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RESEARCH ARTICLE

Inversions of chromosome arms 4AL and 2BS in wheat invert the patterns of chiasma distribution

Adam J. Lukaszewski · David Kopecky · Gabriella Linc

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Abstract In many species, including wheat, crossing over is distal, and the proximal regions of chromosome arms contribute little to genetic maps. This was thought to be a consequence of terminal initiation of synapsis favoring distal crossing over. However, in an inverted rye chromosome arm, the pattern of metaphase I chiasmata was also inverted, suggesting that crossover frequencies were specific to chromosome segments. Here, wheat chromosome arms 2BS and 4AL, with essentially entire arms inverted in reverse tandem duplications (rtd), were studied in the MI of meiosis. Inversion–duplication placed the recombining segments in the middle of the arms. While the overall pairing frequencies of the inverted–duplicated arms were considerably reduced relative to normal arms, chiasmata, if present, were always located in the same regions as in structurally normal arms, and relative chiasma frequencies remained the same. The frequencies of fragment or fragment + bridge configurations in AI and AII indicated that of the two tandemly arranged copies of segments in rtds, the more distal inverted segments were more likely to cross over than the segments in their original orientations. These observations show that also in wheat, relative crossover frequencies along chromosome arms are predetermined and independent of the segment location. The segments normally not licensed to cross over do not do so even when placed in seemingly most favorable positions for it.

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A. J. Lukaszewski (✉)
Department of Botany and Plant Sciences,
University of California,
Riverside, CA 92521, USA
e-mail: adam.lukaszewski@ucr.edu

D. Kopecky
Centre of the Region Hana for Biotechnological and Agricultural Research, Institute of Experimental Botany,
Sokolovska 6,
77200 Olomouc, Czech Republic

G. Linc
Agricultural Research Institute of the Hungarian Academy of Sciences,
2462 Martonvásár, Hungary

Introduction

Even a cursory examination of published images of metaphase I of meiosis shows that a majority of species form distal chiasmata, which create typical textbook shapes of ring and rod bivalents (see John 1990). Among species with distal chiasmata are most grasses, including major crops such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), maize (*Zea mays* L.), oats (*Avena sativa* L) and pasture grasses such as ryegrasses (*Lolium sp.*) and fescues (*Festuca sp.*). In wheat, a quick count on several published MI figures shows that among ca. 40–41 paired arms per pollen mother cell (PMC), ca. 34–35 have terminal chiasmata and each meiocyte has only 1–2 chiasmata that could truly be called intercalary. No proximal chiasmata are present. Chiasmata are cytological expressions of crossing over, formed by a crossover event itself and cohesion of sister chromatids, and are incapable of migrating along the chromosome. Therefore, if chiasmata are distal, crossing over must also be distal. Concentration of crossing over in distal regions of chromosome arms has been shown in many species and by different means: by genetic mapping of physical features of chromosomes in wheat (Lukaszewski and Curtis 1993), by allocation of genetic markers to physically defined bins in wheat (Gill et al. 1996; Werner et al. 1992) and grasses (Kopecky et al.

2010) and by associating genetic markers with translocation breakpoints in barley (Kunzel et al. 2000). The general conclusion from these surveys was that in all species tested, crossing over was concentrated distally; proximal halves of the chromosome arms did not contribute in any significant way to genetic maps.

It has been assumed that in wheat, distal concentration of crossing is a natural consequence of terminal initiation of synapsis which, presumably, favors the establishment of distal chiasmata: first to synapse—first to cross over (Curtis et al. 1991; Lukaszewski and Curtis 1993). As most species, wheat relies primarily on the telomere bouquet for the recognition of homologues, and the initiation of synapsis is terminal (Corredor et al. 2007). There exists a general relationship between bouquet formation, initiation of synapsis and pairing (Bass et al. 1997, 2000). Misalignment at the telomere by structural heterozygosity drastically reduces chiasmate pairing (Moens et al. 1989), with the reduction roughly proportional to the degree of misalignment (Curtis et al. 1991; Lukaszewski 1997). This created an expectation that any part of a wheat chromosome arm would cross over if it was brought sufficiently close to the telomere (Lukaszewski 2003). The experiments of Jones et al. (2002) and Qi et al. (2002) appeared to support this expectation. Disregarding some reduction in multiple crossovers, truncation of a chromosome arm did not affect the pattern of crossover distribution; it was still distal and as high as in a normal arm. However, in a truncated chromosome, the distal region consisted of what is an intercalary region in a normal arm. Hence, intercalary regions brought closer to the telomere had much increased crossover frequencies. It was, therefore, surprising to observe that in an inverted arm of a rye chromosome, the pattern of chiasmata was also inverted (Lukaszewski 2008). The region of the arm which, in a normal chromosome, has the highest concentration of chiasmata (that is, the distal region) retained the highest concentration of chiasmata when, following the inversion, it was placed in the immediate vicinity of the centromere. While the overall chiasma frequency in the inverted arm was reduced, presumably owing to problems with synapsis initiation, the relative chiasma distribution did not change: Regions distal in a normal chromosome still had the highest chiasma frequency even when adjacent to the centromere; regions distal due to inversion (that is, proximal in a normal chromosome) did not cross over at all even in the immediate vicinity of the telomere. These observations were made on a rye (*S. cereale* L.) chromosome in the genetic background of wheat (*T. aestivum* L.). While both species are quite similar in their meiotic chromosome behavior, it was not immediately clear if their behavior could be generalized to wheat or to other species. This article presents observations of the MI pairing pattern in two inverted arms in wheat: 4AL and 2BS.

Materials and methods

In the absence of simple inversions among cytogenetic stocks of wheat, reverse tandem duplications (rtd) were used for this study. Such duplications were discovered among progenies of wide crosses and used to observe breakage–fusion–bridge cycles (Lukaszewski 1995). Original breakpoints which created the inversion–duplications were all in the vicinity of the telomeres, and the duplicated–inverted segments covered ca. 20–30% of the arm length. Among progenies of plants with rtds 2B (S) and 4A (L) (where S and L indicate short and long arms, respectively, involved in the aberration), new rtds were found where essentially entire arms were duplicated and inverted (Figs. 1 and 2).

Using the nomenclature for the description of chromosome segments in wheat proposed by Gill et al. (1991), the original breakpoint that created the duplication–inversion in 2B $(S)_{rtd}$ was in segments 2BS2.7 or 2.8, very close to the telomere; the second break was in segments 2BS1.1 or 1.2, immediately adjacent to the centromere (Figs. 1 and 2). It appears that 90–95% of 2BS is duplicated–inverted. In 4A $(L)_{rtd}$, the breakpoint creating the duplication–inversion was in the distal part of the C-band that constitutes region 4AL2.4; the second break was in the 4AL2.2 segment, immediately adjacent to the centromere (Fig. 1). The first break of the chromosome eliminated the terminal ca. 17% of the long arm.

The long arm of chromosome 4A carries locus *Dne* which, in four doses, severely stunts growth (Lukaszewski 1995) and makes 4A $(L)_{rtd}$ homozygotes difficult to work with. Hence, in this study, only heterozygotes were examined. For $2B(S)_{rtd}$, both homozygotes and heterozygotes were used. For easier identification of the chromosome (bivalent), one of the 2B $(S)_{rtd}$ chromosomes was marked with a rye centromere. Plants with $2B_{rec}$ carrying an introgression of a rye centromere were crossed to homozygotes

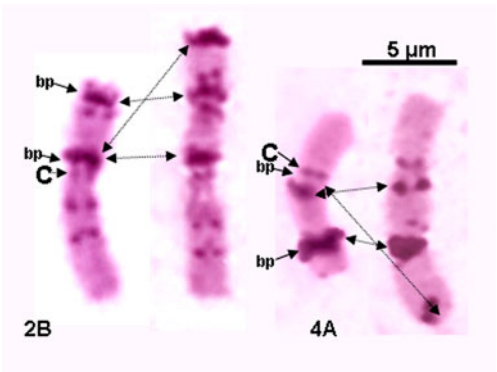

Fig. 1 C-banded chromosomes 2B and 4A and their reverse tandem duplication. In each pair, normal chromosome is on the left; rtd is on the right. *Arrows* point to breakpoints (*bp*) that created the rtds. Distal breakpoints lead to fusion of sister chromatids; proximal breakpoints released the fused chromatids and produced the rtd. *C* centromere

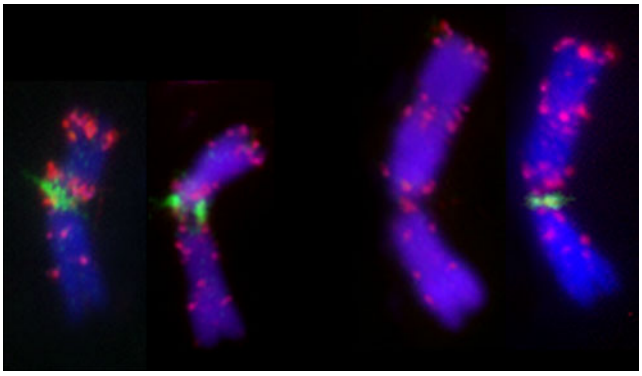

Fig. 2 Chromosomes $2B_{rec}$ (two on the left) and 2B $(S)_{rtd}$ (two on the right). One of the two 2B $(S)_{rtd}$ has the rye centromere (*green*)

of the 2B $(S)_{rtd}$, and the heterozygotes were backcrossed to 2B $(S)_{rtd}$ homozygotes. The resulting progeny were screened by C-banding to identify 2B $(S)_{rtd}$ homozygotes; these, in turn, were screened by in situ probing to identify a chromosome 2B $(S)_{rtd}$ with rye centromere. Along the way, homozygotes 2B $(S)_{rtd}$ and heterozygotes 2B $(s)_{rtd}$ + $2B_{rec}$ were also retained and grown. Chromosome $2B_{rec}$ was produced by repeated cycles of centric misdivision–fusion of wheat 2B with rye 2R (Zhang et al. 2001). The rye centromere in $2B_{rec}$ has no effect pairing on the metaphase I (MI) pairing of the chromosome (Corredor et al. 2007), but owing to its easy hybridization with rye total genomic DNA or the pAWRC.1 rye-centromere-specific probe often referred to in here as the "Francki probe", (Francki 2001), the chromosome can be easily tracked in all stages of the cell cycle, including meiosis.

Tillers were cut from greenhouse grown plants and dissected; anthers judged to be at the desired stages of meiosis (MI, AI and AII) were fixed in a fresh mixture of three parts absolute ethanol to one part glacial acetic acid, stored at 37°C for seven days and frozen at −20°C. Cytological preparations and all in situ probing with labeled DNA were made according to Massoudi-Nejad et al. (2002). The probes used were the rye-centromere-specific probe pAWRC.1 (Francki 2001) and the centromere probe pAct6-09 from *Aegilops squarrosa* which labels all centromeres in wheat, both labeled with DIG-oxygenin and visualized with anti-DIG-FITC; and the GAA microsatellite probe which was labeled with biotin and visualized with avidin-Cy3. The GAA probe was prepared using PCR with $(GAA)_7$ and $(CCT)_7$ primers and wheat genomic DNA as a template, following the protocol of Kubalakova et al. (2000). Counterstaining was with 0.7-μg/ml DAPI in the Vectashield antifade solution (Vector Laboratories, Burlingame, CA, USA). All kits for DNA labeling and detection were from Roche Applied Science (Indianapolis, IN, USA), used according to the manufacturer's instructions. The pAWRC1 and pAct6-09 probes were kindly provided by Dr. B. Friebe, Kansas State University, Manhattan, KS, USA.

Since no chromosome specific means of detection were available for 4A $(L)_{rtd}$, observations of this chromosome were made on meiotic squashes stained with 1.5-μg/ml propidium iodide (PI) in the Vectashield antifade solution, following labeling with probe pAct6-09. Identification of the chromosome was based entirely on the large difference in arm length; the centromeric probe assisted in arm recognition.

All observations were made under a Zeiss Axioscope 20 equipped with epi-fluorescence, recorded with a SPOT RT Color digital camera (Diagnostic Instruments Inc.), and processed using the SPOT Advanced and Adobe Photoshop CS software. All images presented here were manipulated as needed to enhance contrast, remove debris if present, orient the chromosomes in a consistent manner and tone down the background distortion.

Results

The overall pairing levels of the rtd arms here were low, both in homozygotes and heterozygotes. At the same time, pairing frequencies of the arms unaffected by the structural change were typical for wheat chromosome arms (Sallee and Kimber 1978). Therefore, pairing reduction can be attributed to the structural aberrations alone.

Chromosome 4A $(L)_{rtd}$ was not marked by readily identifiable features, and only a fraction of the PMC had bivalents spread well enough for a positive identification of the bivalent. Among 76 PMCs scored at MI, the chromosome was always paired into a bivalent. The short arm was paired in 75 PMCs (98.7%); the long arm was paired in 8 PMCs (10.5%). Whenever there was pairing in the long arm, chiasmata were always positioned at the end of the normal arm and in the middle of the duplicated–inverted arm; in no instance, the terminal end of the duplicated–inverted arm was involved in a chiasmate association (Fig. 3). AI and II were not analyzed.

The attempt to mark chromosome 2B $(S)_{rtd}$ by the introgression of a rye centromere signaled that there was little crossing over between the short arm of $2B_{rec}$ (the chromosome with rye centromere) and the segment of 2BS in normal orientation in 2B $(S)_{rtd}$. Only this type of a crossover can produce chromosome 2B $(S)_{rtd}$ with rye centromere. Among 276 progeny from the cross [2B $(S)_{rtd}$ × $2B_{rec}$] × 2B $(S)_{rtd}$, only a single homozygote $2B(S)_{rtd}$ was found where one $2B(S)_{rtd}$ had the rye centromere (Fig. 2). By accident, one of the retained heterozygotes of 2B $(S)_{rtd}$ had 2B without the rye centromere, for two confirmed crossover events among 276 gametes tested (0.7%).

The overall MI pairing of 2B $(S)_{rtd}$ was lower than that of 4R $(L)_{rtd}$: Among 547 PMCs scored at MI in 2B $(S)_{rtd}$

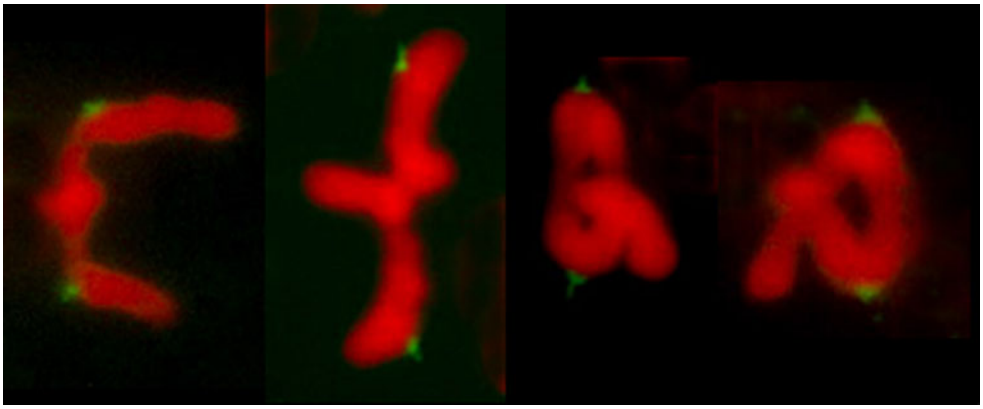

Fig. 3 MI bivalents 4A-4A $(L)_{rtd}$. From left to right, rod paired in S; rod paired in L, the area of the duplication breakpoint; and two rings paired in the area of the duplication breakpoint. Centromeres are labeled *green*

heterozygotes, the short arms were paired with each other in only 6% (33 instances) of PMCs; pairing in L was 84.3%. Analogously, among 186 MI PMCs scored in 2B $(S)_{rtd}$ homozygotes, pairing in S was 5.4% (10 instances), and pairing in L was 74.7%. Both in heterozygotes and homozygotes, chiasmata connecting the two arms were always in the vicinity of the breakpoint that created the duplication–inversion, that is, in the distal region of the normal 2BS and in the middle of the duplicated-inverted arm (Figs. 4 and 5). The single exception to this was a ring bivalent with a bridge-like connection between the telomeric ends of the duplicated arms (Fig. 5). The bridge was labeled by the GAA probe; it appears to have been heterochromatic. It did not look like any other chiasma observed in this or other studies of the authors;

Rtd arms are capable of foldback pairing. Such pairing analyzed only in 2B $(S)_{rtd}$ as 4A $(L)_{rtd}$ could not be identified unequivocally in all PMCs, with bending in the middle of the rtd arm being taken as possible evidence of folding back. In heterozygotes 2B $(S)_{rtd}$ both in univalents and in rod bivalents paired in L, such bending was observed in 75 PMCs (13.7%). In homozygotes, it was almost twice as high (26.3%). Bending in regions away from the middle of the rtd arm was observed only sporadically, in 1–2 cases each. If such bending is interpreted as an interarm crossover, all chiasmata formed in the vicinity of the original breakpoint (Fig. 4); no loops were observed that would suggest foldback pairing with chiasmata in the proximity of the centromere.

Foldback pairing of an rtd creates an acentric fragment in AI and a chromatid bride in AII (Fig. 6). Pairing of the inverted segment of an rtd with the segment of the other chromosome which is in a normal orientation (either in a normal chromosome in a heterozygote or the other rtd in a homozygote) creates a bridge + fragment configuration in AI. Hence, the frequencies of these configurations give a true estimate of crossover frequencies of the two segments in an rtd. In 2B $(S)_{rtd}$ heterozygotes, the frequencies of the AI configurations "bridge + fragment" and "fragment" were 5.4% and 12.8%, respectively, in a sample of 258 PMCs. In AII, among 47 PMCs scored, four (8.5%) had a bridge. In 2B $(S)_{rtd}$ homozygotes, among 249 PMCs scored in AI, 25 (10%) had one or two fragments (6.8%+3.2%, respectively), and 6 (2.4%) had a bridge + fragment. Among 98 PMCs scored in AII, the bridge configuration was present in 9 PMCs (9.2%). This is almost twice the cross-chromosome pairing frequency of the arms but less than one half of the suspected foldback pairing of the duplicated/inverted arms. There is some basis for a suspicion that the 2B $(S)_{rtd}$ homozygote analyzed was heterozygous for another inversion that produced anaphase bridges with low frequencies. It is possible that the results listed here may carry an error of unknown proportion.

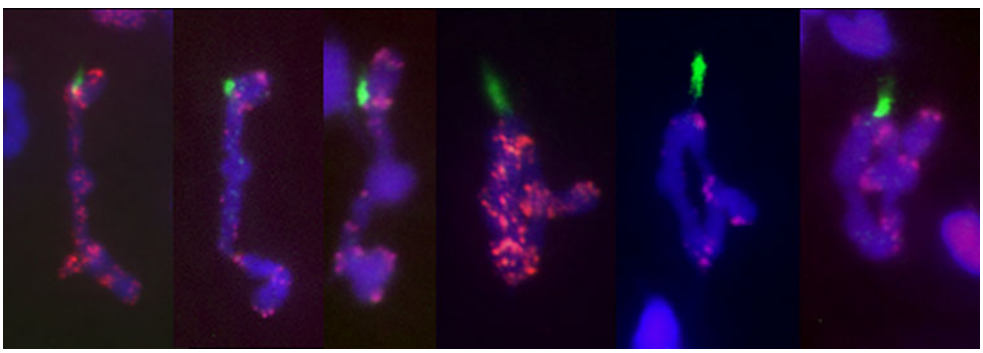

Fig. 4 Bivalents $2B_{rec}$ (*green* centromere) with 2B $(S)_{rtd}$: three on the left are rods paired in the long arms; in two of them, the rtd arm appears to be involved in fold-back pairing in the area of the duplication breakpoint. In all bivalents, $2B_{rec}$ is on top; pairing in S is always of the distal end of the normal arm with the midsection of the duplication

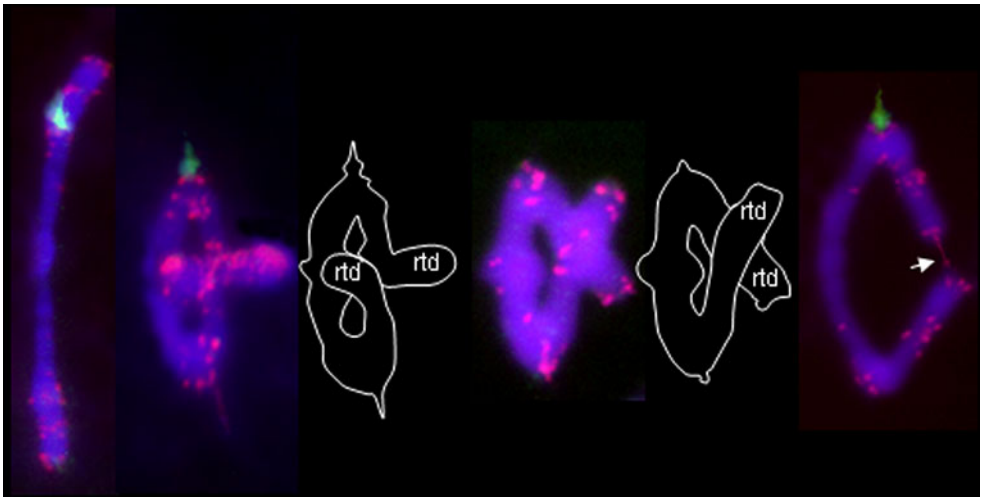

Fig 5 Bivalents in 2B $(S)_{rtd}$ homozygotes. From left to right: rod bivalent paired in L, no fold-back pairing in the rtd arms; two ring bivalents with pairing in the midsection of the rtd arms; far right: the single observed ring bivalent with a heterochromatic thread connecting the telomeric ends of the duplicated arms. Silhouettes of the middle two ring bivalents illustrate the arrangement of the rtd arms (*rtd*)

Discussion

An earlier study has shown that inversion of the long arm of rye chromosome 1R inverted the pattern of chiasma distribution (Lukaszewski 2008). This study attempted to verify if the same system operated in wheat. Simple arm inversions have not been described in wheat; the only stocks suitable for analysis were reverse tandem duplications. Four of such rtds have been described earlier, and their likely origin was illustrated and explained (Lukaszewski 1995). By now, the number of rtds in wheat has grown and includes the two used here, covering most of the arms 2BS and 4AL (Fig. 1). For the sake of this study, it needs to be emphasized that rtds originate by fusion of sister chromatids in the distal part of the arm (distal breakpoints on Fig. 1), followed by breakage of the resulting chromatid bridge in the next mitotic anaphase,

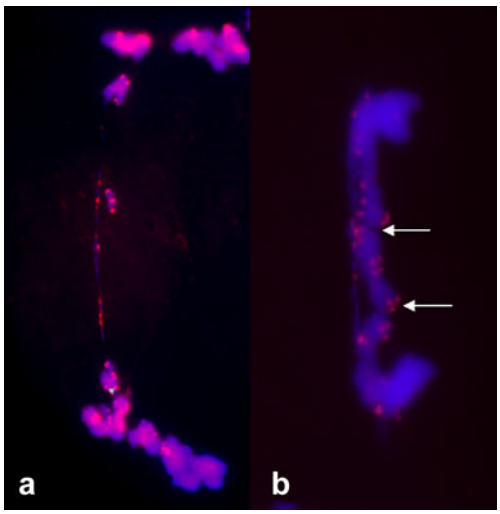

Fig. 6 **a** Bridge and a fragment configuration in anaphase I of meiosis in a heterozygote $2B_{rec}$ + 2B $(S)_{rtd}$. **b** The moment of release of an acentric fragment in early AI of meiosis in a homozygote $2B(S)_{rtd}$. The ends of the fragment are arrowed; the chromatid bridge is on the left of the fragment

here in the immediate vicinity of the centromere (proximal breakpoints; Fig. 1). Therefore, the two segments in each rtd are as similar as sister chromatids, but they are in inverted orientation relative to each other; the inverted segment is distal; the proximal copy retains its normal orientation.

Chiasma distributions in rtds analyzed here show clearly that similarly to the inverted 1R analyzed earlier (Lukaszewski 2008), also in two wheat, the pattern of crossovers in chromosome arms is inverted in arm inversions. In normal chromosomes in both species, crossing over is restricted to the distal about one half of each arm (physically), where it increases exponentially with the proximity to the telomere; the proximal halves do not cross over (Lukaszewski 1992; Lukaszewski and Curtis 1993) and do not contribute to genetic maps (Gill et al. 1996; Werner et al. 1992). All chiasmata in the duplicated–inverted arms 2BS and 4AL in this study were in the vicinity of the duplication/inversion breakpoints (Figs. 3, 4 and 5). These regions are distal in normal, unaltered chromosome arms and contribute essentially entire genetic lengths of the arms. An rtd places them in the middle of the arm, but they still retain their highest crossover frequency. Positioning of a normally proximal chromosome region at the end of an rtd arm does not lead to chiasma formation. Hence, both in wheat and rye, the pattern of crossovers (relative distribution and frequency) along a chromosome arm is a characteristic of specific chromosome segments and independent of the segment's location on the telomere–centromere axis. The single case of a terminal connection between the ends of duplicated–inverted arms was likely a heterochromatic bridge. It is not clear how it might have formed, but heterochromatic connections between homologues are believe responsible for holding pairs of homologues together in achiasmate meiosis (Dernburg et al. 1996; Hughes et al. 2009).

MI pairing levels of the rtds in this study and of the $1R_{inv}$ earlier (Lukaszewski 2008) were reduced relative to structurally unaltered chromosome arms (Sallee and Kimber

1978). This reduction was likely caused by the structural aberration itself which impaired synapsis. However, the relative distribution of chiasmata remained unchanged despite inversion, and there are indications that synapsis still proceeded from the telomere toward the centromere. The pattern of synapsis was not studied here; it will be addressed once the rtds are transferred to the rye background.

Genetic mapping was not performed in this study; the duplicated segments are essentially identical to the original segments, and no genetic variation is available. However, the nature of rtds itself permits a distinction as to which copy of the original arm is involved in crossing over. Foldback pairing with an interchromatid crossover produces an acentric fragment in AI and a bridge in AII; a crossover involving the duplicated–inverted segment of an rtd and its standard-orientation counterpart in the other chromosome produces a bridge + fragment configuration in AI (Fig. 6). When a marked centromere is used, a crossover involving two segments in normal orientation in an rtd heterozygote produces an rtd with a marked centromere and a normal chromosome with a normal centromere. The combinations that are cytologically undetectable can be inferred by subtraction from the frequency of the MI pairing. Following this reasoning, in 2B $(S)_{rtd}$ heterozygotes, crossovers involving two segments in normal orientation accounted for ca. 25% of all events, suggesting that the inverted segment was favored for crossing over. In an rtd, the inverted segments are distal to their normal-orientation counterparts. This is the main indication that the initiation of synapsis was probably still via the telomere bouquet as it is the case in normal chromosomes (Fussel 1987; Harper et al. 2004).

This telomeric initiation of synapsis was thought to be responsible for distal concentration of crossing over in wheat, by offering a priority to distal chiasmata. This assumption appeared to be confirmed when crossover frequencies in distal regions of truncated chromosomes were increased (Jones et al. 2002; Qi et al. 2002). The inversion chromosomes demonstrate that there are limits to such increases: A proximal segment of a chromosome arm will not cross over regardless of its position in the chromosome; distal segments retain their highest crossover rates regardless of their position and orientation. The overall crossover frequencies in inverted arms are reduced, perhaps for structural reasons that complicate synapsis, but relative frequencies remain the same: The most distal segments of the recombining portion of an arm retains its highest crossover frequency in an inversion, when residing next to the centromere (in inverted 1RL) or in the middle of the arm (in rtds). The proximal region of a normal arm which never forms chiasmata in a normal chromosome does not do so even when an inversion places it in the seemingly most favorable distal position. Taken together, observations made in the course of this study and on $1R_{inv}$ earlier (Lukaszewski 2008) and by Naranjo et al (2010) imply that imposed on a chromosome segment are not only the ability/inability to crossover but also specific crossover frequencies. The effect of the centromere–telomere position appears to hold but with a serious limitation: Here, distal copies of the duplicated segments had a 3:1 advantage over the proximal copies in securing crossovers even though they were inverted. This was the case even in heterozygotes, where paring was between a copy in a normal orientation (in the normal chromosome) and the inverted copy (the rtd chromosome) even when immediately adjacent, but more proximal, was a copy in a normal orientation.

Foldback pairing of rtds was higher than pairing with the homologue. Why this was the case is not clear. The chiasmata-forming regions of rtds are in the middle of very long arms and, hence, far away from the standard points of synapsis initiation. With the overall low levels of paring observed here, perhaps random contacts of chromosomes initiate chiasmate pairing. Rapid chromosome movements in early stages of meiosis were observed in yeast (Chikashige et al. 1994) and maize (Sheehan and Pawlowski 2009), but at least in yeast, they appear to be directly related to the telomere movement. An alternative system of homologue recognition is associated with the pairing centers in *Caenorhabditis elegans* and *Droshophila melanogaster* males, where homologue recognition and pairing initiation is brought about by the interactions of specific chromosome sites with cytoskeleton (Tsai and McKee 2011). A non-bouquet-based system was postulated for $1R_{inv}$ (Lukaszewski 2008), where infrequently, but regularly, terminal regions of normal chromosomes found synapsed and crossover with their homologous segments in the inversions chromosomes, located next to the centromeres, hence directly across the entire volume of the nucleus. It does not seem likely that even the movements described by Sheehan and Pawlowski (2009) in maize would account for such long-distance homologue attraction. However, they could facilitate recognition of adjacent homologous segments in the middle of the rtd arms studied here.

The distinction of chromosome segments into crossover-capable and crossover-incapable does not appear irreversible. Under some specific circumstance, chromosome segments which normally do not cross over may do so: Random distribution of chiasmata was observed in a line of interspecific hybrid of rye (Jones 1967), and proximal breakpoints have been observed in homoeologous recombination induced by the absence of *Ph1* locus in wheat (Lukaszewski et al. 2004). Leaving alone the issue of frequencies of such events, recent studies of genome synteny in grasses indicate that in the evolution of current karyotypes, the issue of activation/inactivation of crossing over in some regions of the genome must have been dealt with on at least some occasions. Comparative genomics of Close et al. (2009), Luo et al. (2009) and Mayer

et al. (2011) illustrate that the reduction in chromosome number in grasses was often by insertion of entire chromosomes into other chromosomes. Chromosome 7 of barley offers perhaps the most striking evidence of such insertion, where an entire equivalent of rice chromosome 8 resides in its centromere region, and on the genetic map, it is reduced to a point, hence not undergoing crossing over at all (Close et al. 2009). The real-life rice chromosome 8 crossovers normally, producing a normal genetic map. This and other examples in synteny comparisons among wheat, barley, sorghum, rice and Brachypodium (Close et al. 2009; Luo et al. 2009; Mayer et al. 2011) show that looking at the scale of an entire chromosome, the pattern of crossing is not a function of the gene content or gene concentration, but must be imposed on the chromosome by some other system. A crossover license can be granted under some conditions to otherwise unlicensed regions, such as the two examples in rye and wheat mentioned previously, and perhaps revoked, such as following the insertion of the equivalent of rice chromosome 8 into the centromeric regions of another chromosome. Whether it is a system of allocation of double strand breaks or utilization of such DSB or even the system of DSB repair (sister vs non-sister chromatids), it is not clear at all. At least, as far as the distribution of DSB, published figures from maize (Pawlowski and Cande 2005) suggest that DSB is scattered all over the genome.

This brings in a series of questions on the nature of the crossover licensing system. Given that a single gene can change the chiasma pattern, as in the *Allium* hybrids (Khrustaleva and Kik 1998), the system must be genetic in nature and can probably be controlled and exploited. This system may, in fact, be similar to specific crossover frequencies of the double strand break (DSB) regions on yeast chromosomes. Alien DNA in yeast does not form DSBs and does not recombine, but transfer of a native yeast DSB region into an artificial chromosome not only permits crossing over; this crossing over occurs with a frequency typical for the yeast DNA segment in its native location in the genome (Ross et al. 2000).

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