Independent Wheat B and G Genome Origins in Outcrossing *Aegilops* Progenitor Haplotypes

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The origin of modern wheats involved alloploidization among related genomes. To determine if *Aegilops speltoides* was the donor of the B and G genomes in AABB and AAGG tetraploids, we used a 3-tiered approach. Using 70 amplified fragment length polymorphism (AFLP) loci, we sampled molecular diversity among 480 wheat lines from their natural habitats encompassing all S genome *Aegilops*, the putative progenitors of wheat B and G genomes. Fifty-nine *Aegilops* representatives for S genome diversity were compared at 375 AFLP loci with diploid, tetraploid, and 11 nulli–tetrasomic *Triticum aestivum* wheat lines. B genome–specific markers allowed pinning the origin of the B genome to S chromosomes of *A. speltoides*, while excluding other lineages. The outbreeding nature of *A. speltoides* influences its molecular diversity and bears upon inferences of B and G genome origins. Haplotypes at nuclear and chloroplast loci *ACC1*, *G6PDH*, *GPT*, *PGK1*, *Q*, *VRN1*, and *ndhF* for ~70 *Aegilops* and *Triticum* lines (0.73 Mb sequenced) reveal both B and G genomes of polyploid wheats as unique samples of *A. speltoides* haplotype diversity. These have been sequestered by the AABB *Triticum dicoccoides* and AAGG *Triticum araraticum* lineages during their independent origins.

Introduction

Bread wheat, *Triticum aestivum*, has no direct hexaploid wild progenitor (Morris and Sears 1967; Kimber and Feldman 1987). The species possesses 3 sets of homologous chromosomes, designated as AABBDD, whose origins have differing degrees of certainty. The D chromosomes stem from the wild diploid *Aegilops tauschii* (Kihara 1944) through alloploidization with the wild AABB tetraploid *Triticum dicoccoides*. The A and B chromosomes of that tetraploid derive from an earlier hybridization between the wild AA diploid *Triticum urartu* (Dvorak et al. 1993) and a wild diploid B genome donor: the ultimate source of this B genome is still discussed. A related conundrum is the origin of AAGG *Triticum araraticum*, whose A genome also stems from *T. urartu*, whereby the wild G progenitor is frequently reported to be *Aegilops speltoides* (Rodriguez, Maestra et al. 2000). The B donor is traditionally sought in the *Sitopsis* section of the genus *Aegilops* (Sarkar and Stebbins 1956; Kerby and Kuspira 1988). Previous molecular analyses of single-gene loci for a few accessions are not inconsistent with the view that both the B genome of *T. dicoccoides* (AABB) and the G genome of *T. araraticum* (AAGG) might trace to the *Sitopsis* section, in genetic proximity to wild *A. speltoides* (Blake et al. 1999; Rodriguez, Maestra et al. 2000; Zhang et al. 2002). However, there are caveats.

First, nuclear and cytoplasmically inherited markers yield contrasting results on the issue of B genome origin (discussed in Wang et al. 1997). In addition, ancient allelic diversity among wild ancestors, compounded by the possibility of unrecognized hybridization events, renders inferences of the B progenitor questionable (Huang et al. 2002) in the absence of genome-wide surveys for many loci and accessions. Furthermore, the outcrossing nature of *A. speltoides* (Kimber and Feldman 1987) renders introgression for individual loci difficult to exclude in the absence of extensive lineage sampling. Importantly, cytogenetic evidence does not support the view that *A. speltoides* was the donor of B or G genome, even though such suggestions can be found (Maestra and Naranjo 1998). When synthetic SSAA genomes (S contributed by *A. speltoides* and A by *Triticum*) are crossed to *Triticum durum* (AABB, the domesticated form of *T. dicoccoides*), sterility is observed, pointing to differences between S and B genomes; the same is reported for S and G genomes (Dvorak 1972; Kimber and Athwal 1972). Moreover, B–S pairing in wheat/*A. speltoides* hybrids is comparable to that noted for wheat/*Aegilops longissima* and wheat/*Aegilops sharonensis* hybrids (Fernandez-Calvin and Orellana 1994), suggesting that B chromosomes of polyploid wheats do not pair preferentially to those of *A. speltoides*.

Understanding hexaploid wheat origin would further its genetic improvement (Salamini et al. 2002; Chantret et al. 2005). Here, we report a comprehensive amplified fragment length polymorphism (AFLP) survey of genomic diversity among 1372 individuals from 480 wild B genome progenitor candidates. Through the analysis of Sears's (1954) nulli–tetrasomic (AADDDD) lines, B genome–specific AFLPs were identified. For ~70 domesticated and progenitor lines representing the breadth of wild genomic diversity, haplotypes at nuclear loci *ACC1*, *G6PDH*, *GPT*, *PGK1*, *Q*, *VRN1* and of the chloroplast locus *ndhF* were determined. Comparisons to haplotypes from AA *Triticum boeoticum*, *Triticum monococcum*, and *T. urartu* identified haplotypes specific to the B genome to allow comparison to *Sitopsis* accessions. The data circumscribe molecular diversity among *Sitopsis Aegilops* species and specify the nature of wheat B and G genome origins.

Methods

AFLP Analysis

The 480 *Aegilops* lines used in this study are listed in Supplementary table S1 (Supplementary Material online).

Key words: molecular evolution, *Triticum*, *Aegilops*, hybridization, alloploidization, AFLPs.

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DNA was isolated from freeze-dried or silica-dried leaves of 1372 plants (Supplementary table S2, Supplementary Material online), using the Qiagen (Hilden) DNeasy Kit, and amplified as described by Zabeau and Vos (1993) using the primer combinations E^{ACC}/M^{ACA}, E^{ACC}/M^{AGC}, and E^{ACC}/M^{AGG}. The AFLP bands were scored as 1 or 0 (present or absent). Jaccard (1908) similarities of the 850 individuals with different AFLP patterns (Supplementary table S3, Supplementary Material online) were computed using DistAFLP (Mougel et al. 2002), and Neighbor-Joining (NJ) bootstrap trees were inferred with PHYLIP 3.6 (Felsenstein 2002). DNA from 94 selected *Aegilops*, *T. boeoticum*, *T. urartu*, *T. dicoccoides*, *T. araraticum*, and *T. aestivum* accessions (Supplementary table S4, Supplementary Material online), along with *T. aestivum* Chinese Spring aneuploids: 6 nulliB–tetraD (N1BT1D, N2BT2D, N3BT3D, N4BT4D, N5BT5D, N6BT6D) and 5 nulliB–tetraA (N1BT1A, N2BT2A, N3BT3A, N5BT5A, N7BT7A) (Sears 1954), was amplified using primer combinations E^{ACC}/M^{AGC}, E^{ACC}/M^{AGG}, E^{ACG}/M^{ACC}, E^{ACG}/M^{ACT}, E^{ACG}/M^{AGG}, E^{ACG}/M^{AGT}, E^{AGC}/M^{AGC}, and E^{AGC}/M^{ATA}. NeighborNet (NNet) planar graphs (Bryant and Moulton 2004) of AFLP Hamming distances between individuals were constructed with SplitsTree 4.1 (Huson and Bryant 2006).

Haplotype Analysis

Genes and accessions considered for haplotype analysis are recorded in Supplementary tables S5–S7 (Supplementary Material online). Sixty-seven lines were common to all loci—*T. dicoccoides* (34), *T. dicoccum* (5), *T. durum* (1), *T. araraticum* (5), *Triticum timopheevii* (6), *Aegilops bicornis* (2), *A. longissima* (2), *Aegilops searsii* (2), *A. sharonensis* (2), *A. speltoides* (7), and *A. tauschii* (1). Other sequences from additional lines of those same species, from *T. boeoticum*, *T. monococcum*, *T. urartu*, *T. araraticum*, and *T. timopheevii* (table 2), as well as available published sequences were included. DNA was isolated as described above. Primers (Supplementary table S8, Supplementary Material online) were designed with Primer3 against sequences for *ACC1* (Huang et al. 2002), *G6PDH* (Nemoto et al. 1999), *GPT* (GenBank AF548741), *PGK1* (Huang et al. 2002), *Q* (Faris et al. 2003), *VRN1* (Sherman et al. 2004; Yan et al. 2004), and *ndhF* (Ogihara et al. 2002). Some accessions of *A. speltoides* have 2 copies each of the genes *ACC1* and *PGK1* (Huang et al. 2002); primers for these 2 genes were used allowing the amplification of the same gene in all *A. speltoides*. DNA amplifications were performed in 25 μl containing ~100 ng of leaf DNA, 0.4 μM of each primer, 125 μM of each deoxynucleoside triphosphate (AB gene, Surrey, UK), 3 mM $MgCl_2$, 4% dimethyl sulfoxide, and 1 unit of *Taq* DNA polymerase incubated in a PTC-225 Tetrad Thermal Cycler (MJ Research) as follows: 94 °C for 3 min, 28–33 cycles of 30 s at 94 °C, 20–40 s at 59–65 °C, 50–95 s at 72 °C, and a final extension step of 6 min at 72 °C. Polymerase chain reaction products were sequenced on both strands (Supplementary table S9, Supplementary Material online). Sequences (Supplementary table S10, Supplementary Material online) were processed with Applied Biosystems DNA Sequencing Analysis Software 5.1.1 and manually inspected with BioEdit version 7.0.1 (Hall 1999). The alignments were generated with ClustalW, and the haplotypes were scored manually and with DnaSP (Rozas et al. 2003). For homozygous loci, only one haplotype per line was included in the alignment, both haplotypes for heterozygous loci. Median-joining (MJ) networks (Bandelt et al. 1999) were constructed with the Network 4.1.1.2 program (Fluxus Technology Ltd, Clare, Suffolk, UK). Total number of substitutions per site between populations, D_a (equation 10.21; Nei 1987), using the Jukes–Cantor method was calculated with DnaSP (Rozas et al. 2003).

To obtain A, B, and G genome–specific haplotype sequences for polyploid wheats, three approaches were used. When B genome–specific sequence differences were available, 1) primer combinations were designed and used for haplotype-specific amplification and sequencing; 2) amplification products from A, B, and G genomes were obtained with the A genome primers, but sequenced using genome-specific primers; and 3) in the remaining cases, amplification products for A, B, and G genomes were obtained using nondiscriminating A genome primers, cloned in *Escherichia coli*, and at least 3 sequences per clone obtained, until both haplotypes were identified by comparison to existing A and B genome data for the locus.

Results

Genomic Diversity within the *Sitopsis* Section of *Aegilops*

To survey the molecular diversity among candidate B genome donors, we studied 2–3 plants each from a total of 501 accessions spanning all 5 *Sitopsis Aegilops* species—*A. searsii*, *A. bicornis*, *A. sharonensis*, *A. longissima*, and *A. speltoides*—collected along the Eastern Mediterranean (fig. 1). (We follow Dorofeev et al. [1979] for *Triticum* binomial nomenclature and Van Slageren [1994] for *Aegilops*). Accessions were grown in 2003 and morphologically reidentified, whereby 21 misassigned lines or interspecific hybrids were discarded. DNA was collected from 1372 plants: *A. bicornis* (39 accessions, 105 individuals), *A. longissima* (81, 227), *A. searsii* (97, 285), *A. sharonensis* (112, 327), *A. speltoides* (149, 422) and from the D genome outgroup species *A. tauschii* (2, 6).

A screen using 3 AFLP primer combinations uncovered a total of 70 polymorphic bands across all plants. This revealed 850 individuals with different AFLP patterns: *A. bicornis* (36 accessions, 44 individuals), *A. longissima* (80, 165), *A. searsii* (54, 77), *A. sharonensis* (101, 176), *A. speltoides* (147, 386), and *A. tauschii* (2, 2). The NJ tree of Jaccard (1908) distances is shown in figure 2 and provides an overview of *Sitopsis* genome diversity. The primary screen revealed the breadth of divergence within each species, helping to choose accessions for subsequent fine-scale analyses. In addition, two notable results emerged. First, in all intraspecific pairwise comparisons, AFLP-based genetic similarity was lower in the outbreeder *A. speltoides* than in any of the other members of the section which are inbreeders (Kimber and Feldman 1987) (fig. 2). Second, figure 1 shows that the *Sitopsis* section (with one exception, a plant of *A. bicornis* mapping outside but near the major cluster of the species) does not include other genetically distinct major groups, besides those represented by *A.*

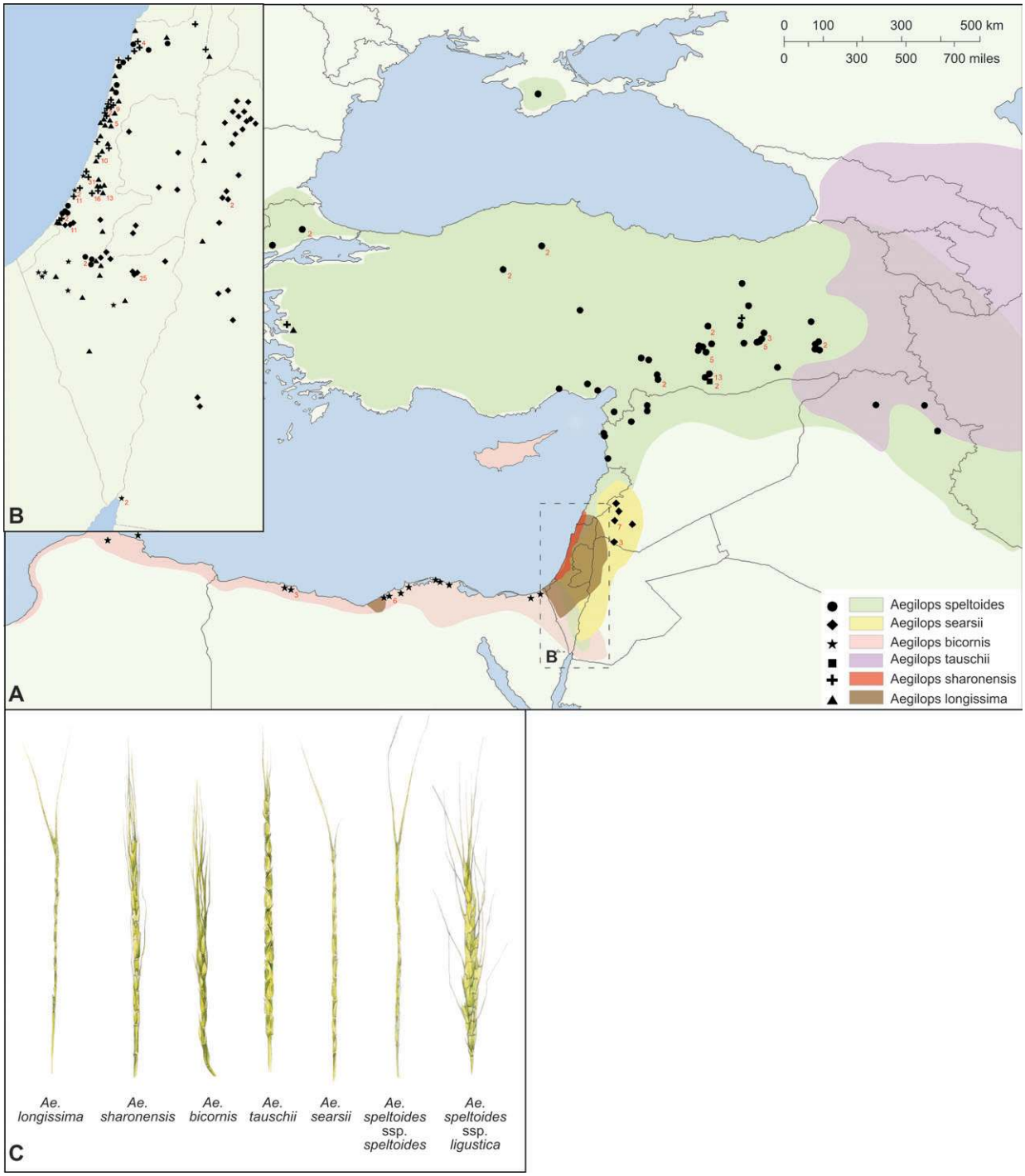


FIG. 1.—Natural distribution of the different *Aegilops* section *Sitopsis* species and *Aegilops tauschii*. (*A*) Distribution in the Eastern Mediterranean region and the Near East (Hall 1999) are indicated by colors, 387 collection sites are drawn with symbols. (*B*) Enlarged Israel region showing 253 collection sites. Numbers in red below the symbol indicate how many accessions were collected from the same site. Among the 480 accessions were analyzed: *Aegilops bicornis* (39 accessions), *Aegilops longissima* (81), *Aegilops searsii* (97), *Aegilops sharonensis* (112), *Aegilops speltoides* (149), and *A. tauschii* (2). Accessions sorted by country: China (1), Egypt (18), Iraq (4), Israel (253), Jordan (37), Libya (2), Portugal (2), Syria (29), Turkey (70), and Ukraine (1), 63 analyzed accessions of unknown origin are not shown in the figure. *Aegilops* species sorted by country: China (1 SPE), Egypt (18 BIC), Iraq (4 SPE), Israel (9 BIC, 69 LOG, 52 SEA, 104 SHA, 20 SPE), Jordan (4 BIC, 5 LOG, 28 SEA), Libya (2 BIC); Portugal (2 SPE), Syria (16 SEA, 13 SPE), Turkey (1 LOG, 2 SHA, 64 SPE, 2 TAU), Ukraine (1 SPE), and unknown (6 BIC, 6 LOG, 1 SEA, 6 SHA, 44 SPE). Country known, but exact collection site unknown within the country 27 (Israel: 3 LOG, 3 SHA, 1 SPE; Jordan: 2 BIC, 2 LOG, 6 SEA; Syria: 2 SEA, 7 SPE; Turkey: 1 SPE). (*C*) Spike morphologies for *Aegilops* species relevant to this study.

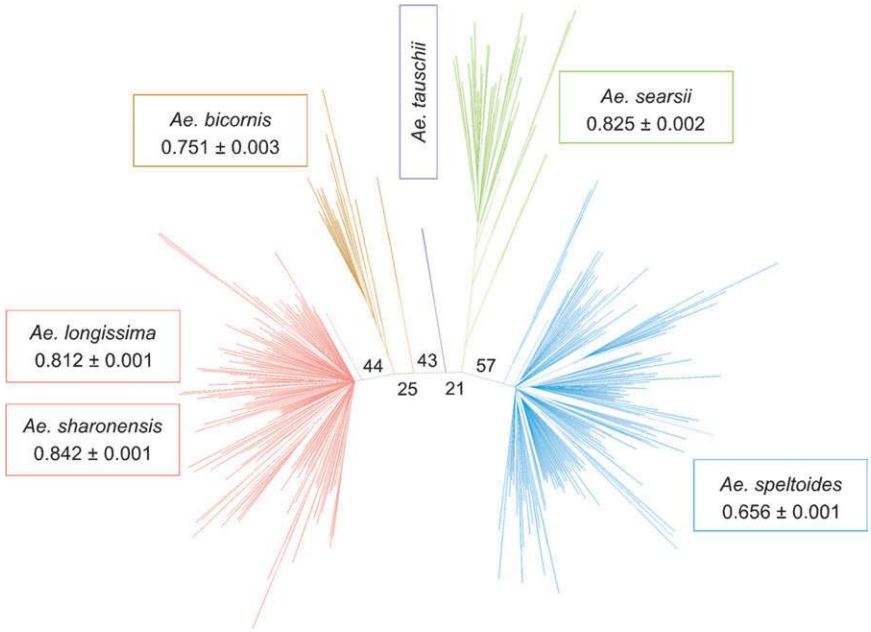


FIG. 2.—Unrooted NJ tree of Jaccard (1908) distances based on AFLP markers describing genetic relationships among 850 individuals of the genus *Aegilops,* section *Sitopsis*, and *Aegilops tauschii*. The 850 genotypes—*Aegilops bicornis* (36 accessions, 44 individuals), *Aegilops longissima* (80, 165), *Aegilops searsii* (54, 77), *Aegilops sharonensis* (101, 176), *Aegilops speltoides* (147, 386), and *A. tauschii* (2, 2)—were selected as unique pattern out of a total of 1372 one-plant samples. A total of 70 AFLP polymorphic loci were generated with primer combinations E36M35, E36M40, and E36M41. Bootstrap proportions for the main internal edges are shown. Numbers within boxes report the average intraspecific value of Jaccard (1908) genetic similarity (±standard error).

bicornis, *A. searsii*, *A. speltoides*, and the *A. sharonensis*–*A. longissima* cluster (the two last species, however, mapped to some degree separately when using more AFLP primer combinations, like in fig. 3*A* and *B*). It is concluded that the *Sitopsis* section does not contain cryptic species molecularly distinct from those currently recognized (Kimber and Feldman 1987), such that our sample appears to span the full breadth and depth of molecular diversity within the section. The inability to distinguish *A. sharonensis* from *A. longissima* individuals in the coarse screen is irrelevant here, as are the low bootstrap proportions for branches separating species at these 70 loci.

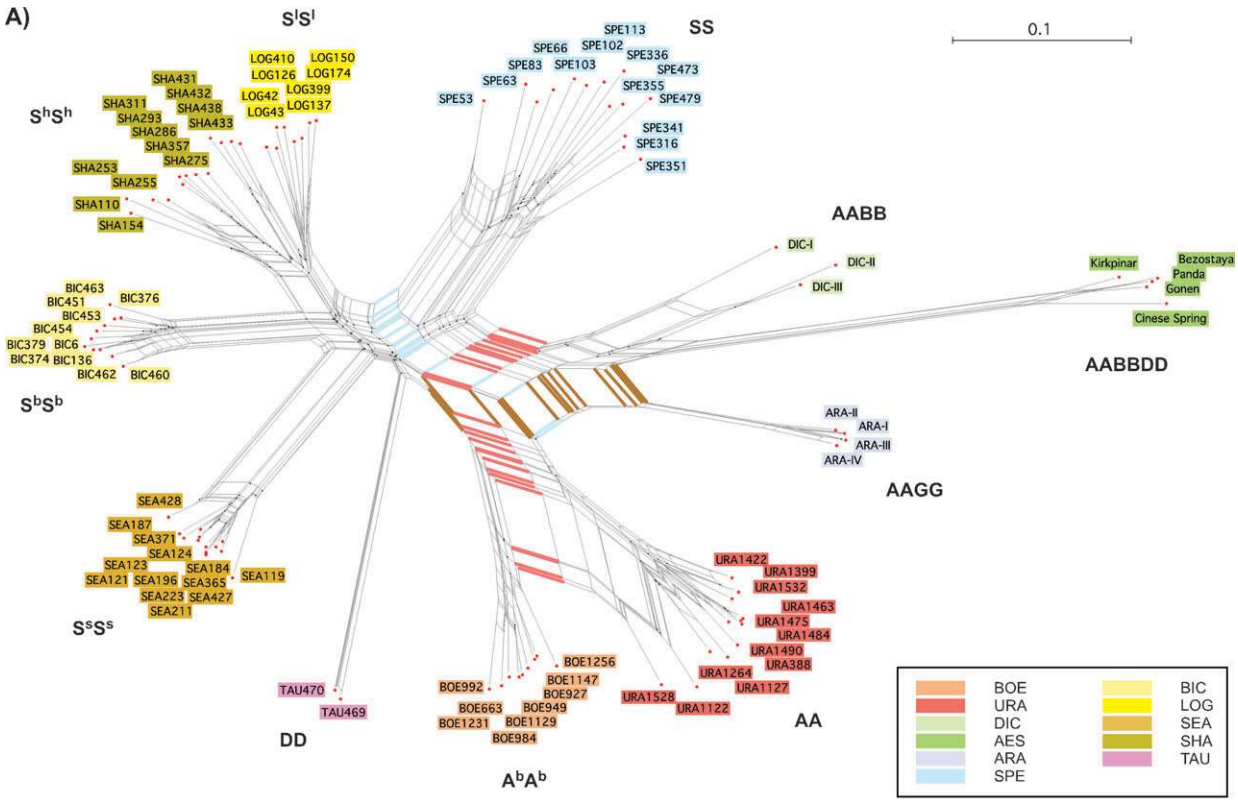
Higher Resolution among Wild and Domesticated Genomes

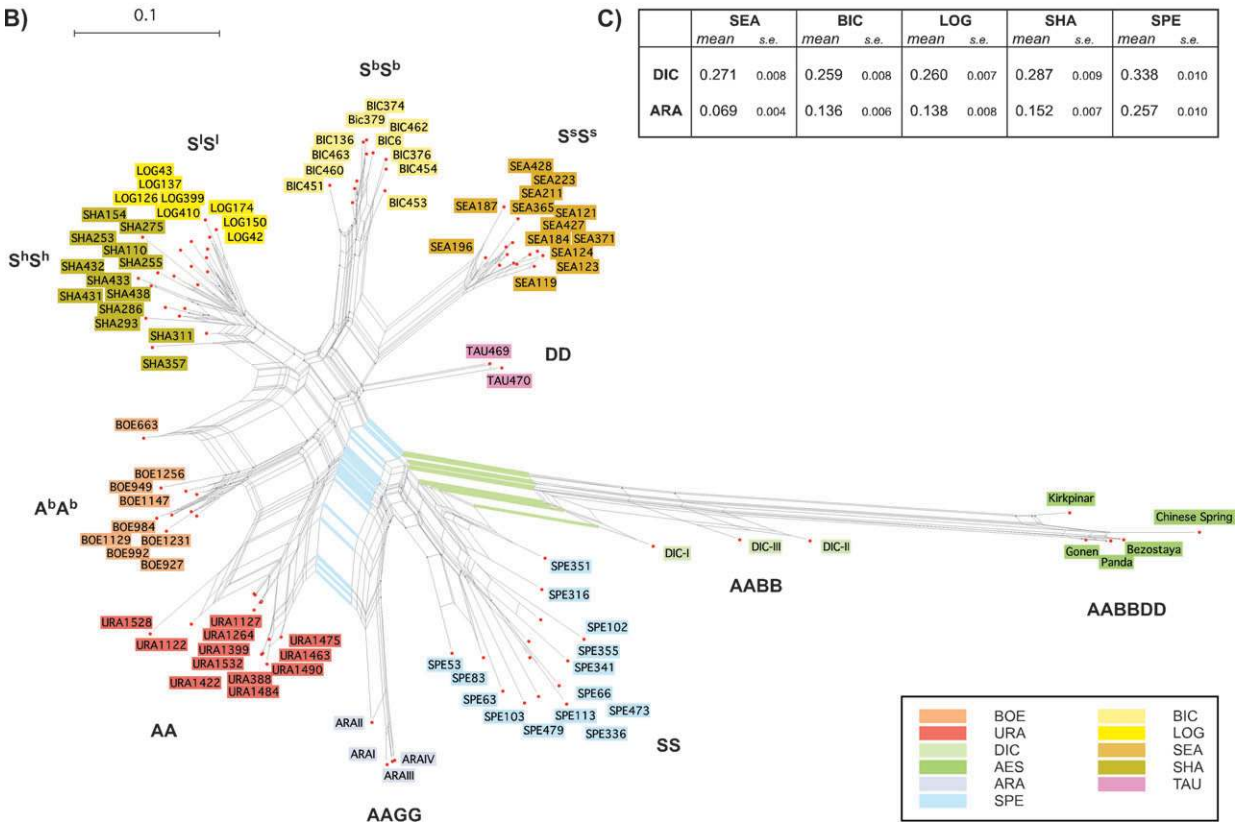
A reduced set of 59 *Aegilops* plants representative for molecular diversity within the section was considered for further AFLP studies, carried out with a higher number of primer combinations. The selected plants were *A. bicornis* (11 individuals), *A. longissima* (8), *A. searsii* (13), *A. sharonensis* (13), and *A. speltoides* (14). Selection of plants within species was carried out, maximizing the average genetic distances among selected plants. Two plants from the *A. tauschii* (D genome) outgroup were included, as were 5 *T. aestivum* cultivars (AABBDD), 3 wild *T. dicoccoides* (AABB), 4 wild *T. araraticum* (AAGG, the progenitor of the domesticated *T. timopheevii*), 9 wild *T. boeoticum* (A^bA^b, the progenitor of the domesticated *T. monococcum*, A^mA^m), and 12 wild *T. urartu* (AA). The choice of the 9 and 12 accessions, respectively, of *T. boeoticum* and *T. urartu* considered the criterion specified above, based on published and unpublished molecular data of the authors. Other *Triticum* accessions were chosen as representative of molecular diversity among the species considered in experiments previously published by the authors (references in Salamini et al. 2002).

Eleven AFLP primer combinations amplified 375 polymorphic bands across these 94 lines, from which NNets were constructed (fig. 3*A*). NNet splits graphs can be interpreted like trees in that they contain splits (branches) with weights (lengths). Parallel lines identify the same split or

→

FIG. 3.—NNets of Hamming distances for AFLP polymorphisms among *Sitopsis* section, *Aegilops* species, and *Aegilops tauschii* (outgroup) with polyploidy wheats. (*A*) NNet for 375 polymorphic loci (11 AFLP primer combinations) and 94 lines. AES, *Triticum aestivum*; ARA, *Triticum araraticum*; BIC, *Aegilops bicornis*; BOE, *Triticum boeoticum*; DIC, *Triticum dicoccoides*; LOG, *Aegilops longissima*; SEA, *Aegilops searsii*; SHA, *Aegilops sharonensis*; SPE, *Aegilops speltoides*; TAU, *A. tauschii*; and URA, *Triticum urartu*. The number of individuals considered was 5 (AES), 4 (ARA), 11 (BIC), 9 (BOE), 3 (DIC), 8 (LOG), 13 (SEA), 13 (SHA), 14 (SPE), 2 (TAU), and 12 (URA). Genome assignments are shown. Relevant splits are highlighted (see text). (*B*) NNets based on 65 AFLP polymorphic loci (11 primer combinations) assigned specifically to B chromosomes of *T. aestivum* using Chinese Spring nulli–tetrasomic lines for the same 94 lines. Other details as in (*A*). (*C*) Jaccard (1908) genetic similarity index (average proportion of identical B genome–specific AFLP bands) among *T. dicoccoides* (DIC)/*T. araraticum* (ARA) lines and *Aegilops* species based on the 65 AFLP B bands in (*B*).





C)

	SEA		BIC		LOG		SHA		SPE	
	mean	*s.e.*	*mean*	*s.e.*	*mean*	*s.e.*	*mean*	*s.e.*	*mean*	*s.e.*
DIC	0.271	0.008	0.259	0.008	0.260	0.007	0.287	0.009	0.338	0.010
ARA	0.069	0.004	0.136	0.006	0.138	0.008	0.152	0.007	0.257	0.010

branch. Boxes indicate support for 2 competing patterns of taxon relationship. NNet splits graphs highlight the predominant phylogenetic signals in the data and the extent to which these signals may or may not be tree-like (Huson and Bryant 2006). In cases of reticulate evolutionary history, hybrid taxa are suggested by the occurrence of incompatible splits (which appear as boxes), often with hybrid taxa being linked by splits to their potential parents. NNet split graphs only display the contradictory splits that can be visualized in a single plane and should not be considered an explicit model of reticulate evolutionary history. Nevertheless, they provide an implicit representation of evolutionary history (Huson and Bryant 2006) and one that is useful for identifying and exploring different signals and their meaning. For allopolyploid species, NNet has an advantage for data visualization over tree-building methods, which assume that the data have evolved on a single bifurcating tree. At this level of genome-wide comparison, the only *Sitopsis* member that shared a split with the AABB, AAGG, and AABBDD polyploids was *A. speltoides*. That split reflects a higher proportion of shared AFLP bands between polyploid wheats and *A. speltoides* as compared with other SS genomes. A second split divides *T. urartu* (AA) from all diploids sampled but excludes *T. boeticum* (A^bA^b), and indeed *T. urartu* is the A genome donor (Dvorak et al. 1993). No split links *A. tauschii* (DD) to the hexaploid AABBDD genome. However, this might be expected because the NNet method can only represent incompatible splits projected onto 2 dimensions (Huson and Bryant 2006). With the D genome absent in the 7 tetraploids sampled, signals from A, B, G, and S genomes will override the weaker signal linking AABBDD and DD genomes. A strong split linking the AA diploids with *T. araraticum* (AAGG) to the exclusion of *T. dicoccoides* (AABB) is also observed, indicating that the AABB and AAGG genomes, both involving *T. urartu*, are the result of independent polyploidization events.

The B Genome

Six nulliB–tetraD and 5 nulliB–tetraA lines of the hexaploid cultivar Chinese Spring (Sears 1954) were included in the AFLP analysis. They identified 65 bands that reside specifically on the B genome. If the split that links *A. speltoides* to polyploids is a historical component of genome similarity, it should become more prominent in the NNet based on the 65 B genome–specific bands. This is observed in figure 3*B*, where the split linking *A. speltoides* to polyploid wheats is highlighted in blue. These B bands were selected by virtue of their occurrence in hexaploid wheat, not by virtue of their character state among tetraploids or diploids. Hence, they do not skew the locus sample systematically toward any potential B genome donor. They represent B genome–specific signals with regard to polyploid origins; competing A- and D-specific signals are diminished, but not abolished, because A, B, G, and D genomes are still related at these loci.

Figure 3*B* reveals that the *A. speltoides* genome is most similar to the B and G genomes of polyploid wheats. And because we have extensively sampled genome diversity across the *Sitopsis* (fig. 2), this indicates that the *A. speltoides* S genome is the extant version of B and G genomes of polyploid wheats. Identical B genome–specific AFLP bands shared between tetraploid wheats and the species of the *Sitopsis* section using the Jaccard (1908) similarity (fig. 3*C*) further support that conclusion.

The NNet shows a strong split linking hexaploid wheat with *T. dicoccoides* to the exclusion of *T. araraticum*, highlighted in green in figure 3*B*. This corresponds to the well-known participation of *T. dicoccoides* in bread wheat origin (Dvorak et al. 1993). Evidence for additional hybridization events was uncovered, namely the strong component of similarity linking few *T. urartu* (AA) accessions to the *T. boeticum* complex (A^bA^b), probably reflecting interspecific crosses. However, even in the B genome–specific data, no *A. speltoides* genome sampled was specifically more similar to all polyploids sampled. Nonetheless, if the B and G genomes stem from within *A. speltoides*, then genome-specific haplotypes from polyploids should provide more detailed evidence for that origin.

Congruent Evidence from Haplotypes

Haplotypes recognized in DNA fragments for the nuclear genes *ACC1*, *G6PDH*, *GPT*, *PGK1*, *Q*, and *VRN1* and in a 719-bp region of the chloroplast gene *ndhF* were determined for tetraploids, *Sitopsis* members, and AA diploids. In total, 0.73 Mb of sequence data were obtained and combined with 80 000 bp from previous studies (Supplementary tables S5–S7, Supplementary Material online) for analysis. At all nuclear loci investigated, the number of net nucleotide substitutions per site between populations (Nei 1987), D_a, revealed that *T. dicoccoides* B genome haplotypes were always more similar to those in *A. speltoides* than those in any other species. The same was true for comparisons of the *T. araraticum* G genome haplotypes (table 1), providing additional evidence for an origin of both B and G genomes from a *A. speltoides* donor. The same was evident for the cytoplasmically inherited *ndhF* gene (table 1).

MJ networks for these loci (fig. 4) reveal higher levels of haplotype diversity within the outbreeder *A. speltoides* than in other wheats. Furthermore, B and G genome haplotypes of the tetraploids were consistently more closely related to *A. speltoides* than to other sources. At *G6PDH*, 8 *A. speltoides* haplotypes were observed: SPE-I is the closest relative of B and G haplotypes, which are monomorphic for *T. dicoccoides* and dimorphic for *T. araraticum*, whereas other *Sitopsis* or A genome haplotypes are distinct by $\geq$20 substitutions. At *ACC1*, *A. speltoides* revealed 7 haplotypes: SPE-I and -II are identical to those found in G genome, SPE-III is the closest relative of the major *T. dicoccoides* B haplotype; no *ACC1* haplotypes are shared between *A. speltoides* and remaining *Sitopsis* species. A genome haplotypes at *ACC1* were much less diverse than B genome homologues. At *GPT*, only one *A. speltoides* haplotype was observed, which is identical to the major *T. araraticum* haplotype and shows only 2 nucleotide differences to the main *T. dicoccoides* B haplotype; other *Sitopsis* or A genome haplotypes were clearly distinct. *Q* was by far the most polymorphic locus sampled: the closest progenitor to the main *T. dicoccoides* B genome haplotype was SPE-I, different by 7 nucleotides to the *T. araraticum* haplotype

Table 1
Sequence divergence between tetraploid wheat and *Sitopsis* section haplotypes (boldface: divergence between S and B or G genomes)

Gene (L)[b]	Genome (n)	$D_a \pm$ SE $\times 10^3$ (n)[a] in comparison with				
		Aegilops bicornis	*Aegilops longissima*	*Aegilops searsii*	*Aegilops sharonensis*	*Aegilops speltoides*
ACC1 (366)	dicA[c] (37)	14 ± 2 (3)	12 ± 2 (5)	17 ± 2 (6)	14 ± 2 (4)	5 ± 1 (23)
	dicB[d] (37)	14 ± 2 (3)	12 ± 2 (5)	17 ± 2 (6)	14 ± 3 (4)	**5** ± 1 (23)
	araA[e] (5)	14 ± 5 (3)	12 ± 4 (5)	17 ± 4 (6)	14 ± 5 (4)	5 ± 1 (23)
	araG[f] (6)	10 ± 5 (3)	8 ± 5 (5)	13 ± 5 (6)	10 ± 5 (4)	**1** ± 2 (23)
G6PDH (537)	dicA (34)	54 ± 8 (2)	53 ± 8 (2)	51 ± 8 (2)	53 ± 8 (2)	43 ± 4 (15)
	dicB (34)	52 ± 8 (2)	52 ± 8 (2)	50 ± 8 (2)	52 ± 8 (2)	**12** ± 3 (15)
	araA (5)	52 ± 19 (2)	52 ± 19 (2)	50 ± 18 (2)	52 ± 19 (2)	39 ± 8 (15)
	araG (6)	48 ± 18 (2)	48 ± 18 (2)	46 ± 17 (2)	48 ± 18 (2)	**10** ± 4 (15)
GPT (673)	dicA (34)	9 ± 1 (2)	14 ± 2 (2)	9 ± 1 (2)	14 ± 2 (2)	21 ± 2 (7)
	dicB (34)	15 ± 2 (2)	17 ± 3 (2)	15 ± 2 (2)	17 ± 3 (2)	**3** ± 0.3 (7)
	araA (5)	9 ± 3 (2)	13 ± 5 (2)	9 ± 3 (2)	14 ± 5 (2)	20 ± 4 (7)
	araG (5)	15 ± 6 (2)	17 ± 6 (2)	15 ± 6 (2)	17 ± 6 (2)	**0** ± 0.4 (7)
PGK1 (665)	dicA (35)	24 ± 5 (2)	21 ± 3 (2)	26 ± 4 (2)	21 ± 3 (2)	12 ± 2 (16)
	dicB (35)	24 ± 5 (2)	19 ± 3 (2)	24 ± 4 (2)	21 ± 3 (2)	**15** ± 4 (16)
	araA (5)	24 ± 10 (2)	21 ± 7 (2)	26 ± 10 (2)	21 ± 7 (2)	12 ± 4 (16)
	araG (5)	19 ± 9 (2)	16 ± 6 (2)	19 ± 7 (2)	16 ± 6 (2)	**15** ± 5 (16)
Q (917)	dicA (36)	111 ± 16 (2)	81 ± 14 (3)	100 ± 15 (2)	94 ± 16 (3)	68 ± 6 (13)
	dicB (36)	77 ± 11 (2)	48 ± 11 (3)	85 ± 12 (2)	55 ± 13 (3)	**20** ± 3 (13)
	araA (5)	113 ± 41 (2)	83 ± 32 (3)	102 ± 37 (2)	96 ± 35 (3)	70 ± 14 (13)
	araG (5)	65 ± 24 (2)	35 ± 18 (3)	75 ± 28 (2)	43 ± 20 (3)	**12** ± 5 (13)
VRN1 (304)	dicA (34)	53 ± 8 (2)	53 ± 8 (2)	56 ± 8 (2)	53 ± 8 (2)	58 ± 5 (11)
	dicB (34)	28 ± 4 (2)	28 ± 4 (2)	35 ± 5 (2)	28 ± 4 (2)	**26** ± 3 (11)
	araA (5)	53 ± 19 (2)	54 ± 19 (2)	57 ± 21 (2)	53 ± 19 (2)	58 ± 11 (11)
	araG (5)	20 ± 7 (2)	21 ± 7 (2)	28 ± 10 (2)	20 ± 7 (2)	**19** ± 5 (11)
ndhF (719)	dic-cp[g] (34)	6 ± 1 (2)	6 ± 1 (2)	6 ± 1 (2)	6 ± 1 (2)	**0** ± 0.2 (7)
	ara-cp (9)	6 ± 2 (2)	6 ± 2 (2)	6 ± 1 (2)	6 ± 1 (2)	**0** ± 0.3 7)

[a]Total number of substitutions per site between populations ± standard error (SE) (Nei 1987) calculated with DnaSP (Rozas et al. 2003); n, number of loci sequenced for each species. [b]Number of sites compared, all gapped sites excluded. [c]*Triticum dicoccoides* A genome. [d]*T. dicoccoides* B genome. [e]*Triticum araraticum* A genome. [f]*T. araraticum* G genome. [g]Chloroplast genome.

and by 17 nucleotides from the major *T. dicoccoides* B haplotype; the other haplotypes were more distant to B and G genomes. At *PGK1*, 13 *A. speltoides* haplotypes were found: SPE-I differed by 2 nucleotides from the main B haplotype of *T. dicoccoides*; the rare SPE-II is not more closely related to the single *T. araraticum* G haplotype than haplotypes found among other *Sitopsis*; for this gene, a greater diversity of *A. speltoides* haplotypes relative to other *Sitopsis* was particularly evident. *VRN1* (Supplementary fig. S1, Supplementary Material online) did not reveal a closer relationship for either *A. speltoides* or other *Sitopsis* to B or G genome. For this gene, the simplest interpretation is that our present lineage sample at *VRN1* did not uncover *A. speltoides* B genome progenitor haplotypes: only a clear distinction between A and B/G genome–specific haplotypes was evident.

The main *A. speltoides ndhF* haplotype was identical with that of tetraploids and hexaploids (fig. 4). The network, while excluding the progenitors of *A. bicornis*, *A. longissima*, *A. sharonensis*, and *A. searsii* as the B female recipients in the cross with the A genome, provides evidence that female gametes of *A. speltoides* generated the AABB and AAGG genomes.

In summary, the MJ networks uncover no *Sitopsis* haplotypes that are more similar to B or G genome than *A. speltoides* haplotypes are, indicating that the *A. speltoides* gene pool participated in the synthesis of AABB and AAGG genomes. Furthermore, loci that are highly polymorphic in *A. speltoides*, such as *PGK1*, underscore the need to sample many lineages to uncover B genome progenitor alleles.

The 2 distinct G genome haplotypes at *ACC1* differing by 4 substitutions, each identical to haplotypes occurring in *A. speltoides*, could, at face value, suggest 2 independent origins for *T. araraticum*. However, for all loci at which the B genome donor was heterozygous—for instance, in unreduced gametes—both alleles should persist in modern tetraploids. This problem is related to the outcrossing nature of *A. speltoides* (table 2): of the 39 loci investigated, 76% were heterozygous as compared with 7.4% for remaining *Aegilops* species, all predominantly inbreeders (Kimber and Feldman 1987). The distinctness of B and G genome haplotypes at all nuclear loci, and the proximity of *A. speltoides* progenitors in most cases, clearly indicates independent alloploidization events underlying *T. araraticum* and *T. dicoccoides* origins, consistent with their divergent positions in the analysis of B genome–specific AFLPs (fig. 3*B*). The results of table 3, which underscore the close relationship of B and G genomes relative to A genome, support this conclusion.

Discussion

Domestication of wheats commenced about 10 000 years ago (Salamini et al. 2002), but the events that gave rise to wild polyploids are older. Estimates for the age of *T. dicoccoides* origin range from >0.5 MYA (Huang et al. 2002), 0.25–1.3 MYA (Mori et al. 1995; Huang

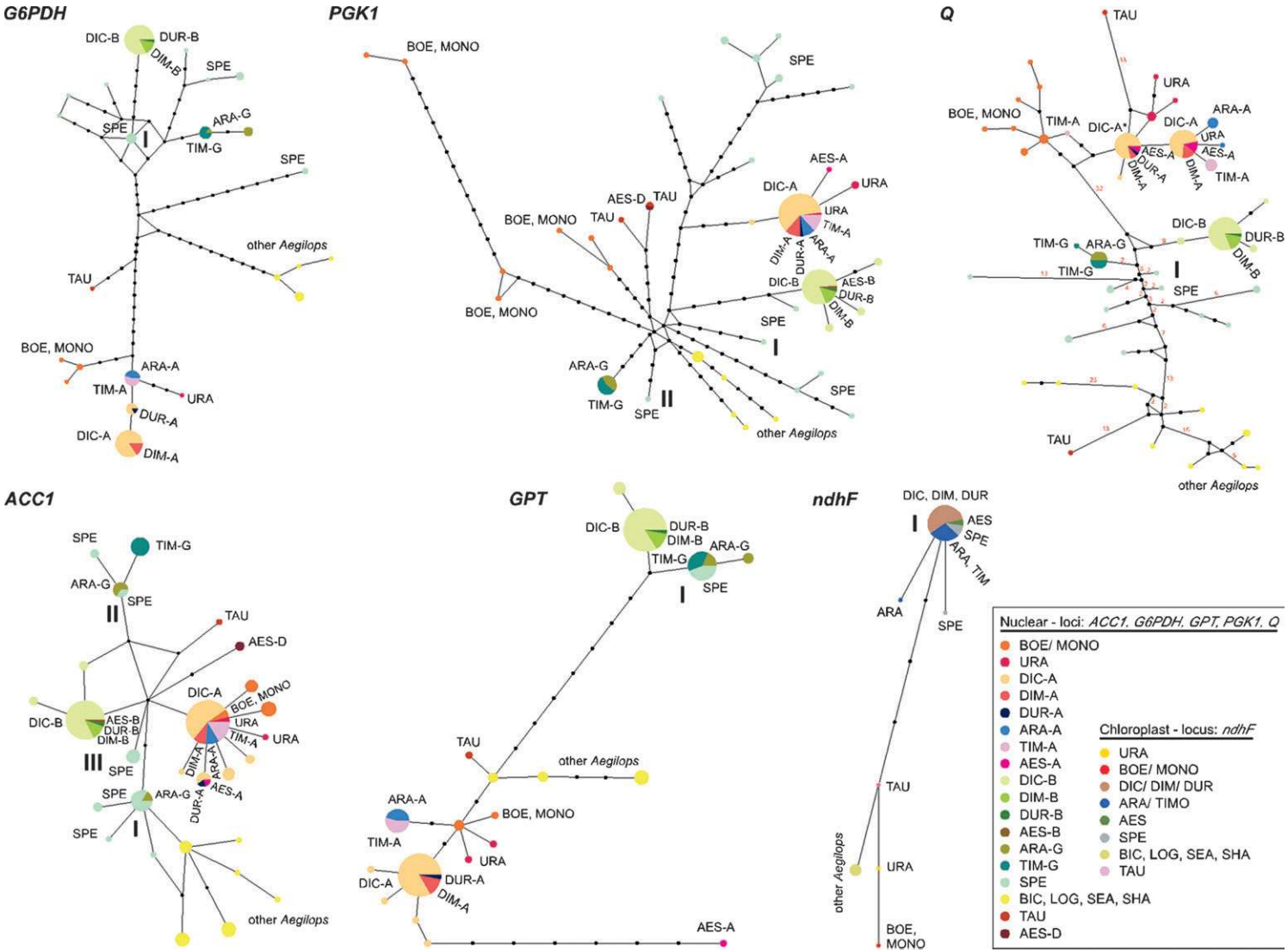


FIG. 4.—MJ networks derived from DNA sequence haplotypes among accessions, involving in all loci *Triticum dicoccoides* (34), *T. dicoccum* (5), *Triticum durum* (1), *Triticum araraticum* (5), *Triticum timopheevii* (6), *Aegilops bicornis* (2), *Aegilops longissima* (2), *Aegilops searsii* (2), *Aegilops sharonensis* (2), *Aegilops speltoides* (7), and *Aegilops tauschii* (1) plus additional haplotype data for each locus as available. Distance between 2 black dots is one nucleotide substitution. AES, *Triticum aestivum*; ARA, *T. araraticum*; BIC, *A. bicornis*; BOE, *Triticum boeoticum*; DIC, *T. dicoccoides*; DIM, *T. dicoccum*; DUR, *T. durum*; LOG, *A. longissima*; MONO, *Triticum monococcum*; SEA, *A. searsii*; SHA, *A. sharonensis*; SPE, *A. speltoides*; TAU, *A. tauschii*; TIM, *T. timopheevii*; and URA, *Triticum urartu*. Species names according to Dorofeev et al. (1979) and Van Slageren (1994). *G6PDH*—For this MJ network, we used 72 lines from this project, for a total of 132 sequences. *ACC1*—116 lines: 79 from this project; 37 from published results of other lines; total of 176 sequences: 139 from this project; 37from published sequences. *GPT*—72: 71; 1; 124: 123; 1. *PGK1*—86: 76; 10; 144: 134; 10. *Q*: 93: 81; 12; 157: 145; 12. *ndhF*—79: 78; 1; 79: 78; 1.

Table 2
Heterozygosity among *Aegilops* accessions at loci sampled

	ACC1			*G6PDH*			*GPT*			*PGK1*			*Q*			*VRN1*		
Species	n[a]	H[b]	h[c]	n	H	h	n	H	h	n	H	h	n	H	h	n	H	h
Aegilops speltoides	8	7	5	8	7	8	7	0	1	8	6	11	8	5	10	7	4	6
Aegilops bicornis	2	0	1	2	0	2	2	0	1	2	0	2	2	0	2	2	0	1
Aegilops longissima	2	1	3	2	0	1	2	0	1	2	0	1	2	1	3	2	0	1
Aegilops searsii	2	0	1	2	0	1	2	0	1	2	0	2	2	0	2	2	0	1
Aegilops sharonensis	2	0	1	2	0	1	2	0	1	2	0	1	2	1	2	2	0	1
Aegilops tauschii	1	0	1	1	0	1	1	0	1	1	0	1	1	1	2	1	0	1

[a]Number of individuals sampled. [b]Number of heterozygous individuals found. [c]Number of haplotypes found (including publically available sequences).

et al. 2002), or 0.36 MYA (Dvorak and Akhunov 2005) but are heavily subject to haplotype sampling variance, as our data underscore, for which reason the B genome donor has remained in issue. Though the source of the B genome has been sought among *Sitopsis* (Sarkar and Stebbins 1956; Kerby and Kuspira 1988), with a focus on *A. speltoides* (Dvorak and Zhang 1990; Huang et al. 2002), genetic data have been equivocal (Dvorak 1972; Kimber and Athwal 1972; Fernandez-Calvin and Orellana 1994) in the absence of studies sampling many loci and accessions.

Our data indicate an origin of the B genome from within *A. speltoides*. First, the analysis of 375 AFLP loci links specifically polyploid wheats with *A. speltoides* to the exclusion of other *Sitopsis* species (fig. 3*A*). In addition, 65 AFLPs specific to the *T. aestivum* B genome link *A. speltoides* even more closely to the B and G genomes (fig. 3*B*). Second, with the exception of *VRN1*, haplotypes from chloroplast and nuclear loci show that *A. speltoides* shares the highest average sequence identity with the B and G genomes (table 3) and reveals specific progenitor-descendant relationships in the MJ networks (fig. 4). These findings can be incorporated into a broader scheme of wheat genome evolution (Supplementary fig. S2, Supplementary Material online) with resolved positions of the B genome relative to S progenitors and G sisters.

AABB and AAGG genome origins have been attributed to the same single hybridization event (Wagenaar 1961; Tanaka et al. 1979; Gill and Chen 1987; Provan et al. 2004) or to separate alloploidization events (Mori et al. 1995; Brown-Guedira et al. 1996; Rodriguez, Perera et al. 2000; Huang et al. 2002). In support of the former view, *T. dicoccoides* and *T. araraticum* have almost identical morphology, but they have F1 hybrids showing 100% sterility (Tanaka et al. 1979) (with normal chromosome pairing, Rao and Smith 1968; Rawal and Harlan 1975; Tanaka et al. 1979). In addition, some lines of *T. araraticum* produce hybrids with a significant level of fertility when crossed to *T. dicoccoides* (Rao and Smith 1968; Rawal and Harlan 1975). Our data resolve this issue. The hybridization events leading to AABB and AAGG genomes occurred independently as evidenced 1) by their distinct positions in AFLP analyses, 2) by the finding that each has sequestered different samples of *A. speltoides* haplotype diversity, and 3) from the comparison of divergence within and among A, B, and G genome haplotypes (table 3). The B and G genomes are clearly distinct, incompatible with the view of a single-hybrid origin (Rodriguez, Maestra et al. 2002).

Wheat is no exception to the rule that specific polyploids arose recurrently during flowering plant evolution (Soltis 2005), accompanied by extensive and rapid genome restructuring (Leicht and Bennett 1997). Alloploidization often involves intergenomic recombination (McGrath et al. 1990; Jang and Gill 1994; Song et al. 1995; Soltis 2005) and rapid loss of DNA (Özkan et al. 2001), whereby subsequent diploidization restores disomic genetics (Levy and Feldman 2002). The genetic control of chromosome pairing provides insights on wheat alloploid evolution. *Aegilops speltoides* forms are known that suppress pairing among homologous chromosomes (*Ph1* activity) (Aghaee-Sarbarzeh et al. 2000). If *Ph1* genotypes participate in polyploidization events, interspecific hybrids acquire a

Table 3
Average between- and within-genome haplotype sequence divergence (boldface: significant divergence between B to A and B to G comparisons)

	$D_a \pm$ SE $\times 10^3$ (n)[a]; *Triticum dicoccoides* B versus		Average sequence divergence within genomes			
Gene (L)[d]	A genome[b]	G genome[c]	*T. dicoccoides* B	Other B[e]	*Triticum araraticum* G	*Triticum timopheevii* G
ACC1 (366)	5.8 ± 0.4 (72)	7.7 ± 1.1 (15)	1.7 ± 1.9 (37)	1.5 ± 1.8 (43)	5.9 ± 5.7 (6)	4.2 ± 5.3 (15)
G6PDH (537)	49.7 ± 1.7 (56)	**19.3** ± 1.6 (11)	0.0 ± 0.0 (34)	0.0 ± 0.0 (41)	1.7 ± 2.1 (5)	2.1 ± 2.1 (11)
GPT (673)	20.2 ± 0.8 (57)	**3.6** ± 0.4 (11)	0.2 ± 0.6 (34)	0.2 ± 0.5 (40)	1.5 ± 1.0 (5)	1.2 ± 1.2 (11)
PGK1 (665)	23.1 ± 1.4 (63)	20.6 ± 1.5 (11)	0.8 ± 1.3 (35)	0.7 ± 1.2 (41)	0.0 ± 0.0 (5)	0.0 ± 0.0 (11)
Q (917)	81.3 ± 2.3 (73)	**21.7** ± 1.5 (11)	0.5 ± 0.8 (36)	0.4 ± 0.7 (43)	0.0 ± 0.0 (5)	0.2 ± 0.5 (11)
VRN1 (304)	55.9 ± 1.8 (64)	**6.8** ± 0.6 (11)	2.6 ± 2.2 (34)	2.5 ± 2.2 (41)	0.0 ± 0.0 (5)	0.0 ± 0.0 (11)

[a]Number of net nucleotide substitutions per site between populations ± standard error (SE) (Nei 1987) calculated with DnaSP (Rozas et al. 2003) using the Jukes–Cantor method; n, number of loci sequenced for each species. [b]A genome of *Triticum monococcum*, *Triticum boeoticum*, *Triticum urartu*, *T. dicoccoides*, *Triticum durum*, *T. dicoccum*, *T. araraticum*, and *T. timopheevii*. [c]*T. araraticum* and *T. timopheevii*. [d]Number of sites compared, all gapped sites excluded. Alignments available as Supplementary Material online. [e]*T. durum*, *T. dicoccum*.

bivalent type of chromosome pairing, the case of *T. dicoccoides* (Sears 1976). Other lines of *A. speltoides* do not show *Ph1*-like activity (Kimber and Feldman 1987), having loci that suppress *Ph1*, thus allowing homologous pairing in interspecific crosses (Sears 1976). The absence of *Ph1* or *Ph1*-like activity favors tetravalent formation and, possibly, intergenomic translocations. *Triticum araraticum* has extensive DNA loss (Özkan et al. 2001) and 6 chromosomal rearrangements relative to *T. dicoccoides* (Rodriguez, Maestra et al. 2000; Rodriguez, Perera et al. 2000), 4 of which are intergenomic G–A translocations. A possibility is that in the AAGG genome synthesis, the *Ph1* allele of *A. speltoides* was suppressed and later on restored via genetic segregation (today *T. araraticum* has an active *Ph1* allele). Similar evolutionary mechanisms may underlie the cytogenetic distinctness of S, B, and G genomes, whose evolutionary relationships are nonetheless revealed by AFLP and haplotype data.

Previous studies suggested that *T. araraticum* inherited a *A. speltoides* cytoplasm (Mori et al. 1997; Wang et al. 2000; Provan et al. 2004) but were conflicting for *T. dicoccoides* (Wang et al. 2000; Provan et al. 2004). Our *ndhF* data assign the *A. speltoides* cytoplasm to both *T. dicoccoides* and *T. araraticum*. This cytoplasm is distinct from that of other *Sitopsis* species.

We identified no *A. speltoides* line that shares greater similarity to all polyploids sampled than any other *A. speltoides* line. Furthermore, the *A. speltoides* haplotypes most similar or identical to B and G genome haplotypes are dispersed across different individual lines. Further sampling within *A. speltoides* might uncover lines that carry the same combination of haplotypes as the B genome donor contained. However, because the species is an outbreeder, it is more likely that no modern *A. speltoides* lines have preserved the B donor genotype in its contiguous ancestral state.

Supplementary Material

Supplementary material mentioned in the text, comprising 10 supplementary tables and 2 supplementary figures are available online at http://en.tecnoparco.org/Default.aspx?tabid=118 and at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org). Sequence data from this article are deposited in GenBank Data library under accession no. provided in Supplementary table S10.

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