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# Painting rye B chromosomes in wheat: interphase chromatin organization, nuclear disposition and association in plants with two, three or four Bs

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**The B chromosomes (Bs) of rye (*Secale cereale*) have been studied at interphase in terms of their chromatin organization, patterns of nuclear disposition and physical association in plants with two, three, and four Bs. The study was made in the Lindström strain of hexaploid wheat, which carries the rye Bs as an addition line, by *in situ* hybridization with a B-specific probe and by genomic *in situ* hybridization (GISH) with rye genomic DNA, enabling whole chromosome painting. Repetitive sequences common to the As and Bs of rye allow for visualization of the rye B at interphase in the wheat background. A B-specific probe enables the orientation of two or more Bs to be determined, and the combination of both probes used together gives information on the disposition of the Bs and on their patterns of physical association within the nucleus. The Bs form linear 'strings', and the ends of their long arms, which can be detected by the B-specific probe, are usually located within the hemisphere of the nucleus that has the least condensed chromatin. There is dose-dependent association, and even numbers (2B, 4B) have a greater preference for association than odd ones (3B).**

**Key words:** B chromosomes, genomic *in situ* hybridization, nuclear architecture, *Secale cereale*, *Triticum aestivum* cv. Lindström

## Introduction

The B chromosome (B) of rye (*Secale cereale*) has been known for more than 60 years (Gotoh 1924). A wealth of knowledge now exists about its occurrence in populations, its transmission properties, phenotypic effects, structure and organization, and its role in the genetic system. The literature up to 1992 has been reviewed by Jones & Puertas (1993), and some additional recent information, together with a general review of Bs in plants, is also given by Jones (1996). The overview is that the rye B is a selfish chromosome maintained by mitotic drive, and that it can be looked upon as having a host–parasite interaction with the A chromosome set. Research growth points are focused on transmission genotypes, to try to interpret the variation found in transmission rates of the Bs in crosses involving different A genotypes, and on its molecular aspect in terms of sequence organization and expression (Wilkes *et al.* 1995). The molecular analysis has thus far uncovered some B-specific as well as some shared A/B repetitive sequences, and there is also work to indicate that the B may affect the activity of the rDNA loci of the A chromosomes by modifying their pattern of condensation (Delgado *et al.* 1995). The present work is a further contribution towards our understanding of the sequence organization and expression of the B of *S. cereale*. We have taken advantage of the Lindström strain of wheat (Lindström 1965), which carries rye Bs in a wheat background, to visualize the interphase disposition and organization of the rye Bs by *in situ* hybridization and by genomic *in situ* hybridization (GISH). Rye Bs have repetitive sequences in common with the As, so whole genome DNA of rye can be used to paint the Bs and to distinguish them from the wheat interphase chromatin. A B-specific probe for a sequence at the distal end of the long arm can also be used to see the interphase orientation. Distinct levels of B chromosome effects in several endo- or exophenotypic characteristics have been correlated with the presence of odd or even B numbers (Jones & Rees, 1982). The hypothesis that differential phenotypic effects of odd and even Bs could be related to modified patterns of Bs' association or organization during interphase (Jones & Rees 1969) is supported by the results of the present work. As most B effects are normally detected in differentiated cells, a comparative study of the disposition and organization of Bs in interphase was performed both in meristematic root-tip cells as well as in cells from the elongating region.

## Materials and methods

### Plant material

Seeds of *Triticum aestivum* cv Lindström were germinated on moist filter paper at 24°C and were then treated to synchronize cell division (Delgado *et al.* 1995). Root tips from each seedling were excised and fixed in 3:1 (v/v) absolute ethanol–glacial

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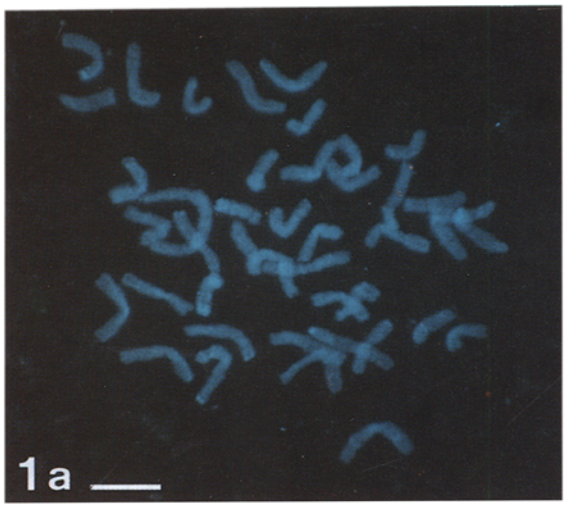


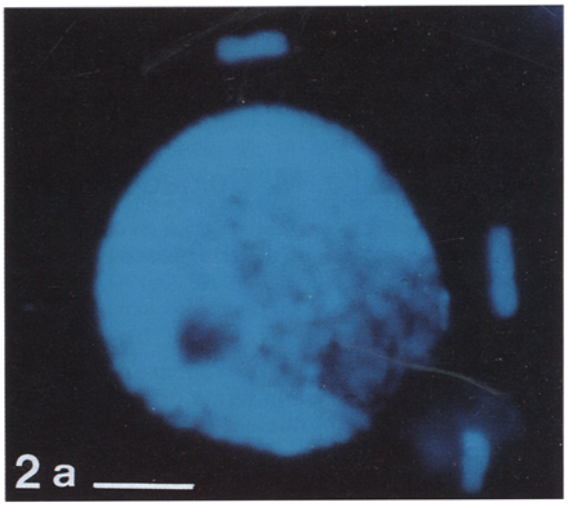


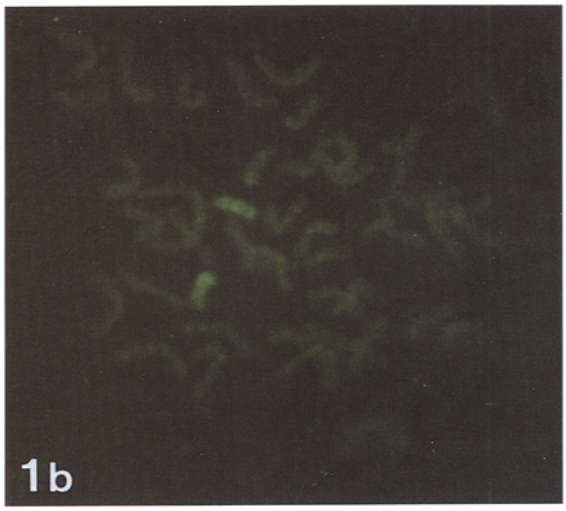


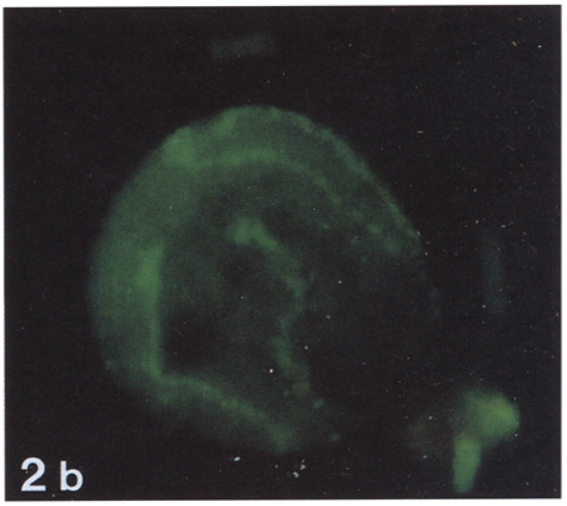


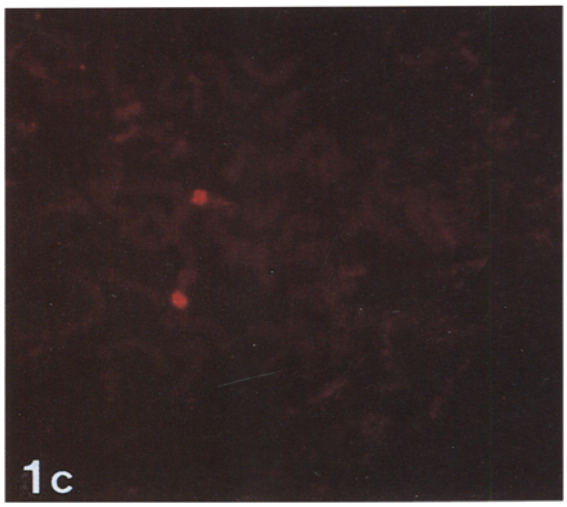


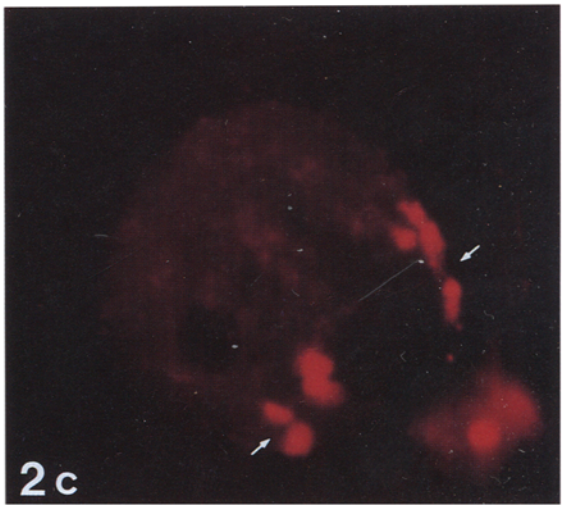


acetic acid for the analysis of interphase cells or pretreated with ice-cold water for at least 24 h to induce c-metaphases, and then fixed. Spreads were made according to Schwarzacher *et al.* (1989). For the isolation of nuclei from the elongating region of the root a 2–5 cm segment adjacent to the meristematic region was used and in this case the tissue was mechanically disrupted after enzymatic digestion on a glass slide to improve isolation.

### *In situ* hybridization

Total genomic DNA was isolated from leaves of *Secale cereale* cv. Centeio do Alto following a procedure from Coen & Carpenter (1986). The isolated DNA was sonicated to 10–12 kb fragments and labelled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation to use as an *in situ* hybridization probe, and was detected by fluoresceinated (FITC) anti-digoxigenin (Boehringer Mannheim). Total genomic DNA from *Triticim aestivum* cv. Chinese Spring was prepared to use as blocking DNA as described by Anamthawat-Jónsson *et al.* (1990). The *in situ* hybridization and detection procedures were adopted from Leitch *et al.* (1991). D1100 probe, kindly supplied by Dr John Forster, University of Wales, Aberystwyth, Institute of Biological Sciences, is a cloned *Eco*RI fragment derived from a 1.1-kb clone (Sandery *et al.* 1990). This probe belongs to a family of highly repeated sequences, which in rye only hybridizes with the distal chromatin of the long arm of the B (Blunden *et al.* 1993). The probe was labelled with biotin-11-dUTP (Sigma) by nick translation and detected with streptavidin–Cy3 conjugate. DNA was counterstained with DAPI in antifade (AF-2, Citifluor Ltd). Interphase and metaphase analyses were performed with an epifluorescence microscope.

## Results

The number of Bs present in each plant was determined in c-metaphases, by double *in situ* hybridization using total genomic rye DNA simultaneously with the D1100 B-specific probe. Three classes of plants were selected, 2B, 3B and 4B. Painting the Bs in this way allows their clear identification in metaphase cells (Figure 1). The D1100 probe hybridizes with the distal region of the long arm of the Bs (Figure 1c). The hybridization pattern of this probe was consistent in all metaphase Bs analysed, showing a small gap of lower fluorescence between two regions of intense fluorescence. Rye genomic DNA hybridization reveals the total length of the metaphase B apart from the terminal heterochromatic region (Figure 1b). In interphase cells the simultaneous use of the two probes in interphase nuclei allowed for visualization of whole Bs as well as for their patterns of organization. The analysis of interphase was only performed on individual nuclei from root tips where the nuclear structure was well preserved, and where it was possible to distinguish the maximum number of Bs by both probes.

### Interphase B-chromatin organization

Interphase B chromosomes show a consistent pattern of organization of their chromatin in a linear and string-like form (Figure 2). The pattern of D1100 label shows most of the Bs having the signal restricted to a small region, and in some cases a region of decondensed label was also observed. When the signal was dispersed the level of condensation of this region was not constant, and different lengths of interphase chromatin could be seen (Figure 2c). In some Bs, in fact, the D1100 label is so dispersed that it corresponds to about one-third of the B interphase length, in contrast with the small fraction that it occupies when condensed. This variable pattern of chromatin condensation revealed by the D1100 probe is not a characteristic of particular nuclei however, as Bs with dispersed or condensed label were detected within the same nucleus; and neither is it reflected at metaphase where the same pattern is found in all Bs. When the D1100 label shows a big dispersion it is possible to observe two regions of high fluorescence with a gap of lower intensity between them (Figure 2c) as can also be seen in metaphase. Individual chromatids of the Bs could not be seen in any of the interphase nuclei from the meristematic cells, and a similar pattern of the label was observed in nuclei from cells of both the meristematic and the elongation region.

### Interphase B-nuclear disposition

In interphase nuclei DAPI staining distinguishes two regions of differential chromatin concentration. The D1100 probe is generally located in the area of lower chromatin concentration at the periphery of the nucleus, with the rest of the B extending towards the area of higher chromatin concentration. In order to study the way in which the Bs are disposed within the nucleus, in all three B-classes of plants, the nuclei were classified in terms of the relative orientation of the Bs. The use of two probes, to delineate the whole B as well as to locate a specific region at one end, allows us to visualize how the interphase Bs are oriented when more than one is present. Two or more Bs were considered as co-oriented when the D1100 label was positioned in the same direction, in relation to the disposition of the whole B within the nucleus, whereas Bs with their D1100 signals in opposite positioning were considered as

**Figure 1.** Metaphase root-tip cell of Lindström wheat with two B chromosomes. **a** DAPI staining for DNA. **b & c** Simultaneous *in situ* hybridization with a digoxigenin-labelled rye total genomic DNA probe detected with anti-digoxigenin FITC conjugate (green), which reveals the entire rye B chromosome apart from the distal region of the long arm (**b**); and a rye B-specific DNA D1100 probe labelled with biotin and detected with streptavidin–Cy3 conjugate (red), which labels the terminal region of the long arm (**c**). Bar = 10 $\mu$m.

**Figure 2.** Interphase root-tip nucleus of Lindström wheat with 4 B chromosomes. **a** DAPI staining for DNA. **b & c** Simultaneous *in situ* hybridization with a digoxigenin-labelled rye total genomic DNA probe detected with anti-digoxigenin FITC conjugate (green), showing interphase rye B chromosomes in a string-like form (**b**) and a rye B specific DNA 1100 probe labelled with biotin and detected with streptavidin–Cy3 conjugate (red), revealing different levels of chromatin condensation and the typical pattern of D1100 label with a gap of lower fluorescence between two strongly labelled regions (arrow) (**c**). Bar = 10 $\mu$m.

**Table 1** Frequencies of nuclei with different patterns of B orientation in plants of Lindström wheat with 2, 3 and 4 B chromosomes

| | Percentage of nuclei with all Bs co-oriented | Percentage of nuclei with non-co-oriented Bs | Total number of nuclei analysed |
|---|---|---|---|
| 2Bs | 93  | 7  | 151 |
| 3Bs | 84  | 16  | 78 |
| 4Bs | 81  | 19  | 132 |

In the nuclei diagrams each B chromosome is represented by a line corresponding to the rye genomic DNA label and a dot that indicates the D1100 label. The nuclei diagrams shown are the most representative of each class.

non–co-oriented. The data on patterns of B-orientation for nuclei with 2, 3, and 4Bs are given in Table 1. In all of the plants analysed there was a high frequency (81–93%) of nuclei in which all of the Bs were co-oriented, and there was no significant difference in these values for plants in the three different B-classes ($P < 0.01$). In the 2B class a comparison was made of the frequency of co-orientation in the elongation and meristematic regions of the roots, and the frequency of 92% co-orientation in the elongating region did not vary significantly from that of the meristem (93%) ($P < 0.01$).

### Association of Bs

The analysis given above indicates a non-random pattern of the disposition of the Bs within the nucleus, with a marked tendency for co-orientation. Besides the relative orientation of the Bs, a degree of association of these chromosomes was also observed,and these observation are summarized in Table 2. Association means co-oriented Bs lying parallel to one another and at a distance that is less than one-quarter of the greater diameter of the nucleus. Co-oriented Bs lying at distances of more than one-quarter of the greater diameter of the nucleus, or being closer but lying in opposite directions, are considered to be positioned independently, i.e. not associated. The frequency of nuclei with associated Bs, that is with at least two Bs associated in the 2B, 3B and 4B classes, are respectively 72%, 69% and 91% (Table 2). In cells from the elongating region of the root tip the level of association of the 2B class (76%) was

**Table 2.** Indexes of B associations in plants of Lindström wheat with 2, 3 and 4 B chromosomes

| | Zero associations | One association | Two associations | Three associations | Number of cells analysed | Percentage of nuclei with associated Bs | Mean degree of association per cell | Degree of association per B (%) |
|---|---|---|---|---|---|---|---|---|
| 2Bs | 42 | 109  | | | 151 | 72 | 0.72 | 72 |
| 3Bs | 24 | 45  | 9  | | 78 | 69 | 0.40 | 50 |
| 4Bs | 12 | 28  | 25  | 49  18  | 132 | 91 | 0.58 | 76 |

In the nuclei diagrams each B chromosome is represented by a line corresponding to the rye genomic DNA label and a dot that indicates the D1100 label. The nuclei diagrams shown are the most representative of each class.

similar to that observed in the meristematic region. It must be realized however that these frequencies cannot be directly compared, as the increase in the B number naturally allows more opportunities for any two Bs present to associate in close proximity in various ways. In order to resolve this problem and to allow a direct comparison of the association levels of the Bs, independently of their number present we have calculated an index of the *mean degree of association of Bs per cell.* This index is a weighted mean, which varies between zero and one, zero being the complete absence of association and one the maximum.

As shown in the diagrams of the nuclei in Table 2, the number of associations per nuclei was scored by considering the association between two Bs as being independent of any other possible association of those Bs with any other.

The weights were attributed as a fraction of the maximum possible number of associations per genotype; that is for 2B plants, zero for no association and 1 for one association; for 3B plants, zero for no association, 1/2 for one association and 1 for two associations; for 4B plants, zero for no association, 1/3 for one association, 2/3 for two associations and 1 for three associations. The degree of association for the 2B class is therefore $(109 \times 1) + (42 \times 0)$ divided by $151 = 0.72$; that for the 3B class is the weighted mean of $(45 \times 1/2) + (9 \times 1) + (24 \times 0)$ divided by $78 = 0.40$ and the 4B is the weighted mean of $(28 \times 1/3) + (74 \times 2/3) + (18 \times 1) + (12 \times 0)$ divided by $132 = 0.58$. The three means are significantly different from one another ($P < 0.01$).

Another way to express the results is in terms of the *degree of association per B,* rather than per cell, which permits us to evaluate the influence of different genotypes on the B itself (Table 2). For this purpose the total number of associated Bs is divided by the total number of Bs present in each class. In the case of 2Bs this is $109 \times 2/151 \times 2 = 0.72$; for 3Bs it is $(45 \times 2) + (9 \times 3)$ divided by $(3 \times 78) = 0.50$; and for 4Bs it is $(28 \times 2) + (25 \times 3) + (49 \times 4) + (18 \times 4)$ divided by $(132 \times 4) = 0.76$. Expressed in this way the dose-dependent association behaviour of the Bs is even more evident.

Whichever way we express the data it also clear that the majority class of association is that involving the Bs in pairs rather than in threes or fours (Table 2). From those nuclei that have associated Bs, the percentage of nuclei showing Bs in pairs is 100% for 2B, 83% for 3B and 64% for 4B.

## Discussion

It is becoming increasingly clear that there is order to the arrangement of chromosomes in interphase nuclei. The application of FISH and GISH has opened up a new avenue of research, and has enabled us to discover that chromosomes lie in single domains and are not intermingled. In humans they occupy certain restricted territories, or compartments (Cremer *et al.* 1993), whereas as in plants the arrangement seems to be 'string-like' with threads running through the nucleus.

In plants it has been convenient to work with alien chromosomes, or alien segments from translocations, and to discriminate the alien material from the background of the recipient parent nucleus. Hexaploid wheat is the favoured host material. One of the first studies of this kind was reported in a review paper on nuclear architecture by Heslop-Harrison & Bennett (1990), who used wheat that included two alien chromosome arms originating as a translocation between chromosome 1 from B genome of wheat and 1R from rye. The two rye arms were found to occupy two quite separate and distinct string-like linear domains. Schwarzacher *et al.* (1992) later studied various alien chromosomes and chromosome arms in wheat, including the 5AS/5RL translocation for the two 5RL arms of rye. These authors likewise found two separate and parallel domains running through the nucleus. Recently Islam-Faridi & Mujeeb-Kazi (1995) have worked with several rye additions and translocations, mostly at metaphase of mitosis, and they also show one exceptionally clear interphase nucleus homozygous for the 1RS arms of rye; and this again reveals two parallel and distinct linear domains. The general opinion is that homologues have their own domains, and there is no evidence thus far from these *in situ* studies in plants that points to any somatic interphase pairing. The situation in yeast appears to be different. Scherthan *et al.* (1994) have performed FISH studies on vegetative nuclei of *Schizosaccharomyces pombe,* using cells before the induction of meiosis, and have found that homologues share a joint territory.

The interphase organization of the rye Bs that we report on here has similar general features to those of the 5R and 1R A-chromosome arms described by others, i.e. the Bs occupy distinct linear and parallel string-like domains running through the nucleus. Notwithstanding the fact that we have also been able to describe the orientation of the Bs, and the particular patterns of association due to the B-specific sequence, there is no obvious difference between the A and the B chromosome interphase organization.

Our material does differ from earlier works, however, in two major respects; firstly, it concerns dispensable supernumerary B chromosomes for which there is no previous information, and secondly because of the very nature of the Bs we have multiple copies (3B, 4B) in excess of 2. This polysomy of the Bs enables us to gain insight into the question of genetic activity in relation to interphase organization and association of chromosomes, which is a topic of some interest in relation to As (Heslop-Harrison & Bennett 1990) as well as Bs.

The idea that the Bs of rye may be associated in pairs at interphase was first proposed by Jones & Rees (1969), in order to explain differential activity of Bs depending on their presence in odd or even numbered combinations. The initial observations for the so-called odd–even effect were made on A chromosome behaviour at

meiosis and on aspects of nuclear physiology. The Bs seemed to be less harmful when present in even numbers. The present results provide some preliminary evidence to suggest that the Bs in rye are not independent at interphase, but rather that they are co-oriented and that they associate in much closer proximity than we would expect from a random pattern of distribution. There is no suggestion of somatic pairing, as far as we can judge with this new method of looking at interphase chromosomes, but clearly there is physical proximity and parallel co-orientation. The *indexes of mean degree of association of Bs per cell* and the *degree of association per B* that we have used also indicate that there are differences in these values in relation to odd and even numbers, in that there is a greater degree of association for the 2B (0.72) and the 4B classes (0.76) than there is for the 3B class (0.50) and, within the nuclei, where there is association a marked tendency for Bs to be associated in pairs is observed, i.e. 100% for the 2Bs, 83% for the 3Bs and 64% for the 4Bs. Where Bs are associated they therefore have a preference for being in pairs. The data are preliminary, and there is a need to look in more detail and to use more B classes, but thus far there are some grounds for believing that the odd–even effect may have a basis in the physical organization of Bs in the interphase nucleus.

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## References

Anamthawat-Jónsson K, Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1990) Discrimination between closely related *Triticeae* species using genomic DNA as a probe. *Theor Appl Genet* **63**: 337–348.

Blunden R, Wilkes TJ, Forster JW *et al.* (1993) Identification of the E3900 family, a second family of rye B chromosome specific repeated sequences. *Genome* **36**: 706–711.

Coen ES, Carpenter R (1986) Transposable elements in *Anthirrinum majus:* generations of genetic diversity. *Trends Genet* **2**: 292–296.

Cremer T, Kurz A, Zirbel R *et al.* (1993) Role of chromosome territories in the functional compartmentalization of the cell nucleus. *Cold Spring Harb Symp Quant Biol* **58**: 777–791.

Delgado M, Morais-Cecílio L, Neves N, Jones RN, Viegas W (1995) The influence of B chromosomes on rDNA organisation in rye interphase nuclei. *Chrom Res* **3**: 487–491.

Gotoh K (1924) Uber die chromosomenzahl von *Secale cereale* L. *Bot Mag Tokyo* **38**: 135–152.

Heslop-Harrison JS, Bennett MD (1990) Nuclear architecture in plants. *Trends Genet* **6**: 401–405.

Islam-Faridi MN, Mujeeb-Kazi A (1995) Visualization of *Secale cereale*. DNA in wheat germ plasm by fluorescent *in situ* hybridization. *Theor Appl Genet* **90**: 595–600.

Jones RN (1996) B chromosomes in plants. Tansley review. *New Phytol* (in press).

Jones RN, Puertas MJ (1993) The B-chromosomes of rye (*Secale cereale*) In: Dhir KK, Sareen TS, eds. *Frontiers in Plant Science Research*, Delhi, India, Bhagwati Enterprises: Chapter 6, 81–112.

Jones RN, Rees H (1969) An anomalous variation due to B chromosomes in rye. *Heredity* **24**: 265–271.

Jones RN, Rees H (1982) *B Chromosomes*. London: Academic Press.

Leitch IJ, Leitch AR, Heslop-Harrison JS (1991) Physical mapping of plant DNA sequences by simultaneous *in situ* hybridization of two differently labelled fluorescent probes. *Genome* **34**: 329–333.

Lindström J (1965) Transfer to wheat of accessory chromosomes from rye. *Hereditas* **54** 149–155.

Sandery MJ, Forster JW, Blunden R, Jones RN (1990) Identification of a family of repeated sequences on the rye B chromosome. *Genome* **33**: 908–913.

Scherthan H, Bähler J, Kohli J (1994) Dynamics of chromosome organization and pairing during meiotic prophase in fission yeast. *J Cell Biol* **127**: 273–285.

Schwarzacher T, Anamthawat-Jónsson K, Harrison GE *et al.* (1992) Genomic *in situ* hybridization to identify alien chromosomes and chromosome segments in wheat. *Theor Appl Genet* **84**: 778–786.

Schwarzacher T, Leitch AR, Bennett MD, Heslop Harrison JS (1989) *In situ* localization of parental genomes in a wide hybrid. *Ann Bot* **64**: 315–324.

Wilkes TM, Francki MG, Langridge P *et al.* (1995) Analysis of rye B-chromosome structure using fluorescence *in situ* hybridization (FISH). *Chrom Res* **3**: 466–472.