Effective chromosome pairing requires chromatin remodelling at the onset of meiosis

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During meiosis, homologous chromosomes (homologues) need to recognise each other and then intimately associate [1]. Meiotic studies have revealed that chromatin is remodelled at the onset of meiosis prior to pairing and recombination [2,3]. However little is known what effect this remodelling has on these processes. We show here in wheat that chromatin remodelling of homologues can only occur if they are identical or nearly identical. Moreover a failure to undergo remodelling results in reduced pairing between the homologues. Thus chromatin remodelling at the onset of meiosis enables the chromosomes to become competent to efficiently pair and recombine.

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Hexaploid wheat possesses three related genomes totally 16,000Mb in size composed of 7 sets of 6 related chromosomes with similar genes orders and vast tracts of related and highly repetitive sequences. The *Ph1* locus in hexaploid wheat ensures that only true homologues pair at meiosis amongst the 6 related chromosomes [4]. At the onset of meiosis in hexaploid wheat, homologues undergo synchronised chromatin remodelling in the presence of *Ph1* when the telomeres cluster as a bouquet and initiate intimate pairing from the telomeres [5]. By visualising the behaviour of homologues carrying different combinations of rye subtelomeric heterochromatin, it is possible to assess the effect that varying homology has on the ability to remodel chromatin and the consequent effect that this has pairing and recombination. A number of wheat lines have been generated in which a wheat chromosome arm has been substituted for homologous rye chromosome arms carrying distinctive subtelomeric heterochromatin. Two of the wheat lines carry a pair of homologues with rye subtelomeric heterochromatin derived from the same variety (either being derived from KingII or Petkus), the third line carries a pair of homologues with similar sized rye heterochromatin regions but derived from two different varieties (one from KingII and the other from Petkus) and the final line carries homologues with different sized rye subtelomeric heterochromatin derived from the two different varieties (Petkus and Imperial).

Prior to meiosis, the telomeres are dispersed around the nuclear periphery. In these premeiotic cells, no change in conformation of the heterochromatin was visualised with these regions remaining compact in all of the meiocytes examined from the different wheat lines (Fig. 1a, d, g). However the subtelomeric heterochromatin behaviour varied from when the telomeres began to cluster in the meiocytes from the different lines. The subtelomeric heterochromatin remained compact during the telomere clustering and bouquet formation in the wheat line carrying homologues with different sized subtelomeric heterochromatin regions (Petkus/Imperial). Moreover the subtelomeric heterochromatin regions were only paired with each other in 30% of the meiocytes at diplotene which then reduced further to only 16% of the meiocytes by metaphase I (See Supplementary Table 1). In contrast, when the subtelomeric heterochromatin regions were identical on both homologues, they were already colocalised together

prior to the telomere bouquet formation in 60% (Petkus/Petkus) and 50% (KingII/KingII) of the meiocytes examined (Fig. 2a, b). During the telomere bouquet stage, these regions then underwent extensive remodelling in all the meiocytes examined (Fig. 1b, c). The remodelled subtelomeric heterochromatin on the homologues extended up to 5μm in length but differed by no more than 30% in length from each other (see Supplementary Information Table 2). The extended subtelomeric heterochromatin then formed a V-shaped paired structure with the telomere sites at the apex before being "zipping up" (Fig 2c). The subtelomeric heterochromatin regions were paired in 98% of the meiocytes examined from diplotene to metaphase I. In contrast to these observations, the subtelomeric heterochromatin regions were not colocalised together prior to the telomere bouquet in the meiocytes derived in the line carrying homologues with similar sized rye subtelomeric heterochromatin derived from Petkus and King II. However at the telomere bouquet stage, both the Petkus and King II subtelomeric heterochromatin did undergo chromatin remodelling but the remodelled regions differed from each other by up to 2-fold in length (See Supplementary Information Table 1). Moreover the extended heterochromatin regions then did not "zip up" as in the parental lines but paired at either end of the heterochromatin regions forming a loop structure (Fig 2d). The loop structure then coalesced so that the remodelled heterochromatin regions were found to be paired in 79% of the meiocytes at diplotene and 56% at metaphase I (See Supplementary Information Table 1).

Previous studies in *Arabidopsis* have shown that the telomere regions can pair homologously without the occurrence of the classical telomere bouquet found in many species [6]. The present study shows in wheat that there can be a significant level of homologue association via telomeres prior to telomere bouquet being fully formed. However this association only happens in those cases where the homologues are identical in their subtelomeric regions. If the homologues are identical then as has been observed previously, they can intimately align in a "zipping up process" from the telomeres. If these regions show divergence, then homologue association occurs within the telomere bouquet and only after the chromatin remodelling has occurred. Moreover intimate alignment between the diverged subtelomeric regions then occurs through "a pegging together and coalescing" process.

Although there is a reduction in the overall level of pairing (and subsequent recombination) between homologues carrying subtelomeric heterochromatin remodelled to different lengths, it is a failure to remodel the subtelomeric heterochromatin which has the most marked effect on the subsequent overall level of pairing and recombination. Moreover in wheat, the ability to remodel chromatin which affects subsequent pairing and recombination is linked to overall relatedness of chromosomes. Previously it has been shown that the *Ph1* locus in wheat is able to block recombination from occurring between diverged chromosome segments on homologues [7,8]. Since *Ph1* affects the ability to coordinate and control chromatin remodelling, it would seem that these issues are all linked [5]. In hexaploid wheat-rye hybrids where there are only related chromosomes (homoeologues- see Supplementary Fig S1), in the absence of *Ph1* locus and true homologues, the rye subtelomeric heterochromatin remodels (Supplementary Fig S2) [5]. The different sized rye subtelomeric heterochromatin regions then associate with each other. In contrast, in the presence of *Ph1* with no true homologues present, rye subtelomeric heterochromatin cannot remodel and thus no pairing and recombination does not occur (Supplementary Fig S2). The *Ph1* locus has recently been defined to be a cluster of *cdk-like* genes [9]. *Cdk2* from humans shows the closest homology to these wheat *cdk-like* genes [10]. *Cdk2* is involved in chromatin remodelling for replication, initiation of meiosis and the pachytene checkpoint [11]. Thus the ability to initiate the onset of meiosis may be intimately linked to the ability to remodel chromatin which is required for the chromosomes to become competent to pair.

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Plant materials

The following wheat-rye translocation lines were exploited in the present study: Gabo 1BL.1RS (Imperial 1RS) x Veery 3 1BL.1RS (Petkus1RS), CS/Holdfast 1BL.1RS (KingII 1RS) x Federation/Kavkas 1BL.1RS (Petkus 1RS), CS/Holdfast 1BL.1RS (KingII 1RS), Federation/Kavkas 1BL.1RS (Petkus 1RS) in a *Ph1* background and Chinese Spring/*Secale cereale* cv.Petkus F1 hybrids with and without the *Ph1* locus (*ph1b* deficiency). Plants have been grown in controlled environmental room under optimized conditions for wheat.

Tissue preparation

Anthers were staged for meiosis by light microscopy after squashing and staining with aceto carmine. Spikes were fixed by vacuum infiltration of freshly prepared 4% formaldehyde in PEM (50 mM Pipes, 5 mM EGTA, 5 mM MgSO4, pH 6.9) for 1 hour as described previously [12]. Tissues were prepared by anther sectioning (50-100µm thickness with vibratome) or by isolating meiocytes onto a γ-aminopropyl triethoxy silane (APTES) coated slide which has been glutaraldehyde activated as described previously [12].

Fluorescence in situ hybridization

Telomere probe was labelled with biotin-16-dUTP by nick translation of PCR-amplified products using the oligomer primers (5'-TTTAGGG-3')5 and (5'- CCCTAAA-3')5 in the absence of template DNA. Rye heterochromatin probe was labelled with digoxygenin -11-dUTP by nick translation of PCR-amplified products of the rye knob sequence pSc250. *In situ* hybridization protocols have been described previously [12].

Microscopy and Imaging

Meiocytes were visualized using a Nikon Eclipse E600 fluorescent microscope connected to a Hamamatsu CCD camera. Stack images of individual cells have been collected using MetaMorph (Universal Imaging Corp.) software. Deconvolutions of images have been processed with AutoDeblur (AutoQuant Imaging). Projections of 3D picture were performed with the public domain program ImageJ.

ACKNOWLEDGEMENTS

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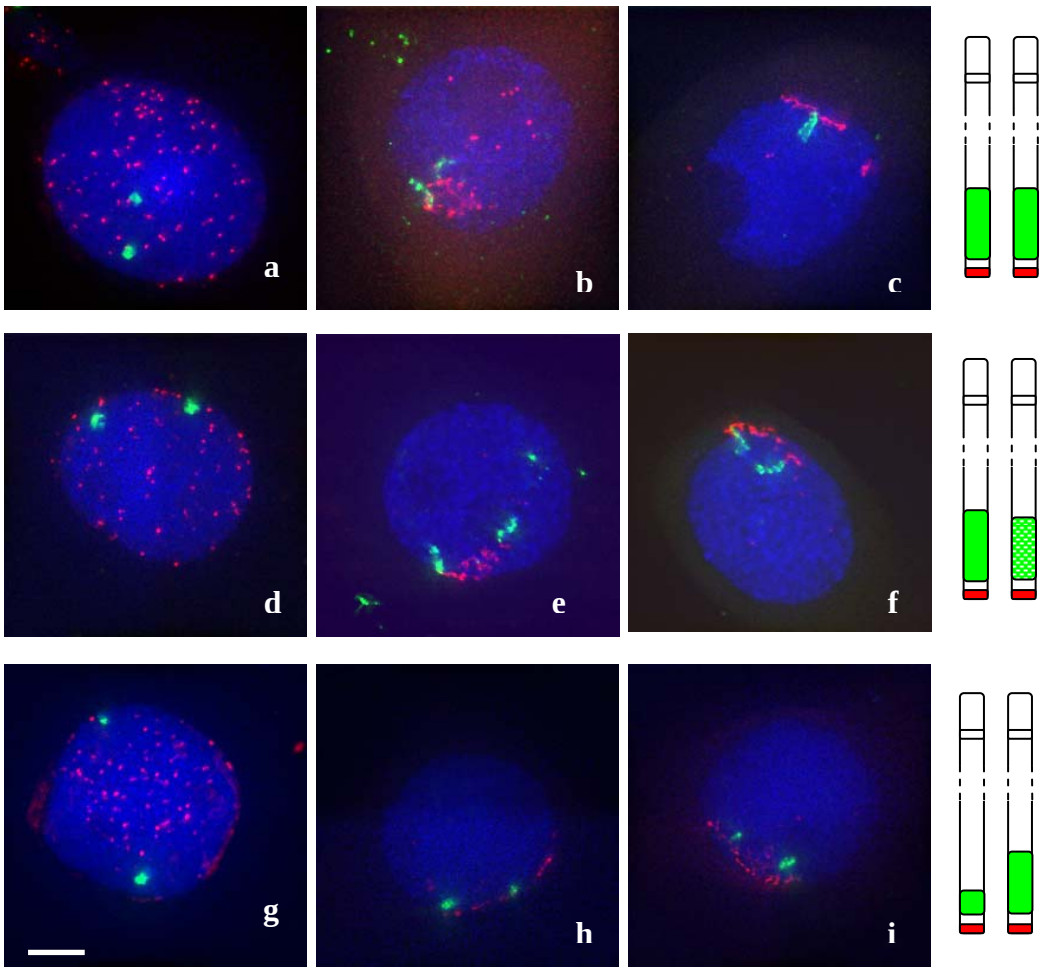


Figure 1: Coordination of heterochromatin remodelling at meiosis: Rye subtelomeric heterochromatin labelled in green and the telomeres in red. KingII/KingII line (identical heterochromatin), (a) Premeiotic nucleus (b) Heterochromatin remodelling during telomere clustering, (c) Heterochromatin association; Petkus/KingII line (similar heterochromatin but not identical) (d) Premeiotic nucleus (e-f) Remodelling during telomere clustering; Petkus/Imperial line (different heterochromatin) (g) Premeiotic nucleus, (h-i) No remodelling during telomere clustering. Schematic representation of the different homologues is shown with subtelomeric heterochromatin in green and telomeres in red. Scale represents ~10µm.

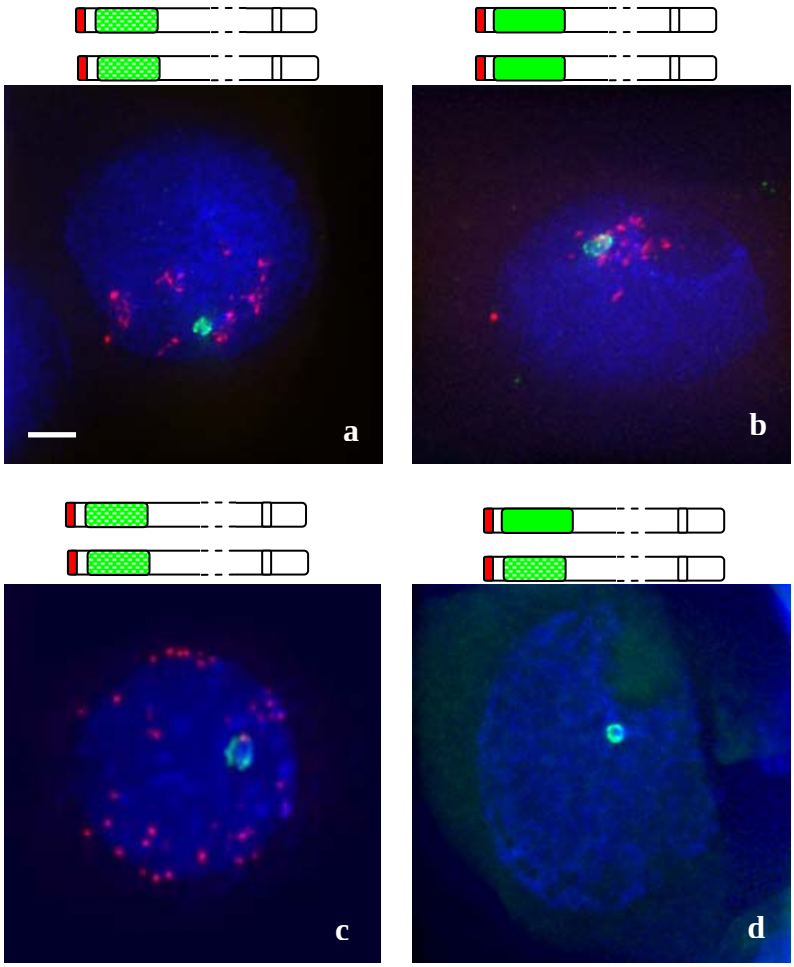


Figure 2: Heterochromatin colocalisation and alignment at meiosis. Rye subtelomeric heterochromatin labelled in green and telomeres in red. The two heterochromatin regions are colocalized prior to the telomere bouquet in the Petkus/Petkus (a) and KingII/KingII lines (b). Homologues are paired from the telomere, forming a fork after telomere clustering in KingII/KingII line (c). Heterochromatin regions associate forming a loop structure in Petkus/KingII line (d). Scale represents ~10µm.

Supplementary Information

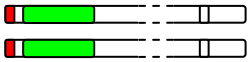
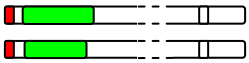
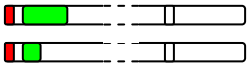
Homologous pairing during meiosis	Late Zygotene	Diplotene	Metaphase I
a 	**98%**	**98%**	**98%**
b 	**82%**	**79%**	**56%**
c 	**44%**	**30%**	**16%**

Table 1: Percentage of meiocytes with paired heterochromatin sites during prophase I: (a) KingII/KingII or Petkus/Petkus lines, (b) Petkus/KingII line and (c), Petkus/Imperial line.

	Identical heterochromatin			Similar heterochromatin from different rye variety		
	Segment 1	Segment 2	Ratio	Segment 1	Segment 2	Ratio
Length of 2 rye segment per cell	5.269	4.509	1.2	4.425	4.093	1.1
	4.217	3.948	1.1	3.893	2.652	1.5
	4.676	3.470	1.3	4.952	3.298	1.5
	5.023	4.899	1.0	5.040	3.938	1.3
	4.053	3.797	1.1	4.106	3.302	1.2
	4.021	3.195	1.3	3.823	3.567	1.1
	3.678	2.896	1.3	3.773	3.752	1.0
	4.333	3.809	1.1	3.034	2.539	1.2
	3.942	3.064	1.3	3.865	3.098	1.2
	3.451	3.451	1.0	5.313	3.524	1.5
	3.936	3.936	1.0	3.463	3.431	1.0
	3.677	3.677	1.0	3.175	2.965	1.1
mean						
t-test			P=0.002			P=0.115

Table 2: Heterochromatin remodelling during the telomere bouquet formation. The length of the remodelled heterochromatin was measured in 3D stacks of meiocytes exhibiting telomere clustering. A t-test was performed for the following null hypothesis that "the length of the two remodelled heterochromatin segments is similar". For the line carrying homologues with identical heterochromatin

(same size, same variety), the null hypothesis can be accepted with 99% confidence but is rejected for the line carrying homologues with similar sized heterochromatin but derived from two varieties.

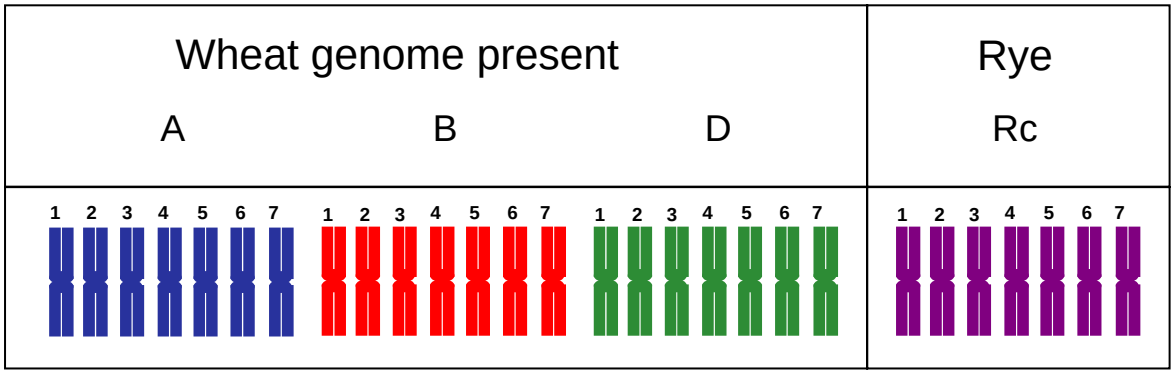


Figure 1: Wheat-rye hybrids: haploid set of 21 chromosomes of wheat and a haploid set of 7 rye chromosomes, 28 homoeologues and no homologues.

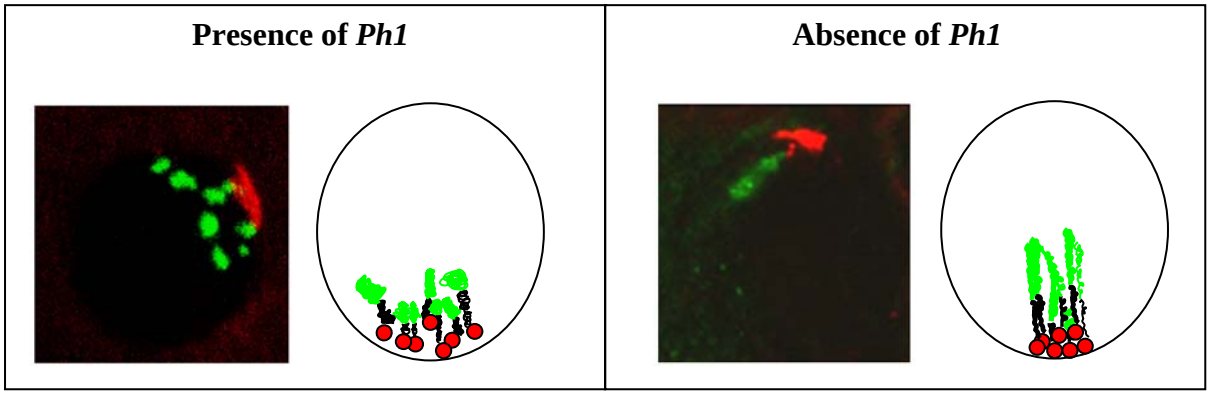


Figure 2: Heterochromatin remodelling in the wheat/ rye hybrid at meiosis: Rye subtelomeric heterochromatin labelled in green, and telomeres in red. In presence of *Ph1*, no remodelling of rye heterochromatin during the telomere cluster. In absence of *Ph1*, the rye heterochromatin remodels during the telomere cluster.

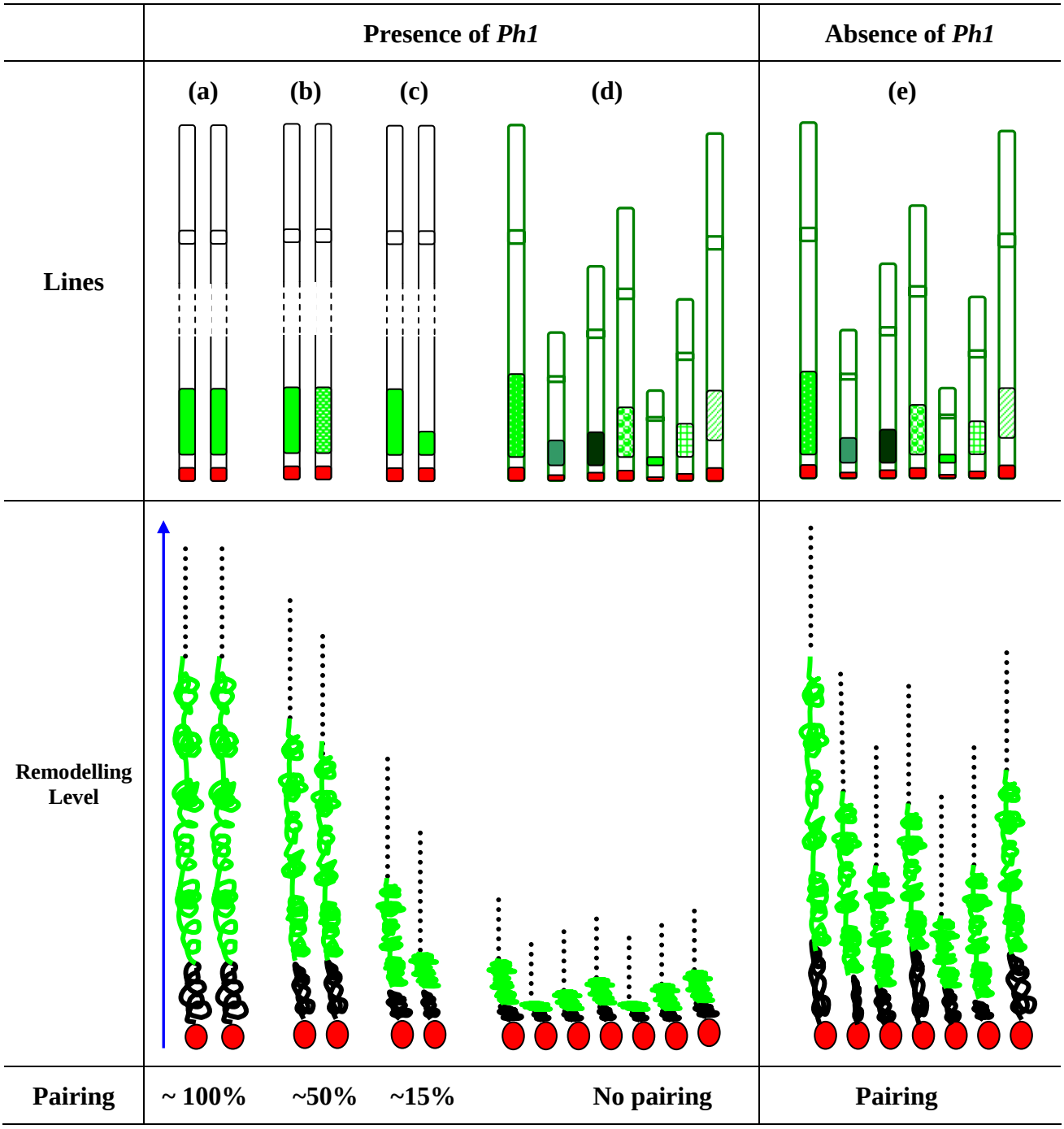


Figure 3: Summary of the ability to chromatin remodel at meiosis: (a) homologues carry identical rye subtelomeric heterochromatin, (b) homologues carry similar sized rye heterochromatin from two different varieties, (c) homologues carry different sized rye heterochromatin from two different varieties, (d), wheat/ rye hybrids in presence of *Ph1*, containing 7 rye segments of different size, (e) wheat/ rye hybrids in absence of *Ph1*, containing 7 rye segments of different size.

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