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E. G. Pestsova · N. P. Goncharov · E. A. Salina

Elimination of a tandem repeat of telomeric heterochromatin during the evolution of wheat

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Abstract An analysis of accessions of *Triticum* and *Aegilops* species (86 diploid, 91 tetraploid and 109 hexaploid) was performed using squash-dot hybridization with the tandem repeat Spelt1 sequence as a probe. The Spelt1 sequence is a highly species-specific repeat associated with the telomeric heterochromatin of *Aegilops speltoides* Boiss. in which its copy numbers vary from 1.5×10^5 to 5.3×10^5. The amounts of Spelt1 are sharply decreased in tetraploid and hexaploid species and vary widely from less than 10^2 to 1.2×10^4. Two tetraploid wheats, *Triticum timopheevii* Zhuk. and *T. carthlicum* Nevski, are exceptional endemic species and within their restricted geographical distributions maintain the amounts of Spelt1 unaltered. The Spelt1 repetitive sequence was localized on the 6BL chromosome of tetraploid wheat *Triticum durum* Desf. cv 'Langdon' by dot-hybridization using D-genome disomic substitution lines. The possible causes of the loss of the telomere-associated tandem repeat Spelt1 in the process of wheat evolution and polyploidization are discussed.

Key words Wheat evolution · Species-specific sequences · Telomere-associated tandem repeats

Introduction

The origin of the cultivated polyploid wheats remains a debatable problem whose solution is important for both theoretical and applied breeding purposes. *Triticum urartu* Thum. and *Aegilops squarrosa* L. have been identified as most probably being the A- and D-genome donors, respectively, to hexaploid wheat *Triticum aestivum* L. However, there is controversy surrounding the donor of the B genome to bread and durum wheats. The putative progenitor is thought to be an *Aegilops* species of the *Sitopsis* section, and the most probable donor appears to be *Aegilops speltoides* Boiss. There is a convincing body of evidence supporting the idea that the S genome of *Ae. speltoides* is closely related to the B genome of polyploid wheats. This evidence is based on karyotype data (Riley et al. 1958), the C-banding of chromosomes (Friebe and Gill 1996), cytological evidence (Kerby and Kuspira 1988), electrophoretic mobilities of proteins (Johnson 1972), the geographical distributions of wild wheat populations (Witcombe 1983) and restriction fragment length polymorphism (RFLP) analyses of low-copy and repetitive DNA sequences (Dvorak and Zhang 1990; Talbert et al. 1995; Sasanuma et al. 1996). The question remains open whether *Ae. speltoides* is the sole source of the B genome or whether the genome resulted from an introgression of several parental species (Zohary and Feldman 1962). With respect to the first of these alternatives, the considerable divergence of the B genome from the progenitor genome is noteworthy (Yen et al. 1996).

Daud and Gustafson (1996) have provided molecular evidence for *Ae. speltoides* as a progenitor of the B genome of polyploid wheats. The dispersed pSp89.XI sequence specific to the *Ae. speltoides* genome is present in the AB genome of *Triticum turgidum* L. and in the ABD genome of *T. aestivum*. The slight decrease in copy numbers of pSp89.XI in the genomes of polyploid wheats suggests that a considerable part of the genome, if not the entire one, originated from *Ae. speltoides*.

The repetitive sequence Spelt1 we used here is also specific to the *Ae. speltoides* genome where it accounts for more than 1% of the genome (Salina et al. 1998). It

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E. G. Pestsova · N. P. Goncharov · E. A. Salina (✉)
Institute of Cytology and Genetics,
Lavrentieva 10, 630090 Novosibirsk, Russia
Fax: + 3832 33 12 78
E-mail: salina@cgi.nsk.su

is arranged in tandem arrays virtually in all the chromosomes and is associated with telomeric heterochromatin. Compared with the pSp89.XI sequence, the copy numbers of the Spelt1 repeat have been decreased 40- to 60-fold in tetraploid wheats *Triticum dicoccum* Schuebl. and *Triticum timopheevii* Zhuk., and the number of localization sites per haploid genome reduced to two in these two species. In bread wheat cv 'Chinese Spring' the copy numbers of Spelt have been decreased more than 1000-fold, and Salina et al. (1997b) were unable to determine its localization site by in situ hybridization.

The aims of the investigation presented here were (1) to analyze the spread of the Spelt1 sequence family with reference to geographical distribution; (2) to identify the chromosomes carrying a large block of the Spelt1 repeated sequence in polyploid wheats; (3) to analyze the repatterning of the tandem repeats associated with telomeric heterochromatin of *Ae. speltoides* during evolution of wheats.

Materials and methods

Plant materials

Plant species were provided by the world collection of the All-Russian Institute of Plant Industry (St. Petersburg, Russia), Small Grain Collection (Aberdeen, USA) and ICARDA (Aleppo, Syria). The species were classified according to Dorofeev and Korovina (1979). The data on *Triticum* and *Aegilops* species are summarized in Table 1. D-genome disomic substitution lines *T. durum* cv 'Langdon' were kindly supplied by Dr. L. R. Joppa (NDSU, Fargo, USA).

Probes

A 150-bp DNA fragment was previously isolated from *Ae. speltoides* var 'ligustica' inserted in the plasmid pBluescript II SK + and designated as Spelt1 (Salina et al. 1997a). Spelt1 was sequenced and the nucleotide sequence data are stored in the EMBL nucleotide sequence database under the accession number Y09217. Spelt1 was used as a probe in this study. The inserted fragment of Spelt1 in the plasmid was amplified by the polymerase chain reaction (PCR). The insert was labeled with α-[^{32}P]-dTAP (Amersham) using the Klenow fragment and M13 primers.

DNA isolation, restriction, gel electrophoresis and blotting

Total DNA was isolated from etiolated seedlings and green leaves as described by Ausubel et al. (1987) and was digested with restricted endonucleases. Restriction fragments were fractionated on 1% agarose gels and blotted onto Hybond-N membranes as specified by the manufacturer (Amersham).

Squash-dot hybridization

We used a rapid screening technique for the detection of repeated DNA sequences in plant tissues (Hutchinson et al. 1985). Seeds were germinated on filter paper at 20°C. After 2–3 days, when the rootlets reached a length of 1.0–1.5 cm, 3 mm of their tips were cut off, and these portions were squashed onto a Zeta-probe blotting membrane. Several root tips from two to three seeds of each accession were squashed onto a membrane to obtain four to nine replicates of the same accession. The DNA of the root tips on the filters was denatured in 0.5 *M* NaOH for 5 min. This was followed by neutralization in 1 *M* TRIS-HCl, pH 7.5, for 5 min, twice, and in 1.5 *M* NaCl, 0.5 *M* TRIS-HCl, pH 7.5, for 5 min, twice. The membranes were then transferred to dry filter paper and air dried. The filters were stored in a plastic bag at 20°C.

Dot hybridization

The amount of Spelt1 in disomic substitution lines of *T. durum* cv 'Langdon' was quantified by dot screening (Kafatos et al. 1979). The conditions of pre-hybridization and hybridization are described below.

Hybridization

Filters were first moistened by floating on 2 × SSC. Pre-hybridization was carried out at 65°C for 4 h in 5 × SSC, 5 × Denhardt's solution, 2% SDS and denatured salmon sperm DNA (200 μg/ml). Hybridization was conducted in the same solution for 16 h. The filters were washed at 65°C in 2 × SSC, 0.5% SDS for 15 min, twice. They were also washed at 65°C in 0.1 × SSC, 0.5% SDS for 15 min twice, when necessary. Estimates of the degree of squash-dot and dot hybridization were based on radioactivity measurements of filter strips comprising one root tip or one spot representing 0.5 and 1 μg of blotted DNA. Based on four to nine replicates of squash-dot hybridization, the mean number of counts of the [^{32}P]-labeled Spelt1 probe was calculated for each accession.

Results

Estimation of the copy numbers of the Spelt1 sequence in *Triticum* and *Aegilops* species

In our previous study, the copy numbers of Spelt1 were determined in 26 species of *Triticum* and *Aegilops* using just 1 accession number for each species (Salina et al. 1998). In this study, a considerably larger number of species and accessions differing in ploidy level were involved in the quantitative estimates (Table 1). We wished (1) to test the significance of the data indicating that the number of telomeric blocks of Spelt1 is reduced in allopolyploids and also to rule out the possibility that in our previous study accession(s) with eliminated Spelt1 might have been chosen by chance and (2) to analyze the spread of the Spelt1 sequence family with reference to geographical distribution.

Squash-dot hybridization enables the rapid examination of a large number of individual plants omitting laborious DNA isolation (Hutchinson et al. 1985). With the squash-dot technique, hybridization to squashed root tips made it possible to estimate the relative copy numbers of Spelt1, where the copy number in 26 *Triticum* and *Aegilops* species were known. Hutchinson et al. (1985) diagnosed repetitive sequences that are

Table 1 Geographic origins of *Triticum* and *Aegilops* accessions used in this study

Species	Genome	Number of accessions	Location
T. monococcum L.	A^b	5	Azerbaijan, Germany, Romania, Turkey, Georgia[a]
T. boeoticum (Boiss.) MK	A^b	4	Ukraine, Turkey, Iraq, Armenia
T. urartu Thum.	A^u	4	Armenia, Georgia, Iraq, Syria
Ae. squarrosa ssp. *straugulata* (Eig) Tzvel.	D	5	Azerbaijan, Armenia
Ae. squarrosa ssp. *eusquarrosa* Holzm.	D	5	Georgia, Daghestan, Kirghizia, Uzbekistan, Kazakhstan
Ae. speltoides var 'ligustica' (Savign.) Coss.	S	13	Turkey, Israel, unknown
Ae. speltoides var 'speltoides' Tausch	S	26	Turkey, Israel, Iraq, Syria, Afghanistan, Turkmenistan, unknown
Ae. bicornis (Forsk.) Jaub. and Sp.	S^b	3	Israel, Egypt
Ae. searsii Feld. and Kis.	S^s	4	Jordan
Ae. longissima Schweinf. and Muschl.	S^l	5	Jordan, Palestine
Ae. sharonensis Eig.	S^l	4	Palestine, Turkey, Israel
Ae. heldrechii Holzm.	M^h	4	Greece, Asia Minor
Ae. caudata L.	C	1	Greece
Ae. uniaristata Vis.	U_n	3	Greece, Japan
T. militinae Zhuk. and Migusch.	A^bG	1	Turkey
T. timopheevii Zhuk.	A^bG	9	Georgia
T. araraticum Jukabz.	A^bG	4	Azerbaijan, Armenia, Iraq
T. turanicum Jukabz.	A^uB	5	Uzbekistan, Turkey, Turkmenistan, Daghestan
T. carthlicum Nevski	A^uB	8	Georgia, Armenia, Daghestan
T. aethiopicum Jukabz.	A^uB	9	Ethiopia
T. polonicum L.	A^uB	3	Georgia, Russia
T. dicoccoides (Koern) Thell.	A^uB	1	Israel
T. dicoccum (Schrank) Thell.	A^uB	9	Russia, Turkey, Germany, Czech Republic
T. turgidum L.	A^uB	7	Russia, Armenia, Uzbekistan, Azerbaijan, Algeria, Spain, China
T. durum Desf.	A^uB	35	Russia, China, Turkey, Greece, Iran, Syria, Palestine, Tunesia, India, Canada, Cyprus, Kazakhstan, Portugal, Italy, Israel, Ukraine
T. compactum Host.	A^uBD	3	USA, Peru
T. sphaerococcum Perc.	A^uBD	1	India
T. spelta L.	A^uBD	16	Spain, Iran, Ukraine, Germany, Azerbaijan, Daghestan, Turkmenistan, Yugoslavia, Poland
T. petropavlovskii Udacz. and Migusch.	A^uBD	3	China
T. aestivum L.	A^uBD	86	Yugoslavia, Canada, Armenia, China, Russia, Czech Republic, Japan, Germany, Romania, Hungary, unknown

[a] Georgia: Caucasian republic of the former USSR

repeated 10^2–10^6 times in the genome, and they observed that, within the range of 3×10^3–1×10^4 copies of repeated sequence, there is virtually a linear relation between the counts per minute of [^{32}P]-labeled probe hybridized to root tips and the amount of the sequence present in the DNA of the plant material. Assuming that there is a linear relation between the amount of hybridization of Spelt1 [^{32}P]-labeled DNA to squashed root tips and the number of Spelt1 copies, we estimate the amount of Spelt1 in a total of 286 wheat and goat grass species.

Diploids

All the *Sitopsis* section species (*Ae. speltoides*, *Ae. sharonensis*, *Ae. longissima*, *Ae. searsii* and *Ae. bicornis*) were squash-dot hybridized using Spelt1 as a probe. The number of Spelt1 copies was determined in 39 accessions of two *Ae. speltoides* varieties, var 'speltoides' (13 accessions) and var 'ligustica' (26 accessions). From 1–5 accessions were from the remaining diploid species (Table 1). Figure 1 is a representative squash-dot autoradiograph. The mean number of [^{32}P] counts for each accession after hybridization, using Spelt1 as a probe, are graphically represented in Fig. 2.

In *Ae. speltoides*, the copy numbers of Spelt1 ranged from 1.5×10^5 to 5.3×10^5 per genome (Fig. 3). In the other diploid species, the copy numbers of Spelt1 are of three orders of magnitude smaller, varying from less than 100 to 500 copies per haploid genome. Thus, our data support the notion that the Spelt1 family is highly species-specific and allow us to follow its fate during the course of wheat polyploidization.

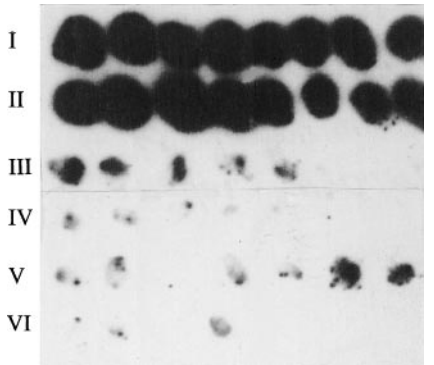

Fig. 1 An autoradiograph showing different degrees of hybridization of the [^{32}P]-labeled Spelt1 probe to meristematic root-tip squashes from different accessions of *Triticum* and *Aegilops* species. *I*, *II Ae. speltoides*, *III T. monococcum*, *IV T. urartu*, *V T. boeoticum*, *VI Ae. longissima*

Tetraploids

The number of copies of the Spelt1 sequence was determined in 91 accessions of tetraploid wheat representing 11 species, of which some are wild and others are cultivated worldwide (Fig. 3). The amount of Spelt1 in the tetraploid genomes varied widely from less than 10^2 to 10^4 copies. It should be noted that the copy numbers of the Spelt1 were high in wheat species with the AG genome. The number of Spelt1 copies was stably high in *T. timopheevii*, a species endemic to West Georgia (the former USSR), with a mean copy number of 5×10^3. The number of Spelt1 copies was low, not more than 250 copies per haploid genome, in another endemic Georgian species, *T. carthlicum*, with the AB genome. This variation in the amount of Spelt1 was wider in tetraploid wheats cultivated over more extensive areas. Thus, the copy numbers of the Spelt1 sequence varied from less than 10^2 to 1.1×10^4 in *T. durum*. However, we were not able to establish a relationship between number of copies of Spelt1 and the distribution areas of cultivated and wild wheats.

Hexaploids

The occurrence of the Spelt1 sequence was analyzed in 109 accessions representing 5 hexaploid species from Europe, Asia, South and North America (Table 1). There were great differences in copy numbers among and within hexaploid species (Fig. 3). The intraspecific variation in the copy numbers of the Spelt1 sequence could be well estimated for *T. aestivum* and *T. spelta* because of their high numbers of accessions, 86 and 16, respectively. The amount of the Spelt1 sequence varied from less than 10^2 to 7.5×10^3 copies in the *T. spelta* accessions and from less than 10^2 to 1.2×10^4 copies in the *T. aestivum* genomes. Cultivar 'Chinese Spring' of common wheat was found to have less than 100 copies of Spelt1 in its genome. Notably, the Spelt1 repeat was not detected by in situ hybridization in this cultivar (Salina et al. 1997b).

Identification of chromosomes with large blocks of the Spelt1 repeats in polyploid wheats

The copy number of Spelt1 in hexaploid wheat cultivar 'Chinese Spring' is low, and this makes the identification of the chromosomes carrying Spelt1 repeats using aneuploid lines of 'Chinese Spring' difficult. D-genome disomic substitution lines of tetraploid wheat *T. durum* cv 'Langdon', kindly supplied by L. R. Joppa (Joppa and Williams 1988), were used for this purpose. The Spelt1 sequence was squash-dot hybridized to root tips taken from seedlings of these lines. The mean numbers of [^{32}P] counts of four to nine replicates are represented in Fig. 4A.

According to the results obtained the mean numbers of counts for several substitution lines were 1.6 times higher than those for the initial line, however, there was a two- to threefold variation in the counts among replicates within a line. We also observed wide variations in the numbers of counts among replicates during our analysis of wheat and *Aegilops* accessions. These variations were obvious in species with Spelt1 present in the genome in copy numbers smaller than 10^3. Using the squash-dot technique we calculated the copy numbers of the Spelt1 sequence in *T. durum* cv 'Langdon' to be about 10^3 per haploid genome. D-genome substitution line 6D(6B) (14″ + t′6BS) lacking the long arm of 6B chromosome has smaller amounts of the Spelt1 repeats. This, however, did not allow us, with certainty, to localize the Spelt1 telomeric block on this chromosome.

For a more precise localization, we dot hybridized the [^{32}P]-labeled Spelt1 sequence to DNA of D-genome disomic substitution lines of *T. durum* cv 'Langdon' to determine the amount of Spelt1 in the lines. The experiments were done in two replicates, using 0.5 and 1 μg DNA. The results of one experiment are shown in Fig. 4B. It was found that the copy number of Spelt1 is twofold smaller in 6D(6B) (14″ + t′6BS) than in the other substitution lines. This indicates that the block of tandem Spelt1 repeat is on the long arm of 6B chromosome in durum wheat, as our results of squash-dot analysis first suggested.

Genomic organization of the Spelt1 sequence

The arrangement of the Spelt1 sequence in tandem arrays was detected in our previous Southern hybridization to the *Hae*III, *Rsa*I and *Tag*I digests of genomic

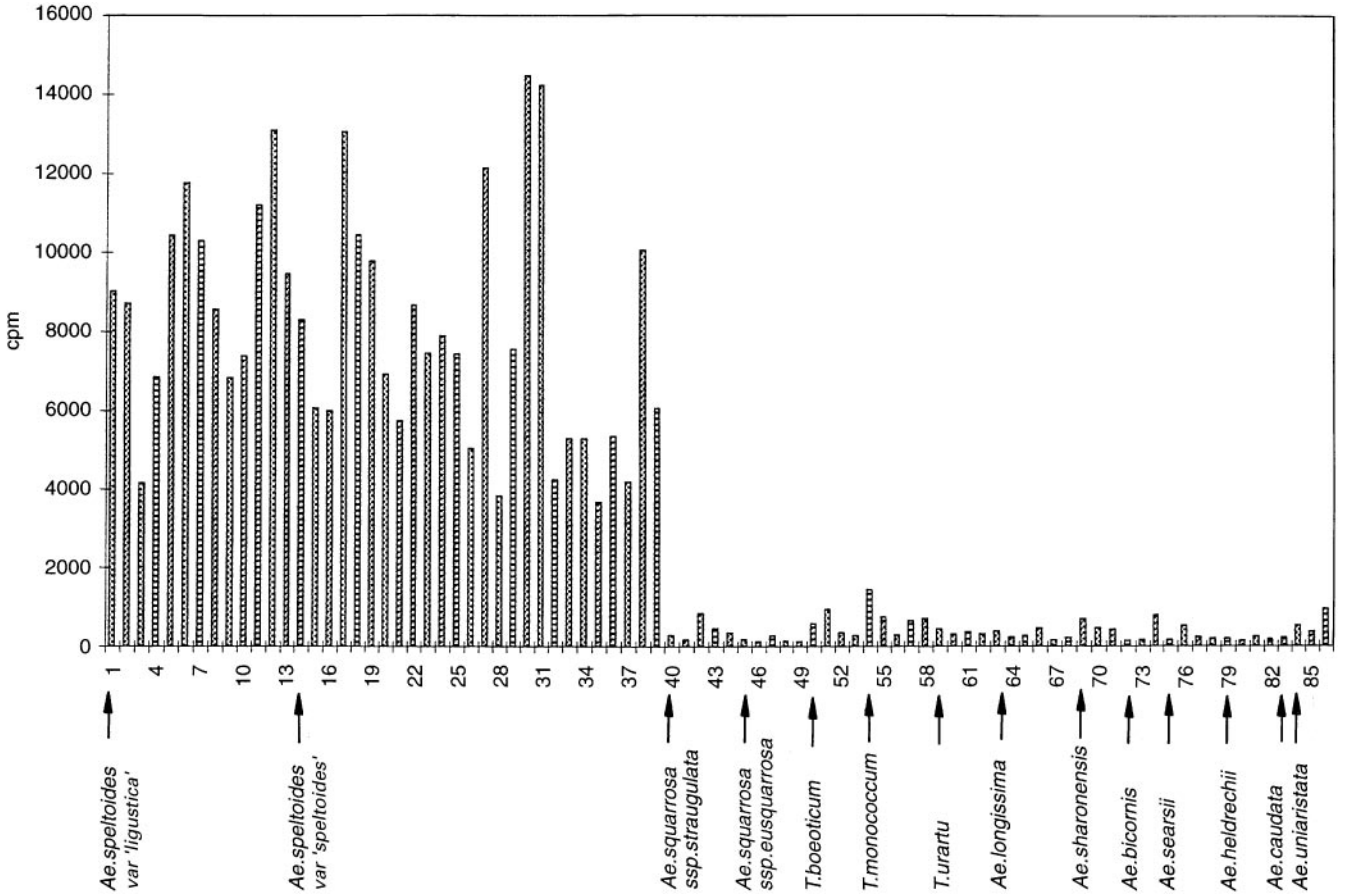

Fig. 2 Hybridization of Spelt1 to squashed root tips from each accession of diploid *Triticum* and *Aegilops* species: *Ae. speltoides* var 'ligustica' (*1* K-21; *2* K-66, *3* K-197, *4* K-198, *5* K-443, *6* K-459, *7* K-461, *8* K-196, *9* K-2280, *10* K-2282, *11* K-1015, *12* K-2302, *13* K-2303); *Ae. speltoides* var 'speltoides' (*14* K-22, *15* K-49, *16* K-68, *17* K-77, *18* K-250, *19* K-392, *20* K-448, *21* K-449, *22* K-450, *23* K-453, *24* K-464, *25* K-1018, *26* K-1276, *27* K-1592, *28* K-1597, *29* K-2275, *30* K-2281, *31* K-2283, *32* K-2366, *33* K-2369, *34* Ae-46599, *35* Ae-46808, *36* Ae-46809, *37* Ae-47115, *38* Ae-49016, *39* Ae-116067); *Ae. squarrosa* ssp. *straugulata* (*40* K-1163, *41* K-750, *42* K-1185, *43* K-79, *44* K-1159); *Ae. squarrosa* ssp. *eusquarrosa* (*45* K-1216, *46* K-637, *47* K-671, *48* K-1358, *49* K-1161); *T. boeoticum* (*50* K-18424, *51* K-14384, *52* K-40118, *53* K-2714); *T. monococcum* (*54* K-18105, *55* PI-35247, *56* PI-306547, *57* PI-554596, *58* PI-418582); *T. urartu* (*59* K-33869, *60* Ae-44829, *61* PI-487272, *62* PI-427328); *Ae. longissima* (*63* Ae-47293, *64* Ae-48606, *65* K-194, *66* Ae-47328, *67* Ae-48613); *Ae. sharonensis* (*68* Ae-47080, *69* Ae-47098, *70* K-1583, *71* K-905); *Ae. bicornis* (*72* K-1311, *73* K-1327, *74* K-2332); *Ae. searsii* (*75* Ae-47303, *76* Ae-47320, *77* Ae-47338, *78* Ae-47353); *Ae. heldrechii* (*79* K-669, *80* K-1600, *81* K-1601, *82* K-2272); *Ae. caudata* (*83* K-2270); *Ae. uniaristata* (*84* K-463455, *85* K-1590, *86* K-2273). The results are the means of four to nine replicates of each accession

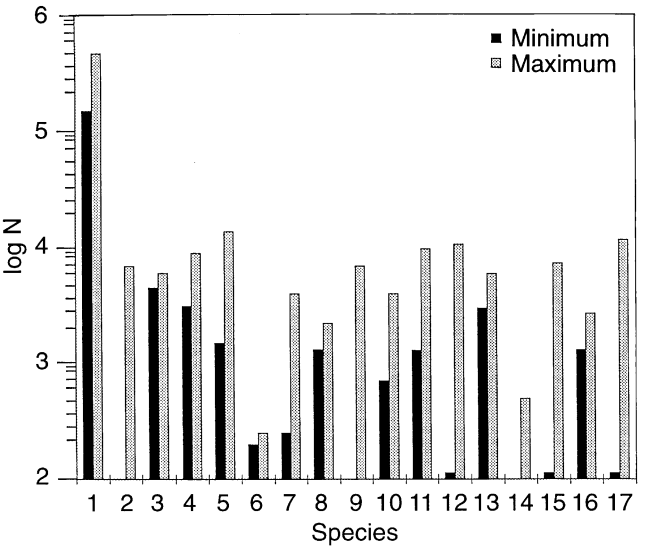

Fig. 3 Intraspecific variation in the mean copy numbers of Spelt1 estimated by squash-dot hybridization. *1 Ae. speltoides* var 'speltoides', *2 T. militinae*, *3 T. timopheevii*, *4 T. araraticum*, *5 T. turanicum*, *6 T. carthlicum*, *7 T. aethiopicum*, *8 T. polonicum*, *9 T. dicoccoides*, *10 T. dicoccum*, *11 T. turgidum*, *12 T. durum*, *13 T. compactum*, *14 T. sphaerococcum*, *15 T. spelta*, *16 T. petropavlovskii*, *17 T. aestivum*. The number of examined accessions is given in Table 1. *N* Copy numbers of repeats

DNAs from 15 different *Triticum* and *Aegilops* species. Many bands in polyploid wheats have been difficult to diagnose and, consequently, analysis has been conducted under conditions used for unique DNA sequences (Salina et al. 1997a). The finding of wide variations in

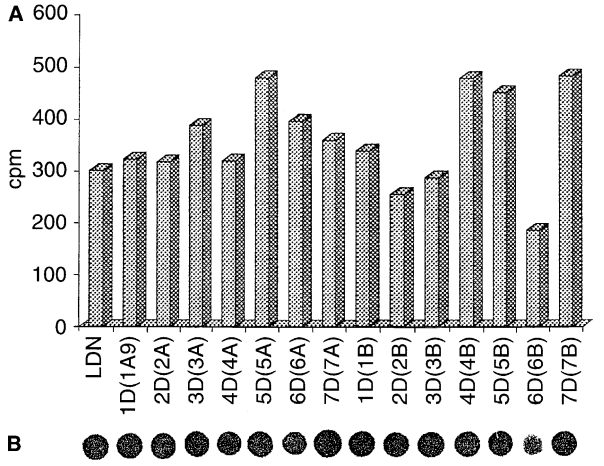

Fig. 4 **A** The mean number of counts of the [^{32}P]-labeled Spelt1 probe hybridized to root tips from each disomic substitution line of *T. durum* cv 'Langdon'. **B** Dot-hybridization of the [^{32}P]-labeled Spelt1 probe hybridized to 1 μg DNA from each of the disomic substitution lines of *T. durum* cv 'Langdon'.

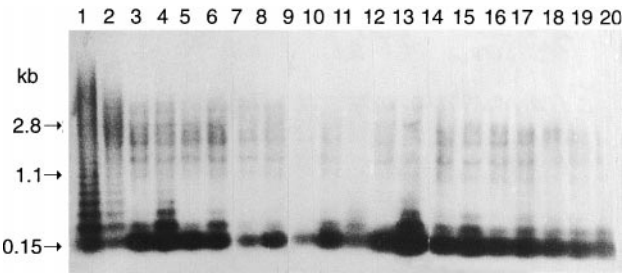

Fig. 5 Blot-hybridization of [^{32}P]-labeled Spelt1 probe to the DNAs of different wheat species digested with *Rsa*I: *T. spelta* (*1* K-53660, *2* K-45364, *3* K-45750); *T. aestivum* (*4* cv 'Codga Bugday', *5* cv 'Caigette 1', *6* cv 'Blue A', *7* cv 'Skorospelka', *8* cv 'Soor Ghanum', *9* cv 'V7', *10* cv 'Red' *11* cv 'San moude Alba', *12* cv 'Lovrin 13', *13* K-26084); *T. durum* (*14* cv 'Souri 484 K-16477', *15* cv 'Nursit K-13332', *16* cv 'Garnovka K-1931'); *T. timopheevii* (*17* K-35916, *18* K-29568, *19* K-29558); *T. turanicum* (*20* K-31693)

copy numbers of the Spelt1 sequence in polyploid wheats prompted us to determine the genomic organization of Spelt1 in species where it is abundant.

The arrangement of Spelt1 in tandem arrays was clearly visible in several accessions of *T. spelta* in blot hybridization to genomic DNAs from tetraploid and hexaploid species digested with restriction endonuclease *Rsa*I (Fig. 5). The ladder and the set of large fragments were the two hybridization patterns consistently seen together in polyploids. When genomic DNA was incompletely digested with *Taq*I (30 min, 65°C), the tandem arrangement of Spelt1 was detected virtually in all the polyploid species analyzed. After a longer digestion with *Taq*I (4 h, 65°C), the tandem arrays were completely restricted and, as a result, 150-bp monomeric repeated subunits prevailed (data not shown).

Thus, blot analyses of polyploid species with high amounts of the Spelt1 sequence did not reveal restriction fragment length polymorphism among the species.

Discussion

Comparative analysis of the DNA repeats of the putative ancestors of hexaploid wheats has demonstrated that 2–3% of the *Ae. speltoides* genome is composed of species-specific sequences (Flavell 1982). 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. 1997a). Of these, two, Spelt1 and pAesKB52, are subtelomeric repeats. The species-specific Spelt1 repeat has been localized to sites of the major blocks of heterochromatin, closer to the chromosomal ends (Salina et al. 1997b). The pAesKB52 is found proximal to the major heterochromatin blocks (Anamthawat-Jonsson and Heslop-Harrison 1993) and, hence, it is proximal to the Spelt1 repeats in *Ae. speltoides*. The pAesKB52 sequence also occurs in the subtelomeric region of the chromosomes in other species of the *Sitopsis* section. The same arrangement of species-specific and common subtelomeric repeat families has been also described for *S. cereale* (Jones and Flavell 1982).

The dispersed repeat pSp89.XI, species-specific to *Ae. speltoides*, proved to be useful in assessing the contribution of *Ae. speltoides* to the formation of the B genome of polyploid wheat (Daud and Gustafson 1996). The assessment has allowed us to conclude that the bulk of the B genome is derived from the *Ae. speltoides*. These molecular data are consistent with other lines of evidence (Dvorak and Zhang 1990; Friebe and Gill 1996; Sasanuma et al. 1996). In contrast to the dispersed pSp89.XI sequence, the amount of the subtelomeric Spelt1 repeat in the tetraploid species has been reduced by 50- to 1000-fold (Fig. 3) and the number of localization sites on the chromosomes decreased from 13 to 2 or more per haploid genome (Salina et al. 1997b). Furthermore, our previous data allow us to exclude the possible influence of the divergence of the primary structure of Spelt1 repeats on the differences we observed for copy numbers between *Ae. speltoides* and other species (Salina et al. 1997a). Thus, there is reason to suggest that the Spelt1 repeat was eliminated in the course of wheat allopolyploidization.

Based on the data in the literature on the structure and arrangement of telomeric regions of chromosomes in the cell nucleus (Gustafson and Bennet 1982; Bennet 1982; Zhimulev 1997), the removal of telomere-associated tandem repeats in allopolyploids may be necessary for (1) the synchronization of replication in hybrid genomes; (2) the reorganization of chromosome

distribution in the nucleus; (3) the establishment of a balance in the DNA content in the cell.

The Spelt1 repeat family is not stable in allopolyploid wheats. The amount of Spelt1 varies widely even within a species, and it is not correlated with the natural geographical distributions of a species. *T. timopheevi* and *T. carthlicum* are exceptional endemic species, their restricted geographical distributions maintain the amounts of Spelt1 unaltered (Fig. 3). Thus, large subtelomeric blocks of the Spelt1 repeats are, for the most part, unstable within *Triticum* and *Aegilops* species and can be eliminated during the evolution and the formation of alloploid wheat forms.

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References

Anamthawat-Jonsson K, Heslop-Harrison JS (1993) Isolation and characterization of genome-specific DNA sequences in *Triticeae* species. Mol Gen Genet 240:151–158

Ausubel ML, Brent R, Kingston RE, Moore DD, Smith JA, Seidman JG, Struhl K (1987) Current protocol in molecular biology. Greene Publishing Assoc, Wiley Interscience, New York

Bennet MD (1982) Nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and the implication of the order for genome evolution and phenotypic variation. In: Dover GA, Flavell RB (eds) Genome evolution. Academic Press, London

Daud HM, Gustafson JP (1996) Molecular evidence for *Triticum speltoides* as B-genome progenitor of wheat (*Triticum aestivum*). Genome 39:543–548

Dorofeev VF, Korovina ON (1979) Flora of cultivated plants. Wheat. Kolos, Leningrad

Dvorak J, Zhang HB (1990) Variation in repeated nucleotide sequences sheds light on the origin of the wheat B and G genomes. Proc Natl Acad Sci USA 87:9640–9644

Flavell RB (1982) Amplification, deletion and rearrangement: major sources of variation during species divergence. In: Dover GA, Flavell RB (eds) Genome evolution. Academic Press, London

Friebe B, Gill BS (1996) Chromosome banding and genome analysis in diploid and cultivated polyploid wheats. In: Jauhar PP (ed) Methods of genome analysis in plants. CRC Press, Baton Rouge, Fla., pp 39–60

Gustafson JP, Bennet MD (1982) The effect of telomeric heterochromatin from *Secale cereale* on Triticale (× *Triticosecale*). I. The influence of the loss of several blocks of telomeric heterochromatin on early endosperm development and kernel characteristics at maturity. Can J Genet Cytol 24:83–92

Hutchinson J, Abbott A, O'Dell M, Flavell RB (1985) A rapid screening technique for the detection of repeated DNA sequences in plant tissues. Theor Appl Genet 69:329–333

Johnson BL (1972) Protein electrophoretic profiles and the origin of the B genome of wheat. Proc Natl Acad Sci USA 69:1398–1402

Jones JDG, Flavell RB (1982) The mapping of highly-repeated DNA families and their relationship to C-bands in chromosomes of *Secale cereale*. Chromosoma 86:595–612

Joppa LR, Williams ND (1988) Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. Genome 30:222–228

Kafatos TC, Jones CW, Efstratiadis A (1979) Determination of nucleic acid sequence homologies and relative concentrations by dot-hybridization procedures. Nucleic Acids Res 7:1541–1552

Kerby K, Kuspira J (1988) Cytological evidence bearing on the origin of the B genome in polyploid wheats. Genome 30:36–43

Riley R, Unrau J, Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49:91–98

Salina EA, Pestsova EG, Vershinin AV (1997a) Spelt1: a novel family of tandemly repeated DNA in cereals. Russ J Genet 33:352–357

Salina EA, Pestsova EG, Alkhimova EG, Heslop-Harrison JS, Vershinin AV (1997b) Variation of telomeric species-specific repeats of *Triticum speltoides* during the evolution of polyploid wheats (Abstr). In: Conf Mol Biochem Physiol Aspects Plant Sci., Moscow

Salina EA, Pestsova EG, Adonina IG, Vershinin AV (1998) Identification of a new family of tandem in *Triticeae* genomes. Euphytica 100:231–237

Sasanuma T, Miyashita NT, Tsunewaki K (1996) Wheat phylogeny determined by RFLP analysis of nuclear DNA 3. Intra- and interspecific variations of five *Aegilops* Sitopsis species. Theor Appl Genet 92:928–934

Talbet LE, Blake NK, Storlie EW, Lavin M (1995) Variability in wheat based on low-copy DNA sequence comparisons. Genome 38:951–957

Witcombe JR (1983) A guide to the species of *Aegilops* L. IBPGR Secretariat, Rome

Yen Y, Baenziger S, Morris R (1996) Genomic constitution of bread wheat: current status. In: Jauhar PP (ed) Methods of genome analysis in plants. CRC Press, Baton Rouge, Fla., pp 359–373

Zhimulev IF (1997) In: Polytene chromosomes, heterochromatin, and position effect variegation. (Advances in genetics, vol 37.) Academic press, London, New York, pp 238–282

Zohary D, Feldman M (1962) Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. Evolution 16:44–61