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Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes

(introgression/*Triticum*/evolution)

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ABSTRACT **A general method based on variation in repeated nucleotide sequences was developed for the identification of diploid species most closely related to a specific genome of a polyploid species. The utility of this method was demonstrated by showing that *Triticum speltoides* is the most closely related extant species to both the B and G genomes of tetraploid wheats.**

Inferences on the phylogeny of polyploid plants were traditionally based on investigation of chromosome pairing in interspecific hybrids (1). In many instances, however, this method failed to generate a conclusive picture. Tetraploid wheats, *Triticum turgidum* (L.) em. Morris et Sears (genomes AABB) and *T. timopheevii* (Zhuk.) Zhuk. (genomes AAGG) are well-known examples. On the basis of chromosome pairing it was concluded that the A genome of both species was contributed by *T. monococcum* L. (2, 3). The discovery of the second diploid species with the A genome, *T. urartu* Thum., created a dilemma, since the chromosomes of *T. monococcum* and *T. urartu* paired about equally well with the chromosomes of polyploid wheats (4, 5). Investigation of polymorphism in several protein or enzyme loci suggested that the source of the A genome may be *T. urartu*, but the question whether *T. urartu* contributed the A genome of one or both tetraploid wheats remained a controversy (6, 7). Dvořák *et al.* (8) hypothesized that the multilocus, multicopy nature of the nuclear repeated nucleotide sequences and their gradual evolutionary turnover are characteristics making them a potentially useful new tool for the studies of the phylogeny of polyploid plants. From the variation in the lengths of restriction fragments in several sequence families, one of them later shown to be interspersed (9), and the intensity of signal in Southern blots, they inferred that the A genome in both tetraploid wheats was contributed by *T. urartu*.

An opposite dilemma was encountered for the second genome of tetraploid wheats. In this case no diploid species has been identified which has chromosomes that could be unequivocally shown to pair with the chromosomes of the B and G genomes of tetraploid wheats. On the basis of morphological evidence it was suggested that the B genome of *T. turgidum* was contributed by a species closely related to *T. speltoides* (Tausch.) Gren. (10). However, the chromosomes of *T. speltoides* pair poorly with the chromosomes of the B genome (11–13), and it was concluded that *T. speltoides* could not be a source of the B genome (12). Since the chromosomes of the G genome of *T. timopheevii* pair, albeit poorly, with the chromosomes of the B genome, the B and G genomes must be very closely related (14, 15). The cytoplasms of the tetraploid wheats were contributed by the B- and G-genome sources, and that of *T. timopheevii* is equivalent to that of *T. speltoides* (16). The nucleotide sequences in the spacers separating the 26S and 18S rRNA genes in *T. speltoides* are the most closely related of all diploid *Triticum* species to those in the B genome (15). These and other data (for review see ref. 17) leave little doubt that the source of the B and G genomes of tetraploid wheats is the section Sitopsis, which includes *T. speltoides*, *T. longissimum* (Sweinf. et Muschl.), *T. searsii* nom. nud., *T. bicorne* Forssk., and *T. sharonense* nom. nud.

In an effort to elucidate the phylogeny of the wheat B and G genomes, we refined the technique reported by Dvořák *et al.* (8) so that the results can be numerically analyzed. The technique is used here to determine which species of Sitopsis has a nuclear genome most closely related to the B and G genomes of *T. turgidum*, *T. timopheevii*, and *T. aestivum*.

MATERIALS AND METHODS

Plants. Genomic DNAs isolated from single plants were used throughout this work. Taxonomical treatment of the genus *Triticum* by Bowden (18) as modified by Morris and Sears (19) is followed whenever possible. A single accession was used for *T. monococcum* ssp. *aegilopoides*, *T. urartu*, *T. tauschii* (Coss.) Schmalh. (DD genomes), *T. muticum* (Boiss.) Hackel, *T. comosum* (Sibth. et Smith) Richter, *T. uniaristatum* (Vis.) Richter, *T. umbellulatum* (Zhuk.) Bowden, and *T. caudatum* (L.) Godron et Gren. In Sitopsis, a number of accessions representing the geographic distribution of each species were used; investigated were 60 accessions of *T. speltoides*, 17 of *T. longissimum*, 18 of *T. searsii*, 18 of *T. bicorne*, and 10 of *T. sharonense*. Tetraploid wheat *T. turgidum* was represented by 21 accessions of wild ssp. *dicoccoides* (Korn ex Schweinf.) Löve and 7 cultivars of *T. turgidum* ssp. *durum* (Desf.) Löve from the U.S.S.R., India, Spain, Portugal, and Egypt. A single accession of cultivated *T. timopheevii* ssp. *timopheevii* (Zhuk.) Löve and 23 accessions of wild ssp. *armeniacum* (Jakubz.) Löve (syn. ssp. *araraticum* Jakubz.) were used. Four ssp. of the hexaploid cultivated wheat *T. aestivum* (genomes AABBDD) were investigated; these were represented by 12 accessions of ssp. *spelta* (L.) Thell., including 5 from Iran, 9 and 4 accessions of ssp. *macha* (Dekapr. et Menabde) Mac Key and ssp. *vavilovii* (Jakubz.) Löve, respectively, and 1 cultivar, 'Chinese Spring,' of bread wheat, *T. aestivum* L. ssp. *aestivum* Löve (genomes AABBDD).

The distribution of repeated nucleotide sequences among the B-genome chromosomes was investigated in disomic substitution lines in which each of the B-genome chromosomes of *T. aestivum* cv. Chinese Spring was individually replaced with the homoeologous chromosome pair of *Lophopyrum elongatum* (Host) Löve (20–22).

Cloning of Repeated Nucleotide Sequences. Genomic DNA was isolated from leaves of *T. urartu* and *T. speltoides* ssp.

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Abbreviations: DB, diagnostic band; DI, diagnostic hybridization intensity; RSI, repeated nucleotide sequence identity.

ligustica (8), purified on a CsCl gradient, digested with the restriction enzyme *Mbo* I, and ligated to *Bam*HI-digested pUC18. To select clones of repeated nucleotide sequences, plasmids (23) were immobilized on Zeta-Probe membrane by using a dot-blot apparatus (Bio-Rad) and hybridized with homologous genomic DNA labeled with ^{32}P by nick-translation. Clones from *T. speltoides* preferentially hybridizing with *T. speltoides* genomic DNA versus genomic DNA of *T. urartu* were selected as reported (8). The objective of selecting these clones was to eliminate confounding effects of the hybridization of the probe with the A-genome DNA.

Experimental Design and Statistical Analyses. *Step 1.* The genomic DNA of one accession of each diploid species of *Triticum* was digested with *Hae* III, *Mbo* I, *Taq* I, *Sst* I, or *Dde* I, electrophoretically fractionated in agarose gels, and blotted on Zeta-Probe membrane (Bio-Rad) according to the manufacturer's specifications. The blots were recurrently probed (24) with ^{32}P-labeled inserts of plasmids (listed in Table 1) and autoradiographed. The profiles of bands in the lanes were compared visually, and if a band was observed in only a single taxon it was recorded (for an example see Fig. 1). These bands were potentially diagnostic bands for the species. Overall hybridization in each lane was also visually evaluated for intensity, and if high in one species but undetectable or lower by about 1 order of magnitude in all other species it was also recorded.

Step 2. A number of accessions of each relevant taxon were investigated to ascertain whether the bands or hybridization intensities recorded in step 1 were diagnostic (for an example see Fig. 2). If a band occurred in all accessions of a taxon but was absent in all accessions of all other taxa, it was concluded to be a diagnostic band (DB). An analogous procedure (for an example see Fig. 3) was used to identify diagnostic hybridization intensities (DIs). Note that DI does not refer to the intensity of individual bands but to the hybridization intensity of all bands.

Step 3. The presence of each DB and DI identified in step 2 was now scrutinized in the polyploid species (for examples see Figs. 2 and 3). The DNAs of a number of accessions of each polyploid (Table 2) were digested with the restriction enzyme that generated the specific DB or DI at the diploid level, and the Southern blots were hybridized with the specific repeated sequence. The presence or absence of the DB or DI was determined by inspecting the profiles of bands of diploids and polyploids on the same Southern blot.

Step 4. The number of DBs plus DIs of a specific diploid that were observed in a polyploid was divided by the total number of DBs plus DIs of the diploid. The resulting fraction was an estimate of the repeated nucleotide sequence identity (RSI) between the diploid and polyploid.

Step 5. The RSIs involving a specific polyploid were ranked, and the significance of the difference between the highest and the second highest was tested by the normal-deviate test using formula 19.11 given by Steele and Torrie (25).

RESULTS

Seven of the 14 repeated sequence families (Table 1) revealed at least one DB (Figs. 1 and 2), and 1 family (Fig. 3) revealed a DI in the species of Sitopsis. Southern blots of DNAs of disomic substitution lines in which each chromosome of the *T. aestivum* B genome was individually replaced with the homoeologous chromosome of *L. elongatum* were hybridized with each of the seven repeated nucleotide sequences to determine chromosomal distribution of DBs and the DI. From the changes in the intensity of DBs in the DNAs of the disomic substitution lines, it was apparent that loci hybridizing with the rRNA probes, pTa250.15 and pTa794, were each in two chromosomes in the B genome. The insert of pTuUCD15 showed a slight reduction of hybridization with the DNAs of disomic substitution lines 1E(1B) and 3E(3B), indicating that chromosomes 1B and 3B carry major quantities of this sequence. No obvious change in the intensity of DBs in the DNAs of the substitution lines was observed with the remaining four sequences, pTsUCD2, pTsUCD4, pTsUCD5, and pAsKSU1. This indicated that these repeated sequences are on a number of B-genome chromosomes.

Table 1. Characteristics of the clones of repeated nucleotide sequences used for identification of DBs or DIs in Sitopsis

Clone*	Insert size, base pairs	Vector	Insertion site	Showed DB or DI in Sitopsis	Source
pTuUCD13	380	pUC18	*Bam*HI†	No	Authors
pTuUCD14	280	pUC18	*Bam*HI†	No	Authors
pTuUCD15	200	pUC18	*Bam*HI†	Yes	Authors
pTsUCD1	220	pUC18	*Bam*HI†	Yes	Authors
pTsUCD2	150	pUC18	*Bam*HI†	No	Authors
pTsUCD3	280	pUC18	*Bam*HI†	No	Authors
pTsUCD4	440	pUC18	*Bam*HI†	Yes	Authors
pTsUCD5	210	pUC18	*Bam*HI†	Yes	Authors
pTa250.15	900	pUC18	*Bam*HI	Yes	Ref. 26
pTa794	500	pBR322	*Bam*HI	Yes	Ref. 27
pAsKSU1	1300	pUC8	*Bam*HI	Yes	Ref. 28
pTbUCD1	290	pBSM13	*Bam*HI†	No	Ref. 8
pLeUCD5	160	pUC18	*Bam*HI†	No	Authors
pEleAcc2	600	pGEM1	*Eco*RI–*Acc* I	No	Ref. 29

*pTu, pTs, pTa, pAs, and pTb specify clones isolated from genomic DNAs of *T. urartu*, *T. speltoides*, *T. aestivum*, *T. tauschii*, and *T. monococcum*, respectively; pLe and pEle specify clones isolated from *Lophopyrum elongatum*.
†The *Bam*HI site was destroyed by the cloning procedure.

A total of 19 DBs and 1 DI were found: 18 DBs and 1 DI in *T. speltoides* and 1 DB in *T. searsii* (Table 2). Additionally, there were 6 DBs that differentiated *T. longissimum*, *T. sharonense*, *T. bicorne*, and *T. searsii* as a group from *T. speltoides*. This distribution of DBs in the section indicated a natural dichotomy in Sitopsis that divided it into *T. speltoides* on one hand and the other four species on the other hand. This dichotomy parallels the division of the section Sitopsis into subsections Truncata Eig, including *T. speltoides*, and Emarginata Eig, including the rest of the species. A single band was found (probe pTsUCD4, 0.47-kb *Taq* I band) that was present in *T. longissimum*, *T. sharonense*, and *T. searsii* but absent from *T. bicorne* and *T. speltoides*.

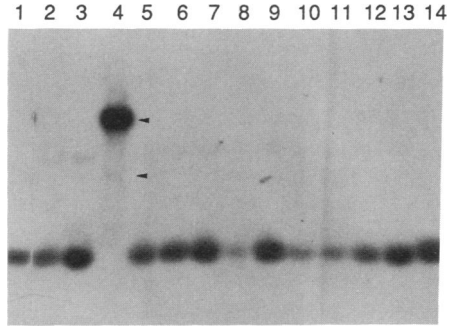

FIG. 1. Genomic DNAs (3 μg) of the following species were digested with restriction enzyme *Mbo* I, Southern blotted, and hybridized with the insert of pTuUCD15: *L. elongatum* (lane 1), *T. urartu* (lane 2), *T. monococcum* ssp. *aegilopoides* (lane 3), *T. speltoides* (lane 4), *T. sharonense* (lane 5), *T. longissimum* (lane 6), *T. bicorne* (lane 7), *T. searsii* (lane 8), *T. muticum* (lane 9), *T. caudatum* (lane 10), *T. comosum* (lane 11), *T. uniaristatum* (lane 12), *T. umbellulatum* (lane 13), and *T. tauschii* (lane 14). Note the 0.33- and 0.66-kilobase (kb) bands unique to *T. speltoides* (arrowheads).

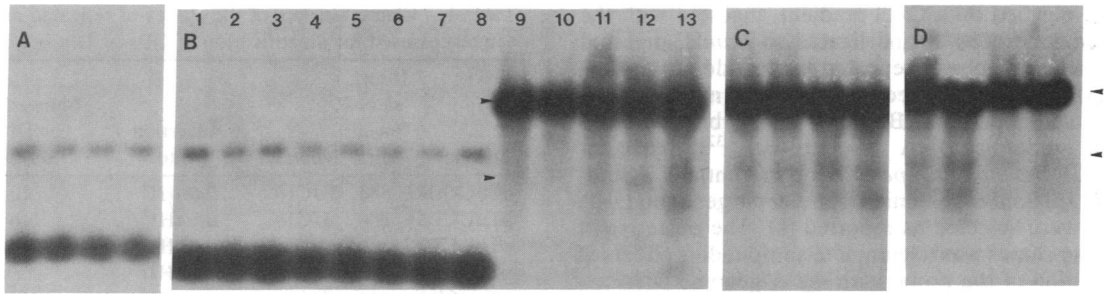

FIG. 2. Three, 6, and 9 μg of genomic DNAs of diploid, tetraploid, and hexaploid species, respectively, were digested with *Mbo* I, and Southern blots were hybridized with the insert of pTuUCD15. Note the absence of the *T. speltoides* diagnostic bands (arrowheads) in the *T. searsii* DNAs (*A*), *T. longissimum* DNAs (*B*, lanes 1–4), and *T. sharonense* DNAs (lanes 5–8) and their presence in *T. speltoides* DNAs (lanes 9–12), *T. aestivum* cv. Chinese Spring DNA (lane 13), *T. timopheevii* ssp. *armeniacum* DNAs (*C*), and *T. turgidum* ssp. *dicoccoides* DNAs (*D*).

The relationships between the diploids of Sitopsis and the polyploid wheats (for examples see Figs. 2–4) were investigated by calculating RSIs, considering each diploid individually or subsections as taxa (Table 3). When each diploid was considered individually, the highest relationship was found between *T. speltoides* and *T. timopheevii* including ssp. *armeniacum* (RSI of 0.95). A less close relationship was found between *T. speltoides* and *T. turgidum*, including ssp. *durum*, and *T. aestivum*, including ssp. *spelta*, *vavilovii*, *macha*, and *aestivum* (RSI of 0.84). Since no DB was found in *T. longissimum*, *T. sharonense*, and *T. bicorne*, RSIs could not be calculated for them individually because the denominator of the RSI fraction was zero. The only other diploid for which RSI could be calculated individually was *T. searsii*, which had RSI of 0.0 with all polyploid taxa.

When the relationships were investigated by using the 6 DBs found in the subsection Emarginata, the RSI of the Emarginata (excluding the 0.47-kb pTsUCD4 *Taq* I band that occurred only in *T. longissimum*, *T. sharonense*, and *T. searsii*) with *T. timopheevii*, including *T. timopheevii* ssp. *armeniacum*, was 0.17, which was lower ($P < 0.01$) than the RSI of 0.95 of *T. speltoides* with *T. timopheevii*. The RSIs of Emarginata with *T. turgidum* (including subspecies) and *T. aestivum* (including subspecies) were the same, 0.33, significantly lower ($P < 0.01$) than the 0.84 of *T. speltoides* with *T. turgidum* and *T. aestivum*. *T. speltoides* appeared more closely related to *T. timopheevii* than to *T. turgidum* and *T. aestivum*. Emarginata appeared more related to *T. turgidum* and *T. aestivum* than to *T. timopheevii*. The RSIs of 0.95 and 0.84 of *T. speltoides* with *T. timopheevii* and *T. turgidum*, respectively, and the difference in the RSIs of the subsection *Emarginata* with *T. timopheevii* and *T. turgidum* were not significantly different.

DISCUSSION

A total of 25 DBs and 1 DI were revealed in the species of the section Sitopsis by 14 repeated nucleotide sequence probes employed in the study. One category of the probes comprised the repeated nucleotide sequences isolated from *T. speltoides*. These clones were selected for preferential hybridization with the DNAs of the Sitopsis species relative to DNA of *T. urartu* with the objective of eliminating potentially confounding effects of bands from the wheat A genome (8). The other category consisted of clones of repeated nucleotide sequences of unknown or known function isolated from other species (*T. urartu*, *T. aestivum*, *T. tauschii*, and *L. elongatum*), and these were not selected here for preferential hybridization with the DNA of the Sitopsis species (for an example see Fig. 1). When the analyses were carried out in the stepwise fashion as described here, the unselected clones were as informative as those selected for preferential hybridization. By first identifying bands that were unique to a single diploid taxon, the confounding or obscuring effects of the other genomes of the polyploid wheats were minimized.

In the study of the phylogenetic relationships between diploid species and polyploid wheats, the RSIs were calculated as fractions of DBs plus DI relative to the total number identified in each diploid. This approach worked well for *T. speltoides* but did not work for *T. longissimum*, *T. sharonense*, and *T. bicorne* and worked poorly for *T. searsii*, since only a single DB was found. This problem was circumvented by treating the four species of the subsection Emarginata as

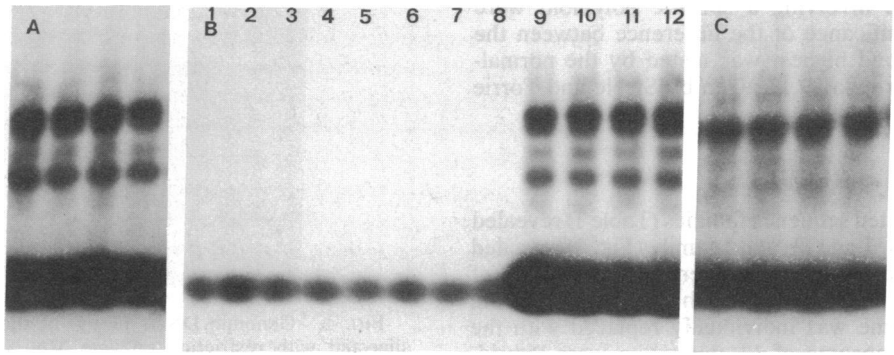

FIG. 3. Three micrograms of genomic DNAs of diploid species and 6 μg of tetraploid species were digested with *Mbo* I, and Southern blots were hybridized with the insert of pTsUCD5. *B* shows hybridization of the probe with DNAs of *T. bicorne* (lanes 1–4), *T. longissimum* (lanes 5–8), and *T. speltoides* (lanes 9–12). Note that the lowest band is much more intense in *T. speltoides*, *T. timopheevii* ssp. *armeniacum* (*A*), and *T. turgidum* ssp. *dicoccoides* (*C*) than in *T. bicorne* and *T. longissimum*, and that the other bands are missing entirely in the latter two species but are of similar intensity in *T. speltoides*, *T. timopheevii* ssp. *armeniacum*, and *T. turgidum* ssp. *dicoccoides*. Hybridization of the probe with *T. sharonense* and *T. searsii* (data not shown) was similar to that with *T. bicorne* and *T. longissimum*.

Table 2. DBs and one DI in diploid species of section Sitopsis and polyploid wheats

Probe	Enzyme	DI or DB, kb	*longissimum* (17)	*searsii* (18)	*sharonense* (10)	*bicorne* (18)	*speltoides* (60)	*dicoccoides* (21)	*durum* (7)	*timopheevii* (1)	*armeniacum* (23)	*aestivum* (1)	*spelta* (12)	*macha* (9)	*vavilovii* (4)
pTuUCD15	*Mbo* I	0.66	−	−	−	−	+	+	+	+	+	+	+	+	+
		0.33	−	−	−	−	+	+	+	+	+	+	+	+	+
pTsUCD2	*Dde* I	0.33	−	−	−	−	+	−	−	+	+	−	−	−	−
	Hae III	1.75	−	−	−	−	+	+	+	+	+	+	+	+	+
	Taq I	1.35	−	−	−	−	+	+	+	+	+	+	+	+	+
		1.12	−	−	−	−	+	+	+	+	+	+	+	+	+
		0.35	−	−	−	−	+	+	+	+	+	+	+	+	+
pTsUCD4	*Mbo* I	0.91*	+	+	+	+	−	−	−	−	−	−	−	−	−
		1.00	−	−	−	−	+	+	+	+	+	+	+	+	+
		1.02*	+	+	+	+	−	−	−	−	−	−	−	−	−
		0.52*	+	+	+	+	−	−	−	−	−	−	−	−	−
		0.75	−	−	−	−	+	+	+	+	+	+	+	+	+
	Taq I	1.27	−	−	−	−	+	+	+	+	+	+	+	+	+
		1.08	−	−	−	−	+	+	+	+	+	+	+	+	+
		1.25*	+	+	+	+	−	−	−	−	−	−	−	−	−
		0.46	−	−	−	−	+	+	+	+	+	+	+	+	+
		0.47	+	+	+	−	−	−	−	−	−	−	−	−	−
		0.88	−	+	−	−	−	−	−	−	−	−	−	−	−
pTsUCD5	*Mbo* I	0.37	−	−	−	−	+	+	+	+	+	+	+	+	+
		DI	−	−	−	−	+	+	+	+	+	+	+	+	+
	Hae III	0.48	−	−	−	−	+	−	−	−	−	−	−	−	−
		0.93	−	−	−	−	+	+	+	+	+	+	+	+	+
		0.43*	+	+	+	+	−	+	+	+	+	+	+	+	+
pAsKSU1	*Taq* I	0.65	−	−	−	−	+	+	+	+	+	+	+	+	+
pTa250.15	*Hae* III	0.50	−	−	−	−	+	−	−	+	+	−	−	−	−
pTa794	*Taq* I	0.50	−	−	−	−	+	+	+	+	+	+	+	+	+
	Mbo I	0.41*	+	+	+	+	−	+	+	−	−	+	+	+	+
Individual species DB + DI			0	1	0	0	19								
Subsections DB + DI					6		19								

Numbers of accessions analyzed are given in parentheses below the species names.
*Diagnostic for subsection Emarginata. Bands without an asterisk are diagnostic for only *T. speltoides*, only *T. searsii*, or all Emarginata except *bicorne*.

a single taxon. Another potential strategy to circumvent this problem could be to use the absence of diagnostic bands (− score in Table 2) in calculating the RSIs. However, such RSIs would be biased because their estimates would be affected by such factors as the number of diagnostic bands considered, their distribution among species, and the number of species investigated. Such a procedure would, consequently, lead to biased inferences and should be avoided.

When the four species of the subsection Emarginata were considered jointly, the analysis showed that *T. speltoides* is the closest extant Sitopsis species to both tetraploid wheat species. *T. speltoides* is closely related to *T. timopheevii* (including ssp. *armeniacum*), which showed 17 of the 18 *T. speltoides* DBs and the single DI. On the other hand, *T. timopheevii* showed only 1 of the 6 DBs for the subsection Emarginata and did not show the single DB of *T. searsii*. These data indicated that *T. speltoides* is the ancestor of the G genome of *T. timopheevii*. This agrees with the chloroplast DNA phylogenetic studies, which showed that the chloroplast genome of *T. timopheevii* is equivalent to that of *T. speltoides* (16). *T. speltoides* also appears to be the closest relative of the B genome of *T. turgidum*, which showed 15 of the 18 DBs of *T. speltoides* but only 1 of the 6 DBs of the subsection Emarginata and did not show the single DB of *T. searsii*. This also agrees with the chloroplast DNA phylogenetic studies, which showed that the *T. speltoides* chloroplast genome is closer to that of *T. turgidum* than to that of any other *Triticum* species (16).

All DBs and the DI of *T. speltoides* that were found in *T. turgidum* were also found in *T. aestivum*. This agrees with the fact that *T. turgidum* and *T. aestivum* have the same B genome; the latter species originated from hybridization of *T. turgidum* with *T. tauchii* (30, 31).

The nuclear and cytoplasmic data fully agree, and both suggest that the G genome of *T. timopheevii* was contributed by *T. speltoides* whereas the B genome of *T. turgidum* was contributed by a species in the evolutionary lineage of *T. speltoides*. One possibility is that the latter species is now extinct or not yet discovered (10). Another possibility is that the two tetraploid wheats originated from hybridization events involving the *T. speltoides* evolutionary lineage that were widely separated in time and, thus, recorded different stages of the evolutionary divergence of the *T. speltoides* lineage from the ancestral Sitopsis (32). Neither the nuclear nor the chloroplast DNA data provide evidence for the

Table 3. RSIs between the species of Sitopsis and polyploid wheats

	*T. timopheevii**		*T. turgidum* and *T. aestivum**	
	RSI	RSI†	RSI	RSI†
T. speltoides	0.95‡	0.95‡	0.84‡	0.84‡
T. longissimum	—	0.17	—	0.33
T. sharonense	—	0.17	—	0.33
T. bicorne	—	0.17	—	0.33
T. searsii	0.0	0.17	0.0	0.33

*There were no differences among subspecies of *T. timopheevii* or between *T. turgidum* and *T. aestivum*.
†RSIs of the subsections Truncata and Emarginata of Sitopsis.
‡RSI value significantly higher at the 0.01 probability level than the next highest RSI value in the column.

modification of the B-genome chromosomes by introgression (33). First, introgression cannot modify cytoplasm because it is uniparentally inherited in *Triticum* (34). If introgression from Sitopsis, or any other *Triticum* species, were responsible for the cytoplasmic differences associated with the wheat B and G genomes, the cytoplasm would have to match one of them. Since this is not the case (16) the cytoplasmic relationships testify to modification of the B genome by evolutionary divergence, not by introgression. Second, if the B genome were modified by extensive introgression from other species of Sitopsis into tetraploid wheats, bands that were diagnostic at the diploid level would appear amalgamated in the two polyploids. This was not found. The only type of introgression that the experimental data do not exclude (but do not provide evidence for, either) is a repeated introgression into tetraploid wheat from the *T. speltoides* lineage at different time points (32).

Two bands of diagnostic restriction fragments of the subsection Emarginata were found in the wheats and could be considered as evidence of introgression. However, it must be kept in mind that the diagnostic nature of restriction fragments or hybridization intensities was determined in modern populations of Sitopsis, but the polyploid wheats originated sometime in the past. The more ancient is their origin the more unlikely it is that the present-day DBs or DIs were diagnostic at the time of their origin, because the spread or elimination of specific repeated sequence variants by homogenization and recombination, making them diagnostic today, may have occurred after the origin of the wheats. From this reasoning, the *T. turgidum* lineage, which shows less similarity to *T. speltoides* and more similarly to the Emarginata subsection than does the *T. timopheevii* lineage, should be more ancient than the *T. timopheevii* lineage, as has been already argued on the basis of other evidence (9).

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