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ORIGINAL PAPER

Forcing the shift of the crossover site to proximal regions in wheat chromosomes

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Abstract
Key message **Terminal deletions obligate the first crossover to be formed in more proximal positions. This increases the recombination rate in intercalary intervals but not in the proximity of the centromere.**
Abstract Crossovers are not uniformly distributed along chromosomes in wheat. They take place preferentially in distal positions. The effect of the chromosomal architecture on crossover positioning has been analyzed from the chiasmate bonds at metaphase I formed by the truncated arms of 51 terminal deletion lines of eight wheat chromosomes. Chromosome 4A and the B genome chromosomes, in their standard or truncated conformation, and their arms, were identified by C-banding. Chromosomes studied show a similar chiasma distribution. Reduction of the size of the truncated arms is accompanied by a gradual decrease of the chiasma frequency in chromosome arms 1BL, 3BS, 3BL, 4BL, 5BS, 5BL, 6BL, 7BS, 7BL and 4AL. In chromosome arm 1BS, most chiasmata are concentrated in the distal half of the satellite and, in 4AS, in the distal 24 %. The arms 2BS, 2BL and 6BS do not show a simple decreasing gradient of the recombination rate, the chiasma frequency increases in subdistal intervals compared to more distal regions. Although terminal deletions usually induce an increase of chiasma frequency in intercalary regions, the level of intact chromosome arms is maintained in only a few deletion lines. Truncated arms containing only the 20 % proximal of the intact arm do not form chiasmata. The relationships of chiasma positioning with chromatin structure and genome organization is discussed.

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Introduction

Chiasmata are the cytological manifestation of crossovers (COs), the exchanges of DNA between homologous chromosomes, in meiotic cells. COs are generated as the outcome of the recombinational repairing process of a fraction of double-strand DNA breaks (DSBs) produced by the topoisomerase-like SPO11 protein at the commencement of meiosis (Keeney et al. 1997). Cohesion between sister chromatids produced after chromatin replication contributes to form chiasmata in sites where COs arise. Chiasmata represent a prominent feature of meiotic prophase I and metaphase I, not only by the generation of new allele combinations, but also because they are responsible of securing a physical connection between the homologous chromosomes that form each bivalent until their segregation at anaphase I. Repair of DSBs is controlled to ensure at least one chiasma (CO) per chromosome pair, referred to as the obligate chiasma (CO). In plants and animals, most of DSBs are repaired by a non-crossover (NCO) pathway, only a minor fraction (5 %) is repaired as COs (Higgins et al. 2014).

Obviously, the positioning of DSBs may condition the distribution of the chiasmata originated later. However, the low CO ratio suggests strict controls that ensure the pronounced preferential chiasma localization observed in many plant species. Chiasma localization is imposed by a

restriction of synapsis to specific chromosome regions, as in the proximal chiasma localization of *Fritillaria* (Newton and Darlington 1930; Darlington 1935; Fogwill 1958). However, in most species, chiasma localization occurs in spite of complete chromosome synapsis. Different patterns of chiasma localization are produced in *Allium* species with large chromosomes that synapse completely along their length. *A. fistulosum* bears most chiasmata proximally located while *A. cepa* exhibits distal to unrestricted chiasma localization (Albini and Jones 1988). Other plant species included important crops such as maize, wheat, barley, or rye, show a pronounced distal chiasma localization (Lukaszewski and Curtis 1993; Künzel et al. 2000; Akhunov et al. 2003a; Anderson et al. 2004; Lukaszewski 2008; Higgins et al. 2012).

The molecular mechanisms responsible of the CO localization are poorly understood. Two aspects of the CO control, the obligate CO, which concerns the probability of a single CO, and CO interference, which concerns the probability of additional COs, are considered to play a relevant role in the decisions performing the fate, CO or NCO, of each DSB repairing event (Jones and Franklin 2006). As a result, COs are well spaced along the chromosomes, with often only a single CO per chromosome arm. Most likely, decisions concerning the site of the first CO are mediated by the restricted location of initiation of homologous interactions, which are confined to a distal region imposed by the formation of the meiotic bouquet (Scherthan 2001; Harper et al. 2004). The ultrastructural analysis of spread silver-stained meiotic nuclei of hexaploid wheat by Holm (1986) revealed that, at the beginning of the zygotene stage, telomeres aggregate and chromosome pairing and SC formation is initiated distally. In nuclei at mid-zygotene, generally the longest SC segments were those joining the distal segments. Likewise, an immunolocalization study of the chronology of overall recombination initiation and progression in meiotic nuclei of barley has demonstrated that recombinational events in distal regions precede those in proximal regions (Higgins et al. 2012). The uneven distribution of heterochromatin and euchromatin along the telomere–centromere axis has suggested also a link between euchromatin and CO formation (Higgins et al. 2014).

The ability of a given chromosome region to form chiasmata has been studied in some chromosomes of wheat and rye using deletions. Hemizygosis for the loss of long terminal segments of wheat chromosome arms 4AL, 2BL and 5BL show a considerable reduction of chiasma frequency in the truncated arm. However, the amount of chiasmate associations at metaphase I is restored to a normal level in the deficient chromosome homozygotes (Curtis et al. 1991). The construction of genetic maps demonstrated that the low chiasma frequency of the region located in the middle of the normal chromosome arms 1BL or 5BL, increased in truncated chromosomes with the distal 25 or 41 % missing, respectively (Jones et al. 2002; Qi et al. 2002). However, no recombination rate increase concerned the proximal region of the truncated chromosome pair. These results suggest that the recombinogenic capacity of a given segment is mainly decided by their position along the telomere–centromere axis. Studies in other chromosomes are however in contradiction with this notion. Chiasmata are infrequent in the proximal third of the 5RL chromosome arm of rye both in normal homozygotes and homozygotes for truncated chromosomes lacking the distal 70 % (Naranjo et al. 2010). This happens regardless synapsis is completed in that region. On the other hand, the positional modification of the recombining region from distal to proximal caused by a paracentric inversion in the rye chromosome arm 1RL and in wheat chromosome arms 4AL and 2BS is accompanied by strict proximal chiasma localization in the affected arm (Lukaszewski 2008; Lukaszewski et al. 2012; Valenzuela et al. 2012). Thus, the chiasma formation and distribution in the arms 5RL, 1RL, 4AL and 2BS is not conditioned by the position but depends on the DNA sequence, or the chromatin organization, present in each chromosome region.

The deletion stocks of hexaploid wheat, with 436 different terminal deletion lines, are excellent tools for physical mapping (Endo and Gill 1996). Deletion lines were isolated after identification of the break point position in C-banded mitotic chromosomes. Wheat chromosomes of the B genome and chromosome 4A can also be identified easily at metaphase I of meiosis (Naranjo et al. 1987). The aim of this paper was to assess the ability to form chiasmata of different chromosomal intervals along the telomere–centromere axis. For this purpose, the frequency of chiasmata was quantified at metaphase I in the truncated arm of deletion lines with variable size relative to the standard structure. Most chromosomes studied showed a gradual reduction of chiasma frequency along the telomere–centromere axis.

Materials and methods

Three or more lines per chromosome arm of the deletion stocks of hexaploid wheat, *Triticum aestivum* cv. Chinese Spring (Endo and Gill 1996) were used for chromosomes 1B, 2B, 3B, 4BL, 5B, 6B, and 4A, and two for chromosome 7B. Because of male sterility caused by homozygosity for deletions of 4BS, no deletion line of this arm was examined. All deletion lines were provided by the National BioResource Project-Wheat, Japan. Seeds were germinated on wet filter paper in Petri dishes at room temperature. Seven days later, plantlets were transplanted into pots and grown in a glasshouse until meiosis.

Table 1 Association frequency (%) of the truncated arm and mean chiasma frequency in different deletions of chromosomes of the B genome and chromosome 4A

Short arm deletions	FL	Association (%)	Bonds/cell[a]	PMCs	Long arm deletions	FL	Association (%)	Bonds/cell[a]	PMCs
1BS-18	Sat, 0.50	16	40.3 ± 0.1	50	1BL-4	1.0	98	40.1 ± 0.2	50
1BS-6	1.04, Nor	14	40.1 ± 0.2	50	1BL-15	0.82	88	39.6 ± 0.2	50
1BS-2	1.06	10	39.7 ± 0.1	50	1BL-14	0.61	78	40.0 ± 0.2	50
1BS-3	0.72	16	39.8 ± 0.2	50	1BL-1	0.47	48	39.8 ± 0.2	50
1BS-15	0.50	8	39.2 ± 0.2	50					
2BS-7	0.89	76	39.8 ± 0.3	50	2BL-6	0.89	84	40.1 ± 0.2	50
2BS-3	0.84	80	39.9 ± 0.2	50	2BL-1	0.69	94	41.1 ± 0.1	50
2BS-12	0.81	96	40.5 ± 0.2	100	2BL-7	0.58	42	40.0 ± 0.2	50
2BS-6	0.56	62	40.9 ± 0.1	50					
3BS-3	0.87	98	40.5 ± 0.1	100	3BL-11	0.81	99	40.2 ± 0.1	100
3BS-7	0.75	66	40.5 ± 0.1	100	3BL-6	0.54	14	23.3 ± 0.6	100
3BS-4	0.55	34	39.4 ± 0.1	100	3BL-3	0.41	12	29.6 ± 0.4	100
3BS-1	0.33	26	39.4 ± 0.2	100					
					4BL-1	0.86	89	40.3 ± 0.2	100
					4BL-3	0.68	41	40.0 ± 0.3	100
					4BL-4	0.43	30	40.5 ± 0.2	100
5BS-6	0.81	73	40.0 ± 0.2	100	5BL-13	0.82	100	40.6 ± 0.3	100
5BS-1	0.67	56	40.2 ± 0.3	100	5BL-14	0.75	89	40.3 ± 0.3	100
5BS-8	0.56	25	40.5 ± 0.2	100	5BL-18	0.66	67	38.5 ± 0.4	100
					5BL-5[b]	0.54	62	37.4 ± 0.4	100
6BS-7	Sat, 0.24	44	39.9 ± 0.1	50	6BL-6	0.79	88	41.3 ± 0.1	50
6BS-2	1.05, Nor	64	40.2 ± 0.2	50	6BL-1	0.70	68	40.8 ± 0.1	50
6BS-5	0.76	34	40.2 ± 0.1	50	6BL-9	0.64	54	40.9 ± 0.1	50
6BS-4	0.46	34	38.7 ± 0.3	50	6BL-3	0.36	28	40.3 ± 0.2	50
7BS-1	0.27	35	40.2 ± 0.3	100	7BL-3	0.86	91	40.6 ± 0.2	100
7BS-3	0.16	0	39.9 ± 0.3	100	7BL-5	0.69	60	40.9 ± 0.2	100
4AS-3	0.76	32	39.7 ± 0.3	50	4AL-6	0.84	90	37.7 ± 0.4	50
4AS-4	0.63	12	39.5 ± 0.2	50	4AL-4	0.80	92	39.8 ± 0.2	50
4AS-1	0.20	0	38.9 ± 0.2	50	4AL-7	0.75	78	39.7 ± 0.2	50

Fraction length (FL) indicates the position of the break point in the arm concerned in each line (Endo and Gill 1996)

[a] Confidence intervals of the mean $= \pm 1.96 \times (\sigma/\sqrt{n-1})$

[b] The 5BL-5 formed on average number of 0.47 multivalents per cell

Emerging spikes were examined to collect anthers with pollen mother cells (PMCs) at metaphase I. Each spikelet has two major flowers, one slightly younger than the other. Each floret contains three anthers approximately synchronous in development. One anther from each major floret was squashed in aceto-carmine to establish the stage of meiosis. When the checked anther was at metaphase I the two sister anthers were fixed in 1:3 glacial acetic acid: ethanol and stored at 4 °C for 1 month. Fixed anthers were squashed and stained according to the C-banding procedure described by Giráldez et al. (1979). A minimum of 50 PMCs per deletion line were scored. The C-banding patterns at metaphase I allowed unambiguous identification of the standard and truncated conformations of chromosome 4A and all of the B genome chromosomes, and their arms, as described by Endo and Gill (1996) and Naranjo et al. (1987).

Results

The average number of bound arms per cell at metaphase I, and the number of PMCs scored in the 51 deletion lines studied are listed in Table 1. Also indicated is the fraction length (FL) which denotes the physical location of the break point in the complete arm as estimated by Endo and Gill (1996). Two lines of chromosome arm 3BL, 3BL-6 and 3BL-3, showed a much lower number of chiasmate bonds (23.3 and 29.6, respectively) than the remaining lines. Univalents and open bivalents were relatively

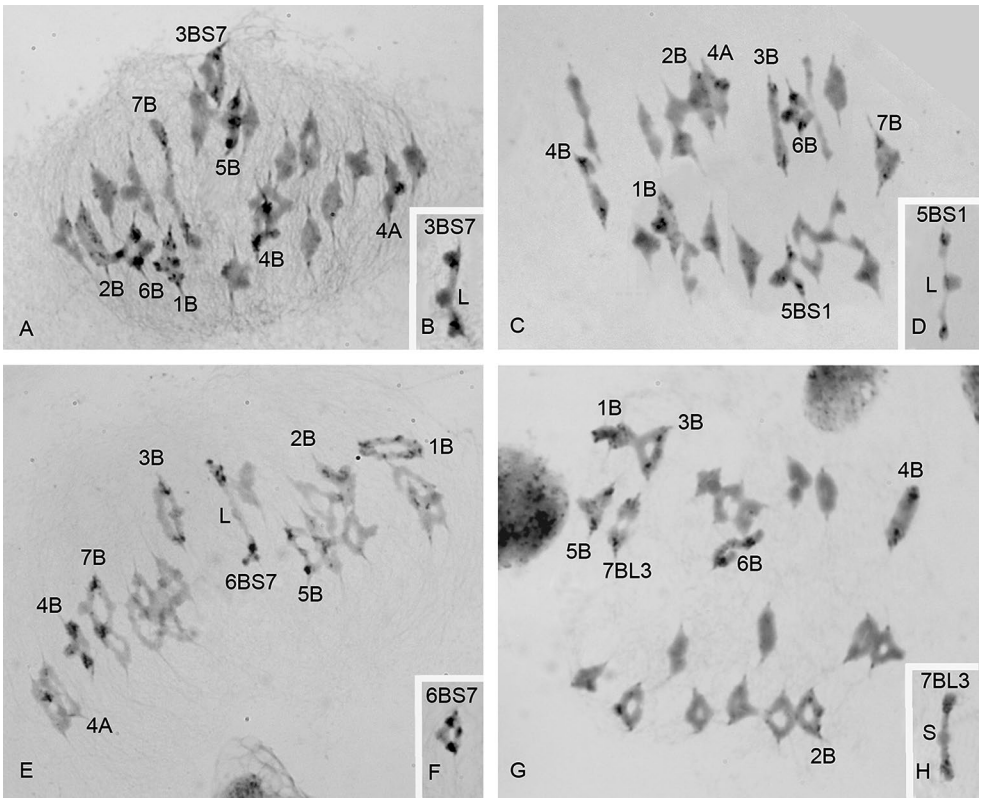

Fig. 1 Identification of the B genome chromosomes and chromosome 4A in C-banded cells at metaphase I of four deletion lines of wheat. **a**, **b** Deletion line 3BS-7. The truncated chromosome 3B forms a ring bivalent in **a** and an open bivalent with the deleted arm not bound in **b**. **c**, **d** Deletion line 5BS-1. Chromosome 5B forms a ring bivalent in **c** while the deleted 5BS arm is not associated in **d**. **e**, **f** Deletion line 6BS-7. Chromosome pair 6B forms an open bivalent with the deleted arms not bound in **e** and a ring bivalent in **f**. **g**, **h** Deletion line 7BL-3. The deleted arms are bound in **g** and not bound in **h**

frequent in these lines denoting failure of homologues to pair, synapse and recombine. In the remaining lines, most of the homologous pairs formed a ring bivalent (Fig. 1) and the mean of bound arms per cell ranged between 37.4 and 41.3. One of these lines, 5BL-5, formed trivalent and quadrivalent configurations with a total frequency of 0.47 multivalents per cell.

All B genome chromosomes and chromosome 4A as well as each truncated arm were identified in all PMCs of all deletion lines (Fig. 1). In addition to the total number of arms being bound, the occurrence of association or not between the deleted homologous arms was scored. The association frequency of each truncated arm is also given in Table 1. The frequency of association of all intact arms of chromosomes studied (Table S1) was obtained from observations in the PMCs of lines 7BS-3 and 7BL-5, in which a high number of anthers at metaphase I could be isolated. Frequencies ranged between 88 % for 5BS and 100 % for 4AL. The distribution of the frequency of association of the standard and truncated chromosome conformations in all of the arms studied is shown in Fig. 2.

Chromosome 1B

This chromosome shows apparent differences between arms in the distribution of chiasmata. In the short arm, most chiasmata are concentrated in the distal half of the satellite. All of the five deletion of this arm formed chiasmata with a frequency of 16 % or lower. The proximal half of 1BS formed chiasmata in 8 % of the PMCs. The 1BL arm shows less pronounced chiasma localization. Deletion of the distal heterochromatin C-bands does not affect chiasma frequency. The proximal half of 1BL forms chiasmata in almost 50 % of PMCs and the chiasma frequency increases with the size of the truncated arm.

Chromosome 2B

Deletion lines of 2BS and 2BL bear relatively high levels of association for the deleted arms. The proximal 56 % of 2BS appears associated in 62 % of PMCs and the proximal 58 % of 2BL in 42 % of PMCs. Both arms show an increase of the chiasma frequency for deletions with break points subdistally located relative to more distal deletions.

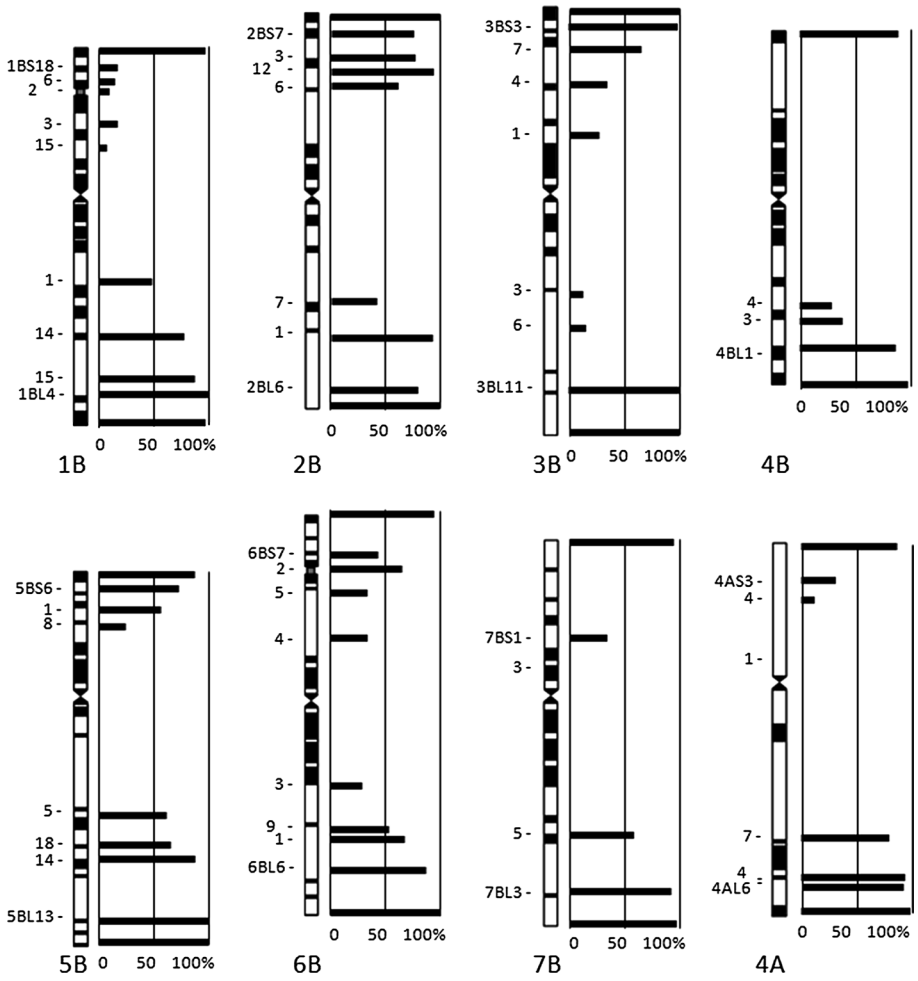

Fig. 2 Frequency (%) of chiasmate bonds at metaphase I for the deleted arms of wheat chromosomes of the B genome and chromosome 4A in 51 deletion lines. *Bars* at the chromosome ends indicate the percentage of association of intact arms. C-banding diagrams of the chromosomes and the positions of the deletion break points (*left*) were taken from Endo and Gill (1996)

Chromosome 3B

Deletion of the distal 13 % of 3BS, as well as of the distal 19 % of 3BL, causes no variation in the ability of these arms to form chiasmata. The progressive reduction of the size of 3BS is accompanied of reduction of the number of chiasmata in such a way that only 26 % of PMCs form chiasmata in its proximal third. The overall reduction of chiasmata produced in deletion lines 3BL-6 and 3BL-3 most likely conditions the low chiasma frequency of the truncated arm in these lines.

Chromosome arm 4BL

In this arm, the chiasma frequency decreases with the size of the deletion. The 43 % proximal segment formed one chiasmate association in only 30 % of PMCs.

Chromosome 5B

5BS is the smallest of all of the arms analyzed and, even carrying the standard structure, shows no chiasma in 12 % of PMCs. The increase of the deletion size is accompanied by a progressive reduction of the chiasma frequency, in such a way that the proximal 56 % of 5BS form chiasmata in only 25 % of PMCs. In the 5BL arm, the loss of the distal 18 % does not affect the chiasma frequency and its subdistal quarter form chiasmata in more than 60 % of the PMCs.

Chromosome 6B

In contrast with 1BS, the 6BS arm forms a considerable number of chiasmata out of the satellite. The frequency of chiasmata even increases when the complete satellite is lost. The proximal half of the chromosome form chiasmata in 34 % of PMCs. The 6BL arm shows a reduction of the chiasma frequency in parallel with the increase of the deletion size. The proximal 36 % of 6BL forms chiasmata in 28 % of PMCs.

Chromosome 7B

The proximal 27 % of the 7BS arm forms chiasmata in 35 % of PMCs but no chiasma is observed when the

truncated arm consists of only the proximal 16 %. The subdistal region of 7BL forms chiasmata in a considerable number of PMCs (91 %) and the proximal 69 % in a lower number (60 %).

Chromosome 4A

Most chiasmata are formed distally in the 4AS arm. The proximal 63 % forms chiasmata in only 12 % of PMCs and the proximal 20 % forms no chiasma. In the long arm, chiasmata show a more spread distribution. The proximal 75 % appears associated in 78 % of PMCs.

Discussion

The frequency of association at metaphase I represents an underestimation of the number of chiasmata per chromosome arm since it is difficult to establish whether two arms being bound have formed or not more than one chiasma. Using the telocentrics of hexaploid wheat, Sallee and Kimber (1978) identified individual chromosome arms and the occurrence of one or more chiasmata as based on the shape of the telocentric bivalent. They estimated an average number of 1.15 chiasmata per chromosome arm. The number of 2.97 chiasmata estimated for chromosome 3B is not quite different from the number of 2.6 COs reported in a fine-scale analysis of recombination (Choulet et al. 2014).

The complete chromosome arm 5BS shows the lowest chiasma frequency of all normal arms studied and appears unpaired in 12 % of PMCs. This behavior may be mediated by the heterobraquial conformation of chromosome 5B. This chromosome shows the highest long arm/short arm ratio of the wheat genome. The size and morphology of submetacentric chromosomes 5R and 6R of rye is similar to that of 5B. The heterobraquial conformation of chromosomes 5R and 6R affects the movement of the telomeres of their short arm, which fail to join the other telomeres during the bouquet formation. Unsuccessful telomere migration is followed of failure of synapsis and recombination of these arms (Naranjo et al. 2010; Naranjo 2014). This might be also the case of chromosome arm 5BS.

Among the sample of deletion lines analyzed only three showed an irregular pattern of chromosome associations at metaphase I. The 5BL-5 deletion caused the formation of multivalents that persisted until metaphase I. The 5BL arm carries the *Ph1* locus, which suppresses the occurrence of homoeologous recombination. This locus is absent in the 5BL-5 deletion (Gill et al. 1993). The deletion lines 3BL-6 and 3BL-3 showed a significant reduction of the total number of chiasmata per cell. A relatively strong pairing promoter gene, whose presence is necessary for normal synapsis and chiasma formation, is located on 3BL (Sears 1954; Kempana and Riley 1962) just in the segment lost in the terminal 3BL-7 deletion (Bassi et al. 2013). The deleted segment is smaller in 3BL-7 than in 3BL-6. Thus, this pairing promoter gene must be situated in the interval between the break points of deletions 3BL-7 (FL = 0.63) and 3BL-11 (FL = 0.81). All of the remaining deletion lines yielded average bound arm frequencies which are consistent with the figure of 40.5 reported in the standard Chinese Spring (Martínez et al. 2001).

The frequency of chiasmate bonds of the truncated arms in the different lines studied show, with some exceptions in 3BS, 3BL and 6BS, a decreasing gradient along the telomere–centromere axis. Only four deletion lines, 1BL1 (FL = 1.0), 3BS3 (FL = 0.87), 3BL11 (FL = 0.81) and 5BL-13 (FL = 0.82) are capable of restoring the level of chiasmate bonds of the intact arms. Reduction of the chiasma frequency is apparent in all of the remaining deletion lines and the level of reduction increases with the proximity to the centromere. The largest deletions studied 7BS-3 and 4AS-1, which have lost the distal 84 and 80 %, respectively, form no chiasma. Such a decreasing gradient is in agreement with previous observation denoting the distal location of chiasmata in wheat chromosomes (Lukaszewski and Curtis 1993; Akhunov et al. 2003a; Lukaszewski 2008) and, therefore, it may represent the general pattern of chiasma distribution in wheat. Wheat chromosomes are capable of forming chiasmata with a relatively high frequency in their distal half. Only the 1BS arm shows an abrupt decrease of the chiasmate bonds frequency in the subdistal half of the satellite, which is not recuperated in more proximal positions.

The frequency of bound arms at metaphase I provides an estimation of the probability of producing at least one chiasma by the arm fraction present in each deletion line. Although the resulting chromosome arm histograms of Fig. 2 fit the general pattern of chiasma distribution, recombination frequencies in intervals bracketed by adjacent break points are most likely different in intact and truncated chromosomes. The following data and previous studies support an increase of the chiasma frequency in terminal regions present in the truncated arms. (1) The histograms of chromosome arms 2BS, 2BL and 6BS show an increase of the chiasma frequency in deletions 2BS-12, 2BL-1 and 6BS-2, whose break points occupy the third, second and second positions, respectively, in the telomere–centromere axis of these arms. (2) Jones et al. (2002) concluded that recombination frequencies of intercalary chromosome segments of the intact 1BL arm increased when placed closer to the telomere in the truncated arm. (3) The FL 0.55-0.59 interval of the 5BL arm undergoes an increased COs frequency in the deletion 5BL-11, where it is distally located, compared to the normal chromosome 5B (Qi et al. 2002). However, the recombination frequency does not change in

the remaining proximal part of the del5BL-11 arm. Accordingly, while the *ph1b* intercalary deletion, which lacks *Ph1,* maps 0.9 cM from the centromere, that is, 1.8 COs are formed in this interval (Sears 1984) the 5BL-5 deletion line, with a break point more proximally located than *Ph1*, form at least one chiasma in 62 % of PMCs. (4) A high resolution COs map constructed for chromosome 3B situates most of exchange events in two intervals occupying the distal 68 Mb of 3BS and the distal 59 Mb of 3BL (Choulet et al. 2014). Such segments represent approximately the distal 20 % of 3BS and the distal 14 % of 3BL, respectively. Thus, they are smaller than the segments missing in deletions 3BS-7 and 3BL-11, respectively. Lines for these two deletions, especially 3BL-11, show very high-recombination frequencies suggesting that chromosome arm shortening increases greatly the recombination in intercalary regions of chromosome 3B.

The decreasing recombination gradient agrees, at least in part, with a model of chiasma distribution along the chromosome based on homologous recognition sites distally located and positive chiasma interference (Jones et al. 2002). Once the first distal CO site is defined, interference reduces the chance of additional COs in each arm. The interference intensity is inversely related to the physical distance between adjacent COs sites (Jones et al. 2002; Saintenac et al. 2009). A more proximal location of the pairing initiation site and absence of interference, since no CO is still formed, are likely conditioning the increase of the recombination rate in normally intercalary regions that become distal after chromosome truncation. Nevertheless, deletions lacking the terminal third of any chromosome arm do not recuperate the level of chiasma frequency reached by the intact chromosomes. This suggests that additional factors are involved in the control of chiasma distribution. The DNA sequence, or chromatin organization, present in each chromosome stretch conditions also the possibility of producing a first CO. The pattern of chiasma distribution in the chromosome studied supports this notion.

Suppression of CO in proximal regions is a common feature of wheat chromosomes (Erayman et al. 2004; Saintenac et al. 2009; Choulet et al. 2014). This happens despite the loss of large chromosome fragments relocating such regions close to the chromosome end as in deletions 4AS-1 (FL = 0.20) and 7BS-3 (FL = 0.16). It is likely that the long arm/short arm ratio modification in the truncated chromosomes may affect migration of the short arm telomere during the bouquet organization and cause failure on homologous recognition and recombination. However, this reasoning becomes inconsistent when chromosomes contain arms of similar size. This is the case of a deleted 5R chromosome (5RS.del5RL) of rye, in which chiasma formation is strongly suppressed in the truncated chromosome arm del5RL despite synapsis is normal (Naranjo et al. 2010). The absence of COs in the pericentromeric region is a phenomenon extended to many other plant species regardless their genome size and complexity (Anderson et al. 2003; Wu et al. 2003; Jensen-Seaman et al. 2004; Drouaud et al. 2006). The pericentromeric region of the B genome chromosomes and chromosome 4A of wheat is especially rich in heterochromatin, which is well known to inhibit CO formation (Gaut et al. 2007). Accordingly, the reduced recombination observed in the terminal region of 3BS has been explained by the high heterochromatin content of the telomeric end of this arm (Saintenac et al. 2009). On the other hand, deletion of the large subtelomeric heterochromatin C-bands of chromosome arm 1BL does not affect the chiasma frequency (Fig. 2). The higher packaging degree of heterochromatin might be a limiting factor to generate CO. Euchromatin histone marks H3k9me3, H3K27me3, H3K4me3 and H4K16ac, are highly abundant in the chromosomal distal regions of barley chromosomes during meiotic prophase I, denoting uneven chromatin remodeling along the telomere–centromere axis (Higgins et al. 2014). On the other hand, the highly recombinogenic distal region of the 1RL arm of rye finds the homologous partner easier than the proximal crossover-poor regions, even though their positions are inverted (Valenzuela et al. 2012). The dissimilar behavior of crossover-rich and crossover-poor regions might be based on differences in the pattern of chromatin remodeling. Relaxation of chromatin organization would facilitate the occurrence of chromosome movements required to find the homologous partner and generate one CO.

A relationship between the recombination rate and the evolution of cereals genome organization has emerged in the last decade. Studies revealed that duplication-derived loci accumulated preferentially in distal high-recombination regions of wheat chromosomes whereas the ancestral copies remained proximally located (Akhunov et al. 2003a). This finding suggests that recombination has played a central role in the evolution of wheat genome structure and that gradients of recombination rates along chromosome arms promote more rapid rates of genome evolution in distal, high-recombination regions, than in proximal, low-recombination regions. Assessment of the synteny degree along homoeologous chromosomes of the A, B, and D genomes of wheat revealed synteny erosion caused by insertions and deletions of loci, which preferentially occur in high-recombination regions (Akhunov et al. 2003b). Comparative studies of wheat–rice or wheat–sorghum chromosome relationships confirmed the erosion of synteny and the accumulation of evolutionary chromosome rearrangements in the distal recombination-rich region of wheat chromosomes (See et al. 2006; Luo et al. 2009). High-recombination regions of wheat chromosome 3B are enriched in gene categories related to adaptation such

as resistance to pathogens, which suggests that meiotic recombination generates variability in these important evolutionary chromosomal domains (Choulet et al. 2014).

Recombinational events are mainly confined to gene-rich regions of wheat and barley chromosomes (Künzel and Waugh 2002; Erayman et al. 2004; Mayer et al. 2011). However, a substantial proportion (more than 30 %) of the gene complement is outside of these regions. Elucidating the basis for chiasma localization, particularly in the cereals, has implication on the possibility of manipulating recombination in poor-crossover regions, which is potentially important for breeding-related programs. Paracentric inversions invert the distal–proximal polarity of chromosome segments, but this change seems to be inefficient in redistributing the chiasma localization (Lukaszewski 2008; Lukaszewski et al. 2012; Valenzuela et al. 2012). The results presented here support that, with some exception, the loss of terminal regions increases the recombination rate in adjacent intercalary regions. However, this effect is not extended to the proximal part of the chromosomes, where CO is suppressed. An alternative strategy for modifying chiasma distribution is suggested by the effect of temperature in barley. Comparison of the chiasma distribution in plants exposed to 22 and 30 °C during meiosis revealed a significant decrease in the distal/proximal COs ratio at the higher temperature (Higgins et al. 2012).

Author contribution statement TN conceived, designed and performed the experiments, analyzed the data and wrote the manuscript.

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Conflict of interest The author declares that he has no conflict of interest.

Ethical standard The author states that the experiments comply with the current laws in Spain where they were performed.

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