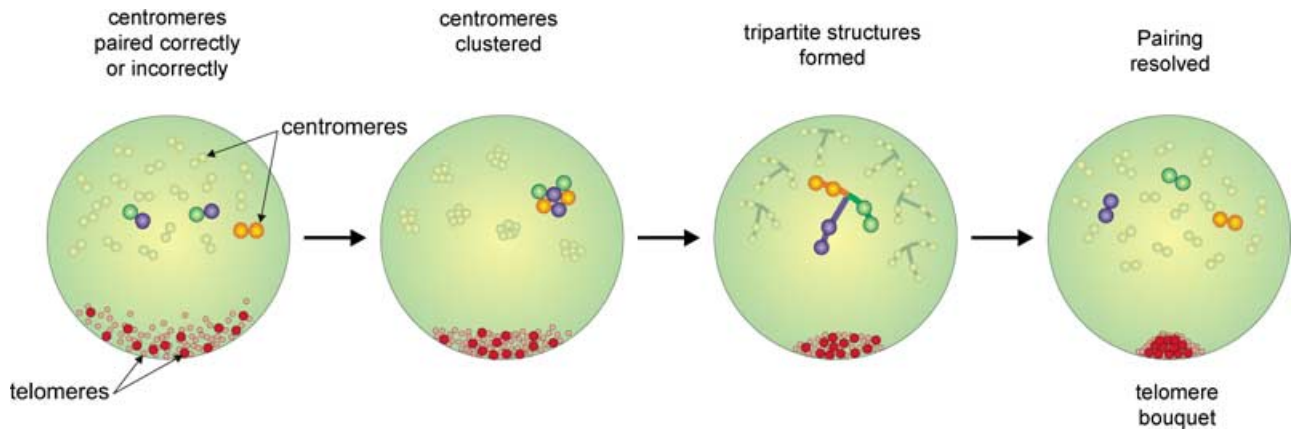


Figure 6. A model for centromere pairing in hexaploid wheat. The centromeres pair either correctly or incorrectly before meiosis during anther development. At meiosis, just before the telomere bouquet is fully formed, the paired centromeres cluster in seven diffuse groupings. These clusters then form tripartite structures, indicating three-way pairing. Finally, the 'parts' of the tripartite structures resolve leaving the 21 elongated centromere sites, which then condense. The model diagrams the centromeres (in yellow, green and blue) and telomeres (in red) of three pairs (homoeologues) of chromosomes.

all these centromere structures as in the process of resolving because the centromeres of the meiocytes at later stages are observed as separate elongated structures. For example, in hexaploid wheat, 21 elongated centromere structures are revealed corresponding to the 21 paired chromosomes at meiosis (Figure 5). In contrast to the cen-

tromeres in meiocytes at early stages (Figures 2–4), these paired centromeres are no longer on the nuclear periphery. Thus, the seven diffuse centromere structures elongate and then the various parts are resolved. In tetraploid wheat, elongated or V-shaped bipartite structures are seen, which resolve into two paired sites (Figure 2h). In the

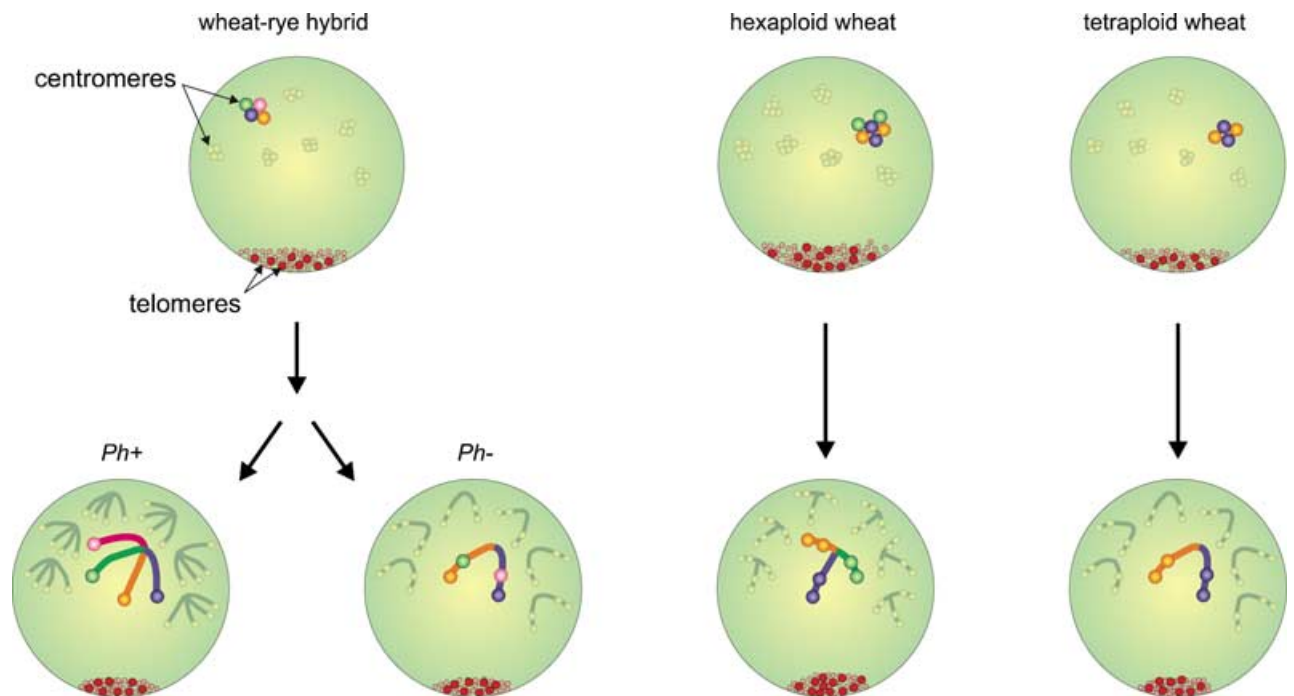


Figure 7. Resolution of the centromere clusters. The figure provides a model of how centromere clustering resolves in the different lines studied. Centromere clusters in hexaploid wheat resolve as tripartite structures while the *Ph1* mutant-rye hybrid and tetraploid wheat resolve as bipartite structures. However, the centromere clusters in the hexaploid wheat-rye hybrid resolve as complex structures (in this case, quadrupartite structures are shown). The centromeres (yellow, green and blue) and telomeres (in red) are indicated. A haploid set of homoeologues is diagrammed in the wheat-rye hybrid lines, while the diploid set of homoeologues is diagrammed in hexaploid and tetraploid wheat.

wheat-rye hybrid, complex structures (including quadripartite structures; Figure 2k) are seen (Figures 2j and 3c,d). These structures are resolved into mostly unpaired centromere sites, as judged by the number of sites seen (Figure 3d). No quadripartite or complex structures have been observed in the *Ph1* mutant-rye hybrid at this stage, instead elongated or V-shaped bipartite structures are observed (Figure 2l).

The Ph1 locus affects the resolution of centromere clusters

The formation of tripartite structures in hexaploid wheat and bipartite structures in tetraploid wheat suggests that there are three- and two-way pairings, respectively, between the centromere sites. A model for the pairing process in hexaploid wheat is shown in Figure 6. This conclusion is further supported by the observations from the wheat-rye hybrid, where there are no homologues. Prior to meiosis, the centromeres mostly form pairs (Martinez-Perez *et al.*, 2001). At early meiosis, these pairs form seven clusters (Figure 2j). As the hybrid possesses 28 centromeres, each cluster is likely to be composed of four centromeres. The observation of complex structures (including quadripartite), rather than bipartite structures as in the tetraploid, implies that the interactions between the centromere pairs are not maintained in the cluster structure and the centromeres are resolved into unpaired sites at this stage (Figure 3d). Thus, associations made premeiotically between centromeres can be resolved at this stage. However, in wheat-rye hybrids lacking the *Ph1* locus, paired centromeres are observed at late stages of the telomere bouquet (Martinez-Perez *et al.*, 2001). We have not observed the complex/quadripartite structures in this hybrid, instead elongated or V-shaped bipartite structures occur during the telomere bouquet stage, implying that *Ph1* is involved in the ability to correct pairing (Figure 2l). A model for how the centromere clusters are resolved in the different lines is shown in Figure 7. We have shown previously that the *Ph1* locus cannot prevent centromeres from pairing incorrectly. In fact, it is now clear from the present study that centromeres engage in multiple interactions, which need to be resolved. Hexaploid wheat lacking the *Ph1* locus still forms the seven clusters, which resolve into 21 sites (Figure 2c,d; Table 1). So, the mechanics of the pairing process is similar to that in wild-type situation. However, the specificity of the resolution process is altered. Previous studies have shown that, when wheat chromosomes synapse during meiosis, half the chromosomes synapse as bivalents while half the chromosomes are engaged in pairing with two or more other chromosomes (Holm, 1986, 1988). Given that synapsis occurs later in the pairing process after the initial interactions, it is quite possible that not only do the centromeres of a cluster engage in multiple interactions with each other, but also

that there are interactions at other sites along the chromosome. For a polyploid such as wheat, the resolution of such interactions is a critical step in the ability to finally achieve homologue pairing. This may represent a fundamental difference between chromosome pairing in cereal polyploids and their diploid progenitors. The key issue for polyploids is to reduce the extensive interactions made early in meiosis. We have shown previously that, in the presence of *Ph1*, incorrectly paired centromeres are resolved, while in the absence of *Ph1*, at equivalent stages, the paired centromeres are unresolved (Martinez-Perez *et al.*, 2001). Other studies have shown that incorrect pairing is also maintained at other chromosomal sites in the absence of *Ph1* (Holm, 1986, 1988; Martinez *et al.*, 2001). Thus, in wheat in which this elaborate mechanism for associating chromosomes is present, the importance of *Ph1* becomes clear in providing a critical role in the resolution of the interactions made. It has been proposed from studies of tetraploid cotton that the resolution of incorrect pairing is connected with the rates at which related chromosomes are condensed (Brown, 1954). If homoeologous chromosomes are condensed at different rates compared to homologous chromosomes, then pairing between homologues within the chromosome cluster may be more stable than that between homoeologues. It remains to be determined whether *Ph1* is directly involved in this resolution process or is simply involved in the timing of the process so that chromosomes interacting at multiple sites have sufficient time to complete resolution before entering metaphase I.

Experimental procedures

Plant material

The following plant lines were used in this study: *T. aestivum* cv. 'Chinese Spring'; a *T. aestivum* cv. 'Chinese Spring' mutant (*ph1b*) lacking the *Ph1* locus; *T. durum*; *T. aestivum* cv. 'Chinese Spring'/*Secale cereale* cv. Petkus F1 hybrid and a *T. aestivum* cv. 'Chinese Spring' mutant (*ph1b*)/*Secale cereale* cv. 'Petkus' F1 hybrid lacking the *Ph1* locus.

Fluorescence in situ hybridisation

The centromere (CCS1) and telomere (TTTAGGG repeat) probes used, the tissue sectioning and specimen preparation, *in situ* hybridisation and probe preparation have all been described previously by Martinez-Perez *et al.* (1999).

Fluorescence microscopy and image processing

Confocal optical sections stacks were collected using a Leica TCS or SP2 confocal microscope as described in 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, Maryland, USA. Final Figures were prepared using Adobe Photoshop.

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