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Variation in the distribution of a genome-specific DNA sequence on chromosomes reveals evolutionary relationships in the *Triticum* and *Aegilops* complex

P. Zhang, B. Friebe, and **B. S. Gill**

Wheat Genetics Resource Center, Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS, USA

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Abstract. The present study analyzed the distribution pattern of the *Ae. speltoides*–derived repetitive clone pGc1R-1 in the *Triticum*/*Aegilops* complex. Fluorescence in situ hybridization analysis showed that clone pGc1R-1 is a S-genome-specific repetitive sequence that hybridized to the S-genome of three species in the section *Sitopsis*, *Aegilops speltoides* (S), *Ae. longissima* (S^l), and *Ae. sharonensis* (S^{sh}), but not to *Ae. bicornis* (S^b) and *Ae. searsii* (S^s), nor to any other diploid *Aegilops* species. This clone also hybridized to the very closely related G-genome of *T. timopheevii* subsp. *armeniacum* and *T. timopheevii* ssp. *timopheevii*, but not to the B-genome of *T. turgidum* and *T. aestivum*. Hybridization also was observed in the polyploid *Aegilops* species, *Ae. kotschyi* (U^kS^k), *Ae. peregrina* (U^pS^p), and *Ae. vavilovii* ($X^{va}D^{va}S^{va}$). Large inter- and intraspecific variations were observed. Our results confirm that the S genome is related more to the S^l and S^{sh} genomes than to the S^b and S^s genomes; there is a greater affinity between the G and S genomes than between the B and S genomes. Mechanisms to account for the variation in the FISH pattern with different genomes include sequence amplification and deletion. Variation in the distribution of this genome-specific DNA sequence, pGc1R-1, on chromosomes can be used to reveal evolutionary relationships in the *Triticum* and *Aegilops* complex.

Key words: *Aegilops*, *Triticum*, fluorescence in situ hybridization, pGc1R-1, genome-specific repetitive sequence, evolution.

Introduction

Genetic studies have revealed that polyploid wheat has two evolutionary lineages. *Triticum turgidum* L. ($2n = 4x = 28$, AABB) and *T. aestivum* L. ($2n = 6x = 42$, AABBDD) comprise one lineage (the emmer-dinkel group), and *T. timopheevii* (Zhuk.) Zhuk. ($2n = 4x = 28$, A^tA^tGG) and *T. zhukovskyi* Menabde & Ericzjan ($2n = 6x = 42$, $A^tA^tA^tA^tGG$) comprise the timopheevi group. Jiang and Gill (1994a) reported different species-specific chromosome translocations indicating a diphyletic origin of the emmer-dinkel and timopheevi groups. This hypothesis was supported by phylogenies based on restriction fragment analyses of chloroplast (Ogihara and Tsunewaki 1988, Miyashita et al. 1994) and mitochondrial DNA (Terachi et al. 1990). Furthermore, these results suggested that the origin of each species was separated in time with *T. turgidum* arising earlier than *T. timopheevii*.

The B- and G-genome chromosomes are well differentiated with respect to constitutive heterochromatic content and chromosomal translocations (Feldman 1966, Hutchinson and Miller 1982, Hutchinson et al. 1982, Chen and Gill 1984, Gill and Chen 1987, Badaeva et al. 1994, 1995, Friebe and Gill 1996).

Aegilops is the most closely related genus to *Triticum* and both share an annual growth habit. The genus *Aegilops* is comprised of 11 diploid and 12 polyploid species including tetraploids and hexaploids. The genus *Triticum* is composed of four groups (einkorn, emmer, timopheevi, and dinkel wheat), and the genus *Aegilops* consists of six sections (*Polyeides*, *Cylindropyrum*, *Comopyrum*, *Amblyopyrum*, *Sitopsis* and *Vertebrata*) (van Slageren 1994).

The section *Sitopsis* includes the diploid species *Ae. longissima* (Schweinf. & Muschl. in Muschl.) Eig, *Ae. sharonensis* Eig, *Ae. searsii* Feldman & Kislev ex K. Hammer, *Ae. bicornis* (Forsskal) Jaub. & Spach., and *Ae. speltoides* Tausch.

Evidence based on morphological studies (Sarkar and Stebbins 1956), karyotype data (Riley et al. 1958, Kerby and Kuspira 1988), meiotic chromosome pairing analysis in interspecific hybrids (Feldman 1966, Dvorák and Appels 1982), C-banding analysis (Friebe and Gill 1996), electrophoretic mobilities (Witcombe 1983), nuclear DNA analyses (Gill and Appels 1987, Dvorák et al. 1989, Dvorák and Zhang 1990, Jiang and Gill 1994b, Mori et al. 1995, Daud and Gustafson 1996, Sasanuma et al. 1996) and fluorescence in situ hybridization (Badaeva et al. 1996a, 1996b) suggested that the B genome is most closely related to the S genome of *Ae. speltoides* and that the G genome is virtually identical to the S genome of *Ae. speltoides*. Plasmon analysis also pointed to *Ae. speltoides* as the B-genome donor (Tsunewaki and Ogihara 1983, Tsunewaki et al. 1991). In addition, the results from phenetic and organellar DNA analyses suggest that among all the plasmons of diploid *Aegilops* species, the S plasmon of *Ae. speltoides* is most closely related to the B and G plasmons (Tsunewaki 1994, Tsunewaki 1996).

The genomes of the Triticeae species include relatively large amounts of DNA, with the 1C DNA amount ranging from 5.5×10^9 bp in *T. uratu* Thum. (AA), 12×10^9 bp in *T. turgidum*, and 16×10^9 bp in *T. aestivum* (Bennett and Smith 1976, Bennett and Leitch 1995). One of the characteristics of Triticeae genomes is that more than 75% of their DNA consists of repetitive DNA sequences (Flavell et al. 1974, Flavell 1985, Bennetzen and Freeling 1997). Changes in repetitive DNA are responsible for most of the changes in chromosome and genome size during the evolution and divergence of species (Flavell 1983). Some repetitive DNA sequences are common between several species, whereas others are species-specific. Several genome- or species-specific repetitive sequences have been identified in Triticeae (Bedbrook et al. 1980, Appels and Moran 1986, Rayburn and Gill 1986b, Tsujimoto and Gill 1991, Anamthawat-Jónsson and Heslop-Harrison 1993, Daud and Gustafson 1996, Salina et al. 1997).

Comparative analysis of the repetitive DNA sequences in *Ae. speltoides* with those of hexaploid wheats has demonstrated that about 2–3% of the *Ae. speltoides* genome is composed of species-specific sequences. To date, at least three nucleotide sequences from different repeated families of the S-genome of *Ae. speltoides* have been cloned (Anamthawat-Jonsson and Heslop-Harrison 1993, Daud and Gustafson 1996, Salina et al. 1997, Nasuda 1999).

In the present study, we used FISH with the highly repetitive *Ae. speltoides*-derived DNA sequence pGc1R-1, to analyze intraspecific and interspecific genome differentiation among all 11 diploid *Aegilops* species, two tetraploid *Aegilops* species, three tetraploid *Triticum* species, one hexaploid *Aegilops* species, and two hexaploid *Triticum* species. These data provide useful information on genome differentiation in the *Triticum*/*Aegilops* complex.

Materials and methods

Plant material. In the present study, 11 diploid *Aegilops* species analyzed include *Ae. tauschii*,

Ae. speltoides, *Ae. sharonensis*, *Ae. longissima*, *Ae. bicornis*, *Ae. searsii*, *Ae. mutica*, *Ae. umbellulata*, *Ae. caudata*, *Ae. uniaristata*, *Ae. comosa* var. *comosa* and var. *subventricosa*. In addition, different accessions of tetraploid *T. turgidum*, *T. timopheevii* ssp. *timopheevii* and ssp. *armeniacum*, *Ae. kotschyi*, *Ae. peregrinum*, and hexaploid *T. aestivum* and *Ae. vavilovii* were analyzed. Genome constitution, chromosome number, country of origin are given in Table 1. The nomenclature follows that of van Slageren (1994) except that we consider *Ae. mutica* as belonging to the genus *Aegilops*. All materials are maintained at the Wheat Genetics Resource Center at Kansas State University.

Fluorescence in situ hybridization analysis. Slide preparation and pretreatment, hybridization conditions, post-hybridization washes, and imaging were as described by Zhang et al. (2001). Clone pGc1R-1 is a 258-bp fragment of a tandem repetitive element cloned from a wheat-*Ae. speltoides* T2B-2S translocation line and hybridizes to telomeric and subtelomeric regions of most *Ae. speltoides* chromosomes (Nasuda 1999, Friebe et al. 2000). The repeat was inserted into the *Eco*RI/*Mse*I restriction site of the pT-Adv plasmid vector (Clontech, Palo Alto, CA). This clone has 98% sequence homology to the 5′-end of the S-genome-specific repetitive element pAesKB52 isolated by Anamthawat-Jónsson and Heslop-Harrison (1993). One μg of pGc1R-1 plasmid DNA was labeled with fluorescein-11-dUTP (Amersham Biosciences Corp., Piscataway, NJ) using nick translation according to the manufacturer's protocol. Chromosomes were counterstained with propidium iodide (PI) in Vectashield (Vector Laboratories, Burlingame, CA). Slides were analyzed with an epifluorescence Zeiss Axioplan 2 microscope. Images were captured using a SPOT CCD (charge-coupled device) camera operated with SPOT 2.1 software (Diagnostic Instruments, Sterling Heights, MI) and processed with Adobe Photoshop v5.5 (Adobe Systems Inc., San Jose, CA).

Results

Diploid species. FISH using pGc1R-1 as probe did not detect hybridization site in any of the diploid *Aegilops* species except for three species, *Ae. speltoides*, *Ae. longissima*, and *Ae. sharonensis*. Hybridization was not observed in the remaining two *Sitopsis* species, *Ae. bicornis*, and *Ae. searsii*. Different hybridization patterns were observed between different accessions of a given species and, in *Ae. speltoides*, between homologous chromosomes within the same plants. Most of the pGc1R-1 FISH sites were located in telomeric or subtelomeric regions (Fig. 1a–c).

A large degree of intraspecific polymorphism in FISH patterns was observed within the seven *Ae. speltoides* accessions. In four *Ae. speltoides* accessions, pGc1R-1 FISH sites were observed on all seven chromosome pairs (TA1770, TA1773 (Fig. 1a), TA1789, and TA1793), and on only six chromosome pairs in the remaining three accessions (TA1777, TA1778, and TA1783). Different pGc1R-1 FISH patterns between homologous chromosomes were observed in two accessions (TA1770 and TA1778).

Two accessions of *Ae. longissima* were analyzed, and pGc1R-1 FISH sites on four chromosome pairs were observed in TA1910 (Fig. 1c), whereas in TA1912 only three chromosome pairs were labeled.

Five (TA1996) and six (TA1995) (Fig. 1b) pairs of pGc1R-1 FISH sites were observed in *Ae. sharonensis*. The locations were similar to those in *Ae. speltoides* and restricted to telomeric and subtelomeric chromosome regions. The SAT chromosome pair in the accession TA1995 lacks pGc1R-1 FISH site, and its morphology suggests that it is either $1S^{sh}$ or $6S^{sh}$.

Polyploid wheat. None of the A-, B-, and D-genome chromosomes of *T. aestivum* or the A- and B-genome chromosomes of *T. turgidum* had pGc1R-1 FISH sites.

Two accessions of *T. timopheevii* ssp. *timopheevii* were analyzed and in only one accession (TA103, Fig. 1e) we observed a very small pGc1R-1 FISH site in the short arm of one chromosome pair. No labeling was observed in the accession TA140. One bright pair of pGc1R-1 FISH sites was observed in all four accessions of *T. timophevii* ssp. *armeniacum*

Table 1. Plant material used in this study

Species	Accession	Genome	Country of Origin
Ae. speltoides Tausch	TA1770	2n = 2x = 14, SS	Iraq
	TA1773		Turkey
	TA1777		Turkey
	TA1778		Turkey
	TA1783		Israel
	TA1789		Iraq
	TA1793		Syria
Ae. longissima (Schweinf. & Muschl. *in* Muschl.) Eig	TA1910	2n = 2x = 14, S^lS^l	Israel
	TA1912		Israel
Ae. sharonensis Eig	TA1995	2n = 2x = 14, $S^{sh}S^{sh}$	Turkey
	TA1996		Israel
Ae. bicornis (Forsskal) Jaub. & Spach.	TA1942	2n = 2x = 14, S^bS^b	Egypt
	TA1954		Egypt
Ae. searsii Feldman & Kislev *ex* K. Hammer	TA1841	2n = 2x = 14, S^sS^s	Jordan
	TA2355		Israel
Ae. tauschii Coss.	TA2452	2n = 2x = 14, DD	Iran
	TA2462		Iran
	TA2507		Turkey
Ae. mutica Boiss.	TA2753	2n = 2x = 14, TT	Russia
	TA2754		Armenia
	TA2755		Armenia
Ae. caudata L.	TA1908	2n = 2x = 14, CC	Unknown
	TA1909		Turkey
	TA2093		Turkey
Ae. comosa Sm. *in* Sibth. & Sm. var. *comosa*	TA2104	2n = 2x = 14, MM	Greece
	TA2731		Turkey
Ae. comosa Sm. *in* Sibth. & Sm. var. *subventricosa* Bioss.	TA1965	2n = 2x = 14, M^hM^h	Turkey
	TA1968		Greece
Ae. uniaristata Vis.	TA2688	2n = 2x = 14, NN	Greece
	TA2696		Turkey
	TA2768		Greece
Ae. umbellulata Zhuk.	TA1825	2n = 2x = 14, UU	Turkey
	TA1831		Iran
	TA1851		Unknown
T. timopheevii (Zhuk.) Zhuk. ssp. *armeniacum* (Jakubz.) MacKey	TA12	2n = 4x = 28, A^tA^tGG	Iraq
	TA934		Iraq
	TA1475		Turkey
	TA1557		Azerbaijan
T. timopheevii (Zhuk.) Zhuk. ssp. *timopheevii*	TA103	2n = 4x = 28, A^tA^tGG	Yugoslavia
	TA140		USA
T. turgidum L., Langdon	TA3028	2n = 4x = 28, AABB	USA
Ae. kotschyi Bioss.	TA2665	2n = 4x = 28, $U^kU^kS^kS^k$	Jordan
	TA2679		Jordan
Ae. peregrina Hackel	TA2681	2n = 4x = 28, $U^pU^pS^pS^p$	Jordan
	TA2775		Israel
T. aestivum L., Chinese Spring	TA3008	2n = 6x = 42, AABBDD	China
Ae. vavilovii (Zhuk.) Chennav.	TA2340	2n = 6x = 42, $X^{va}X^{va}D^{va}D^{va}S^{va}S^{va}$	Israel
	TA2655		Jordan

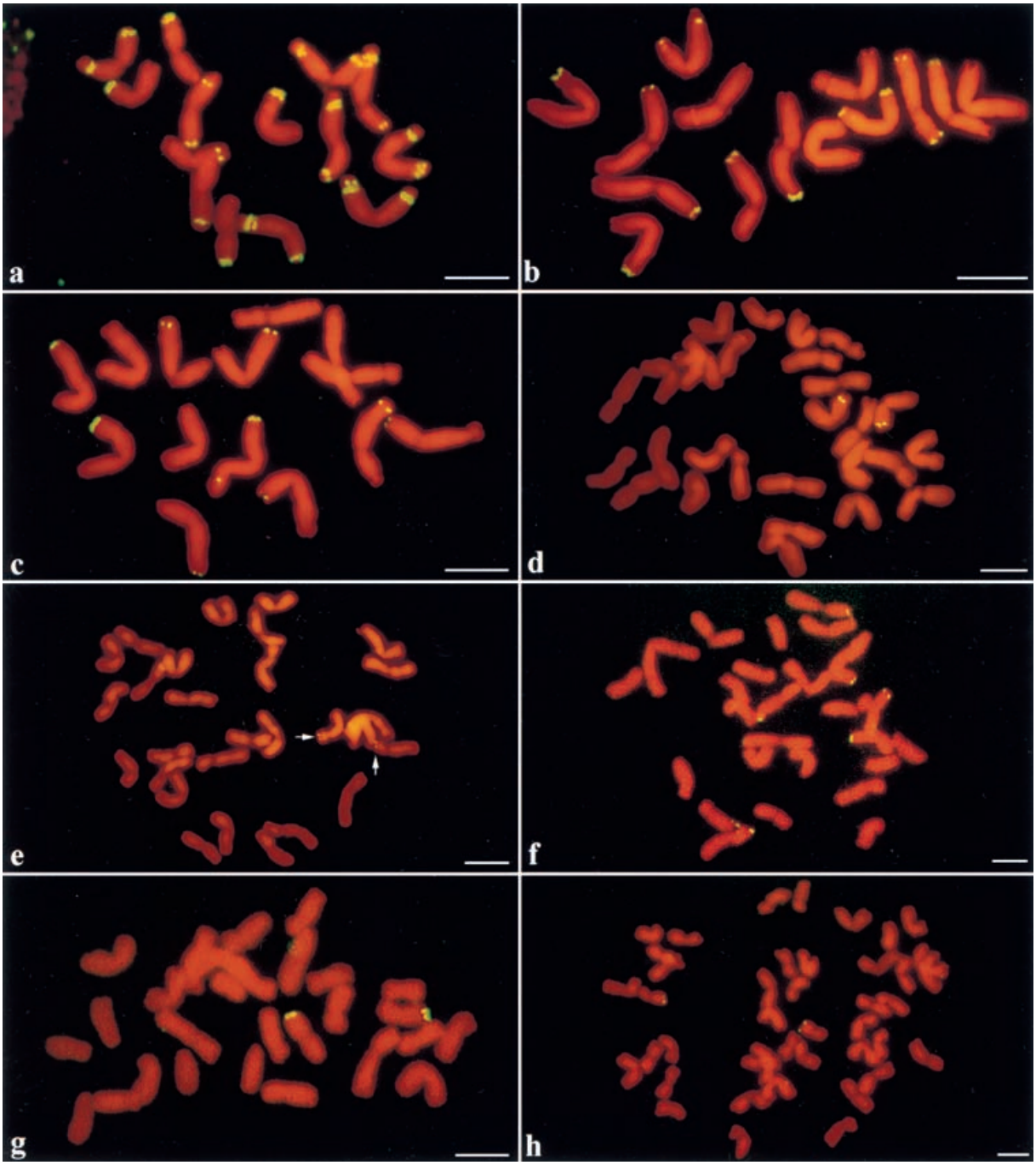

Fig. 1. pGc1R-1 FISH patterns of mitotic metaphase chromosomes of (**a**) *Ae. speltoides* (TA1773), (**b**) *Ae. sharonensis* (TA1995), (**c**) *Ae. longissima* (TA1910), (**d**) *T. timopheevii* ssp. *armeniacum* (TA934), (**e**) *T. timopheevii* ssp. *timopheevii* (TA103) (arrows point to the hybridization sites), (**f**) *Ae. kotschyi* (TA2665), (**g**) *Ae. kotschyi* (TA2679), and (**h**) *Ae. vavilovii* (TA2655). Bars are equal to 10 μm

analyzed, however, the chromosome location was different. In accession TA12, it mapped to a subtelomeric region of the long arm of a SAT chromosome, whereas in TA934 it mapped to the short arm of a non-SAT chromosome (Fig. 1d).

Polyploid *Aegilops* species containing S-genome. Clone pGc1R-1 also hybridized to *Ae. kotschyi* and *Ae. peregrinum*, which have *Ae. longissima* as the donor species of the S^k and S^p genomes, respectively. In addition, clone pGc1R-1 hybridized to *Ae. vavilovii*, of which the X^{va} genome may have come from an extinct ancestor of the section *Sitopsis*, probably closely related to *Ae. speltoides* (Dubcovsky and Dvorák 1995, Dvorák 1998, Badaeva et al. 2002). Intraspecific polymorphisms in FISH patterns also were observed within different accessions of *Ae. kotschyi*, *Ae. peregrina*, and *Ae. vavilovii*. In the two accessions of *Ae. kotschyi* analyzed, three pairs of chromosomes in accession TA2665 (Fig. 1f) and one pair of chromosomes in accession TA2679 (Fig. 1g) had hybridization signals. In the two accessions of *Ae. peregrina* analyzed, three pairs of chromosomes of the accession TA2681 were labeled with the pGc1R-1 probe, whereas no hybridization signal was observed in the accession TA2775. The two accessions of *Ae. vavilovii*, TA2340 and TA2655 (Fig. 1h), have hybridization signals on two and one pair(s) of chromosomes, respectively. Signals on these three species are relatively weak compared with those on *Ae. speltoides*, *Ae. longissima*, and *Ae. sharonensis*.

Discussion

The present study confirms that clone pGc1R-1 does not hybridize to any of the B-genome or A- and D-genome chromosomes of *T. turgidum* and *T. aestivum* (Friebe et al. 2000). Clone pGc1R-1 has 98% sequence homology to the 5′ -end of the S-genome-specific repetitive element pAesKB52 and represents more than 1% of the *Ae. speltoides* genome (Anamthawat-Jónsson and Heslop-Harrison 1993). Previously, Anamthawat-Jónsson and Heslop-Harrison (1993) reported that clone pAesKB52 hybridized to subtelomeric and telomeric chromosome regions of *Ae. speltoides*, *Ae. sharonensis*, and *Ae. longissima* but no hybridization was observed in *Ae. tauschii*, *Ae. umbellulata*, *Ae. ventricosa* Tausch, *T. monococcum* L., *T. turgidum*, *T. timopheevii*, and *T. aestivum* using Southern blot analysis. However, because only a few *Triticum*/*Aegilops* species and only one accession per species were analyzed no detailed information about the intra- and interspecific variation of this sequence was obtained. In the present study the pGc1R-1 FISH patterns of all diploid *Aegilops* species and all S-, G-, and B-genome polyploids were analyzed, which allowed to establish genome affinities and revealed evolutionary relationships of the *Triticum*/*Aegilops* complex.

Among all the diploid *Aegilops* species studied, only *Ae. speltoides*, *Ae. longissima*, and *Ae. sharonensis* belonging to the section *Sitopsis* had pGc1R-1 FISH sites. The pGc1R-1 FISH sites were located in telomeric and subtelomeric chromosome regions. A high level of intraspecific polymorphism in the pGc1R-1 FISH pattern was observed in *Ae. speltoides*, both between homologous chromosomes within plants and between different plants of a given accession. To the contrary, only minor intraspecific variation in pGc1R-1 FISH pattern was observed in *Ae. longissima* and in *Ae. sharonensis*. The differences in the amount of pGc1R-1 polymorphism can be attributed to the differences in breeding behavior of the species involved, *Ae. speltoides* being allogamous and *Ae. longissima* and *Ae. sharonensis* being autogamous.

Clone pGc1R-1 hybridized to the S^k genome of *Ae. kotschyi* and the S^p genome of *Ae. peregrina*. Our data further confirmed that neither *Ae. searsii* nor *Ae. bicornis* contributed the S genome to *Ae. kotschyi* and *Ae. peregrina* as suggested by restriction fragment analysis of chloroplast DNA (Ogihara and Tsunewaki 1988, Siregar et al. 1988).

We observed pGc1R-1 FISH sites in both accessions of *Ae. vavilovii* tested. *Ae. vavilovii* originated from the hybridization of tetraploid *Ae. crassa* Boiss., section *Vertebrata* ($2n = 4x = 28$, $X^{cr}X^{cr}D^{cr1}D^{cr1}$), with *Ae. searsii* (Badaeva et al. 2002). Because clone pGc1R-1 did not hybridize to *Ae. searsii*, the FISH hybridization sites in *Ae. vavilovii* most likely are located on the X^{cr} genome. This result is in

agreement with analyses of repeated nucleotide sequence (Dubcovsky and Dvorák 1995, Dvorák 1998) and the distribution of rDNA loci (Badaeva et al. 2002). These data suggest that the X^{cr} genome may have come from an extinct ancestor of the section *Sitopsis*, probably closely related to *Ae. speltoides*.

We observed distinct pGc1R-1-FISH sites in some of the G-genome chromosomes of the timopheevii wheats, generally the FISH sites in *T. timopheevii* ssp. *armeniacum* were brighter than those observed in the cultivated form *T. timopheevii* ssp. *timopheevii*. These results indicate that the S genome of *Ae. speltoides* is closer related to the G genome of *T. timopheevii* than to the B genomes of *T. turgidum* and *T. aestivum*. Anamthawat-Jónsson and Heslop-Harrison (1993) did not observed hybridization signals using the related clone pAesKB52 in *T. timopheevii* using Southern blot analysis, probably because only one accession of this species was analyzed and not the whole range of polymorphism within this species was detected. Our data are in agreement with meiotic pairing analysis (Shands and Kimber 1973, Maestra and Naranjo 2000, Rodriguez et al. 2000), the sequence homoeology of organellar DNA (Tsunewaki 1996), and the analysis of repetitive DNA sequences (Dvorák and Zhang 1990). Previous studies suggested a diphyletic origin of *T. turgidum* and *T. timopheevii* (Jiang and Gill 1994a). The distribution of the pGc1R-1 FISH pattern on B- and G-genome chromosomes further supports this hypothesis.

A large proportion of the cereal genome is composed of repetitive DNA. Although some repetitive DNA sequences are shared between several cereal species (Jiang et al. 1996, Aragon-Alcaide et al. 1996), others are species-specific (Bedbrook et al. 1980, Rayburn and Gill 1986a, McIntyre et al. 1990, Anamthawat-Jónsson and Heslop-Harrison 1993, Daud and Gustafson 1996, Salina et al. 1997, Nasuda 1999). In addition to clones pAesKB52 and pGc1R-1 two more repetitive sequences were isolated from *Ae. speltoides*. Clone pSp89.XI contains a dispersed repetitive DNA sequence (Daud and Gustafson 1996). This sequence is speltoides-specific because it was not detected in any other genomes of the *Sitopsis* section and only a minor degree of intergenomic homoeology existed in either A or D genomes of wheat. The sequence is present also in the genomes of tetraploid and hexaploid wheats, with the relative abundance decreasing from *Ae. speltoides* to *T. turgidum*, and to *T. aestivum*. The clone Spelt1 is a species-specific tandem repeat associated with the telomeric heterochromatin of *Ae. speltoides*, which accounts for 2% of the DNA of *Ae. speltoides*. The amount of Spelt1, the copy number, and the number of localization sites per haploid genome greatly decrease in tetraploid and hexaploid species (Salina et al. 1997, 1998).

Many of the repetitive DNA sequences are present as tandem repeats, which are known to undergo rapid changes via amplification and deletion events. Several possible mechanisms have been proposed for amplification of tandem repetitive sequences, such as replication slippage, unequal crossing over and recombination of chromosome strands within tandemly arrayed heterochromatic repeats, and rolling-circle amplification (Hourcade et al. 1973, Smith 1976, Bedbrook et al. 1980, Dover 1982, 1986, 1993, Cuzzoni et al. 1990, Strand et al. 1993), as reviewed by Charlesworth (1994) and Charlesworth et al. (1994).

There are two possible explanations for our observations. First, the sequences of the pGc1R-1 family were amplified in *Ae. speltoides*, *Ae. longissima*, and *Ae. sharonensis*, eliminated in *T. turgidum*, and reduced significantly in *T. timopheevii*, *Ae. kotschyi*, *Ae. peregrina*, and *Ae. vavilovii* during the polyploidization process. Second, this sequence amplification is a more recent and gradual event. However, because this sequence exists in several species, the amplification event had to occur in all of them which is unlikely. Therefore, we think that most likely the sequence elimination occurred during or after the polyploidization event.

Similarly, Salina et al. (1997, 1998) found the elimination of the Spelt1 repeat, another

highly species-specific repeat from *Ae. speltoides*, in the course of wheat evolution and allopolyploidization. Using newly synthesized amphiploids, Liu et al. (1998a, 1998b) showed that chromosome- or genome-specific sequences were rapidly eliminated from one of the parental genomes. This elimination led to molecular diploidization resulting in accentuated differentiation of homoeologues and, thereby, may have contributed to their diploid-like cytological behavior.

The organization and evolution of repetitive sequences are important in understanding long-range genome organization and the types of change that can occur during speciation. Clone pGc1R-1 also is useful for monitoring the transfer of *Ae. speltoides* chromatin with useful traits in wheat improvement.

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Address of the authors: Peng Zhang, Bernd Friebe (e-mail: friebe@ksu.edu), Bikram S. Gill, Wheat Genetics Resource Center, Department of Plant Pathology, 4024 Throckmorton, Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA.