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RESEARCH ARTICLE

Unexpected behavior of an inverted rye chromosome arm in wheat

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Abstract Distal location of chiasmata in chromosome arms is thought to be a consequence of the distal initiation of synapsis. Observations of meiotic behavior of a rye chromosome with an inverted arm show that patterns of chiasma distribution and frequency are also inverted; therefore, the patterns of synapsis and chiasma distribution are independent, and recombination frequency along a chromosome is position-independent and segment-specific. Since cases of random distribution of chiasmata and recombination are known in rye, a genetic mechanism must be present that licenses specific chromosome regions for recombination. Large differences in the metaphase I pairing of the inversion in various combinations of two armed and telocentric chromosomes confirm the major role of the telomere bouquet in early homologue recognition. However, occasional synapsis and chiasmate pairing of the distal regions of normal arms with the proximal regions of the inversion suggest that an alternative mechanism for juxtaposing of homologues must also be present. Synapsis in inversion heterozygotes was mostly complete but in the antiparallel orientation, hence defying homology, but non-homologues never synapsed. Instances of synapsis strictly limited to the chiasma-capable segments of the arm suggest that, in rye, both recombination-dependent and recombination-independent mechanisms for homologue recognition must be present.

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Introduction

Rye (*Secale cereale* L.) has been a favorite subject in early studies on meiotic chromosome pairing and, especially, on the frequency and distribution of chiasmata (Rees and Thompson 1956, 1958; Sybenga 1965, 1966a; Jones 1967, 1978; Orellana and Giraldez 1981; De Jong et al. 1991), mostly for its low number (n = 7) of very large chromosomes. It is a typical example of a species with distal chiasmata where crossing takes place near the telomeres and distal from the centromeres. Even a quick scan of published figures and of any MI preparation immediately shows a majority of chiasmata to be so distal that they are often called end-to-end (Jones 1978, 1987), even though the prominent terminal heterochromatic blocks (telomeric C-bands) do not participate in crossing-over (De Jong et al. 1991). Intercalary chiasmata in rye are infrequent. Even when present, the intercalary chiasmata appear to be located in the distal quarter of chromosome arms; those located in the middle of the arms seem rare; proximal chiasmata are non-existent. Interestingly, even in a haploid of rye, rare metaphase I chiasmata were located in the immediate vicinity of the telomeric C-bands (De Jong et al. 1991). It has been concluded that the distal distribution of chiasmata in rye is under genetic control: among progeny of an interspecific hybrid *S. dighoricum* × *S. turkestanicum*, both of which have distal chiasmata, Jones (1967) identified one family with random chiasma distribution. Some of these chiasmata were localized in the proximal halves of the arms.

Distal distribution of chiasmata is common among Gramineae: A pattern similar to rye is present in wheat and its close relatives in the genus *Aegilops*, in barley, oats, *Lolium*, *Festuca*, and probably many others. This distal concentration of chiasmata results in distal concentration of crossing-over. In rye, wheat, and barley, the distal halves of

the arms contribute almost the entire lengths of genetic maps (Curtis and Lukaszewski 1991; Gill et al. 1996; Künzel et al. 2000; Lukaszewski 1992; Lukaszewski and Curtis 1993). Distal distribution of chiasma/crossing-over in wheat was believed to be a consequence of terminal initiation of synapsis and strong positive chiasma interference (Lukaszewski and Curtis 1992a). Exercises with genetic mapping in truncated wheat chromosomes suggested that, given an opportunity for the initiation of synapsis, as present in the vicinity of the telomere, any part of a wheat chromosome would be capable of high crossing-over (Jones et al. 2002; Qi et al. 2002; Lukaszewski 2003). This article presents evidence that the pattern of chiasma distribution in a rye chromosome arm is independent of the pattern of synapsis progression: in an inverted arm of a rye chromosome in wheat, synapsis still appears to be initiated distally in a majority of cases, but chiasmata are formed in the same region as before the inversion, now in the vicinity of the centromere. Even the chiasma frequency distribution within the chiasma-capable portion of the arm is preserved following the inversion. The observations presented in this paper also reinforce the notion of a major role of the leptotene bouquet in the search for a homologous pairing partner but at the same time imply that some alternative mechanism for homologue search and alignment must be present.

Materials and methods

During centric fission–fusion exercises with the 1RS.1BL wheat–rye translocation in wheat cv. 'Pavon 76' (Lukaszewski 1997a), a chromosome 1R was found in which the long arm underwent an inversion of most of its length (Fig. 1). One of the inversion breakpoints was in the vicinity of the telomere, between C-bands once labeled L2 and L3 (Lukaszewski 1992) where L3 is the telomeric and L2 the subtelomeric C-bands on 1RL (Fig. 1); the other breakpoint was within the centromeric region, very close to the primary

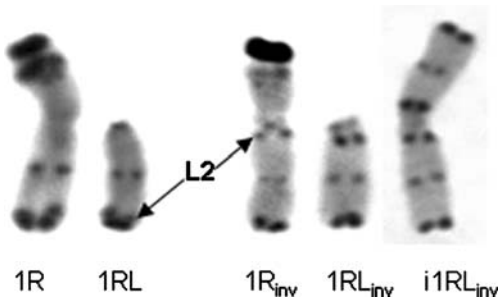

Fig. 1 Normal and inversion chromosomes 1R, their telocentrics (1RL and 1RL$_{inv}$), and isochromosome i1RL$_{inv}$ following C-banding. L2 is a subtelomeric C-band that inversion placed adjacent to the centromere

constriction. In a similar chromosome 1R, the physical distance between bands L2 and L3 was estimated at 1% of the chromosome length (Lukaszewski 1992) or about 1.7% of the long arm's length. It appears, therefore, that disregarding the length of the terminal C-band, about 90–95% of the arm is inverted.

Chromosome 1R with inverted long arm, henceforth designated 1R$_{inv}$, was present as a monosomic substitution for wheat chromosome 1A. Following self-pollination, a disomic substitution of 1R$_{inv}$(1A) was selected. This substitution was crossed to euploid Pavon 76, and among progeny of the resulting double monosomics, telocentric 1RL$_{inv}$ and isochromosomes (i1RL$_{inv}$) were identified, selected, and grown. Their progenies were screened to select ditelosomics (Dt). To observe the meiotic behavior of the inverted arm in heterozygotes, the 1R$_{inv}$(1A) and Dt1RL$_{inv}$(1A) lines were crossed to Pavon1R(1A) where 1R originated from a wheat line E12165 and to two substitutions1R(1D) in Pavon: of 1R from triticale ANOAS (1R$_{an}$) and of an engineered chromosome 1R.1D$_{5+10}$–2 (Lukaszewski and Curtis 1992b). Telocentric chromosome 1RL$_{an}$ was produced by centric misdivision from 1R$_{an}$ and crossed to Pavon 1R$_{inv}$ (1A) and 1RL$_{inv}$(1A).

In each generation, samples of progenies from self-pollination and crosses were screened by C-banding using a standard protocol (Lukaszewski and Xu 1995), and plants with desired chromosome constitutions were selected and grown in the greenhouse at the University of California, Riverside, campus. At meiosis, one of the three anthers in each tested flower was live-stained in 2% acetocarmine and checked under a microscope. If the desired stages of meiosis were present, the remaining two anthers of the flower were fixed in a 3:1 mixture of glacial acetic acid and absolute ethanol at 37°C for 7days, stained in acetocarmine for 2h, fixed again, and frozen at −18°C until needed. For fixation, anthers were selected with pollen mother cells (PMCs) in the pachytene–diplotene and metaphase I (MI). Meiotic analyses were performed on material collected during three consecutive growing seasons: spring 2006 and 2007, and fall 2006.

Squash preparations of the PMCs and all in situ probing with labeled DNA were made according to Massoudi-Nejad et al. (2002). The probes were the total genomic DNA of rye, rye-specific centromeric probe pAWRC.1 (Francki 2001), and clone pTa71 with the 18S-5.8S-26S cluster of wheat ribosomal RNA genes (Gerlach and Bedbrook 1979). Total genomic DNA and clone pAWRC.1 were labeled with digoxigenin by digoxigenin (DIG)-nick translation and visualized with anti-DIG-flourescein isothiocyanate (FITC). Clone pAWRC.1 was also directly labeled with rhodamine. DIG and rhodamine labeling and the anti-DIG fluorescein kits were from Roche Applied Science (USA), used according to the manufacturer's instructions. The pAWRC1

was kindly provided by Dr. B. Friebe of Kansas State University, Manhattan, KS; pTa71 was provided by Dr. K. Gill, then also of KSU, Manhattan, KS. All counterstaining was with 1.5μg/ml propidium iodide in the Vectashield antifade solution (Vector Laboratories).

The probes were used either in single or double labeling experiments. In single labeling, all probes were DIG-labeled. In double labeling, DIG-labeled total genomic DNA of rye was used in a combination with directly rhodamine-labeled centromeric probe pAWRC.1, or both total genomic rye DNA and the centromeric probe were labeled with DIG–oxygenin, but the total genomic rye probe was used at about 25% of its standard working concentration. Low concentration of the FITC-labeled total genomic probe in combination with red counterstaining produced yellow labeling of the entire chromosome and bright green labeling of the terminal C-band on the short arm, while the centromeric probe used in a normal concentration produced bright green labeling of the centromere. These probes, used in various combinations, permitted clear identification of individual arms of the studied chromosome.

In probing with total genomic DNA, the standard probe to block ratio was about 1:150; in probing with pAWRC1, herring sperm DNA fragmented to ca. 200–300bp was used, usually in a ratio of 1:100. Observations were made under a Zeiss Axioscope 20 equipped with epi-fluorescence, recorded with a Spot RT Color digital camera (Diagnostic Instruments) and processed using the Spot Advanced and Adobe Photoshop CS software. All images presented in this paper were manipulated to enhance contrast.

On each preparation, all pollen mother cells (PMCs) available for and accessible to analysis were scored. The proportions of PMCs with paired or non-paired arms in various combinations were compared by the χ^2 test.

Results

The genome-wide levels of the MI chromosome pairing in the tested wheat lines were normal and did not appear to be affected to any appreciable extent by the presence of the rye chromosome pair or by the season. The short arms of 1R were not the target of the focus of this study, but their pairing was scored as a control measure. They paired with variable frequencies, ranging from 51.9% in the $1R_{inv}$ + $1R_{\#2}$ combination to 80.3% in $1R_{inv}$(1A). This pairing frequency appeared to be combination-specific; it was higher for identical vs. non-identical 1RS arms and was not related to pairing of the long arms. It will not be further discussed.

Pairing of normal 1RL arms was always high and showed little variation from year to year or from combination to combination. Therefore, the frequencies for pairing of the 1RL arm for all three sources were combined, giving the mean of 94.4%; for Dt $1RL_{an}$, it was 91.2%, and for the complete plus telosomic, it was 92.6% (Table 1). The average MI pairing frequency for normal 1RL in the entire study was 93.4%, and chiasmata were always distal. In the sample of 499 PMCs scored only one clearly intercalary chiasma was observed, and it appeared to be located in the distal half of the arm.

The MI-pairing frequencies of the inverted arm were variable and depended primarily on the structure of the pairing partner and the position of the centromere. More importantly, two positions of chiasmata, terminal and proximal, were evident in all combinations where at least one arm was inverted (Fig. 2b, left). The terminal chiasmata were likely formed in the non-inverted segment of the arm, distal to the distal inversion breakpoint, and in the immediate vicinity of the terminal C-band. Among the total of 2,587 PMC scored in combinations involving

Table 1 Meiotic metaphase I pairing configurations of the normal (n) and inverted (inv) long arms of rye chromosome 1R in wheat

Chromosomes present	Number of PMCs scored	Frequencies of the 1RL arms associations		
		Telomere-to-telomere (%)	Centromere-to-centromere (%)	Telomere-to-centromere (%)
$1R_n$ + $1R_n$	289	94.4	0.0	0.0
$1R_{inv}$ + $1R_{inv}$	691	1.7	57.6	0.0
$1R_{inv}$ + $1R_n$	680	2.1	0.0	2.5
$1RL_n$ + $1RL_n$	80	91.2	0.0	0.0
$1RL_{inv}$ + $1RL_{inv}$	469	2.6	78.0	2.3
$1RL_{inv}$ + $1RL_n$	195	0.5	0.0	55.9
iso$1RL_{inv}$	59	0.0	84.7[a]	0.0
$1R_n$ + $1RL_n$	135	92.6	0.0	0.0
$1R_{inv}$ + $1RL_{inv}$	164	1.8	12.8	0.0
$1R_{inv}$ + $1RL_n$	141	0.0	0.0	9.2
$1R_n$ + $1RL_{inv}$	606	2.3	0.0	43.7

Frequencies for $1R_n$ + $1R_{inv}$ and $1R_n$ + $1RL_{inv}$ represent means for chromosomes 1R from three sources: $1R_e$, $1R_{an}$, and 1R#2; frequencies for $1RL_n$ + $1RL_n$ and $1R_n$ + $1RL_n$ are for $1R_{an}$ only.

[a] Frequency of iso$1RL_{inv}$ bent at the centromere

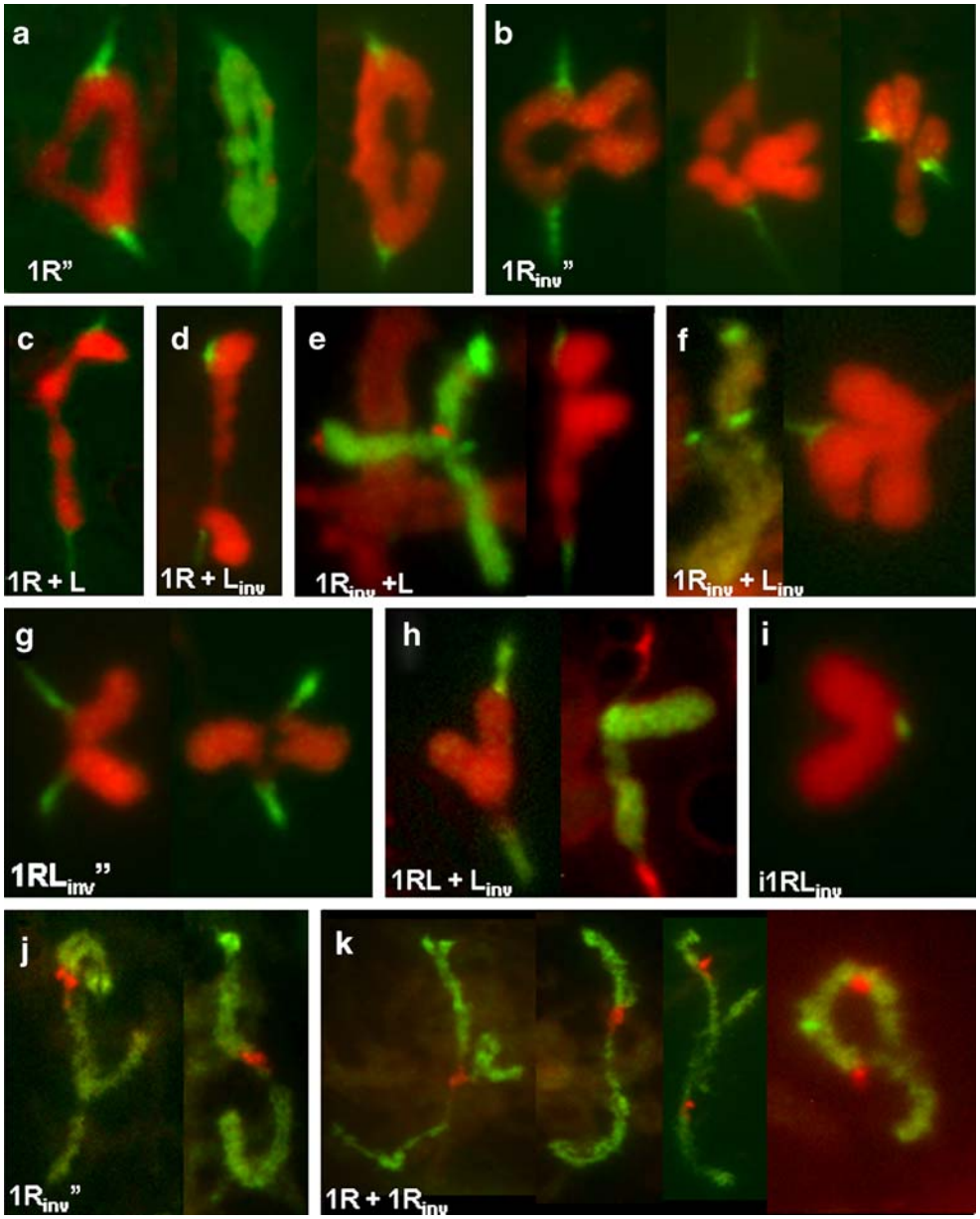

Fig. 2 Chiasmate and synaptic configurations of rye chromosome 1R with inverted long arm ($1R_{inv}$) visualized by genomic in situ hybridisation (GISH)/flourescent in situ hybridization. On all photographs, *green staining* of the entire chromosomes is by GISH with rye total genomic probe labeled with DIG–FITC; centromeres are visualized with the rye-centromere-specific pAWRC.1 probe labeled either with DIG–FITC (*green*) or directly labeled with rhodamine (*red*). **a** Typical ring bivalents 1R; in each, the short arms are to the left; **b** bivalents of $1R_{inv}$ homozygotes, short arms are to the left. In each bivalent, there is a chiasma in L adjacent to the centromere; **c–f** heteromorphic bivalents of two-armed chromosome with a telocentric; **c** normal chromosomes, terminal chiasma; **d** in 1R + $1RL_{inv}$ the latter is paired by the centromeric region to the telomere region of two-armed chromosome; **e** early diplotene (*left*) and MI (*right*) of $1R_{inv}$ + 1RL; distal region of the telocentric is paired with the centromere region of $1R_{inv}$; **f** $1R_{inv}$ + $1RL_{inv}$; chromosomes are completely synapsed in the diplotene (*left*) but chiasma in MI is adjacent to the centromere; **g**, **h** in ditelosomics, chiasmata adjacent to the centromere in $1RL_{inv}$″ (**g**) and antiparallel orientation in $1RL_{inv}$ + 1RL (**h**); **j** isochromosome $1RL_{inv}$ bent at the centromere, presumably because of a proximal chiasma; **k** and **l** *bright green* signal identifies the short arm; **k** synapsis of the long arm of the $1R_{inv}$ homozygote can either cover only the portion of the arm capable of crossing-over (*left*) or the entire arm (*right*); **l** synaptic configurations of the inversion heterozygote, *from left to right*: no synapsis in L, complete hence antiparallel synapsis in L, synapsis in L limited to the segment capable of recombination

the inverted arm, the mean frequency of such terminal chiasmata was 2.01%, ranging from 0 in a sample of 141 PMCs in the combination $1R_{inv}$ + 1RL to 2.6% in a sample of 469 PMC in ditelosomic $1RL_{inv}$ (Table 1). Given that the segment L2-terminal C-band, in which the inversion breakpoint is located was mapped genetically at 2.9cM in rye and 2.0cM in triticale (Lukaszewski 1992), the breakpoint is likely located in the distal half of this segment,

physically less than approximately 1% of the euchromatic portion of the arm away from the terminal C-band. Since the MI pairing frequency of this non-inverted segment appears to be constant across all combinations studied, it will be ignored in subsequent considerations.

In homozygotes/disomics for a normal 1RL arm, the MI pairing frequency attributable to the segment involved in the inversion ranged from 89% to 92%, and all chiasmata were in the distal regions of the arm. In inversion homozygotes, the same segment paired at 57.6% in the two-armed chromosome and at 78% in a ditelosomic (Table 1), and chiasmata were in the centromere region (Fig. 2). Therefore, relative to a normal arm, the inversion-reduced MI chiasmate pairing by approximately 35% in a two-armed chromosome and by approximately 12% and in a ditelosome. Absence of the short arm significantly (χ^2 = 51.92, $p > 0.001$) increased pairing frequency of the inverted arm. The inversion also changed the position of chiasmata, from distal/terminal in a normal arm to near-centromeric in the inversion.

In inversion heterozygotes, the MI pairing was highly combination-dependent. When the $1R_{inv}$ was present with normal 1R, there was essentially no pairing in the long arms. On rare occasions (2.5%), a chiasma bound the telomeric region of the long arm of one chromosome, assumed to be the normal arm, to the centromeric region of the second chromosome, assumed to be the inversion chromosome. The assumption is based on the position of chiasmata in disomics for normal and inverted arms. In combinations involving a two-armed chromosome with a telocentric, pairing frequency of the long arms was greatly affected by the combination: When the two armed chromosome carried the inversion and the telocentric was normal, MI pairing was 9.2% and in all instances, a chiasma bound the distal end of the telocentric with the centromere area of the two-armed inversion chromosome. However, when the two-armed chromosome was normal and the telocentric was inverted, the MI pairing was 43.7%, and it was of the distal end of the two-armed chromosome to the centromere region of the inversion telocentric. This difference in pairing frequency is so large that no χ^2 test was deemed necessary. In the combination of a normal with an inverted telocentric, pairing was 55.9% and it was always in antiparallel orientation, the telomere region of one telocentric to the centromere region of the other. Anaphase I configurations typical for inversion heterozygotes were beyond the scope of this study and will not be discussed.

In isochromosome $1RL_{inv}$, chiasmata at the ends of the arms or in intercalary positions would have produced easily recognizable MI figures: a ring univalent or a small ring with two crossed arms. No such figures were observed in the sample of 59 PMCs scored. Presence of chiasmata in the immediate vicinity of the centromere was difficult to ascertain. Such a location of a chiasma would presumably bend the chromosome at the centromere. In the scored sample, 50 PMCs (84.7%) had the $i1RL_{inv}$ bent at the centromere (Fig. 2j). Whether this represents the actual chiasmate pairing of the arms in the immediate vicinity of the centromere or a combination of chiasmate pairing and natural bending of a univalent is not clear. Among 35 wheat univalents encountered in the analyzed PMCs, four (11.4%) were bent and 31 were straight, suggesting that natural bending of univalents was infrequent. However, these were wheat univalents 1A, a rather small chromosome; $1RL_{inv}$ is a much larger chromosome, and perhaps, it would bend more frequently even without a chiasma. Still, it appears likely that a large proportion of bending of $i1RL_{inv}$ was due to a crossover located very close to the centromere. No chiasmata in intercalary positions in $i1RL_{inv}$ were observed.

Synaptic configurations were observed in pachytene and early diplotene. For 1RS, there was a good correspondence between pachytene synapsis and MI pairing in that 86.3% of the arms were synapsed, while 80.3% were paired in MI. For 1RL, in all combination with reduced MI pairing, such as in inversion heterozygotes, synapsis frequency was always significantly higher than the MI pairing frequency.

In a disomic for a normal 1R, the long arm was synapsed along its entire length in 48 out of 49 PMCs scored (Table 2). Frequencies of synapsis for the inversion chromosome varied both as to frequency and location. In $1R_{inv}$ homozygotes, the long arms were synapsed in 81.2% of PMCs analyzed, while in MI the two were paired 59.3%. More striking is the difference in distribution of synapsis: The two arms were synapsed along their entire length in 65.6% of PMCs and only in the proximal region of the arms in another 14.6% of PMC (Fig. 2k), while all chiasmate pairing was only in the proximal region. In inversion homozygotes when one chromosome was two-armed and the other was telocentric ($1R_{inv}$ + $1RL_{inv}$), the long arms synapsed in 50% of PMCs (either along the entire length or some segment of it), but the MI pairing was 14.6%, a significant reduction both in synapsis and MI pairing over a normal chromosome or a disomic for two-armed inversion chromosomes, but the reduction in MI pairing was far greater. In inversion heterozygotes with two-armed chromosomes, most synapsis of the long arms was parallel along the entire length of both arms (Fig. 2l; Table 2).

Discussion

The behavior of the inverted 1RL arm in synaptic and chiasmate stages of meiosis raises several interesting questions regarding two basic phenomena. One group of questions deals with the genetic control of chiasma

Table 2 Frequencies of synapsis (pachytene and early diplotene) of the normal (n) and inverted (inv) long arms of rye chromosome 1R in wheat. In some instances, the orientation of the arms in synapsed bivalents was scored in sub-samples of cells from double-labeled preparations

Chromosomes present	Number of PMCs scored	PMCs with complete synapsis (%)	PMCs with only proximal synapsis (%)	PMCs with only distal synapsis (%)	Synapsis telomere to-centromere (%)
$1R_n + 1R_n$	49	98.0	0	0	0
$1R_{inv} + 1R_{inv}$	96	65.6	14.6	1.0%	0
$1R_{inv} + 1R_n$	229	32.6	0	0	5.8%
$1RL_{inv} + 1RL_{inv}$	16	75.0	12.5	0	0
$1RL_{inv} + 1RL_n$	173	24.3	0	0	10.8%
$1R_{inv} + 1RL_{inv}$	26	46.2	3.8	0	0
$1R_{inv} + 1RL_n$	253	21.9	0	0	15.1%[a]

[a] 11% distal region of telocentric synapsed to centromere of inversion; 4.1% telomere of inversion synapsed to centromere region of telocentric

distribution along the chromosome arm, including an apparent division of the arm into regions where recombination is permitted and where it is not, and within the region where it is permitted, on the control of the chiasma, hence crossover, frequency distribution. The other set of questions deals with pre-synaptic stages of homologue search and recognition.

The MI behavior of the inverted 1RL arm clearly shows that it has a region capable of crossing over, hence chiasma formation, and a region that is not. In a normal configuration, the chiasma-capable region spans the distal about one half of the arm, probably less. In disomics for normal, two-armed chromosomes, this produces bivalents with shapes typical for distal chiasmata (Fig. 2a). When the arm is inverted, the region capable of chiasma formation is positioned next to the centromere, and this is where chiasmata are now formed, producing bivalents with shapes rather unusual for rye (Fig. 2b). This change in chiasma pattern/bivalent shape in inversion homozygotes illustrates that the ability or inability of a segment to crossovers is independent of its position on the telomere–centromere axis.

No less interesting is the pattern of chiasma frequency distribution in the recombining segment of the arm itself. While no specific technique was used to monitor precise chiasma/crossover locations and their frequencies, it was obvious, and Fig. 2 illustrates this clearly, that in the inverted arm the chiasma frequency distribution is inverted as well. In a normal 1RL, chiasmata concentrate in the distal region of the chiasma-capable segment and in the euchromatic segment adjacent to the terminal C-band, and rarely form in the most proximal reaches of the chiasma-capable segment, that is, in the middle of the arm. Consequently, recombination frequencies are the highest in the most distal region of the arm (Lukaszewski 1992). In the inverted arm, a great majority of chiasmata were directly adjacent to the centromere, and none were observed in the middle of the arm (Fig. 2). These two observations, that the ability or inability of a region to recombine is segment-specific and does not change with the change of segment's position on the centromere–telomere axis, and that chiasma frequency in the recombining region is also independent of the orientation and position of the segment imply two levels of chiasma control. One affects the ability/inability to crossover, and the other controls the specific crossover frequency. Both these types of control appear to be independent of the pattern of synapsis.

The pattern of synapsis in $1R_{inv}$ will be a focus of a separate study (T. Naranjo, personal communication), but there is no reason to suspect that the general pattern of the telomeric synapsis initiation was abandoned in this arm. There were numerous indications that inversion or not, synapsis still followed the general pattern for rye, originating in the vicinity of the telomere and progressing toward the centromere (Gillies 1985). Several late zygotene PMCs in inversion homozygotes and heterozygotes showed synapsis of almost the entire chromosome except for the centromeric regions of various lengths. So, if synapsis in the inverted arm was initiated distally and then proceeded toward the centromere, and all indication point to this, and had the pattern of synapsis dictated chiasma distribution in the recombining portions of the inverted arm, most chiasmata should have formed in about the middle of the arm, that is, in the first synapsed region of the chiasma-capable segment of the arm. Instead, chiasmata concentrated adjacent to the centromere, in the region that very likely synapsed last. This is a clear confirmation that there is no relationship between the pattern of synapsis and chiasma distribution in rye as postulated already 40 years ago, based on the observations of a population of interspecific rye hybrids with random chiasma distribution (Jones 1967).

At this point there are no indications as to what mechanism may render a large portion of a chromosome arm incapable of recombination. Absence of recombination cannot be an intrinsic characteristic of chromatin itself but rather it must be an imposition of some kind on a segment of a chromosome. In the above-mentioned population of rye, Jones (1967) observed random distribution of chiasmata,

including some in the vicinity of the centromere. In induced wheat-rye recombination, translocation breakpoints very close to the centromere were recovered, albeit with very low frequency (see Fig. 3 in Lukaszewski et al. 2004). Therefore, the proximal regions of rye chromosome arms have the capacity to recombine; they are prevented from doing so by some mechanism that may break down in a wide hybrid. A genetic system allocating chiasmata to the proximal chromosome regions operates in some *Allium* species (Albini and Jones 1987; Khrustaleva and Kik 1998). In interspecific hybrids, it is inherited in a Mendelian fashion, so presumably, in its absence, the same chromosomes are capable of distal recombination. A change from a distal chiasma distribution in the parents to a random distribution of homoeologous recombination was noted in a *Lolium multiflorum* × *Festuca pratensis* hybrid (Zwierzykowski et al. 1999). A change to more random chiasmata pattern has also been observed in *L. perenne* upon inbreeding (Karp and Jones 1983), implying a genetic control of chiasma allocation. Presumably, the system of chiasma allocation to chromosome segments in wheat is either the same as that in rye, or they are very similar. 1R in wheat studied here behaved exactly the same as it does in rye, and in the same fashion as wheat chromosome 1B (Lukaszewski, unpublished).

Chiasmate pairing is indispensable for regular disjunction of chromosomes in anaphase I of meiosis and for a proper reduction of the chromosome number, hence efficient production of gametes. Eukaryotic chromosomes contain very large amounts of DNA, and the total numbers of chromosomes present can also be high. Still, with precision approaching 100%, homologues find each other in the early stages of meiosis and enter metaphase I bound by chiasmata. The complexity of the problem in selecting the correct pairing partner (the homologue) is alleviated to some degree by the polarity of the nucleus, the so-called Rabl's orientation (Fussel 1987) with the centromeres congregating on one pole of the nucleus and the telomeres on the other. In many species, in the early meiotic prophase, the telomeres congregate even further, forming a tight knot on the nuclear envelope, known as the telomere (or leptotene) bouquet (Dawe 1998; Harper et al. 2004). It is a common feature in organisms undergoing meiosis and is believed to be responsible for bringing homologous chromosomes into sufficient proximity for homology recognition and the initiation of synapsis (Bass et al. 2000; Bass 2003; Harper et al. 2004; Scherthan 2007).

In yeast, precise homologue alignment is preceded by the early stages of crossing-over: the formation of double strand breaks (DBS) and subsequent double strand invasion by single-strand overhangs (Gerton and Hawley 2005). Consequently, synaptic abnormalities do not preclude crossing-over. Many eukaryotes appear to have a DBS-independent process of homologue alignment (Gerton and Hawley 2005). How this is accomplished is not clear: if synapsis precedes the DBS formation and double-stranded DNA invasion, what juxtaposes homologues for such a precise alignment that crossing-over can follow? Since the synaptonemal complex itself is indifferent to homology (Loidl 1990), it cannot be held responsible for the recognition of homologues. On the other hand, the bouquet stage brings all telomeres together, not just the homologous ones. If the synaptonemal complex is fully capable of synapsing non-homologous chromosomes (Gillies 1974; De Jong et al 1991) or non-homologous parts of homologues (Moses et al. 1982) and all telomeres are brought together, why do non-homologues not synapse?

It is the presence of the telomeric repeat that dictates the movement toward the bouquet; in maize, insertion of the telomeric repeat in an intercalary position will direct such an intercalary segment to the bouquet (Carlton and Cande 2002). True to this, a telocentric chromosome, which to be stably passed through generations must have a telomeric repeat at its broken centromere, enters the bouquet with its both ends (Carlton and Cande 2002; Maestra et al. 2002). In this study, the ability of the centromere end of the $1RL_{inv}$ telocentric to enter the bouquet significantly increased the MI chiasmate pairing over two-armed chromosomes; there was an almost fivefold increase in the MI pairing frequency in heterozygotes with one complete and one telocentric chromosome (Table 2). The centromere of $1RL_{inv}$, by entering the bouquet, places its chiasma-capable segment in the same location and orientation as its corresponding segment in the distal end of a normal arm of the two-armed chromosome. In the reciprocal combination, when inversion was in a two-armed chromosome and its chiasma-capable region was in the vicinity of the centromere, hence on the opposite pole of the nucleus, it was always far away from its corresponding segment in the vicinity of the telomere of the normal telocentric, and the MI pairing was low. At the same time, migration of the centromere of the telocentric to the bouquet in the combination of $1R_{inv}$ + $1RL_{inv}$ drastically reduced chiasmate pairing over that in the inversion disome ($1R_{inv}''$) and ditelosome ($1RL_{inv}''$) (Table 1), probably by separating the chiasma capable segments. This behavior underscores the importance of the leptotene bouquet in the initiation of chiasmate pairing.

While the differences in the MI pairing in different combinations of two-armed and telocentric chromosomes are readily explained by the role of the telomere bouquet, the observations also suggest that a mechanism other than the bouquet must exist for searching of the corresponding pairing segment. In combinations where one of the chiasma-capable segments was in the vicinity of the centromere pole of the nucleus, such as in a two-armed inverted chromosome, while the other must have been in

the telomere pole, such as in a normal two-armed chromosome or in a normal or an inverted telocentric, the two segments did manage to establish chiasmate pairing with an up to 9.2% frequency (Table 1). No migration of the centromeres of two-armed chromosomes into the telomere bouquet has been reported in recent studies testing the centromere role in pairing initiation in wheat (Corredor et al. 2007) or in maize (Carlton and Cande 2002; Bass et al. 2000). Perhaps, this phenomenon reflects relatively rare instances when telomeres fail to integrate into the bouquet and remain randomly scattered throughout the nucleus or a bouquet-independent mechanisms for homology search as postulated by Harper et al. (2004), such as mechanisms in yeast capable of scanning the entire genome (Zickler 2006). Such whole-genome sweeps better explain frequent detection of various chromosome aberrations in different locations in the genome than standard initiation of pairing at the telomere. With the apparent general indifference of the SC to homology (Loidl 1990) and initiation of pairing at the telomere, intercalary aberrations would have to be routinely ignored and fail to pair with their homologous counterparts in original positions and orientation.

Chromosome pairing, defined here as the stage of recognition and juxtapositioning of homologues, precedes synapsis so that synapsis is of already pre-recognized, verified, and pre-aligned segments (Pawlowski and Cande 2005). In this context, the inverted 1RL arm behaves in an odd fashion. In inversion homozygotes, synapsis is complete in most PMCs, even though the distal regions of the arms are incapable of crossing-over and never form chiasmata. Whether other recombination events take place, this study was not capable of detecting them. Even more surprising were inversion heterozygotes: The most frequent synapsis was along the entire arm's length in an antiparallel fashion (Fig. 2; Table 2), hence completely defying homology at the local level along the entire length of the arm. Yet this synapsis was always with the homologous arm. In no instance was the inverted arm observed to synapse with a non-homologue, and such non-homologous univalents were frequently available (univalents 1A or 1D, in hybrids with $1R_{an}$ and $1R_{\#2}$). This suggests that either, in most cases, synapsis of the arm is indeed de-coupled from the early stages of crossover formation or that there is another licensing mechanism that controls homologue alignment. Pawlowski et al. (2004) postulated in maize a "continuous coordination between progression of pairing and recombination" and that "homology recognition is linked to this coordination…." Different synaptic configurations of the inverted chromosome observed here suggest a homology recognition system that is de-coupled from crossing-over.

While the homology defying synapsis appeared to be the main operating procedure for the chromosomes involved, another option was exercised with a low frequency, where synapsis was with full regard to homology, in proper orientation of the two arms, and restricted to the segment capable of crossing-over (Fig. 2l). This appears to indicate that two different mechanisms of homology search in preparation for synapsis operate in the material analyzed. One is crossover independent, and it appears to be the main system that somehow recognizes a homologous chromosome but is completely indifferent to the site-specific actual DNA sequence similarity. The other system is crossover-dependent and permits synapsis only of chromosome parts capable of crossing-over even if they are inverted relative to each other and in the opposite ends of the arm.

It could be argued that the behavior of the inversion chromosome in this study illustrates the role of the centromere in homologue recognition, as postulated by Martinez-Perez et al. (2001). However, Corredor et al. (2007) have shown that centromeres of homologues associate only as a consequence of synapsis progression from the distal ends of the arms. Moreover, even when connected by a common centromere, perfectly homologous arms (such as those originating from sister chromatids) of asymmetrical isochromosomes did not pair when they differed by deficiencies (Lukaszewski 1997b). The behavior of the inverted arm in this study could also be viewed as an extreme example of synaptic adjustment. This phenomenon was first observed in mouse (Moses et al. 1982) and later in many other species, where synapsis could proceed in an anti-parallel fashion in inversion heterozygotes or other structural aberrations. Here, the inversion was not of a complete arm. At the telomere, a very small segment remained in the standard orientation. Therefore, the inverted and normal arms must have had properly aligned segments of homology in the immediate vicinity of the telomere (distal to the inversion breakpoint). Whether this was sufficient to produce perfectly synapsed normal and inversion arms in a majority of cases is unclear. There may be no synaptic adjustment in plants: Maguire (1981) searched for it in maize and did not find it; in rye heterozygous for telomeric C-bands, unsynapsed segments of single lateral components were present in all chromosomes, as expected (Gilles and Lukaszewski 1989). It is also unlikely that antiparallel synapsis of the long arms in inversion heterozygotes was a carry-over from the short arms. In several PMCs, complete synapsis of the long arms and absence of synapsis of the short arms were observed.

This study was of a single arm of a rye chromosome introgressed into wheat and so does not provide a comfortable basis for generalizations. However, rye is a very typical example of a species with distal chiasmata, and it does not differ in this respect from many species, whether closely or distantly related. The same pattern of distal crossing-over/chiasmata is well known in wheat (Gill et al. 1996; Lukaszewski and Curtis 1993) in barley (Künzel et al. 2000; Close et al. 2007), and given the similarity of

genetic maps, may be common for all Gramineae. There are good indications that in wheat, as in rye, proximal stretches of chromosomes are excluded from recombination. A survey of over 500 bivalents 1B in wheat failed to detect even a single chiasma in the proximal halves of the arms; midget chromosomes made of proximal regions of wheat and rye chromosomes do not pair and hence are difficult to maintain (Lukaszewski 1997c and unpublished data); wheat chromosomes with reverse tandem duplications involving almost complete arms 2BS and 4AL never formed chiasmata in their distal ends but do so, albeit infrequently, in the vicinity of the duplication breakpoints, that is in the middle of the duplicated-inverted arms (Lukaszewski 1995 and unpublished data) and wheat telocentrics (or, for that matter, telocentrics in any species with distal chiasmata) have never been reported to form proximal chiasmata (Salee and Kimber 1978). If it was the timing of synapsis on the centromere–telomere axis that dictated chiasma distribution, telocentrics, with their ability to enter the leptotene bouquet with both ends, ought to form proximal chiasmata as often as the terminal ones, but they do not. Distal pairing initiation is also typical for maize. Burnham et al. (1972) established that the distal 40% of chromosome arms were responsible for the entire pairing initiation. Therefore, the patterns observed here, in a study of a single chromosome arm, may have wider implications than for rye or wheat. They may revive the concept of "zygomeres" in rye (Sybenga 1996b) or "pairing centers" in maize (Maguire 1986) as specialized regions responsible for the establishment of chiasmate pairing and may help to explain high recombination rates in disproportionately small segments of chromosome arms (Künzel et al. 2000; Faris et al. 2000) that do not appear to posses any clear characteristics differentiating them from the adjacent segments with low recombination rates (Yao et al. 2002).

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