Physical mapping of 5S and 18S–25S rDNA and repetitive DNA sequences in *Aegilops umbellulata*

A. Castilho and J.S. Heslop-Harrison

Abstract: An accurate physical map of the location of the 5S and the 18S–5.8S–25S rRNA genes and a repetitive DNA sequence has been produced on *Aegilops umbellulata* Zhuk., ($2n = 2x = 14$) chromosomes by in situ hybridization. Chromosome morphology together with the hybridization pattern of pSc119.2, a DNA sequence from rye, allowed identification and discrimination of different chromosomes; pSc119.2 hybridizes with all *Ae. umbellulata* chromosomes at the telomeres, except for the short arm of chromosome 6U, and shows intercalary sites on the long arms of chromosomes 6U and 7U. The 5S and 18S–25S rDNA have been mapped physically only on the short arms of chromosomes 1U and 5U. On chromosome 1U the order of the genes is 5S rDNA subterminal and 18S–25S rDNA more proximal, while on chromosome 5U the position of the genes is reversed. The relative order of the genes, together with the hybridization pattern of the pSc119.2, is useful in identifying whole chromosomes or chromosome segments from *Ae. umbellulata* in recombinant or addition lines with wheat. The data help link the physical organization of chromosomes to the genetic map. Other members of the Triticeae vary in the presence and order of the 5S and 18S–25S rDNA sequences on groups 1 and 5, indicating multiple and complex evolutionary rearrangements of the chromosome arms.

Key words: *Triticum umbellulatum*.

Résumé : Une carte physique précise de la localisation des gènes d'ARNs ribosomiques 5S et 18S–5.8S–25S ainsi que d'une séquence d'ADN répétée a été produite pour les chromosomes du *Aegilops umbellulata* Zhuk. ($2n = 2x = 14$) par hybridation in situ. La morphologie chromosomique combinée avec l'hybridation de la sonde pSc119.2, une séquence d'ADN du seigle, a permis d'identifier et distinguer certains chromosomes. Cette sonde hybride avec les régions télomériques de tous les chromosomes à l'exception du bras court du chromosome 6U de même qu'avec des sites internes sur les bras longs des chromosomes 6U et 7U. La localisation physique des ADNr 5S et 18S–25S n'a été réalisée que sur les bras courts des chromosomes 1U et 5U. Sur le chromosome 1U, l'ADNr 5S est en position subterminale et l'ADNr 18S–25S est en position proximale tandis que sur le chromosome 5U, l'ordre des gènes est inversé. L'ordre relatif des gènes, combiné au motif d'hybridation obtenu avec la sonde pSc119.2, permet d'identifier des chromosomes entiers ou des segments de chromosome du *Ae. umbellulata* présents dans des lignées d'addition ou de recombinaison avec le blé. Ces données facilitent l'intégration des cartes physiques et génétiques. D'autres membres des Hordées varient quant à la présence et l'ordre des séquences d'ADNr 5S et 18S–25S sur les chromosomes des groupes 1 et 5 ce qui indique de multiples et complexes réarrangements au niveau des bras de chromosomes au cours de l'évolution.

Mots clés : *Triticum umbellulatum*.

[Traduit par la rédaction]

Introduction

Aegilops umbellulata Zhuk. is an important wild diploid wheat species that includes many genes of actual and potential agronomic value. The transfer of leaf rust resistance from *Ae. umbellulata* was among the first alien gene introductions made to bread wheat (Sears 1956) and this source of resistance is still important today. As well as disease resistance, quality characters are of great interest to wheat breeders. There is an exceptionally high molecular weight glutenin present in *Ae. umbellulata* (Law and Payne 1983) that may be useful for improving wheat breadmaking quality.

The method of in situ hybridization is proving to be very important for following the transfer of alien chromosomes or chromosome segments into wheat. As with many other alien species, the use of total genomic DNA as a

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Fig. 1. Double fluorescence in situ hybridization of a *Ae. umbellulata* ($2n = 2x = 14$) somatic metaphase cell showing the rDNA hybridization sites. The micrograph was taken with a triple filter allowing the simultaneous visualization of the DAPI-stained chromosomes (blue), the hybridization sites of the 18S–25S rDNA labelled with Fluorored (red bands), and the 5S rDNA labelled with digoxigenin-11-dUTP (green dots). **Fig. 2.** *Ae. umbellulata* somatic metaphase cell after in situ hybridization. (*a*) DAPI-stained chromosomes. (*b*) Hybridization sites of the repetitive DNA sequence (pSc119.2) labelled with digoxigenin-11-dUTP (yellow–green dots). (*c*) Diploid karyotype allowing the identification of the seven chromosome pairs by morphological and pSc119.2-hybridization pattern analysis.

probe enables *Ae. umbellulata* chromosomes to be identified in a wheat background, but is is also valuable to have probes that enable chromosomes, or parts of chromosomes, to be identified to homoeologous group. Furthermore, *Ae. umbellulata* is an interesting target species in which to examine syntenic or collinear arrangements of genes within all Triticeae species (Bennetzen and Freeling 1993; Moore et al. 1993), because of its relatively close relationship to bread wheat, its ease of crossing, and its useful genes (Law and Payne 1983). There are already good idiograms showing the morphology of the chromosomes (Heslop-Harrison and Bennett 1983; Kimber 1967). In a wider context, examination of the physical distribution of repetitive genes and other sequences on the different chromosomes of Triticeae cereals is useful for understanding the evolution of and the relationships between the species and their chromosomes, and for seeing the types of genomic change that might be possible during a plant breeding programme.

In the present work, we aimed to produce accurate physical maps of the locations of 5S and 18S–25S rRNA genes and a repetitive sequence on *Ae. umbellulata* chromosomes. These data will be useful for identifying whole chromosomes or chromosome segments in recombinant lines, and will also enable comparisons of chromosomal organization to be made within the Triticeae.

Materials and methods

Plant material and chromosome preparation

Seeds from *Aegilops umbellulata* Zhuk. ($2n = 2x = 14$) were germinated in distilled water on filter paper at 25°C for about 2 days. When root tips were a few millimeters long the seedlings were transferred to a new petri dish with a 0.001% (w/v) hydroxyurea solution and incubated at 25°C for 18 h in the dark (to stop DNA replication). After being washed in distilled water, the seedlings were allowed to grow in distilled water for 5 h at 25°C to allow recovery and to allow cells to enter metaphase. To shorten the chromosomes, the seedlings were treated for 3 h in a 0.05% (w/v) colchicine solution at 25°C and the excised root tips were transferred to ice water at 0°C for 24 h, and finally were fixed in 100% ethanol – acetic acid, 3:1(v/v).

Chromosome preparation followed the method described by Schwarzacher et al. (1989).

DNA probes

The DNA probes used were (*i*) pSc119.2, a tandem repeat sequence containing a 611 base pair (bp) *Hin*dIII fragment isolated from *Secale cereale* L. (Bedbrook and Flavell 1980) and subcloned into the plasmid pUC18 (McIntyre et al. 1990). (*ii*) pTa71, a highly repeated sequence containing a 9 kb *Eco*RI fragment of the rDNA isolated from common wheat, *Triticum aestivum* L. emend Thell (Gerlach and Bedbrook 1979), recloned into pUC19; it contains the coding sequences for the 5.8S, 18S, and 25S genes and spacer sequences. (*iii*) pTa794, a highly repeated sequence containing a 410 bp *Bam*HI fragment of the 5S rDNA isolated from embryos of common wheat (Gerlach and Dyer 1980), cloned into pBR322; it contains a 120 bp coding sequence and the nontranscribed spacer.

In experiments using simultaneous in situ hybridization to map the physical location of the 5S and 18S–25S rRNA genes, the pTa794 clone was amplified and labelled by PCR with digoxigenin-11-dUTP (Boehringer Mannheim, Germany) and the pTa71 clone was labelled with Fluorored (Rhodamine-4-dUTP, Amersham, U.K.) by nick translation.

The karyotype of *Ae. umbellulata* chromosomes was examined after hybridization with the pSc119.2 clone labelled with digoxigenin-11-dUTP by nick translation.

Nonradioactive in situ hybridization

Before hybridization, chromosome preparations on slides were treated with 100 μg/mL DNAase-free RNase in a 2× SSC (0.15 M NaCl plus 0.15 M sodium citrate) solution at 37°C for 1 h in a humid chamber, washed 2× 5 min in 2× SSC at room temperature, and incubated in a 500 μg/mL pepsin in 0.01M HCl solution for 10 min at 37°C. After two washes in 2× SSC, the slides were treated in a prehybridization fixative solution of freshly depolymerized 4% (w/v) paraformaldehyde in water at room temperature for 2× 5 min. Finally, the slides were dehydrated in a graded ethanol series and air-dried.

Prior to hybridization, probes were mixed in a solution containing 50% (w/v) high grade formamide, 5% (w/v) dextran sulphate, 0.1% (w/v) SDS in 2× SSC and 5 ng/μL of autoclaved salmon sperm DNA (hybridization stringency of 76%). The probe concentration (per 30 μL of hybridization mixture in each slide) was 75 ng for pTa71 and 150 ng for pTa794 and pSc119.2. The hybridization mixture was denatured in a 70°C water bath for 10 min and incubated on ice for 5 min before being loaded onto the slide preparations and covered with plastic coverslips.

The chromosomes together with the probe were denatured for 5 min at 70°C using a modified programmable temperature controller (Hybaid; see Heslop-Harrison et al. 1991).

The hybridization was carried out overnight at 37°C in a humid chamber. After hybridization, the slides were washed in 2× SSC at 42°C until the coverslips floated off and were then given a stringent wash in 20% (v/v) formamide in 0.1× SSC at 42°C for 5 min. The slides were washed 2× 5 min in 2× SSC at 42°C and another 2× 5 min in 2× SSC and 4× SSC / 0.2% (v/v) Tween 20 at room temperature.

The rDNA probe (pTa71) with a directly labelled nucleotide (Fluorored) does not need detection steps. The

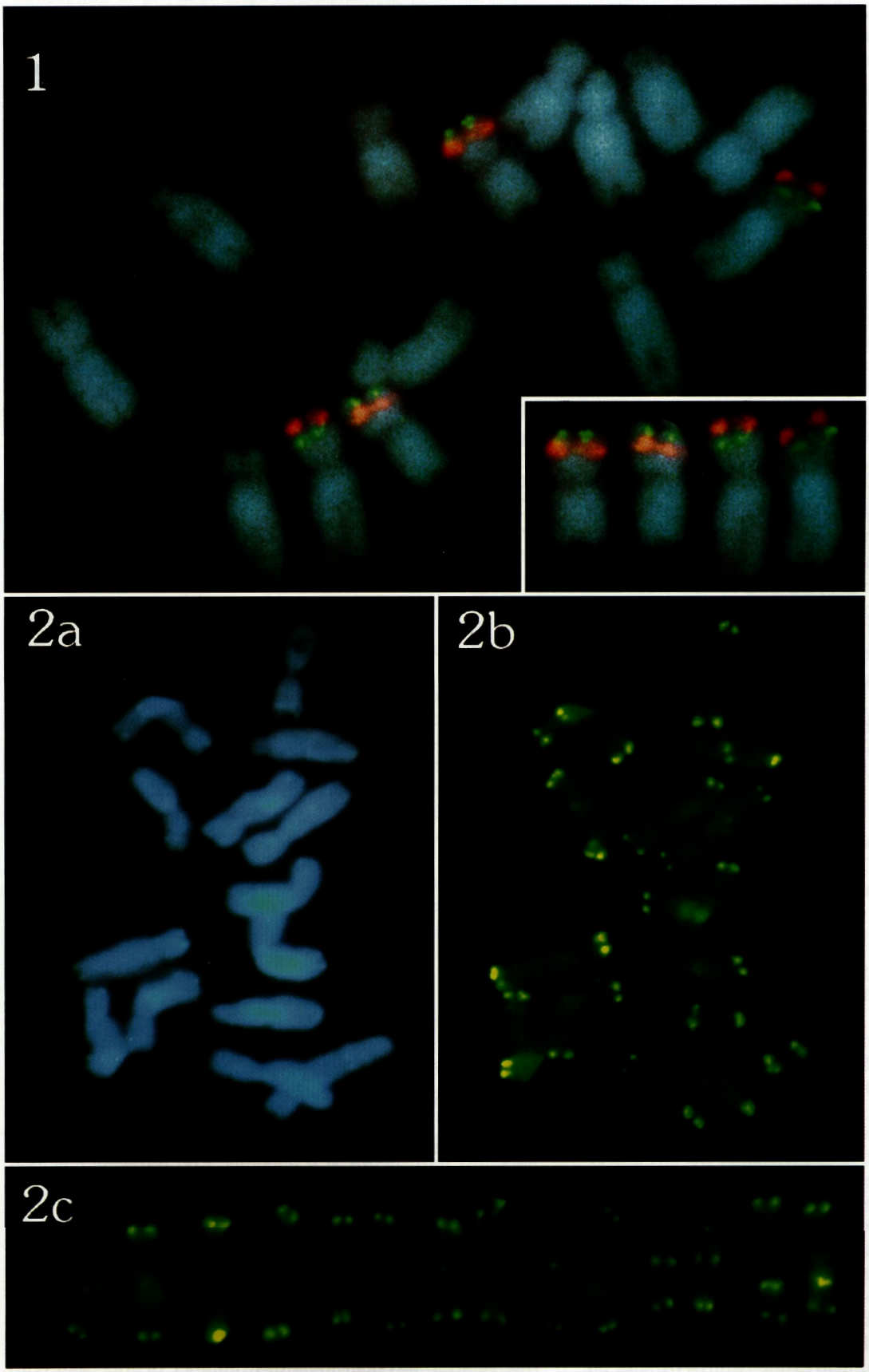
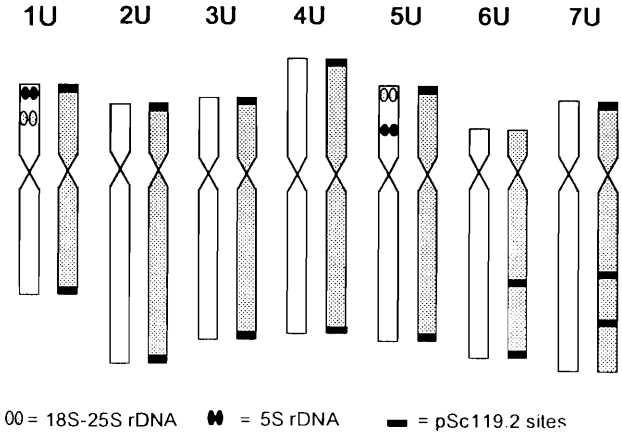

1
2a
2b
2c

Fig. 3. Idiogram of *Ae. umbellulata* chromosomes showing the physical location of the 5S and the 18S–25S rDNA sites (unshaded chromosomes) and the pSc119.2-hybridization pattern (shaded chromosomes).



hybridization sites of the 5S rRNA (pTa794) and the repetitive DNA sequence (pSc119.2) digoxigenin-labelled probes were detected with sheep antidigoxigenin-FITC (fluorescein isothiocyanate, Boehringer Mannheim). Slides were blocked in a 5% (w/v) BSA (bovine serum albumin) in 4× SSC / Tween 20 solution for 5 min at room temperature. Each slide had 100 μL of the detection solution (20 μg/mL of sheep antidigoxigenin-FITC in 5% BSA) added to it and slides were then incubated at 37°C for 1 h in a humid chamber.

After washing the slides in 4× SSC / Tween 20 several times at room temperature, the slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (2 μg/mL in McIlvaines citrate buffer, pH = 7) for 10 min at room temperature. Finally, the slides were mounted in antifade solution (AF1, Citifluor) to reduce the fading of the fluorescence. Probe hybridization sites were visualized with a Leiptz epifluorescence microscope following excitation using appropriate light filters. Photographs were taken with Fujicolor Super HG400 colour print film.

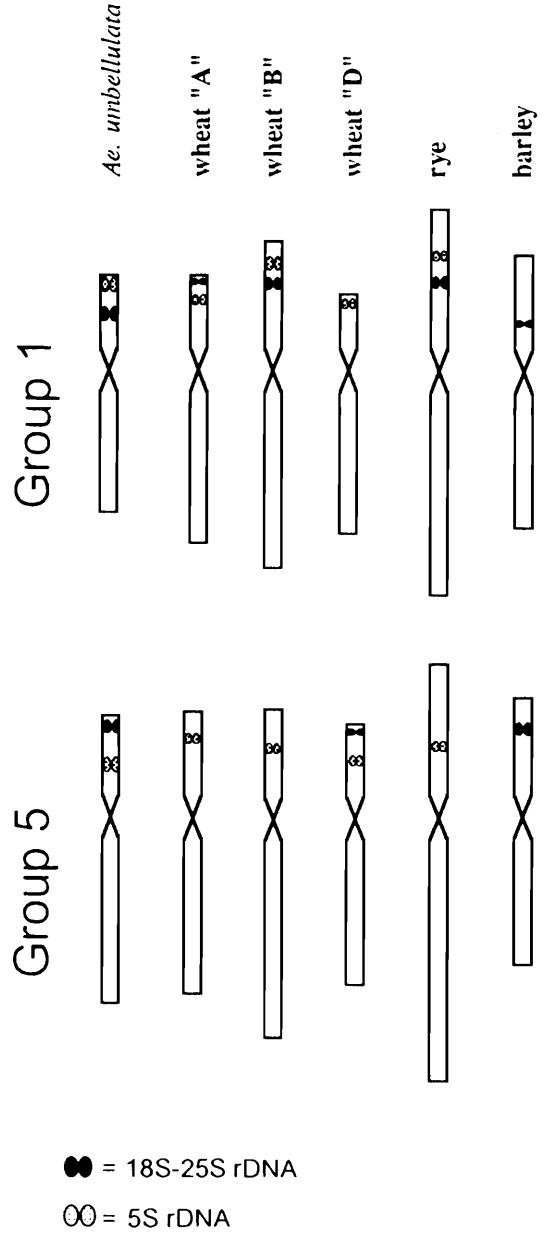
Results

The double target in situ hybridization with the Fluorored-labelled pTa71 and the digoxigenin-labelled pTa794, revealed two sites of both 5S and 18–25S rDNA (Fig. 1). These genes are located on chromosomes 1U and 5U. On chromosome 1U the 5S rDNA appears more distal than the 18S–25S rDNA, while on chromosome 5U the order is reversed.

Figure 2 shows the hybridization pattern of digoxigenin-labelled pSc119.2, a repetitive sequence, on the *Ae. umbellulata* chromosomes. The sequence hybridizes with all chromosomes on both arms, except on the short arm of chromosome 6U which, like chromosome 7U, also shows an intercalary hybridization on the long arm. There are some polymorphisms in size of sites between homologous chromosomes.

Idiograms of the hybridization pattern of the repetitive DNA sequence, the morphology of the chromosomes stained with DAPI, and the physical map of the two rDNA sites are shown in Fig. 3.

Fig. 4. Schematic representation of the group 1 and group 5 chromosomes in wheat (Mukai et al. 1990, 1991), rye (A. Cuadrado, N. Jouve, and J.S. Heslop-Harrison, submitted for publication), barley (Leitch et al. 1992, 1993), and *Ae. umbellulata* (this work) showing the locations of 5S and 18S–25S rDNA loci.



Discussion

Ribosomal RNA genes

The 5S and 18S–25S rRNA genes are highly repeated in the genome and, in the Triticeae, occur in many thousands of copies, most of which are not expressed (see Flavell et al. 1993). Because the sequence variation between loci is often as great as that within loci, and because of the potentially large number of loci, the genes are difficult to map by RFLP or genetic methods. Nevertheless, knowledge of the relative physical locations, number of loci, and copy number at each locus is important and useful in the

construction of physical maps of chromosomes and in phylogenetic studies. Although there are complex regulatory mechanisms for controlling the activity of these genes (Flavell et al. 1993; N. Neves, J.S. Heslop-Harrison, and W. Viegas, submitted for publication), there is no evident reason why they are so variable or present in so many copies. Members of the Triticeae show wide variation in the number of 18S–25S rDNA sites; barley has six sites on six of its seven chromosome pairs (Leitch and Herslop-Harrison 1992; Pedersen and Linde-Laursen 1994), while *Psathyrostachys stoloniformis* has seven sites on five of its seven chromosome pairs (Orgaard and Heslop-Harrison 1994*a*). Using a similar sensitivity of in situ hybridization, rye shows only one pair of rDNA sites, while, as shown here, *Ae. umbellulata* has only two sites, both present as clearly recognizable segments in Feulgen stained chromosome preparations (Kimber 1967), one at a satellite region of a chromosome and the other more terminal.

Figure 4 shows the locations of 5S and 18S–25S rDNA sites on wheat, rye, barley, and *Ae. umbellulata* group 1 and group 5 chromosomes. It is probable that the species arose from a single ancestral genome, but no simple phylogenetic tree can account for the relative rearrangements of the closely related wheat, rye, and *Ae. umbellulata* genomes if the assumption is made that each inversion or deletion/insertion event occurred only once. Thus the rearrangements are presumably complex. Adding further species would not enable such a minimal tree to be generated, but might highlight any events that have happened more than once. Interestingly, in extensive studies of homoeology using RFLP maps of barley, wheat, and rye, only evolutionary translocations have been detected (Devos et al. 1993*a*, 1993*b*). There are no inversions or major deletion or insertion events involving more than one locus evident in the short arms of group 1 and 5 chromosomes, although, for the reasons of copy number and polymorphisms discussed above, 5S rRNA genes have not been mapped genetically. The comparison in Fig. 4 shows that molecular cytogenetic methods are able to detect small chromosomal rearrangements that are impossible to identify using genetic maps, even when these have relatively dense markers.

Localization of the repetitive sequence pSc119.2

The sequence pSc119.2 originates from rye, where it accounts for some 1–2% of the genome (Bedbrook and Flavell 1980). Southern hybridization or dot blotting has shown that the sequence is widespread in the Triticeae (McIntyre et al. 1988; Gupta et al. 1989; Orgaard and Heslop-Harrison 1994*b*) and presumably existed in any common ancestral taxa, although it is absent from *Hordeum* species with the I genome, including *H. vulgare*. There is an alternative possibility that the sequence was distributed by horizontal transfer. The sequence pSc119.2 is proving to be an extremely useful probe for chromosome identification in the Triticeae following in situ hybridization (Leitch et al. 1991), because of its high copy number (allowing easy detection) and polymorphic chromosomal location in many species. Figures 2 and 3 show that the locations of the sequence in *Ae. umbellulata* which has strong terminal pSc119.2 sites are on all long chromosome arms, six of the seven short arms, and two intercalary sites, both of similar strength to the terminal sites. There are some polymorphisms in size of sites, reflecting differences in copy number of the repetitive sequence between homologous chromosomes and indicating rapid evolution of the sequence. In rye, all seven short chromosome arms and six of the long arms have pSc119.2 sites; there are considerable differences in strength among sites (i.e., copy number of the sequence), sites on the long arms being generally small, while there are variably-sized intercalary sites on six chromosomes (Heslop-Harrison et al. 1991). In wheat, all B genome chromosomes show intercalary as well as terminal sites, while all six sites on different A and D genome chromosomes are terminal (Mukai et al. 1993). The presented data extend the pSc119.2 idiograms available to include a new species, and should be useful for identifying whole chromosomes and for detecting translocation breakpoints or deletions in wheat – *Ae. umbellulata* breeding lines. The pSc119.2 probe is a valuable second label to use with low copy probes for chromosome identification.

In situ hybridization of repetitive probes is proving to be a valuable method for examining chromosomal evolution in the Triticeae and gives a complementary view to that obtained from plant morphology, chromosome pairing, or map-based analysis. Furthermore, understanding the physical organization of chromosomes is becoming increasingly important, because of the need to link genetic and physical maps, to transfer known chromosome segments carrying useful genes between species, and to identify regions of enhanced recombination in the genome.

In situ hybridization of defined probes uniquely enables the detailed physical analysis of large scale chromosome structure that underpins such studies.

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