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Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale

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Abstract The physical distribution of translocation breakpoints was analyzed in homoeologous recombinants involving chromosomes 1A, 1B, 1D of wheat and 1R of rye, and the long arms of chromosome 7S of *Aegilops speltoides* and 7A of wheat. Recombination between homoeologues was induced by removal of the *Ph1* gene. In all instances, translocation breakpoints were concentrated in the distal ends of the chromosome arms and were absent in the proximal halves of the arms. The relationship between the relative distance from the centromere and the relative homoeologous recombination frequency was best explained by the function $f(x)=0.0091e^{0.0592x}$. The pattern of recombination in homoeologous chromosomes was essentially the same as in homologues except that there were practically no double exchanges. Among 313 recombinant chromosomes, only one resulted from a double crossing-over. The distribution of translocation breakpoints in translocated arms indicated that positive chiasma interference operated in homoeologous recombination. This implies that the reduction of the length of alien chromosome segments present in translocations with wheat chromosomes may be more difficult than the production of the original recombinants.

Key words Wheat · Homoeologue · Recombination *Ph1* gene

Introduction

Genetic mapping of physical attributes of chromosomes has shown that recombination in tetraploid wheat (*Triticum turgidum* L.), hexaploid wheat (*T. aestivum* L.), and in rye (*Secale cereale* L.) was concentrated in the distal ends of chromosome arms and was practically absent from the proximal halves of the arms (Dvorak and Appels 1986; Lukaszewski 1992; Lukaszewski and Curtis 1993). A similar conclusion can be inferred from the deficiency mapping of genetic markers (Snape et al. 1985; Werner et al. 1992; Gill et al. 1993).

Concentration of recombination in the distal regions of chromosome arms does not appear to result from an inherent inability of the proximal regions to form chiasmata. When present in a homozygous condition, wheat chromosomes deficient for their distal halves paired in meiotic metaphase-I with frequencies comparable to those of normal arms (Curtis at al. 1991). On the other hand, premeiotic applications of weak colchicine solutions changed the pattern of recombination in favor of the proximal regions (Curtis and Lukaszewski 1992). Both of these indicate that the proximal regions of wheat chromosomes are capable of normal levels of recombination. It was speculated (Lukaszewski and Curtis 1993) that the distal concentration of recombination in wheat, and possibly in other species with distal chiasmata, was a consequence of telomeric initiation of chromosome pairing which created favorable conditions for the establishment of distal chiasmata. Strong positive chiasma interference would then reduce the probability of crossing over in the proximal regions, especially in physically short chromosome arms.

Non-random distribution of recombination along chromosome arms has implications in breeding and genetics. It implies that tight linkages may be due not only to the close physical proximity of genes. Genes located in the proximal, non-recombining, regions of chromosomes may be completely linked, even though they can be at a considerable physical distance from one another. Such linkages may be difficult to break. The pattern of the distribution of recombination is also of major importance in experiments whereby segments of chromosomes with desirable genes are transferred from one species to another via induced homoeologous recombination. Given a sufficient level of homoeology to assure some meiotic pairing, any gene located in the distal, recombining, region of a chromosome arm would be relatively accessible to manipulation. The proximal location of such genes, however, would make them

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very difficult to access. In such cases, long proximal segments from the centromere to past the gene would need to be transferred. This would necessarily involve many genes with undesirable agronomic consequences.

This paper summarizes data on the distribution of translocation breakpoints in recombination between homoeologous chromosomes observed in three experiments designed to transfer segments of chromosomes from alien species to bread wheat and vice versa. Recombination between homoeologues was induced either by the *ph1b* mutation or by the removal of chromosome 5B with the *Ph1* gene.

Materials and methods

The data were collected in three different experiments. The first was designed to transfer the *Glu-D1* gene from the long arm of chromosome 1D of bread wheat to the long arm of rye chromosome 1R in hexaploid triticale (*Triticosecale* Wittmack) (Lukaszewski and Curtis 1992); the second to exchange small chromosome segments between the short arms of rye chromosome 1R and wheat homoeologous group-1 chromosomes (A. J. Lukaszewski, unpublished); and the third to transfer leaf rust, greenbug, and black-point resistance genes from chromosome 7S of *Aegilops speltoides* to hexaploid wheat (A. J. Lukaszewski, unpublished).

The first experiment was conducted in hexaploid triticale 'Rhino'. Chromosome substitution 5D(5B) was used to induce homoeologous recombination between the long arms of normal rye chromosome 1R and a centric wheat-rye translocation 1RS.1DL. In addition to the selection of the $1R.1D_{5+10}$–1 chromosome, essentially a rye chromosome 1R with a proximal segment of wheat chromosome arm 1DL in the long arm, data were also collected on the distribution of translocation breakpoints in translocations of the 1RL arm with 1AL, 1BL, and 1DL, and of the 1BL arm with 1AL and 1DL.

Both bread wheat experiments were conducted in *T. aestivum* cv 'Pavon'. Homoeologous recombination was induced by the *ph1b* mutation, originally produced in 'Chinese Spring' wheat by Sears (1984), and transferred to Pavon by backcrosses to 5B monosomics. In the experiment involving the short arm of rye chromosome 1R, plants of Pavon were created with the chromosome constitution 19″+5B*ph1b*′+(1RS.1BL+1B)″, and their progeny, obtained following self-pollination, were screened by C-banding for the presence of translocations. In addition to the exchanges involving the 1RS arm, data were also collected on the distribution of translocation breakpoints in homoeologous recombinants of the long arms of all group-1 chromosomes except for the 1AL–1DL pair.

Chromosome 7S of *Ae. speltoides* was identified in a sample of wheat, labelled "Amigo", obtained from Dr. B.S. Gill, Kansas State University, and transferred to Pavon through backcrosses. This is the same chromosome as described by Friebe et al. (1991). It has a short, presumably terminal, segment of chromosome 7A in the short arm. The precise physical location of the translocation breakpoint is not known but the C-banding pattern indicates that it must be located in the distal 15% of the short arm. In the presence of the *Ph1* gene the 7A segment in chromosome 7S supports complete pairing with telocentric chromosome 7AS of Chinese Spring; the long arm of 7S does not pair with telocentric 7AL or any other wheat chromosome (A. J. Lukaszewski, unpublished). Chromosome 7S in Pavon was combined with the *ph1b* mutation to produce plants with the chromosome constitution 19″+5B*ph1b*′+(7S+7A)″. These plants were backcrossed as male to Pavon, and the resulting progeny were screened by C-banding to identify recombinants.

As in the study on the distribution of recombination in B-genome homologues in wheat (Lukaszewski and Curtis 1993), translocation breakpoints in recombined chromosomes were assigned to segments of chromosome arms based on the differences in C-banding patterns between the arms involved, and assuming the lowest number of cross-over events necessary to produce the observed C-banding pattern. However, unlike recombination involving homologues, the position of a cross-over involving homoeologues can be expressed in terms of the physical or genetic features of either one of the chromosomes involved, with both being equally valid. For the purpose of this study, the breakpoints were assigned to the segments of those arms from each homoeologous pair which had a larger number of C-bands, especially in the distal regions. Larger numbers of C-bands subdivided the arms into larger numbers of shorter interband regions and allowed for a more precise analysis of the distribution of breakpoints. Following these rules, breakpoints in transfers from chromosome arms 1AS and 1DS to 1RS, and from 1RS to 1AS, were assigned to segments of 1RS, while those from 1RS to 1DS were assigned to segments of 1DS. All exchanges between arms 1RS and 1BS were assigned to segments of 1BS, and those of 1RL with 1AL, 1BL and 1DL were assigned to 1RL. Breakpoints in transfers of segments from 1BL to 1AL and from 1AL or 1DL to 1BL were assigned to segments of 1BL; breakpoints in transfers from 1BL to 1DL were assigned to segments of 1DL. No exchanges between 1AS or 1DS with 1BS were observed, and exchanges of 1AS with 1DS and 1AL with 1DL could not be identified. All exchanges involving chromosome 7S were assigned to segments of that chromosome.

All calculations and statistical procedures were the same as used previously for the analysis of recombination in homologues (Lukaszewski and Curtis 1993). Briefly, a coefficient of relative recombination frequency (proportion of recombination in a given segment of a chromosome arm in the total number of chromosomes recombined in that arm) per unit of relative segment's length was calculated for each of the 33 segments analyzed. These coefficients were plotted against the relative distances from the centromere of the segments' distal endpoints using SAS non-linear regression (PROC NLIN). C-band nomenclature, the positions of C-bands relative to the centromere, and the relative lengths of the interband regions in wheat chromosomes were according to Gill et al. (1991); those of rye chromosome arm 1RL were as in Lukaszewski (1992).

Results

In the course of the three studies involving the induction of recombination between chromosomes of wheat and their alien homoeologues, a large number of recombined chromosomes from all seven homoeologous groups were observed. For this analysis, all identified translocations of homoeologous group-1 chromosomes and of chromosome 7S were selected because of their relatively high frequency and because the C-banding patterns of at least one chromosome of each pair allowed monitoring of recombination along the entire lengths of chromosome arms. The data include 49 recombinant chromosomes of the 1RL arm with 1AL, 1BL, and 1DL (Fig. 1), and 46 recombinants of the 1BL arm with 1AL or 1DL arms, observed among 336 F_2 triticale plants. In the wheat experiment involving the 1RS.1BL translocation, there were 36 recombinants of the 1RS arm with the short arms of group-1 wheat chromosomes (Fig. 2), and 168 recombinants of the 1BL arm with 1AL and 1DL arms, identified among 4266 F_2 plants. In the wheat experiment involving chromosome 7S, there were 12 recombinants of the short arms and 13 recombinants of the long arms of chromosomes 7S with 7A (Fig. 3), and one recombinant of the long arm of chromosome 7S with the long arm of chromosome 7B, identified among 165 BC_1 plants. The total number of recombined chromosomes identified in the three experiments was 325. Recom-

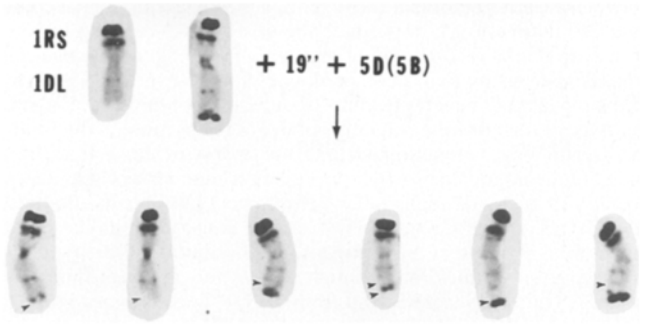

Fig. 1 Recombinants of the long arms of chromosomes 1D of wheat (here in a 1RS.1DL translocation) and 1R of rye induced by a 5D(5B) substitution in hexaploid triticale. Translocation breakpoints indicated by *arrowheads*. Chromosome 1R.1D$_{5+10}$–1 is the last on the right, bottom row

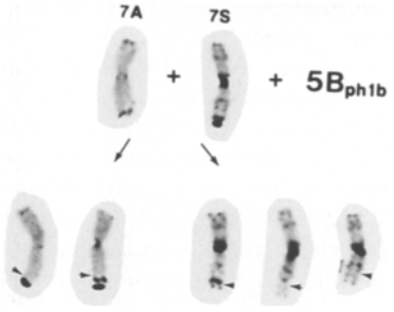

Fig. 3 Recombinants of the long arms of chromosomes 7A of wheat and 7S of *Aegilops speltoides* induced by the *ph1b* mutation in wheat. Translocation breakpoints indicated by *arrowheads*

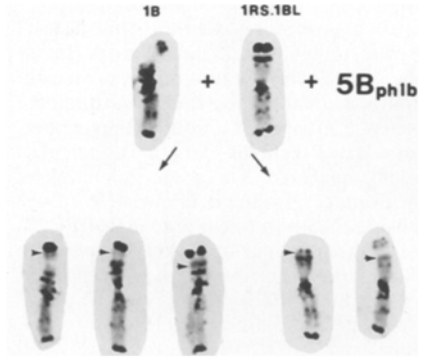

Fig. 2 Recombinants of the short arms of chromosomes 1B of wheat and 1R of rye (here in a 1RS.1BL translocation) induced by the *ph1b* mutation in wheat. Translocation breakpoints indicated by *arrowheads*

bination frequencies ranged from 0.4% for the 1RS arm in wheat to 8.8% for the 1BL arm in triticale. Crossing-over was monitored in 33 segments which were distributed over the entire lengths of chromosomes 1B, 1D and 1R, and the long arm of chromosome 7S.

With one exception, all recombined chromosomes identified in this study resulted from single cross-over events. The exception was a 1D.1R recombinant in triticale in which the long arm had clearly identifiable C-bands 1.3 and 1.5 of 1DL, band L2 of 1RL, and a very short euchromatic terminal segment of the 1.6 region of 1DL (Fig. 1). This arm resulted from a double cross-over: one in the proximal area of the 1.6 region of 1DL and proximal to the L2 band on 1RL, and the other one at the distal end of 1RL, between bands L2 and L3. The identity of segments and the positions of translocation breakpoints were confirmed in subsequent generations (data not shown).

Apart from the short arms in the 7A–7S pair, translocation breakpoints were concentrated in the most distal segments of the chromosome arms in all combinations analyzed (Fig. 4). Of the 326 breakpoints observed in 325 recombined chromosomes, 90.1% were located in the terminal interband segments of chromosome arms. The proportion of the translocation breakpoints located in the subterminal segments was considerably lower (6.7%), and only one breakpoint was located in the middle of a chromosome arm (a translocation involving the long arms of chromosomes 1BL and 1DL).

The short arms in the 7A–7S pair did not conform to this general pattern of concentration of recombination in the most distal segment of an arm. Because polymorphic markers were missing, recombination in the homologous region of the translocated short arm of chromosome 7S could not be monitored. Six translocation breakpoints were located in each of the two adjacent intercalary segments in which homoeologous recombination was monitored. These two segments recombined with corresponding segments of chromosomes 7A with a much higher frequency than any segment of comparable location in other combinations of homoeologues (Fig. 4). Because the short arm of the 7S chromosome was translocated, with the terminal segment being homologous and the proximal segment being homoeologous to 7A, it is possible that the recombination pattern in this arm was governed by different principles than those involved in strictly homoeologous exchanges. For this reason, the 12 recombinants of this arm were not included in the analyses of the distribution of recombination.

Excluding the combination of the short arms of chromosomes 7S and 7A, there were no statistically significant differences in the distribution of the translocation breakpoints among the analyzed chromosome arms. When the results from all arms were combined, a statistically significant relationship, $f(x)=0.0091e^{0.0592x}$, was found between homoeologous recombination frequency, expressed as a relative frequency of translocation breakpoints per unit of relative arm's length, and the relative distance from the centromere (Fig. 5). Approximately 89% of variation in the data is explained by this function ($R^2=0.892$). A somewhat lower proportion of the data's variation ($R^2=0.885$) is explained by the relationship $y=0.8008-0.0622x+0.0009x^2$.

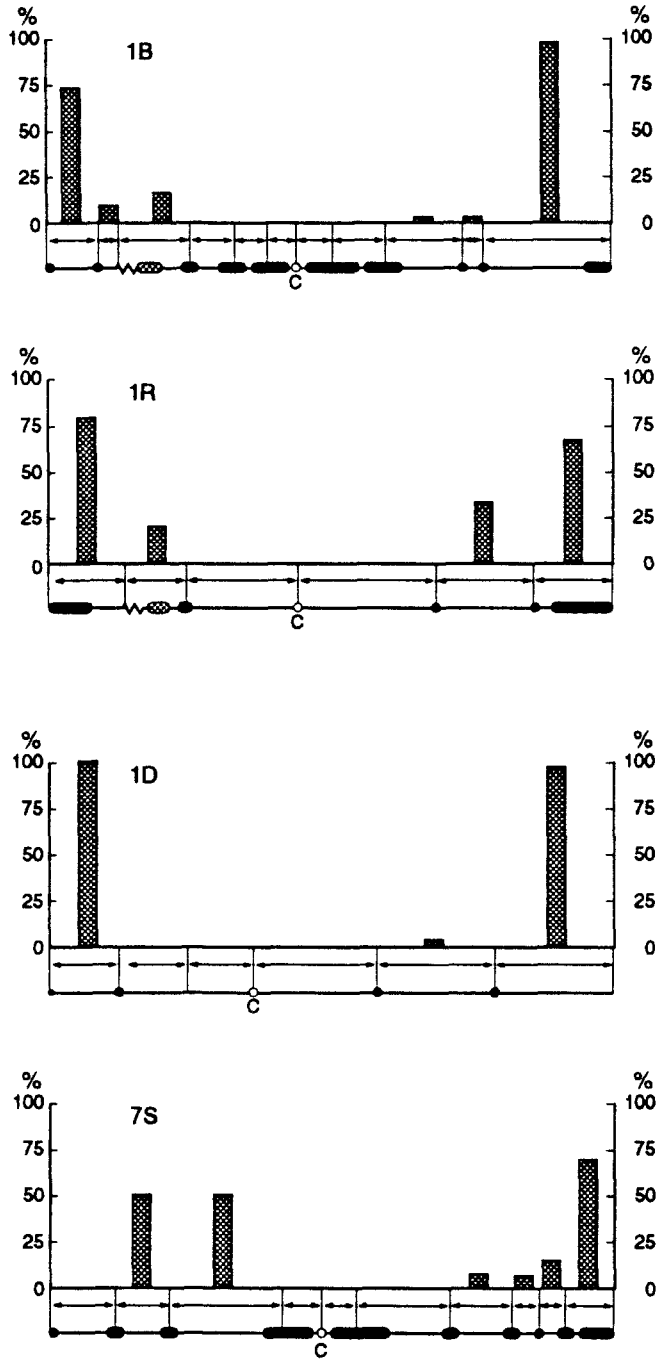

Fig. 4 Distribution of the translocation breakpoints along chromosome arms in the homoeologous recombinants of chromosomes 1A, 1B, 1D and 1R, and of chromosome 7S in wheat and triticale. In each graph, short arms are on the left, long arms on the right. *C* centromere. Positions of C-bands used to determine the location of translocation breakpoints and the lengths of chromosome segments in which homoeologous recombination was monitored are shown schematically under each horizontal axis

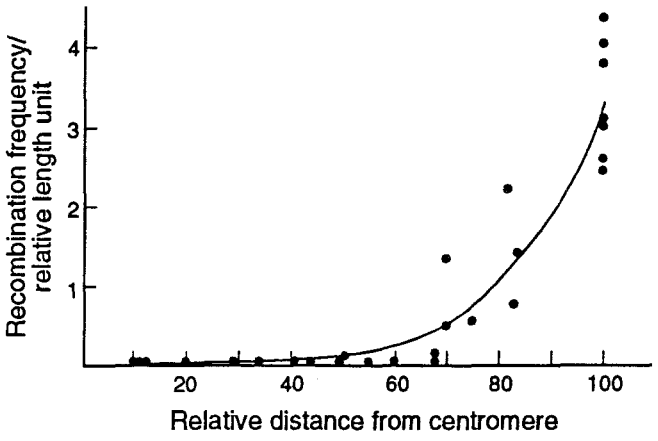

Fig. 5 Relationship between the relative recombination frequency per relative chromosome arm length unit and the relative distance from the centromere in homoeologous recombination in wheat and triticale described by the function $f(x)=0.0091e^{0.0592x}$ ($R^2=0.892$)

Discussion

The overall recombination frequency between the group-1 homoeologues observed in this study was within the ranges expected on the basis of pairing affinities of these chromosomes in the absence of the *Ph1* gene (Naranjo et al. 1987; Naranjo and Fernandez-Rueda 1991). The long arm of chromosome 7S recombined with the long arms of wheat chromosomes 7A and 7B with a combined frequency of 8.5%, which was similar to the recombination frequency of the 1BL arm with 1AL and 1DL arms.

The general pattern of the distribution of translocation breakpoints in homoeologous recombinants was very similar to the distribution of recombination in homologues of the B genome in tetraploid wheat, *T. turgidum* L. (Lukaszewski and Curtis 1993). As between homologues, no homoeologous recombination was detected in the proximal regions of chromosome arms, low levels of recombination were observed in the middle regions of the arms, and the recombination frequency increased exponentially with the proximity toward the telomere. The most proximal translocation breakpoint observed in this study was located in a segment ending 50.1% of the relative chromosome arm length from the centromere. A similar concentration of translocation breakpoints in the distal regions of chromosome arms was observed by Dvorak and Gorham (1992) in recombinants of chromosomes 4B and 4D induced by the *ph1c* mutation in tetraploid wheat.

The major difference between the distribution of recombination between homoeologues and homologues appears to be in the lack of multiple cross-over events. In homologues of the B genome, about 25% of chromosomes analyzed were products of double exchanges and several triple exchanges were observed (Lukaszewski and Curtis 1993). The first cross overs were almost always located in the distal ends of chromosome arms, whereas the location of the second cross overs was governed by the interference distance. A clear relationship was established between physical distance and the interference value. Positive chiasma interference reached a value of 1 at a distance of about 1 μm, and dropped to zero at about 3 μm (Curtis and Lukaszewski 1992). As a consequence of the positive chiasma interference, there was little room for a second chi-

asma in the physically short arms, whereas the physically long arms formed second, interstitial, chiasmata with a high frequency. In this study, which involved 325 recombined homoeologues, only one resulted from a double cross over. This establishes that while multiple exchanges between homoeologous chromosomes can occur, their probability is negligible. It is possible that some double crossover events, if not separated by a C-band, were not detected by the sequence of the procedures employed (preliminary screening by C-banding, followed by screening for the presence of genetic markers, data not shown). However, with the recombination frequency ranging from 0.4% to 8.8% there could not have been many of these.

The only chromosome arm which did not conform to the general pattern of the distal concentration of homoeologous recombination was the short arm of chromosome 7S. This arm is translocated with the proximal minimum 85% of the arm length being from the short arm of 7S and the remaining terminal segment being from the short arm of 7A. In the presence of the *Ph1* gene, either in wheat or in triticale, the 7S segment of the short arm has never been observed to recombine with 7A. In the absence of the *Ph1* gene the 7S segment recombined with a corresponding segment of 7A with a frequency of 7.3%. This is similar to the 8.5% homoeologous recombination frequency of the non-translocated long arm of 7S. However, the translocation breakpoints in the short arm were much more proximal than in the non-translocated long arm, or in any other pair of strictly homoeologous arms (Fig. 4).

A similar change to a proximal location of recombination in a translocated chromosome arm was observed when homoeologous recombination was induced between the 1DL wheat segment in chromosome 1R.1D$_{5+10}$–1 and a corresponding rye segment of a normal long arm of rye chromosome 1R (A. J. Lukaszewski, unpublished). The structure of the long arm of chromosome 1R.1D$_{5+10}$–1 is very similar to that of the short arm of chromosome 7S. It consists of a proximal segment of about 80% of wheat arm 1DL and a distal segment of about 20% of rye arm 1RL, with most of the rye segment being heterochromatic. Translocation breakpoints of all ten recovered secondary recombinants of the long arm of 1R.1D$_{5+10}$–1 with 1RL were located in the vicinity of the 1DL 1.5 C-band, and were at a considerable distance from the original translocation breakpoint. No recombination in such a proximal position was observed when normal arms 1DL and 1RL were induced to pair. Translocation breakpoints in 16 of 18 (89%) recombinants involving normal, non-translocated arms 1DL and 1RL were located in the terminal segment of the 1RL arm between C-bands L2 and L3, and the remaining two breakpoints were located in the subterminal segment (Fig. 4). In both the 7S and the 1R.1D$_{5+10}$–1 experiments, the frequency of homoeologous recombination in the translocated and non-translocated arms was similar. However, the presence of homologous terminal segments in the translocated arms shifted homoeologous recombination to much more proximal positions.

In the presence of the *Ph1* gene the terminal 7A segment of the translocated short arm of chromosome 7S supports complete pairing with the short arm of normal chromosome 7A while the terminal 1RL segment in chromosome 1R.1D$_{5+10}$–1 supports at least 80% pairing with normal 1RL. Some pairing reduction in the absence of the *Ph1* gene could be expected but in a great majority of instances one chiasma must be present in the homologous regions of the translocated arms. Consequently, the chiasmata producing homoeologous exchanges would be the second ones in the arm. The location of these second chiasmata is likely dictated by the positive chiasma interference resulting in a more proximal location of crossing-over than between non-translocated homoeologous arms.

It has been observed in bread wheat that different genomes have similar chiasma frequencies despite large differences in chromosome length (Sallee and Kimber 1978). Also, an analysis of the physical distribution of chiasmata in wheat bivalents suggested considerable differences in the interference distance between different genomes (A. J. Lukaszewski, unpublished). Chiasma density was greater, and the chiasma interference distance appeared shorter, in the D-genome than in the B-genome chromosomes. It is not clear which level of chiasma interference operates in heteromorphic bivalents involving homoeologues from these two genomes. Physical location of translocation breakpoints in recombinants involving the long arms of chromosomes 1R.1D$_{5+10}$–1 and 1R suggests that the shorter interference distance, that of the D-genome, was in effect. However, much larger samples of recombined chromosomes need to be produced to determine the actual interference distance in homoeologous exchanges.

The observation that positive chiasma interference may operate in homoeologous exchanges suggests that engineering of small segments of alien chromosomes in wheat may be impractical. Chiasmata would form preferentially in the homologous segments of the translocated arms while the positive chiasma interference would reduce the probability of exchanges within the alien segment. While this requires experimental proof, it seems that it would perhaps be more efficient to select greater numbers of the original reciprocal single exchange products with translocation breakpoints as close to the gene or region of interest as possible. As demonstrated by Sears (1981), complementary pairs of single exchange translocations can be used to produce chromosomes with small inserts of alien segments. Such an approach could perhaps be more effective and efficient than attempts to reduce the lengths of alien segments by repeated rounds of *ph1b*-induced homoeologous pairing. On the other hand, positive chiasma interference operating in the translocated arms would aid attempts to reduce the lengths of long proximal alien segments, like those in the short arm of chromosome 7S or in the long arm of chromosome 1R.1D$_{5+10}$–1, and could be used to make the proximal regions of chromosome arms more accessible to cytogenetic manipulations.

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