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New 18S · 26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats

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Abstract. Three new 18S·26S rRNA gene loci were identified in common wheat by sequential N-banding and in situ hybridization (ISH) analysis. Locus *Nor-A7* is located at the terminal area of the long arm of 5A in both diploid and polyploid wheats. Locus *Nor-B6* is located in N-band 1BL2.5 of the long arm of chromosome 1B in *Triticum turgidum* and *Triticum aestivum*. ISH sites, similar to *Nor-B6*, were also detected on the long arms of chromosomes 1G in *Triticum timopheevii* and 1S in *Aegilops speltoides*, but their locations on the chromosomes were different from that of *Nor-B6*, indicating possible chromosome rearrangements in 1GL and 1BL during evolution. The third new locus, *Nor-D8*, was only found on the short arm of chromosome 3D in the common wheat Wichita. The loss of rRNA gene locus *Nor-A3* and gain of repetitive DNA sequence pSc119 on the terminal part of 5AS suggest a structural modification of 5AS. Comparative studies of the location of the 18S·26S rRNA gene loci in polyploid wheats and putative A and B (G) genome progenitor species support the idea that: (1) *Triticum monococcum* subsp. *urartu* is the donor of both the A and A^t genome of polyploid wheats. (2) *Ae. speltoides* is closer to the B and G genome of polyploid wheats than *Aegilops longissima* and is the most probable progenitor of these two genomes.

Introduction

Recently, non-radioactive in situ hybridization (ISH) techniques were successfully used for mapping multigene families in plants (Mukai et al. 1990, 1991; Leitch and Heslop-Harrison 1992, 1993). Using this technique, not only can the genes be physically mapped to a specific chromosomal area, but also, in several cases, new minor loci have been identified. It is difficult to identify these minor loci by conventional Southern analysis because they may lack polymorphisms and/or are masked by the major loci.

In common wheat (*Triticum aestivum* L. em Thell, $2n = 6x = 42$, AABBDD), the 18S·26S rRNA genes, usually located in nucleolus organizing regions (Nor), are found on chromosomes 1A, 1B, 6B, and 5D based on their nucleolar activity and satellite morphology (Crosby 1957; Bhowal 1972). The chromosomal locations have also been confirmed by both DNA/rRNA hybridization (Liang et al. 1977) and ISH analysis (Appels et al. 1980; Hutchinson and Miller 1982). Mukai et al. (1991) reported a new locus, *Nor-D4*, on chromosome 7D. In the present paper, we report the identification of three additional new 18S·26S rRNA gene loci in *T. aestivum*. In addition, the presence of these new loci in tetraploid and diploid progenitor species of common wheat was investigated. We also demonstrate that the location of the 18S·26S rRNA genes provides excellent chromosomal landmarks to investigate the evolution of polyploid wheats.

Materials and methods

The sources of species used in the study are given in Table 1. The Wichita monosomic 3D line was used to confirm the location of a new 18S·26S rRNA gene locus on 3DS. All materials are maintained in the Wheat Genetics Resources Center at Kansas State University, Manhattan.

The 18S·26S rRNA gene probe contains a single wheat 18S·26S rRNA gene repeat unit that originated from plasmid pTa71 (Gerlach and Bedbrook 1979). Clone pSc119 contains a 120 bp highly repetitive DNA sequence that was isolated from rye and is also present in *Triticum* (Bedbrook et al. 1980). The chromosomal location of the rRNA gene loci was determined by a sequential modified N-banding and ISH technique (Jiang and Gill 1993). For multicolor fluorescence ISH analysis (Leitch et al. 1991), the 18S·26S rRNA gene probe was labeled with digoxigenin-11-dUTP and detected with rhodamine-conjugated anti-digoxigenin (Boehringer Mannheim). Probe pSc119 was labeled with biotin-11-dUTP and detected with fluorescein avidin D (Vector Laboratory). Color photos were taken by double exposure using

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Table 1. Species of *Triticum* and *Aegilops* used in this study

Species	WGRC accession no.	Genome	Geographic origin
T. monococcum subsp. *boeoticum*	TA188	AA	Iran
T. monococcum subsp. *boeoticum*	TA203	AA	Iraq
T. monococcum subsp. *urartu*	TA708	AA	Turkey
T. monococcum subsp. *urartu*	TA765	AA	Lebanon
Ae. speltoides subsp. *ligustica*	TA1770	SS	Iraq
Ae. speltoides subsp. *ligustica*	TA1777	SS	Turkey
Ae. speltoides subsp. *aucheri*	TA1773	SS	Turkey (Diyarbakir)
Ae. speltoides subsp. *aucheri*	TA1778	SS	Turkey (Cerikli)
T. timopheevii subsp. *timopheevii*	TA140	A^tA^tGG	Unknown
T. timopheevii subsp. *araraticum*	TA12	A^tA^tGG	Iraq
T. turgidum var. *durum* cv. Langdon	–	AABB	
T. aestivum cv. Wichita	–	AABBDD	
T. aestivum cv. Cheyenne	–	AABBDD	
T. aestivum cv. Chinese Spring	–	AABBDD	
Wheat-rye 1B/1R translocation line 79–3435	–	AABBDD	

Kodak EKTAR 1000 Film. Black and white photos were taken using Kodak technical Pan film 2415.

Results

Using sequential N-banding and ISH analysis, the 18S·26S rRNA genes were detected on six pairs of Chinese Spring (CS) chromosomes, 1A, 5A, 1B, 6B, 5D, and 7D (Figs. 1,2). In addition to the previously reported loci *Nor-A1* on 1AS, *Nor-B1* on 1BS, *Nor-B2* on 6BS, *Nor-D3* on 5DS, and *Nor-D4* on 7DL, two new loci were detected on the long arms of chromosomes 1B and 5A.

The new locus on 5A was located at the terminal area of the long arm. Chromosome 5A can be identified by its characteristic N-band near the centromere on the long arm (Fig. 1f). This new locus is designated as *Nor-A7*. By comparing the N-banding and ISH patterns, the new locus on 1BL is localized in band 1BL2.5 (Fig. 1b, for banding nomenclature see Gill et al. 1991). This locus is designated as *Nor-B6*. Based on ISH signal intensity, the order of copy numbers of the rRNA gene loci in CS is 6BS > 1BS > 5DS > 1AS > 7DL > 5AL > 1BL.

Nor-B6 and *Nor-A7* were also detected at the same positions on 1BL and 5AL of durum (*Triticum turgidum* L. var. *durum*) wheat Langdon (Fig. 1c,g) and common wheat cultivars Wichita, Cheyenne, and a wheat-rye T1BL·1RS translocation line 79–3435 (Fig. 2, also see Table 2). *Nor-A7* was found on the long arm of chromosome $5A^t$ in both *Triticum timopheevii* Zhuk. subsp. *timopheevii* and *T. timopheevii* subsp. *araraticum* (Fig. 1h). A minor hybridization site, similar to *Nor-B6*, was detected on the long arm of 1G in *T. timopheevii* subsp. *araraticum* line TA12 (Fig. 1d). The location of this site is at the middle of the arm rather than the subterminal position of *Nor-B6* on 1BL. However, this site was not clearly visible in *T. timopheevii* subsp. *timopheevii* line TA140.

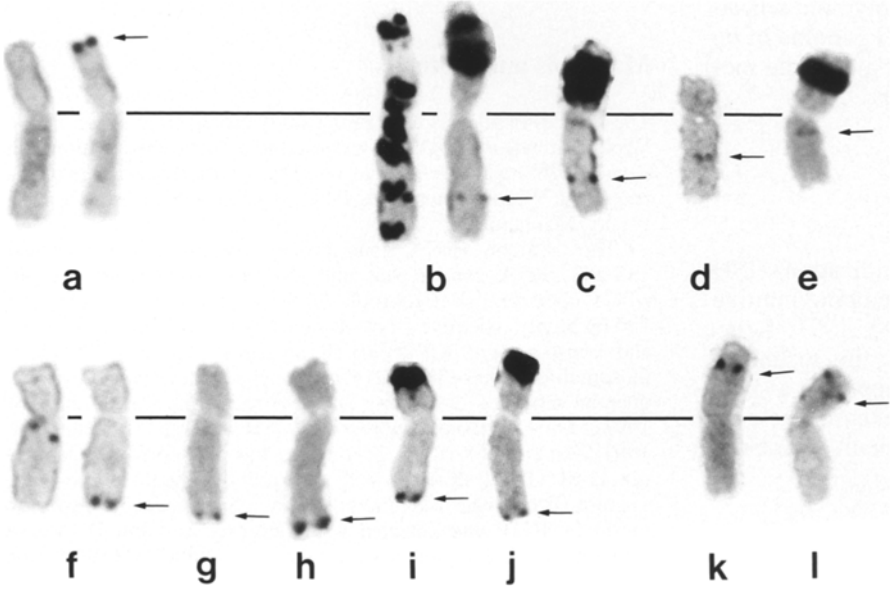


Fig. 1a–l. New 18S·26S ribosomal gene loci (*arrowed*) in diploid and polyploid wheats. **a** *Nor-D8* on 3DS of Wichita (*left* N-banding; *right* in situ hybridization, **b** *Nor-B6* on 1BL of Chinese Spring (*left* N-banding; *right* ISH); **c** *Nor-B6* on 1BL in Langdon; **d** *Nor-B6* on 1GL of *Triticum timopheevii* subsp. *araraticum* line TA12; **e** *Nor-B6* on 1SL of *Aegilops speltoides* line TA1777; **f** *Nor-A7* on 5AL of Chinese Spring (*left* N-banding; *right* ISH); **f)** *Nor-A7* on 5AL of Langdon; **h** *Nor-A7* on $5A^tL$ of *T. timopheevii* subsp. *timopheevii* line TA140; **i** *Nor-A7* on 5AL in *Triticum monococcum* subsp. *boeoticum* line TA203; **j** *Nor-A7* on 5AL of *T. monococcum* subsp. *urartu* line TA765; **k** *Nor-A5* on putative 7A of *T. monococcum* subsp. *boeoticum* line TA188; **l** *Nor-A6* on an unidentified chromosome of *T. monococcum* subsp. *boeoticum* line TA188

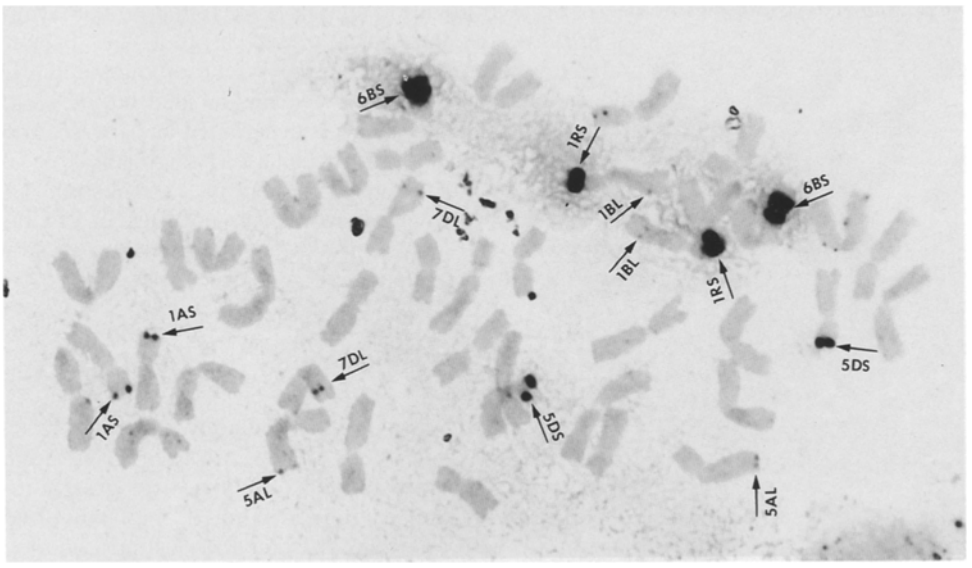


Fig. 2. In situ hybridization of a complete somatic metaphase cell of wheat-rye T1BL·1RS translocation line 79–3435 using an 18S·26S rRNA gene as a probe. The rRNA gene loci on 1RS, 1BL, 6BS, 1AS, 5AL, 5DS, and 7DL are identified

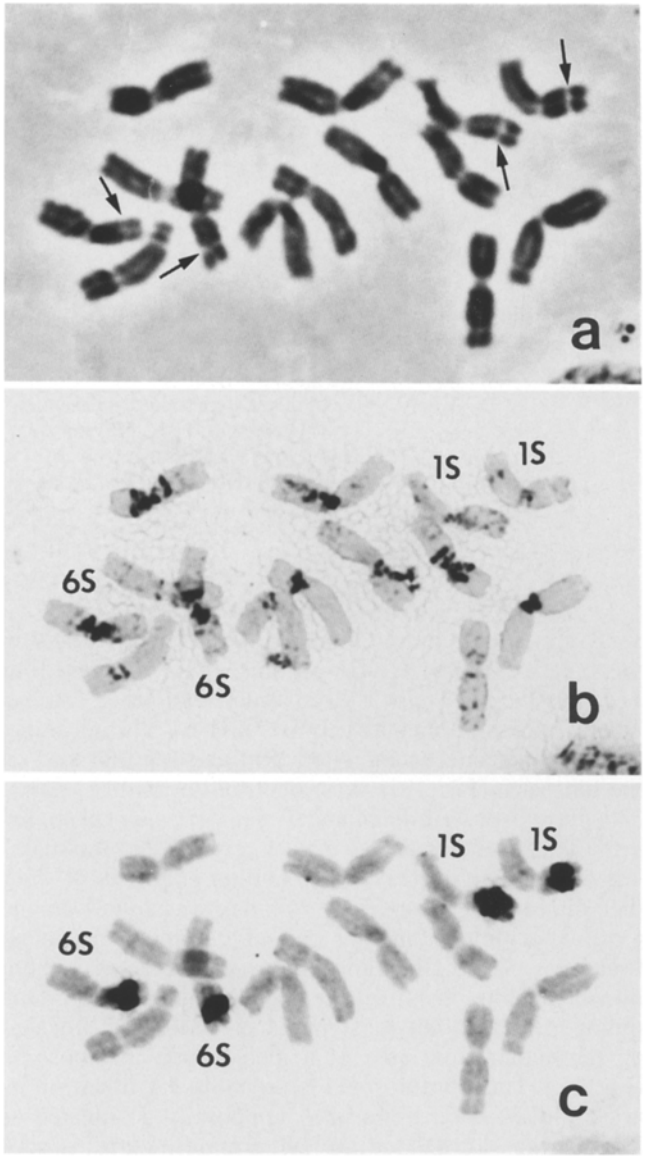
Table 2. Summary of 18S·26S rRNA gene loci in diploid and polyploid wheats

Locus	Chromosome	Species	Reference
Nor-A1	1AS	*T. aestivum*	Mukai et al. (1991)
	1AS	*T. turgidum*	Present study
	$1A^tS$	*T. timopheevii*	Present study
	1AS	*T. monococcum*	Miller et al. (1983)
Nor-A3	5AS	*T. monococcum*	Miller et al. (1983)
Nor-A7	5AL	*T. aestivum*	Present study
	5AL	*T. turgidum*	Present study
	$5A^tL$	*T. timopheevii*	Present study
	5AL	*T. monococcum*	Present study
Nor-Ax	Putative 7A	*T. monococcum* subsp. *boeoticum*	Present study
Nor-Ay	Unknown	*T. monococcum* subsp. *boeoticum*	Present study
Nor-B1	1BS	*T. aestivum*	Appels et al. (1980)
	1BS	*T. turgidum*	Hutchinson and Miller (1982)
	$6A^tS$	*T. timopheevii*	Present study
	1SS	*Ae. speltoides*	Present study
Nor-B2	6BS	*T. aestivum*	Appels et al. (1980)
	6BS	*T. turgidum*	Hutchinson and Miller (1982)
	6GS	*T. timopheevii*	Present study
	6SS	*Ae. speltoides*	Present study
	6SS	*Ae. longissima*	Friebe et al. (1993)
Nor-B6	1BL	*T. aestivum*	Present study
	1BL	*T. turgidum*	Present study
	1GL	*T. timopheevii*	Present study
	1SL	*Ae. speltoides*	Present study
Nor-D3	5DS	*T. aestivum*	Appels et al. (1980)
	5DS	*Ae. squarrosa*	Mukai et al. (1991)
Nor-D4	7DL	*T. aestivum*	Mukai et al. (1991)
	7DL	Ae. squarrosa	Mukai et al. (1991)
Nor-D8	3DS	T. aestivum cv. 'Wichita'	Present study

To investigate the presence of *Nor-A7* and *Nor-B6* in the putative A and B (G) genome donor species, four *Triticum monococcum* L. and four *Aegilops speltoides* Tausch lines were analyzed by ISH. A chromosome, with a major site on the short arm and a minor site on the long arm (Fig. 1i,j), was identified in both *T. monococcum* subsp. *boeoticum* and *T. monococcum* subsp. *urartu*. This chromosome is most likely 5A and the major site on the short arm is *Nor-A3* (Miller et al. 1983). Another major site, most likely *Nor-A1*, was found on the terminal region of another pair of chromosomes in all four lines. Two extra minor sites were detected in both *boeoticum* lines from different geographical areas. One minor site, temporally designated as *Nor-Ax*, was located on the subterminal area of a metacentric chromosome (Fig. 1k). This chromosome is probably 7A based on its arm ratio. Another minor site, temporally designated as *Nor-Ay*, was located on the subterminal area of a submetacentric chromosome (Fig. 1l). These two minor sites were not detected in the two *urartu* lines.

In *Ae. speltoides*, two major sites were found on the two satellite chromosomes in all four lines analyzed (Fig. 3). Based on their N-banding patterns, the satellite chromosomes are most likely 1S and 6S, respectively. The N-banding pattern of 6S was very close to 6B of common wheat. Multicolor fluorescence ISH analysis using pSc119 and the 18S·26S rRNA genes as probes suggested that these two satellite chromosomes cannot be 2S, 4S, 5S, or 7S because the pSc119 hybridization patterns of these chromosomes were very close to 2B (2G), 4B (4G), 5B (5G), and 7B (7G) in polyploid wheats (Fig. 4). A minor site was found in the proximal region of the putative 1S chromosome (Fig. 1e) in some cells of all four *Ae. speltoides* lines. The detection of this minor site was inconsistent in different cells and preparations, probably due to the low copy numbers compared with other loci.

In Wichita wheat, in addition to the rRNA genes on 1A, 5A, 1B, 6B, 5D, and 7D, another new hybridization site was detected on a chromosome lacking N-bands



(Fig. 1a). Hybridization signals were found on the terminal areas of the short arms of six chromosomes in normal Wichita but only five in the Wichita monosomic 3D line, indicating that this new site is located on the short arm of 3D. This new locus is designated as *Nor-D8*. Based on the intensity of hybridization signal, the size of *Nor-D8* is similar to that of *Nor-A1* on chromosome 1A in Chinese Spring wheat. *Nor-D8* is not present in Chinese Spring and Cheyenne wheat; the latter is a closely related line of Wichita based on its breeding pedigree.

Discussion

The present study revealed that the copy number of each 18S·26S rRNA gene locus in wheat varies in different populations. The copy number of *Nor-A1* was greatly reduced since it had been incorporated into polyploid wheats. In addition, the size of *Nor-A1* in Langdon durum is much less than that in four common wheat lines analyzed. The size of *Nor-A7* in two *urartu* lines is small compared with those in two *boeoticum* lines and is difficult to detect. Variation was found among different plants in Wichita wheat. The size of *Nor-A1* in a normal Wichita line is as large as *Nor-D3* in Chinese Spring. However, the size of *Nor-A1* in a Wichita monosomic 3D line is similar to *Nor-A1* in Chinese Spring.

Our failure to detect *Nor-A3* in the polyploid wheats cannot be explained by variation in copy number because none of the polyploid wheat lines in the present study showed a hybridization signal on 5AS. A nonreciprocal

Fig. 3a–c. Sequential N-banding and ISH analysis on *Aegilops speltoides* line TA1778. **a** Morphology of somatic metaphase chromosomes before N-banding. The secondary constrictions on the satellite chromosomes are *arrowed*. **b** N-banding pattern on the same metaphase cell. **c** ISH pattern on the same metaphase cell

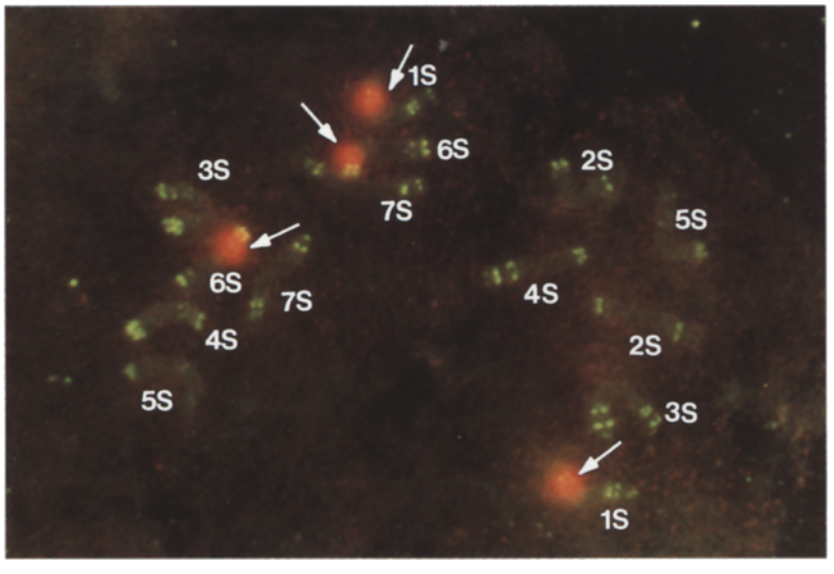


Fig. 4. Simultaneous detection of 18S·26S rRNA genes (*red color*) and repetitive DNA sequence pSc119 (*green color*) on metaphase chromosomes of *Aegilops speltoides* line TA1770 by multicolor fluorescence ISH analysis. The pSc119 hybridization patterns of 2S, 4S, 5S and 7S are similar to those of 2B (2G), 4B (4G), 5B (5G), and 7B (7G) in polyploid wheats (see Rayburn and Gill 1985; Jiang and Gill 1994). The two chromosomes with major 18S·26S rRNA gene clusters (*arrowed*) are most likely 1S and 6S, respectively

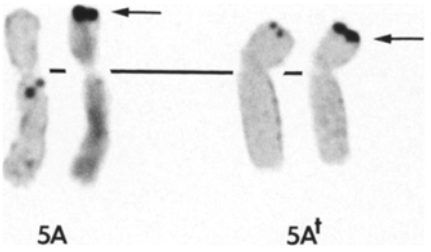


Fig. 5. Sequential N-banding (*left*) and ISH using pSc119 as a probe (*right*) on chromosome 5A of Langdon wheat and 5A^t of *Triticum timopheevii* subsp. *araraticum* line TA12. An ISH site (*arrowed*) is located on the terminal areas of the short arms on both 5A and 5A^t

translocation between 5A and an unknown chromosome probably occurred during the evolution of polyploid wheats, resulting in the loss of *Nor-A3*. Good evidence for this hypothesis is that chromosome 5A in both *T. turgidum* and *T. timopheevii* has a hybridization site on the terminal area of the short arm with the repetitive DNA sequence probe pSc119 (Fig. 5, see also Jiang and Gill 1994). This sequence is present on all chromosomes, mainly at telomeric and subtelomeric areas of *Ae. speltoides*, but not on chromosomes of *T. monococcum* (data not shown). In Langdon durum wheat, 4A and 5A were the only A genome chromosomes that had a pSc119 hybridization site (Jiang and Gill 1994). The pSc119 site on 4AL was derived from 7B (Jiang and Gill 1994) because of the 4A-5A-7B cyclic translocation (Naranjo et al. 1987; Naranjo 1990; Liu et al. 1992). The pSc119 site on 5AS presumably was also translocated from a B genome chromosome. Another possibility for the loss of *Nor-A7* is from a spontaneous deletion at the terminal area of 5AS. The gain of the sequence pSc119 could be from a separate translocation event.

Chromosomes 4A, 5A, and 7B were involved in a cyclic translocation in tetraploid and hexaploid wheats (Naranjo et al. 1987; Naranjo 1990; Liu et al. 1992). The terminal segment of the long arm of 5A was derived from the terminal part of 4AL. However, the tip of 5AL seems to be unmodified based on the location of *Nor-A7* on 5A chromosomes in both diploid and polyploid wheats. This result supports the hypothesis proposed by Naranjo et al (1987) that an initial 5AL/4AL translocation took place in the diploid wheat and a second translocation 4AL/7BS occurred in tetraploid wheat (Fig. 6). However, the possibility that a 5AL/4AL translocation involved only the distal end of *Nor-A7* cannot be excluded at present.

Although the copy number of each 18S·26S locus varied in different populations, the locations of most of the loci were conserved in different diploid and polyploid wheat species. Therefore, the 18S·26S rRNA genes provide excellent cytological markers to study the genome evolution of wheat. Recently, restriction fragment length polymorphism (RFLP) analysis using single copy or repetitive DNA sequences revealed that *T. monococcum*

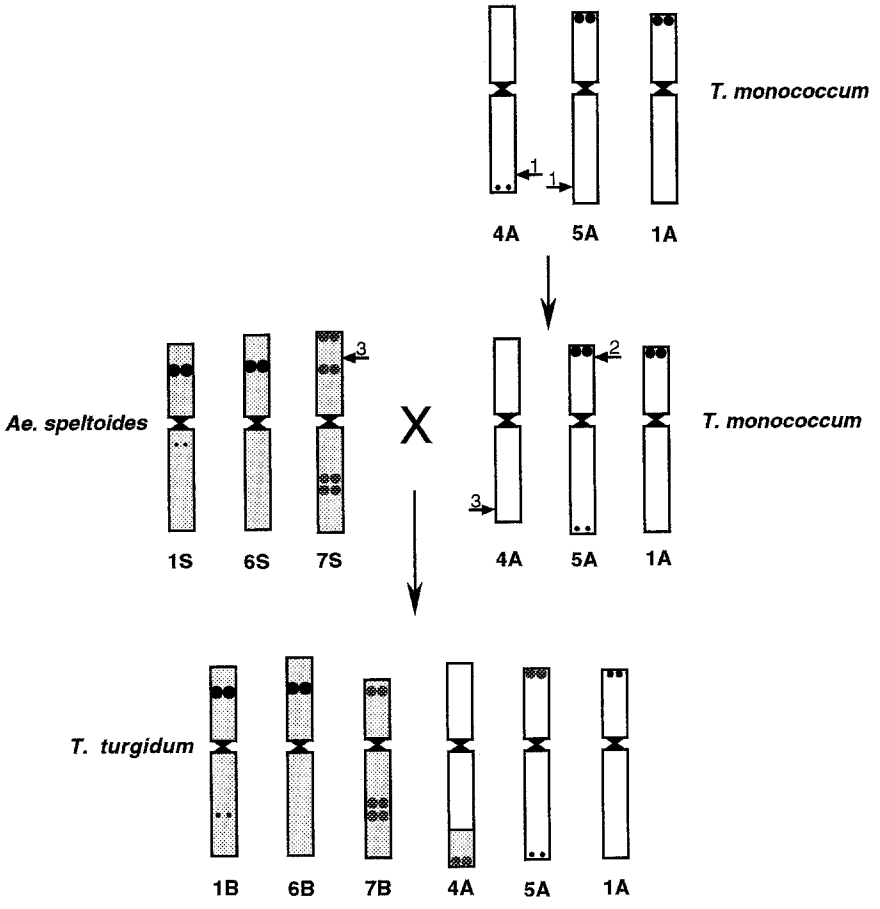


Fig. 6. Chromosomal structural modifications of *Triticum turgidum* (AABB) during evolution. **1** A reciprocal translocation between 4AL and 5AL occurred in the diploid *Triticum monococcum* subsp. *urartu*. **2** The terminal area of 5AS was modified either by a spontaneous deletion or an unknown translocation. **3** The short arm of 7S and the long arm of 4A were involved in a reciprocal translocation (Naranjo et al. 1987; Liu et al. 1992). About 30% of the long arm of 4A was derived from the short arm of 7S. The A genome segment on 7BS is too small to be identified by genomic ISH (see Jiang and Gill 1994). *Solid dots* represent 18S·26S ribosomal RNA gene locations. *Shaded dots* represent the locations of repeated sequence pSc119. The *arrows* do not point to the exact physical breakpoints

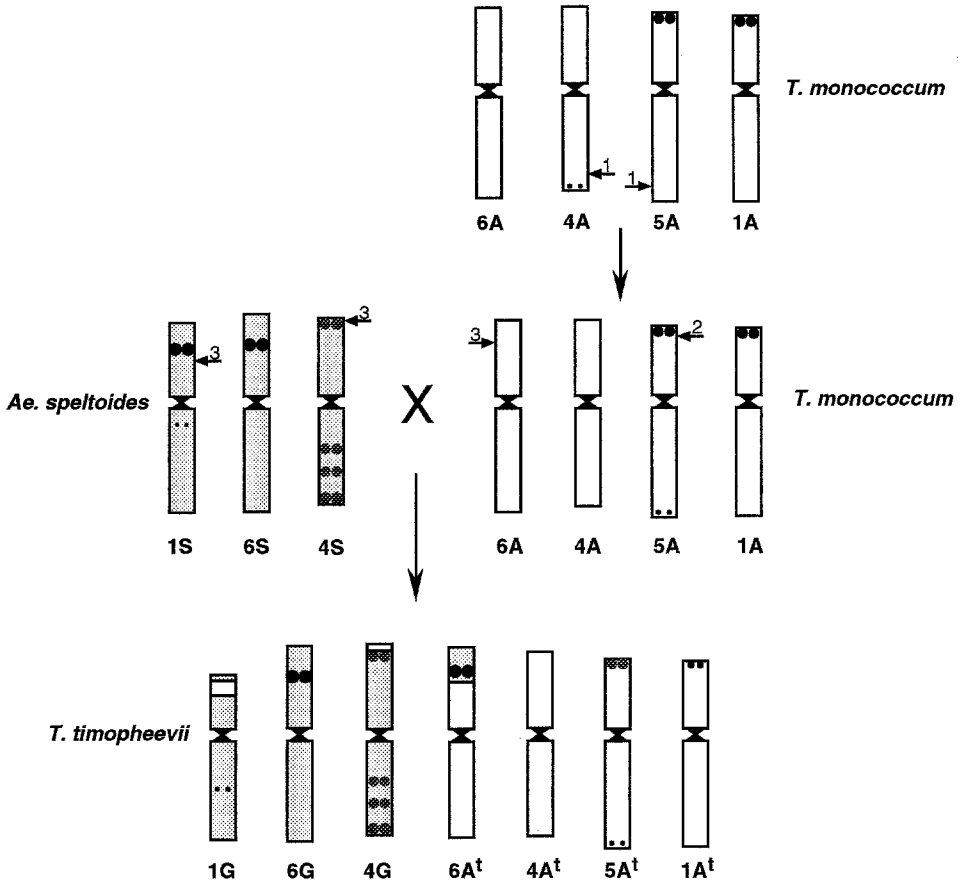


Fig. 7. Chromosomal structural modifications of *Triticum timopheevii* (A^tA^tGG) during evolution. **1** A reciprocal translocation between 4AL and 5AL occurred in the diploid *Triticum monococcum* subsp. *urartu*. **2** The terminal area of 5AS was modified either by a spontaneous deletion or an unknown translocation. **3** Chromosomes 1S, 4S and 6A involved in cyclic translocation, resulting in three A^t/G intergenomic translocation chromosomes in *Triticum timopheevii*: 1G, 4G, and $6A^t$ (for details of the cyclic translocation, see Jiang and Gill 1994). *Solid dots* represent 18S·26S ribosomal RNA gene locations. *Shaded dots* represent the locations of repeated sequence pSc119. The *arrows* do not point to the exact physical breakpoints

subsp. *urartu* is the A genome donor of polyploid wheats (Dvořák et al. 1993; Takumi et al. 1993). In the present study, all the 18S·26S rRNA gene loci, except *Nor-A7*, detected in two *urartu* lines are present in polyploid wheats. In *T. monococcum* subsp. *boeoticum*, two extra minor loci were found. These two minor loci were not present in all polyploid wheat species analyzed. This result supports *T. monococcum* subsp. *urartu* as the donor of A and A^t genomes of polyploid wheats.

The donor of the B genome of polyploid wheats is uncertain. However, a large number of studies using different strategies suggest that *Aegilops longissima* Schweinf. & Muschl. and *Ae. speltoides* are the species most closely related the B genome. In *Ae. longissima*, the major rRNA gene clusters were located on the short arms of chromosomes 5S and 6S, respectively. Only a minor site was found at the middle of the 1S short arm (Friebe et al. 1993). Recently, we analyzed a different *Ae. longissima* accession and the same results were obtained (data not shown). These results indicate that the 18S·26S rRNA gene locations in *Ae. longissima* are not compatible with those on B genome chromosomes (1B and 6B).

ISH analysis on four different *Ae. speltoides* lines indicated the major 18S·26S rRNA gene clusters are located on chromosomes 1S and 6S, respectively. The chromosome morphology of 1S and 6S, including the arm ratios and satellite size, are very close to chromosomes 1B and 6B in polyploid wheats (Fig. 3). In addition, a minor site was found on the long arms of both 1S and 1B chromosomes. Therefore, based on the location of 18S·26S rRNA genes, *Ae. speltoides* is more closely related to the B genome than *Ae. longissima*. In *T. timopheevii*, major 18S·26S rRNA gene clusters are located on the short arms of chromosomes $6A^t$ and 6G. However, the satellite and Nor region of $6A^t$ were derived from 1G (Gill and Chen 1987; Jiang and Gill 1994, Fig. 7). A minor site was also found on the long arm of 1G. Thus, the locations of the 18S·26S rRNA gene of *Ae. speltoides* are also compatible with those of the G genome chromosomes. The ribosomal DNA spacer sequences from *Ae. speltoides* were also closer to those from both AABB and A^tA^tGG species than the spacer sequences from other diploid S genome species, including *Ae. longissima* (Gill and Appels 1987).

The locations of the minor 18S·26S rRNA gene site on the long arms of chromosomes 1S, 1G, and 1B were all different. There are two different possibilities for this result. First, a chromosomal structural rearrangement may have occurred in 1GL and 1BL during the evolution of polyploid wheats, resulting in the shift of the location of this minor site on 1GL and 1BL. Second, the location of this minor site may be polymorphic in different *Ae. speltoides* populations and chromosomes 1G and 1B were derived from different types of 1S. This seems unlikely because a location difference was not found in four *Ae speltoides* lines from different geographical areas.

Cytoplasmic differences and the presence of different

species-specific chromosome translocations suggested that emmer wheat (genomically AABB) and timopheevi wheat (genomically A^tA^tGG) had a diphyletic origin involving two independent hybridization events (Tsunewaki and Ogihara 1983; Jiang and Gill 1994). In the present study, we found that both chromosome 5A from emmer wheat and $5A^t$ from timopheevi wheat had lost the *Nor-A3* locus and gained the repetitive DNA sequence pSc119. This may be due to reciprocal introgression between the two species. The geographical distribution of the wild form of emmer wheat, *T. turgidum* subsp. *dicocoides*, and the wild form of timopheevi wheat, *T. timopheevii* subsp. *araraticum* overlaps in northern Iraq. The possibility of two independent events leading to modification of 5A and $5A^t$ cannot be excluded at present.

The origin of locus *Nor-D8* on the Wichita chromosome 3D is not known. In the diploid D genome species *Aegilops squarrosa* L., rRNA genes were only detected on chromosomes 5D and 7D (Mukai et al. 1991). *Nor-D8* is not present in Cheyenne, a closely related line of Wichita. This locus is possibly derived from other wheat chromosomes, such as 1A and 5D. ISH analysis of other wheat lines in the Wichita pedigree may provide more information on the origin of *Nor-D8*.

At present, we have no information on the nucleolar activity of the newly detected rDNA loci. The possibility that the detected signals are from intergenic sequences rather than coding regions of rDNA cannot be completely ruled out either. Nevertheless, the detected ISH sites have been conserved during a long span of parallel evolution between diploid progenitors and derived polyploid species and can be used as excellent chromosomal landmarks for phylogenetic analysis.

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