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Molecular Phylogenetics and Evolution 39 (2006) 70–82
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the **A**, **B**, and **D** genomes of common wheat (*Triticum aestivum*)

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Received 17 May 2005; revised 8 December 2005; accepted 12 January 2006

Abstract

Common wheat (*Triticum aestivum*) has for decades been a textbook example of the evolution of a major crop species by allopolyploidization. Using a sophisticated extension of the PCR technique, we have successfully isolated two single-copy nuclear genes, DMC1 and EF–G, from each of the three genomes found in hexaploid wheat (**BA^{u}D**) and from the two genomes of the tetraploid progenitor *Triticum turgidum* (**BA^{u}**). By subjecting these sequences to phylogenetic analysis together with sequences from representatives of all the diploid Triticeae genera we are able for the first time to provide simultaneous and strongly supported evidence for the **D** genome being derived from *Aegilops tauschii*, the **A^{u}** genome being derived from *Triticum urartu*, and the hitherto enigmatic **B** genome being derived from *Aegilops speltoides*. Previous problems of identifying the **B** genome donor may be associated with a higher diversification rate of the **B** genome compared to the **A^{u}** genome in the polyploid wheats. The phylogenetic hypothesis further suggests that neither *Triticum*, *Aegilops*, nor *Triticum* plus *Aegilops* are monophyletic.
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Keywords: Allopolyploidy; Phylogeny; *Triticum*; *Aegilops*

1. Introduction

Common wheat (*Triticum aestivum*) has since the pioneering studies by Kihara (1924) been the textbook example of the evolution of a major crop species by allopolyploidization. According to generally accepted interpretations (Cox, 1998) common wheat is an allohexaploid (genomic constitution **BBA^{u}A^{u}DD**) derived through hybridization between a domesticated form of tetraploid, wild emmer, *Triticum turgidum* ssp. *dicoccoides* (genomic constitution **BBA^{u}A^{u}**), and the diploid *Aegilops tauschii* (genomic constitution **DD**). Wild emmer itself is supposed to be an allotetraploid derived through hybridization between two wild diploids: *Triticum urartu* contributing the **A^{u}** genome and possibly *Aegilops speltoides* contributing the **B** genome. However, despite decades of intensive research the origin of the **B** genome has remained controversial (e.g., Huang et al., 2002a) and accordingly the genome of *Ae. speltoides* is usually not designated **B** but **S** (Cox, 1998; Huang et al., 2002a,b; Wang et al., 1996). Generally, the **S** genome is shared by a group of species (*Aegilops* L. section *Sitopsis* (Jaub. & Spach) Zhuk.), which in addition to *Ae. speltoides* (**S**) includes *Ae. bicornis* (**S^{b}**), *Ae. longissima* (**S^{l}**), *Ae. searsii* (**S^{s}**), and *Ae. sharonensis* (**S^{l}**) (Slageren, 1994).

The origin of *T. aestivum* and other polyploid wheat species has been subject of numerous studies and the above scenario is the accepted consensus based on all evidence. The literature on the subject is immense, but only papers using an explicit phylogenetic method will be cited here. This includes papers using parsimony, maximum

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1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.01.023

likelihood, and Bayesian inference, but not neighbor joining, UPGMA, or other phenetic approaches, which are poor estimators of phylogeny (e.g., Farris, 1983). An unfortunate large proportion of papers are based on neighbour joining and/or UPGMA analyses (e.g., Büren, 2001; Galili et al., 2000; Giorgi et al., 2002; Goryunova et al., 2004; Ishii et al., 2001; Provan et al., 2004; Sasanuma et al., 2004; Sourdille et al., 2001; Ünlü and Sümer, 2005; Vakhitov et al., 2003; C. Wang et al., 2000; G.-Z. Wang et al., 2000; Wang et al., 2000b). Most of the published phylogenetic analyses suffer from a limited, biased taxon sampling, either including polyploid wheat and its *a priori* assumed progenitors (Buchner et al., 2004) or only a few additional *Aegilops* and/or *Triticum* L. species (Blake et al., 1999; Huang et al., 2002a,b; Zhang et al., 2002). Rarely are all diploid species of *Aegilops* and *Triticum* included (Sallares and Brown, 2004) and few include more than one or a few other Triticeae species—often *Hordeum vulgare* L. and/or *Secale cereale* L.—as outgroups. This becomes a problem as two of the many areas of disagreement among recent Triticeae phylogenies are the potential monophyly of *Triticum* plus *Aegilops* and monophyly of *Aegilops* itself (Helfgott and Mason-Gamer, 2004; Hsiao et al., 1995; Kellogg and Appels, 1995; Kellogg et al., 1996; Mason-Gamer, 2001, 2005; Mason-Gamer and Kellogg, 1996; Mason-Gamer et al., 1998, 2002; Petersen and Seberg, 1997, 2000, 2002; Seberg and Frederiksen, 2001; Seberg and Petersen, in press). Present phylogenetic analyses all show that *Hordeum* L. and *Secale* L. are poor choices of outgroups. Hence, the taxon sampling in the majority of studies of *Triticum*/*Aegilops* phylogeny inevitable restricts results to the expected.

The present study intends to remedy this restricted taxon sampling primarily in an attempt to elucidate the origin of tetraploid and hexaploid wheats. Sequences from plastid and nuclear genes obtained from both tetraploid and hexaploid wheats are included in phylogenetic analyses together with sequences from all diploid species of *Aegilops* and *Triticum* (Slageren, 1994) and representatives of all genomes traditionally recognized in diploid Triticeae (Wang et al., 1996). This broad taxon sampling simultaneous provides new evidence about the phylogeny of *Aegilops*. We use partial nucleotide sequences from two single-copy nuclear genes, DMC1 (disrupted meiotic cDNA) and EF–G (translation elongation factor G), and one plastid gene, *ndh*F (NADH dehydrogenase subunit F). Allotetraploid and allohexaploid species ideally have two or three copies of each nuclear gene (disregarding potential allelic variation that could produce four or six copies) each received from the diploid ancestors. To pick them up successfully from the genome, we consider an experimental approach employing copy-specific PCR primers to be the most appropriate, because recombination among PCR-generated sequence fragments is negligible (Cronn et al., 2002). The nuclear genes were chosen because they have been used previously for phylogenetic reconstruction of the diploid Triticeae genera (Aagesen et al., 2005; Petersen and Seberg, 2000, 2002; Seberg and Petersen, in press) and successfully elucidated the origin of two tetraploid species of *Hordeum* (Petersen and Seberg, 2004). Previous phylogenetic analyses of the diploid Triticeae genera include the plastid genes *rbc*L and *rpo*A (Aagesen et al., 2005; Petersen and Seberg, 1997; Seberg and Petersen, in press), but here we have chosen *ndh*F because of its higher variability. Preliminary results from the present study have been published as proceedings from the 5th International Triticeae Symposium (Petersen and Seberg, 2005).

2. Materials and methods

Taxon sampling was based on previous phylogenetic analyses of the diploid Triticeae (Aagesen et al., 2005; Petersen and Seberg, 1997, 2000, 2002; Seberg and Petersen, in press), but in addition 12 accessions of diploid species of *Aegilops*/*Triticum*, five accessions of tetraploid *T. turgidum*, and one accession of hexaploid, common wheat, *T. aestivum* cv. Kadet, were included. For a complete taxon list, incl. GenBank accession numbers, see Table 1. With a single exception (see Table 1), voucher specimens are deposited at C. Genomic designations follow Wang et al. (1996).

PCR and sequencing of *ndh*F were performed using the primers ndhF1318 and ndhF2110R (Olmstead and Sweere, 1994). PCR was performed under standard conditions using standard *Taq* polymerase (Amersham Bioscience) and the products were purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Cycle sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Applied Biosystems), and the products were purified using the DyeEX Spin kit (Qiagen) according to the manufacturer's instructions. DNA fragments were separated on an ABI 377 (Applied Biosystems) automated sequencer, and sequence editing was conducted using Sequencher 4.2.2.

In the diploid species PCR and sequencing of the nuclear genes, DMC1 and EF–G, were performed as described by Petersen and Seberg (2000, 2003). Amplification and isolation of each of the gene copies of EF–G and DMC1 from the polyploid species largely followed the procedure described by Petersen and Seberg (2004) except that cloning was entirely avoided. Initially, PCR and sequencing were performed using the same primers as for the diploid species but under less stringent conditions, resulting in sequences showing clear signs of polymorphisms. Inspection of these sequences allowed the construction of genome specific primers. These primers were designed following the MAMA technique (Cha et al., 1992) (mismatch amplification mutation assay), which deliberately incorporates a mismatching nucleotide at the ultimate or penultimate 3′ position of the primer. Mismatch at both the ultimate and the penultimate 3′ positions effectively prevent amplification of the undesired sequences. The technique makes cloning superfluous and avoids PCR artefacts such as chimeric products, which by their potential abundance may otherwise obscure interpretation of results completely

Table 1
Specimens and GenBank accession numbers of the Triticeae plus two species of *Bromus* used as outgroups

Accession No.	Species; voucher information[a]	*ndh*F	DMC1	EF–G
C618	*Bromus arvensis* L.	DQ247873	DQ247821	AY836186
OSA420	*Bromus sterilis* L.	DQ247874	AF277264	AY836187
H6602	*Aegilops bicornis* (Forssk.) Jaub. & Spach; Egypt, Slageren et al. 19-04-1989, ICARDA	DQ247904	DQ247822	DQ247802
H6677	*Aegilops caudata* L.; Turkey, L. Morrison 84TK159	DQ247911	DQ247829	DQ247855
H6673	*Aegilops comosa* Sm. in Sibth. & Sm.; Turkey, Univ. California Riverside G1290	DQ247879	AF277242	AY836193
H6606	*Aegilops comosa*; Turkey, Metzger & Jana 15-08-1979	DQ247906	DQ247824	DQ247850
H6679	*Aegilops longissima* Schweinf. & Muschl.; Israel, Univ. California Riverside G1306	DQ247912	DQ247830	DQ247856
H6605	*Aegilops searsii* Feldman & Kisselev ex K. Hammer; Jordan, Bourgois 15-05-1981	DQ247905	DQ247823	DQ247849
H6683	*Aegilops searsii*; Israel, Univ. California Riverside G3526	DQ247914	DQ247832	DQ247858
H6680	*Aegilops sharonensis* Eig; Israel, Univ. California Riverside G1315	DQ247913	DQ247831	DQ247857
H4523	*Aegilops speltoides* Tausch; Turkey, Petersen & Ørgaard 23-08-1991	DQ247901	AF277256	DQ247847
H6797	*Aegilops speltoides*; L. Morrison s.n. (GH)	DQ247915	DQ247833	DQ247859
H6668	*Aegilops tauschii* Coss.; Iran, Univ. California Riverside G1279	DQ247893	AF277235	AY836207
H6609	*Aegilops umbellulata* Zhuk.; Syria, Bourgois 15-06-1980	DQ247907	DQ247825	DQ247851
H6675	*Aegilops uniaristata* Vis.; Turkey, Univ. California Riverside 1297	DQ247910	DQ247828	DQ247854
H4349	*Agropyron cristatum* Gaertn.	DQ247875	AF277241	AY836188
H5572	*Amblyopyrum muticum* Eig	DQ247876	AF277243	AY836189
H6771	*Australopyrum pectinatum* (Labill.) Á. Löve	DQ247877	AF277252	AY836190
H6723	*Australopyrum retrofractum* (Vickery) Á. Löve	AF267662	AF277251	AY836191
H6724	*Australopyrum velutinum* (Nees) B.K. Simon	—	AF277253	AY836192
H4200	*Australopyrum velutinum*	DQ247878	—	—
H5558	*Crithopsis delileana* (Schult.) Roshev.	DQ247895	AF277240	AY836209
H5561	*Dasypyrum villosum* (M. Bieb.) Maire	DQ247881	AF277238	AY836195
H5552	*Eremopyrum distans* (K. Koch) Nevski	DQ247882	AF277236	AY836196
H5553	*Eremopyrum triticeum* (Gaertn.) Nevski	DQ247883	AF277237	AY836197
H6511	*Festucopsis serpentinii* (C.E. Hubb.) Melderis	DQ247884	AF277247	AY836198
H5556	*Henrardia persica* (Boiss.) C.E. Hubb.	DQ247885	AF277255	AY836199
H5557	*Heteranthelium piliferum* Hochst. ex Jaub. & Spach	DQ247886	AF277238	AY836200
H1942	*Hordeum brachyantherum* Nevski ssp. *californicum* (Covas & Stebbins) Bothmer, N. Jacobsen & Seberg	DQ247887	AF277260	AY836201
H1150	*Hordeum erectifolium* Bothmer, N. Jacobsen & R.B. Jørg.	DQ247888	AF277259	AY836202
H299	*Hordeum marinum* Huds. ssp. *gussoneanum* (Parl.) Thell.	DQ247889	AF277257	AY836203
H801	*Hordeum murinum* L. ssp. *glaucum* (Steud.) Tzvelev	DQ247890	AF277258	AY836204
H3139	*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.	DQ247891	AF277262	AY836205
H6692	*Lophopyrum elongatum* (Host) Á. Löve	DQ247892	AF277246	AY836206
H5575	*Peridictyon sanctum* (Janka) Seberg, Fred. & Baden	DQ247894	AF277244	AY836208
H917	*Psathyrostachys fragilis* (Boiss.) Nevski ssp. *fragilis*	DQ247896	AF277261	AY836210
H4372	*Psathyrostachys fragilis* ssp. *villosus* Baden	DQ247897	AF277263	AY836211
H9182	*Psathyrostachys stoloniformis* Baden	DQ247898	AF277264	AY836212
H9082	*Pseudoroegneria spicata* (Pursh) Á. Löve	DQ247900	AF277245	AY836214
H4342	*Secale strictum* (C. Presl) C. Presl	DQ247899	AF277248	AY836213
H10254	*Taeniatherum caput-medusae* (L.) Nevski	DQ247902	AF277249	AY836216
H6725	*Thinopyrum bessarabicum* (Saÿvul. & Rayss) Á. Löve	—	AF277254	AY836217
H6729	*Thinopyrum bessarabicum*	DQ247903	—	—
H4547	*Triticium monococcum* L., Turkey, Petersen & Ørgaard 31-08-1991	DQ247880	AF277250	AY836194
	Triticum aestivum L. cv. Kadet	DQ247921	DQ247844 (B)	DQ247870 (B)
		n.a.	DQ247845 (A)	DQ247871 (A)
		n.a.	DQ247846 (D)	DQ247872 (D)
H6840	*Triticum turgidum* L. ssp. *dicoccoides*; Turkey, B. Johnson, GRIN PI 428051	DQ247918	DQ247836 (B)	DQ247862 (B)
	(Körn. ex Asch. & Graebn.) Thell.	n.a.	DQ247841 (A)	DQ247867 (A)
H6841	*Triticum turgidum* ssp. *dicoccoides*; Israel, E. Nevo, GRIN PI 470944	DQ247919	DQ247837 (B)	DQ247863 (B)
		n.a.	DQ247842 (A)	DQ247868 (A)
H6838	*Triticum turgidum* ssp. *durum* (Desf.) Husn.; Jordan, S. Kohli, GRIN PI 371823	DQ247916	DQ247834 (B)	DQ247860 (B)
		n.a.	DQ247839 (A)	DQ247865 (A)
H6842	*Triticum turgidum* ssp. *durum*; Iran, H. Springfield, GRIN PI 208908	DQ247920	DQ247838 (B)	DQ247864 (B)
		n.a.	DQ247843 (A)	DQ247869 (A)
H6839	*Triticum turgidum* ssp. *turgidum*; Iran, C. Qualset, GRIN PI 624727	DQ247917	DQ247835 (B)	DQ247861 (B)
		n.a.	DQ247840 (A)	DQ247866 (A)
H6664	*Triticum urartu* Tumanian ex Gandilyan; Iran, Univ. California Riverside G1545	DQ247908	DQ247826	DQ247852
H6665	*Triticum urartu*; Turkey, Univ. California Riverside G1956	DQ247909	DQ247827	DQ247853

GenBank Accession Nos. DQ247821–DQ247921 are new submissions.
n.a., not applicable.
[a] Voucher information is only included if not previously published (Petersen and Seberg, 1997, 2000). Vouchers are deposited at C unless indicated otherwise.

(Cronn et al., 2002). A list of the primers used can be found in Table 2, but future studies may benefit from the now existing sequences enabling design of additional genome specific primers. All sequences were aligned manually. Alignment of *ndh*F sequences was trivial, and introduction of new DMC1 and EF–G sequences into existing matrices for the Triticeae (Petersen and Seberg, 2000; Seberg and Petersen, in press) was unproblematic as well. The matrix is available at TreeBASE (study Accession No. SN2595).

Phylogenetic analyses were made for each of the three individual genes and on a combined matrix including all sequences. The gene trees constructed from the separate analyses of DMC1 and EF–G sequences were used to infer homology of the two or three copies of each of the genes found in the polyploids. Hence, in the combined analysis the individual copies of each nuclear gene of the polyploids were matched in accordance with inferred homology. E.g., when a copy of DMC1 from *T. aestivum* was found to have the same sister group relationships as a copy of EF–G, the two sequences were considered derived from the same progenitor and matched accordingly. Similarly, the position of the polyploid taxa on the *ndh*F gene tree was used to determine which of the nuclear gene sequences to combine with each *ndh*F sequence, on the assumption that the *ndh*F sequence from a polyploid groups with its maternal progenitor. Nuclear sequences inferred to have been donated by the paternal parent would not be matched by any *ndh*F sequence, and were accompanied by missing data in the *ndh*F character set. The DMC1 sequences include a small number of MITEs (Petersen and Seberg, 2000), which were excluded from the analyses. Analyses were performed using both PAUP*, version 4.0b8 (Swofford, 2001) and WinClada, version 1.00.08 (Nixon, 2002), spawning the matrix to NONA version 2.0 (Goloboff, 1993). PAUP*, version 4.0b8 has been preferred to the most recent version 10, as the latter version under some circumstances outputs erroneous tree lengths and an excessive number of tree islands. Uninformative sites were excluded, and informative characters were equally weighted and treated as unordered. Gaps were treated as ambiguous data (?). Initial attempts to code the gaps following the procedure of Simmons and Ochoterena (2000) only increased incongruence among the data partitions and we choose not to apply gap coding. Branches were collapsed when their minimum length were zero (amb-). Analyses performed in PAUP* were using heuristic search, 100 random addition sequences, holding five trees at each step, TBR swapping, and Steepest Descent. In WinClada the matrices were executed using the heuristic search options hold100, mult*100, max*, and hold/10. Support for individual clades was calculated as jackknife values in WinClada running 1.000 replicates, each with the options mult*100, max*, hold/10, and keeping max. 1.000 trees.

The number of character changes occurring within the $\mathbf{A^u}$ genome clade and the **B** genome clade on each of the nuclear gene trees was calculated using PAUP* under both ACCTRAN and DELTRAN optimization.

3. Results

In all polyploid wheats, we successfully amplified the expected two or three copies of each nuclear gene. Sequences obtained using the genome specific primers did not contain polymorphic sites, so we conclude that each of the sequence copies has no allelic variation.

The DMC1 matrix includes 1497 aligned positions of which 168 are phylogenetically informative. Phylogenetic analysis resulted in 12 equally parsimonious trees of length 331 (ci = 0.67, ri = 0.87). One of the 12 trees is shown as Fig. 1. Within the $\mathbf{A^u}$ genome clade only two character changes occur; both within the subclade including sequences extracted from the polyploid taxa. In the **B** genome clade 11 or 12 character changes occur depending on the chosen optimization. Four changes occur in the subclade including the diploid specimens of *Ae. speltoides*, and seven (ACCTRAN optimization) or eight (DELTRAN optimization) changes occur in the subclade including sequences extracted from the polyploid taxa.

The EF–G matrix includes 916 aligned positions of which 175 are phylogenetically informative. Phylogenetic analysis resulted in four equally parsimonious trees of length 313 (ci = 0.68, ri = 0.90). One of the four trees is shown as Fig. 2. Within the $\mathbf{A^u}$ genome clade six character changes occur; four within the subclade including sequences extracted from the polyploid taxa, two within the subclade including the two specimens of *T. urartu*. In the **B** genome clade 13 or 14 character changes occur depending

Table 2
Primers used for PCR and sequencing of DMC1 and EF–G

DMC1:		EFG:	
TDMC1E13	CTGGCACAAATGCTGTCCCG	cMWG699T3-3	CTGCTGACATACTGGAACATCTCGG
TDMC15R	AGCCACCTGTTGTAATCTGG	cMWG699T7-3	TTTGGGTGATGTTATTGGTGACTTG
TDMC1E10R	TGGTTGGTGATGTACACTGCA	cMWG699T3-2	AACTGTTTTCTCATTTGTGA
TDMC1E10	TGCCAATTGCTGAGAGATTTG	cMWG699T7-2	AAGTGTCCTTGCCTTCCAAA
tritDMC1CGF	TTCCGTGTTGATTTCAGTGGCG	EFGT1A	TTAAGCAGTACTCCTTTATGA
tritDMC1TGF	TTCCGTGTTGATTTCAGTGGTG	EFGT2TR	CTACACTCAGAATTAGTACATCT
tritDMC1AGR	GCAATCTTTGTAAGGCGGGAAG	TEFGDR	CAGACAGCAGATCCTGGC
tritDMC1CGR	GCAATCTTTGTAAGGCGGGACG	TEFG417BF	GCCGAGCATCTGTAATTTAGTC
TDMC1GGF	GATCTCAACTGTGCTCATGTGG	TEFG530AR	GCAAAGCAAGTTCAATTCTCCG
TDMC1A409CR	AATGCAAGCATTCGACTACAC	TEFG530AF	CTGCTGTCTGAAATATTTCTGC
TDMC1D409CR	GCAAGCAGCATTCGACTACTC		

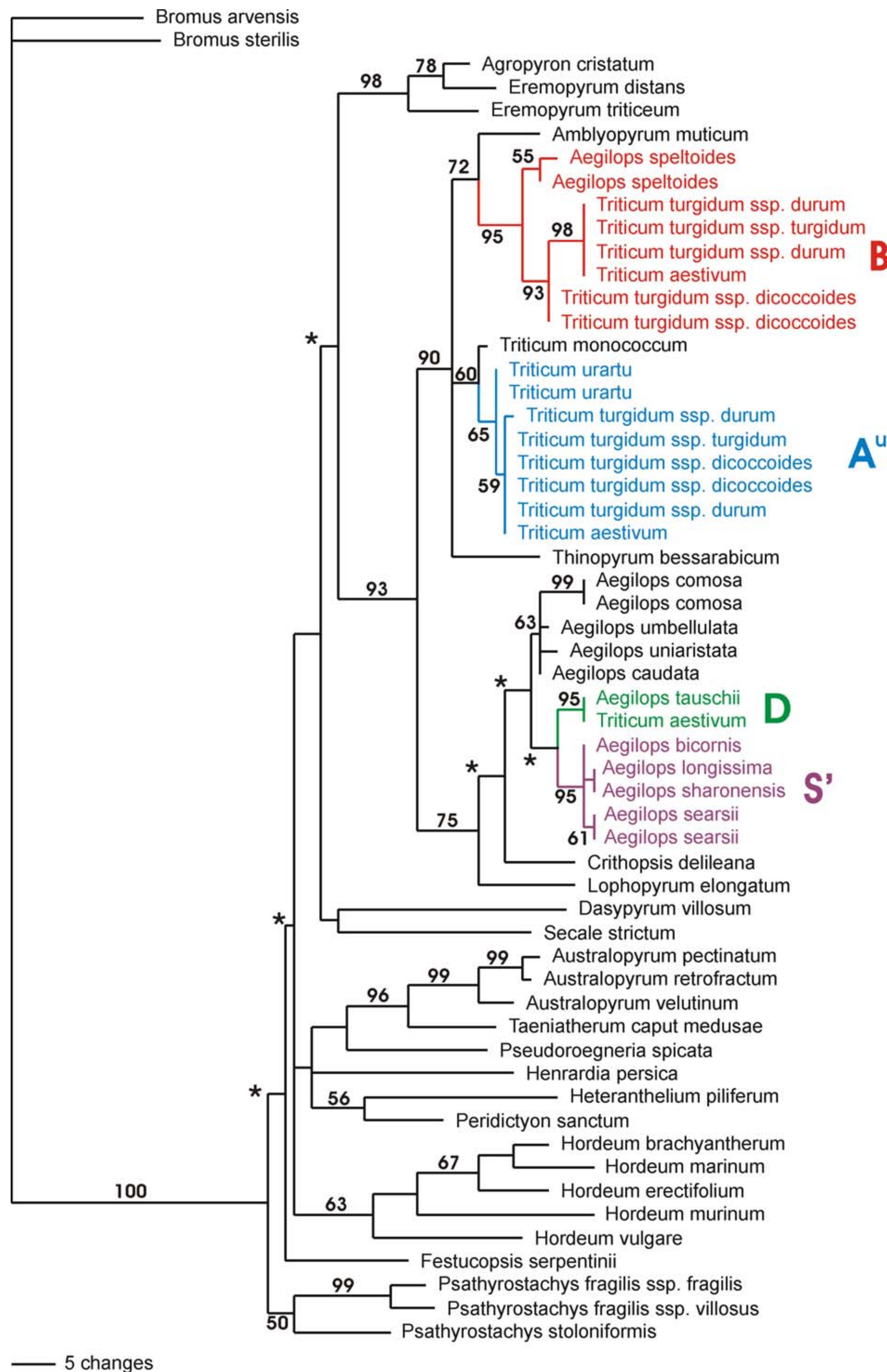

Fig. 1. One of 12 equally parsimonious gene trees (length 331, ci = 0.67, ri = 0.87) derived from phylogenetic analysis of sequence data from the nuclear gene DMC1. Branches that collapse in the strict consensus tree are marked with an *. Numbers above or below branches are jackknife proportions.

on optimization procedure. Eight changes occur in the subclade including sequences extracted from the polyploid taxa, and five (ACCTRAN optimization) or six (DELTRAN optimization) changes occur in the subclade including the diploid specimens of *Ae. speltoides*.

The *ndh*F matrix includes 777 positions of which 49 are phylogenetically informative. Phylogenetic analysis resulted in three equally parsimonious trees of length 65 (ci = 0.83, ri = 0.94). One of the trees is shown in Fig. 3.

Though the individual gene trees are incongruent they show exactly the same sister group relationships of the sequences from tetraploid and hexaploid wheats. Hence, combining the sequences in a total evidence analysis was unproblematic. Combined analysis of all data sets resulted in eight equally parsimonious trees of length 783 (ci = 0.63, ri = 0.85). The strict consensus tree is shown in Fig. 4. WinClada and PAUP* consistently gave the same results.

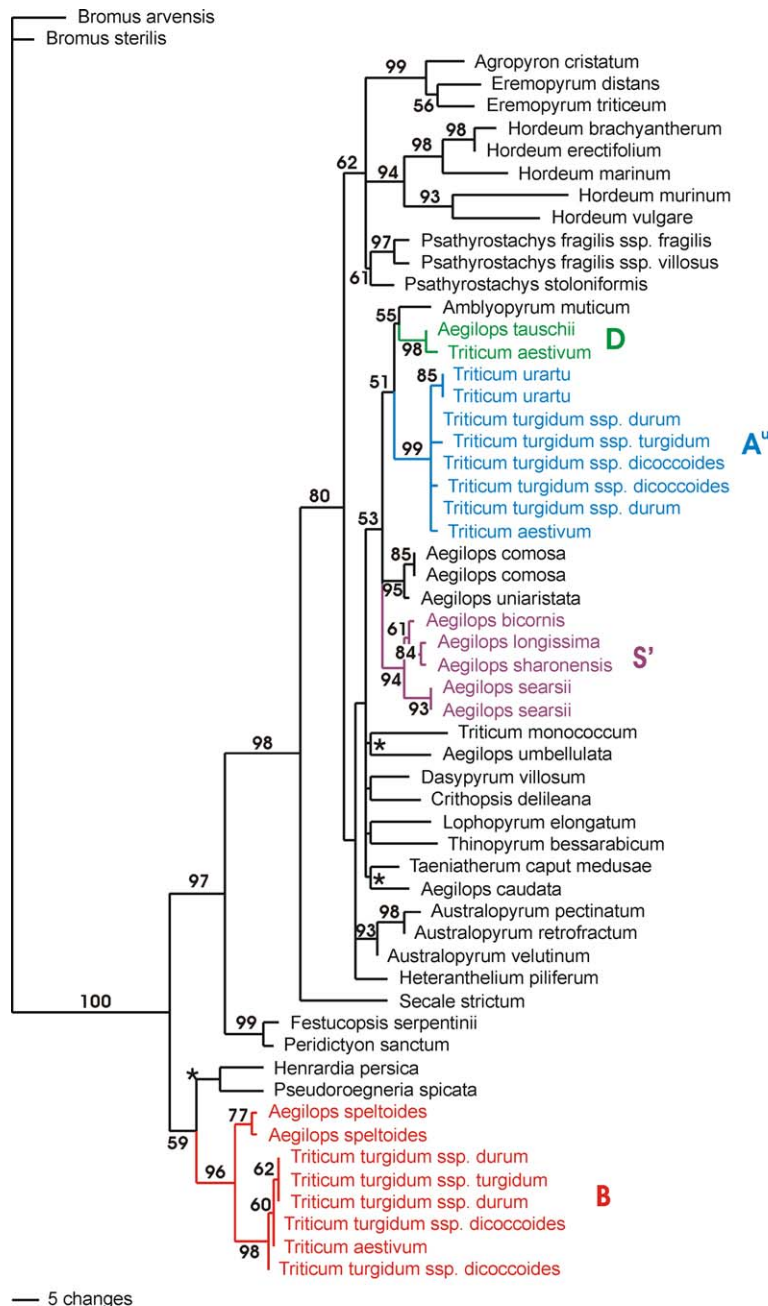

Fig. 2. One of four equally parsimonious gene trees (length 313, ci = 0.68, ri = 0.90) derived from phylogenetic analysis of sequence data from the nuclear gene EF–G. Branches that collapse in the strict consensus tree are marked with an ∗. Numbers above or below branches are jackknife proportions.

4. Discussion

The total evidence analysis presented here provides new evidence about Triticeae phylogeny. However, the phylogeny of the entire tribe has already been discussed in numerous papers (e.g., Hsiao et al., 1995; Kellogg and Appels, 1995; Kellogg et al., 1996; Mason-Gamer, 2005; Mason-Gamer and Kellogg, 1996; Mason-Gamer et al., 1998; Petersen and Seberg, 1997, 2000, 2002; Seberg and Frederiksen, 2001; Seberg and Petersen, in press) and is subject to ongoing research. Hence, the following discussion will concentrate on the phylogenetic relationships within and between *Aegilops* and *Triticum*. In the discussion reference will be made to other published phylogenetic analyses. However, interpretation of the results is often hampered by the limited taxon sampling (see above).

4.1. Is Aegilops plus Triticum monophyletic?

Aegilops and *Triticum* are occasionally considered congeneric (e.g., Bowden, 1959; Dvořák and Zhang, 1992; Yen et al., 2005), and monophyly of the group would conveniently solve the nomenclatural problems caused by the presence of allopolyploid species making one of the

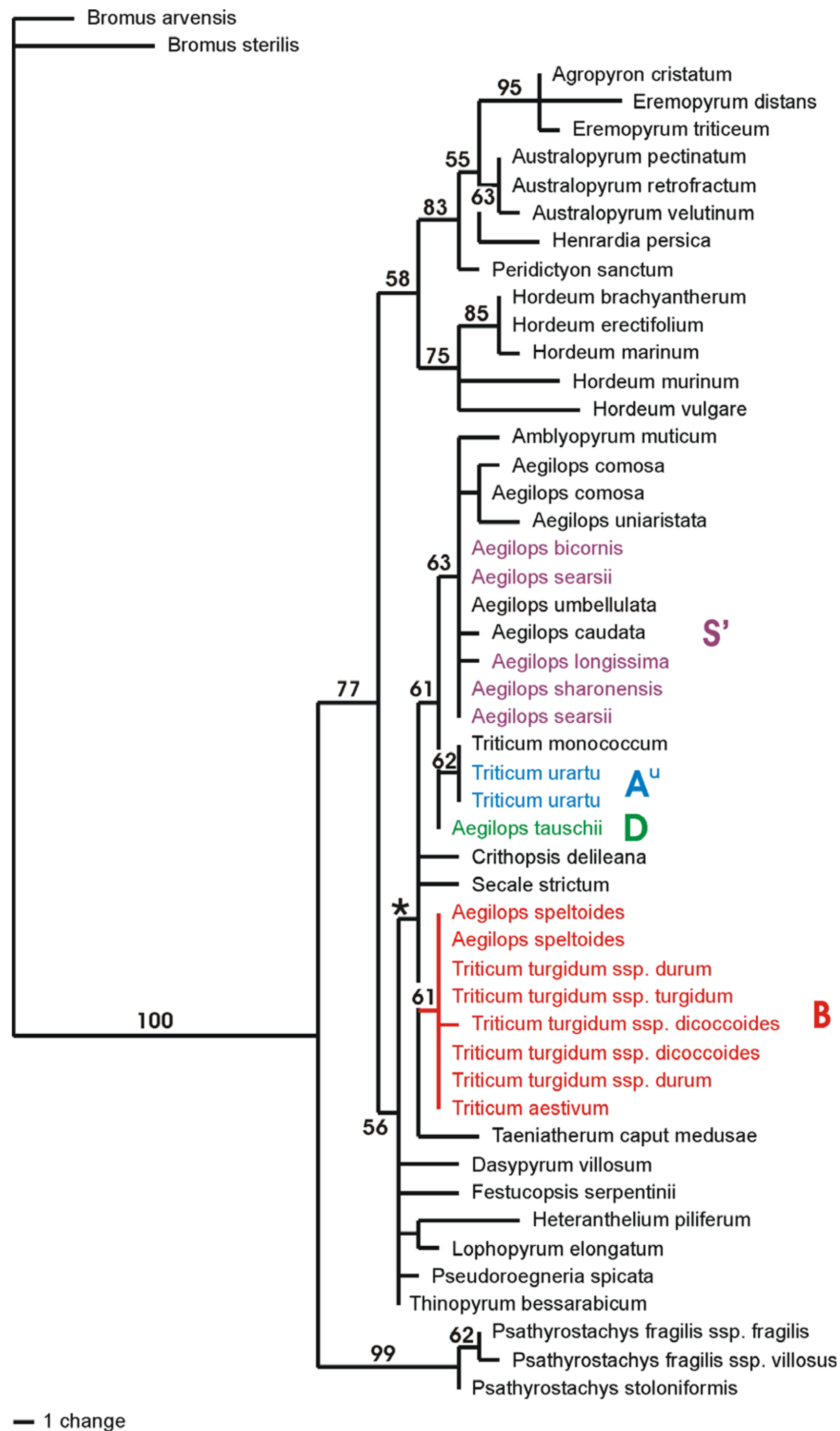

Fig. 3. One of three equally parsimonious gene trees (length 65, ci = 0.83, ri = 0.94) derived from phylogenetic analysis of sequence data from the plastid gene *ndh*F. Branches that collapse in the strict consensus tree are marked with an ∗. Numbers above or below branches are jackknife proportion.

genera paraphyletic. However, the present phylogenetic hypothesis refutes monophyly of *Aegilops* plus *Triticum* (Fig. 4) in agreement with most other phylogenetic analysis of the Triticeae. In our analysis monophyly is severely violated by the position of *Ae. speltoides* and the **B** genome copies of polyploid wheats in a basal trifurcation within the Triticeae. Even if this is ignored a monophyletic group including *Aegilops* and *Triticum* would still have to include *Amblyopyrum* (Jaub. & Spach) Eig, *Thinopyrum* Á. Löve, *Lophopyrum* Á. Löve, and *Crithopsis* Jaub. & Spach. Monophyly of *Aegilops* plus *Triticum* has been refuted by many other molecular data (Kellogg and Appels, 1995; Mason-Gamer, 2001, 2004, 2005; Petersen and Seberg, 1997; Sallares and Brown, 2004; Seberg and Petersen, in press) and by morphological data (Seberg and Frederiksen, 2001).

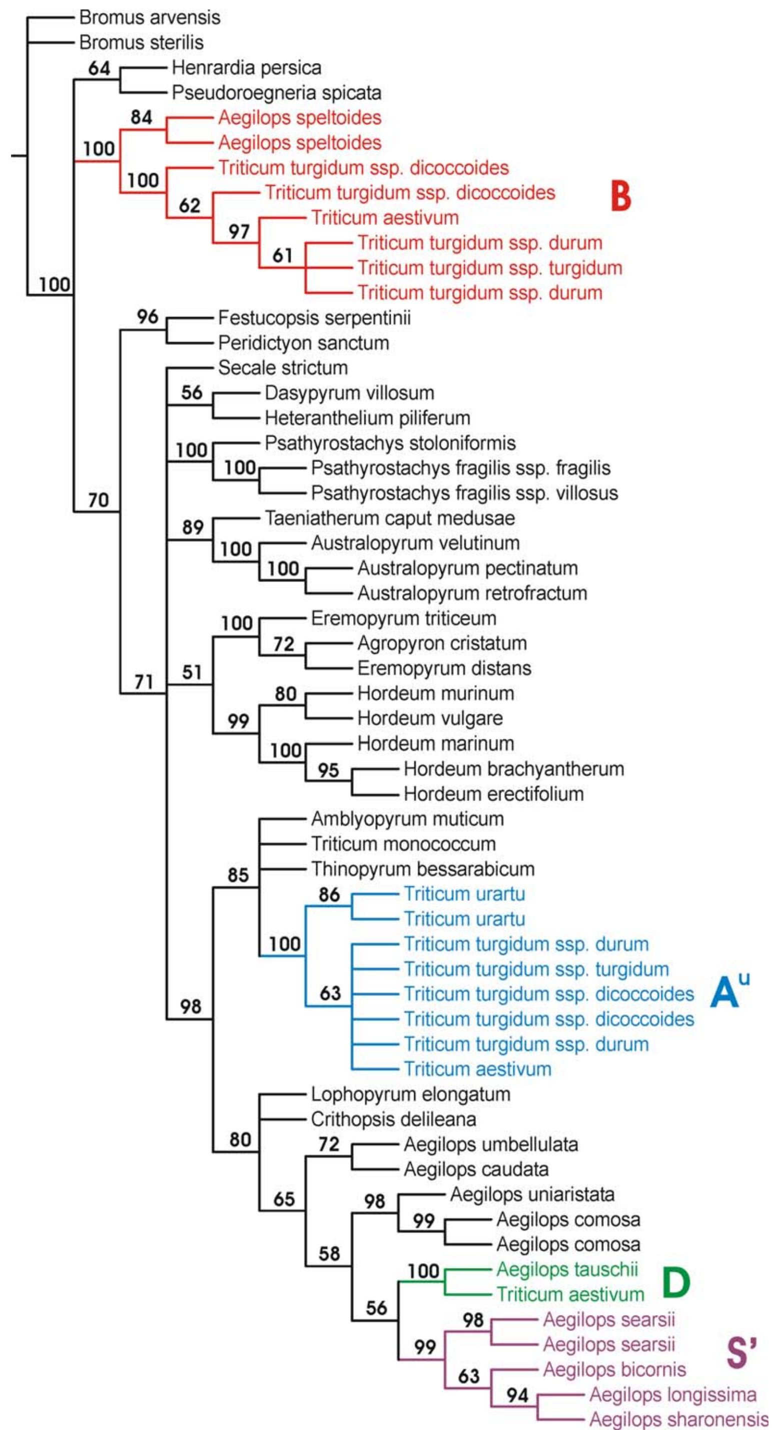

Fig. 4. Strict consensus tree based on eight equally parsimonious trees (length 783, ci = 0.63, ri = 0.85) derived from combined analysis from two nuclear genes, DMC1 and EF–G, and one plastid gene, *ndh*F. Numbers above branches are jackknife proportions.

However, some molecular data do show a monophyletic *Aegilops* plus *Triticum* clade. Hsiao et al. (1995) analysing ITS sequence data found the group monophyletic, but did not include *Amblyopyrum* in their analysis, and only recovered the group after weighting of the data—reanalysis of the unweighted data refuted monophyly (Kellogg et al., 1996). Kellogg and Appels (1995) analysing short spacers of 5S RNA genes also found the group monophyletic, but *Amblyopyrum* and *Ae. speltoides* were not included. Mason-Gamer and Kellogg (1996) and Mason-Gamer et al. (1998, 2002) analysing plastid RFLP data, nucleotide sequences from the granule-bound starch synthase gene, and three different plastid data sets, respectively, recovered the group as monophyletic or potentially monophyletic, but *Amblyopyrum* was still not included in any of the analyses and *Crithopsis* was not included in the latter two. Addition of

other Triticeae taxa to the granule-bound starch synthase gene data set made the group non-monophyletic (Mason-Gamer, 2004). Hence, to various degrees the results from these analyses are not conclusive.

4.2. Is Aegilops monophyletic?

Given the basal position of *Ae. speltoides* within the Triticeae the genus *Aegilops* is not monophyletic in the present analysis (Fig. 4). Monophyly is also violated by the position of the **D** genome of *T. aestivum* within a weakly supported *Aegilops* clade containing all species of *Aegilops* except *Ae. speltoides*. The special problems related to the allopolyploid wheats will be dealt with in further detail below and are not discussed further here.

Previously published phylogenetic analyses based on other molecular data also mostly refute monophyly of *Aegilops*. Plastid and nuclear RFLP data showed diploid species of *Triticum* embedded within *Aegilops* (Dvořák and Zhang, 1992; Mason-Gamer and Kellogg, 1996). Plastid *rpo*A and *rbc*L sequence data showed that *Aegilops* as a minimum would have to include *Triticum* and *Amblyopyrum*, too, though monophyly potentially could be restored by the exclusion of *Ae. speltoides* (Petersen and Seberg, 1997; Seberg and Petersen, in press). Combined analysis of three plastid data sets (*rpo*A, three tRNA gene spacers, RFLPs) supported inclusion of *Triticum* (*Amblyopyrum* is not sampled) and *Ae. speltoides* as the sister to the *Triticum–Aegilops* clade (Mason-Gamer et al., 2002), whereas combined analysis of only two of the data sets (*rpo*A, three tRNA gene spacers) showed *Ae. speltoides* plus *Taeniatherum* Nevski as the sister group to the remaining species of *Aegilops* (Mason-Gamer, 2004). However, in the latter analysis neither *Amblyopyrum* nor *Triticum* were included.

Whereas plastid data consistently refutes monophyly of *Aegilops*, other nuclear sequences not included in the present study give different results. Data from the long spacer units of 5S RNA genes excluded *Ae. speltoides* from a monophyletic group of four other species of *Aegilops*, but the short spacer units of the 5S RNA genes did show a monophyletic *Aegilops* (Kellogg and Appels, 1995). However, the latter analysis did not include *Ae. speltoides*. The analysis of ITS data by Hsiao et al. (1995) resulted in a monophyletic *Aegilops*, but only two species (*Ae. speltoides* and *Ae. tauschii*) were included and *Amblyopyrum* was not sampled. In another analysis of ITS sequence data including more species of *Aegilops*, but only *Triticum* and *Amblyopyrum* as representatives of other Triticeae genera, *Amblyopyrum* was grouped within *Aegilops* (Wang et al., 2000a). Granule-bound starch synthase gene sequences supported *Aegilops* (incl. *Ae. speltoides*) as monophyletic, but *Amblyopyrum* and *Taeniatherum* were not sampled (Mason-Gamer et al., 1998). Addition of *Taeniatherum* plus many more taxa from the Triticeae made *Aegilops* non-monophyletic, with the four species included being located in two groups (*Ae. speltoides*, *Ae. caudata* and *Ae. tauschii*, *Ae. uniaristata*) which together can only be made monophyletic by inclusion of *Triticum*, *Dasypyrum* (Coss. & Durieu) T. Durand, *Lophypyrum*, and *Thinopyrum*. Recent data from β-amylase genes confirmed non-monophyly of *Aegilops*, but the phylogenetic relationships suggested by the data may be strongly influenced by a varying number of paralogs found in different species included in the study (Mason-Gamer, 2005). Not even morphological data used in the circumscription of the genus *Aegilops* clearly support monophyly of *Aegilops*, which is included in an unresolved clade together with *Amblyopyrum* (Seberg and Frederiksen, 2001). Morphology, however, leaves no doubt about the inclusion of *Ae. speltoides* in the clade.

Hence, the majority of evidence seems to suggest that *Aegilops* is not monophyletic. However, it is possible that monophyly can be restored just by the exclusion of *Ae. speltoides*. To maintain only monophyletic higher level taxa *Ae. speltoides* would have to be removed from *Aegilops* and transferred to the genus *Sitopsis* (Jaub & Spach) Á. Löve. *Sitopsis* was originally formed to comprise all members of *Ae*. section *Sitopsis* (Löve, 1982, 1984), but in the proposed circumscription the genus would be monotypic.

Within *Aegilops* Slageren (1994) recognized five sections all of which include diploid species. Three sections include only one diploid species (*Ae. umbellulata* (**U**) in section *Aegilops*; *Ae. caudata* (**C**) in section *Cylindropyrum* (Jaub. & Spach) Zhuk.; *Ae. tauschii* (**D**) in section *Vertebrata* Zhuk. emend. Kihara) together with one or more polyploid species. The remaining sections only include diploid species: *Ae. comosa* (**M**) and *Ae. uniaristata* (**N**) belong to section *Comopyrum* (Jaub. & Spach) Zhuk. and *Ae. speltoides* (**S**), *Ae. bicornis* ($\mathbf{S^b}$), *Ae. longissima* ($\mathbf{S^l}$), *Ae. searsii* ($\mathbf{S^s}$), and *Ae. sharonensis* ($\mathbf{S^l}$) belong to section *Sitopsis*. Whereas section *Comopyrum* is strongly supported (98% jackknife) as monophyletic by the present analysis section *Sitopsis* is not (Fig. 4). In the latter section the position of *Ae. speltoides* as dealt with above violates monophyly, but the remaining species do form a strongly supported monophyletic group (99% jackknife). Hence, it seems appropriate to maintain **S** as the genomic designation for species of section *Sitopsis*, whereas we agree with Sallares and Brown (2004) that the genome of *Ae. speltoides* should be designated **B** rather than **S**. However, the study by Huang et al. (2002a) showed that some, but not all specimens of *Ae. speltoides*, formed a monophyletic group together with other species of section *Sitopsis*, suggesting that further studies are needed to clarify the relationships of *Ae. speltoides*. Apart from *Ae. caudata* section *Cylindropyrum* comprises only one tetraploid species, *Aegilops cylindrica* Host with the genomic composition **CD**. As *Ae. caudata* and *Ae. tauschii* are not sister taxa section *Cylindropyrum* is non-monophyletic. Section *Vertebrata* including *Ae. tauschii* comprises tetraploid and hexaploid species also including the **N**, the **U**, and some "unknown" genomes (Wang et al., 1996). The present analysis shows section *Sitopsis* (excl. *Ae. speltoides*) as the sister to *Ae. tauschii* (and the **D** genome of *T. aestivum*), hence, section *Vertebrata* is also non-monophyletic. The same is true for section *Aegilops*, where the tetraploid and hexa-

ploid species combine the **U** genome with the genomes **M**, **N**, **C**, and **S**.

Few other phylogenetic analyses have included a sufficient number of species to bear evidence on *Aegilops* phylogeny. However, Mason-Gamer and Kellogg (1996) and Sallares and Brown (2004) both confirmed that *Ae. speltoides* is distinct from other species of the genus, but that the remaining species of section *Sitopsis* constitute a monophyletic group. In contrast, Dvořák and Zhang (1992) recovered the entire section *Sitopsis* as monophyletic, but their analysis only included species of *Aegilops*, *Triticum*, *Amblyopyrum*, and *Lophopyrum*. Two analyses based on RFLP data confirmed monophyly of section *Comopyrum* and recovered the relationship between *Ae. caudata* and *Ae. umbellulata* found in the present study (Dvořák and Zhang, 1992; Mason-Gamer and Kellogg, 1996). These two relationships are further supported by sequence data from the granule-bound starch synthase gene (Mason-Gamer et al., 1998), whereas the data did not support section *Sitopsis* even in a restricted sense. Given that the genomic designations of polyploid species of *Aegilops* correctly depict the evolutionary history of the species it seems futile to maintain the sectional division of the genus. However, phylogenetic analyses of the polyploid species are still needed to confirm or reject the suggested genome relationships.

4.3. Is *Triticum* monophyletic?

The genus *Triticum* consists of only two diploid and four allopolyploid species (Slageren, 1994). Disregarding the position of the polyploid species, the present phylogenetic hypothesis suggests that the diploid species, *Triticum monococcum* (**A^m^**) and *T. urartu* (**A^u^**), may form a monophyletic group (Fig. 4). However, *Amblyopyrum* may have to be included in the group. Many other phylogenetic analyses have confirmed monophyly of the diploid species (Huang et al., 2002a; Mason-Gamer, 2001, 2004; Mason-Gamer and Kellogg, 1996; Mason-Gamer et al., 1998; Sallares and Brown, 2004; Zhang et al., 2002), but unfortunately only one of these studies have included *Amblyopyrum*.

The phylogenetic relationships of tetraploid *T. turgidum* (**BA^u^**) and hexaploid *T. aestivum* (**BA^u^D**) obviously violate monophyly of *Triticum* (Fig. 4). *T. turgidum* has received its nuclear genome from *T. urartu* (100% jackknife) and *Ae. speltoides* (100% jackknife) (Figs. 1 and 2) with the latter acting as the plastid donor (Fig. 3), and the additional genome of *T. aestivum* has been paternally derived from *Ae. tauschii* (Figs. 1–4). The phylogenetic relationships of the three wheat genomes are all strongly supported by the jackknife (100%). Accordingly, our data strongly confirm the traditional scenario of the evolution of cultivated wheat (e.g., Cox, 1998), as do most other recent phylogenetic studies of the origin of polyploid wheats. The study of Sallares and Brown (2004) based on external transcribed spacers of the 18S rRNA gene is consistent with the above hypothesis. *Ae. tauschii* was confirmed as the **D** genome donor of *T. aestivum* and *Ae. speltoides* as the **B** genome donor of *T. turgidum*. The relationship between *T. turgidum* and *Ae. speltoides* was also confirmed by Zhang et al. (2002) based on studies of ITS sequence data, but *T. monococcum* rather than *T. urartu* was found as the closest relative of the **A^u^** genome of *T. turgidum*. As representatives of *Aegilops* the study included only members of section *Sitopsis* and *Ae. tauschii*, hence, its conclusiveness is limited. The same restricted sampling of *Aegilops* species was used by Huang et al. (2002a,b), but they included multiple specimens of most species allowing for a test of intraspecific variation and species monophyly. Accordingly, the results of their studies based on sequence data from two nuclear genes, acetyl-CoA carboxylase (ACC) and 3-phosphoglycerate kinase (PGL), are more complex. Both genes recovered *Ae. tauschii* as the closest relative of the **D** genome of hexaploid wheat, but one specimen of *Ae. tauschii* was not member of the clade on the ACC gene tree (Huang et al., 2002a; Fig. 1). However, the position of this specimen on the neighbor-joining tree is not supported on the corresponding strict consensus tree derived from parsimony analysis and monophyly of a group consisting of both specimens of *Ae. tauschii* plus the **D** genome sequence of *T. aestivum* was not contradicted. Considering only clades supported by parsimony the closest relatives to the **A** genome sequences of both tetraploid and hexaploid wheat species were the two diploid species of *Triticum*, but the resolution of the trees did not allow for a distinction between them (Huang et al., 2002a). The relationship of the **B** genome sequences of *T. turgidum* and *T. aestivum* was unresolved both on the ACC and PGK gene trees (Huang et al., 2002a). A sister group relationship to *Ae. speltoides* is among the solutions, but only for a subset of specimens of *Ae. speltoides*. *Ae. tauschii* and the species of section *Sitopsis* were also the only representatives of *Aegilops* included in a study based on sequences from 14 assumed homologous nuclear loci by Blake et al. (1999). A combined analysis of all loci revealed *Ae. speltoides* as the sister to *T. aestivum*, though some individual loci were in conflict. Buchner et al. (2004) analysing sequence data from two sulfate transporter genes confirmed the relationship of *T. aestivum* to *Ae. tauschii* and *T. urartu*, but only one of two gene trees confirmed the relationship to *Ae. speltoides*. As the study included no other diploid species of *Aegilops* and *Triticum*, its conclusiveness is very limited. Hence, the vast majority of phylogenetic evidence confirms the traditional interpretation of the origin of cultivated wheat. Some authors (e.g., Zhang et al., 2002) finding a sister group relationship between *Ae. speltoides* and polyploid wheats have concluded that *Ae. speltoides* either was the **B** genome donor or was the most closely related species to a possibly extinct donor species. However, we find no justification for invoking extinct species as genome donors to the polyploid species of wheat. Only when the sister group to a polyploid species is found to be a clade including two or more species is it become meaningful to speak about an extinct donor.

Given the phylogenetic relationships of the polyploid species of *Triticum* involving two species of *Aegilops*—one

of which might even better be referred to a separate genus *Sitopsis*—the genus *Triticum* is obviously not monophyletic. Hence, if only monophyletic genera are to be accepted all other Triticeae species, except perhaps *Henrardia persica* and *Pseudoroegneria* spp., would have to be included in one genus. This is hardly a practically acceptable solution, though suggested previously (Stebbins, 1956). The basal position of *Ae. speltoides* (and the **B** genome in polyploid wheats) may be regarded as spurious, but even if future studies show it to be incorrect a monophyletic group including diploid and polyploid species of *Aegilops* and *Triticum* will also have to include *Amblyopyrum*, *Thinopyrum*, *Lophopyrum*, and *Crithopsis* (Fig. 4). This would cause extensive name changes (or rather uses of names—most species of Triticeae have previously been included in *Triticum*). However, in the Triticeae is has been widely accepted to recognize paraphyletic genera caused by the occurrence of wide allopolyploids, and these allopolyploids have been referred to separate polyphyletic genera (see Kellogg, 1989). Hence, the diploid species of *Triticum* has been referred to the genus *Crithodium* Link, the $\mathbf{BA^u}$ polyploids to *Gigachilon* Seidl, and hexaploid, $\mathbf{BA^uD}$ *T. aestivum* is the only species left in *Triticum* (Löve, 1984).

4.4. Future phylogenetic studies

Many of the ambiguities emerging from analysis of single or a few data sets can hopefully be solved by combined analysis of more data sets. Hence, future phylogenetic studies of *Aegilops* and *Triticum* may benefit from the many data sets already present, but a substantial amount of new data should be gathered to minimize missing entries in a combined matrix. To provide conclusive results we recommend that the taxon sampling of such studies as a minimum includes representatives of all genera or genome groups within the Triticeae. To take the potential nonmonophyly of species into account it is desirable to include more accessions of the species that a particular phylogenetic analysis is focused on.

4.5. Differential diversification rates among the $\mathbf{A^u}$ and **B** genomes

It has previously been shown that the **B** genome of polyploid wheats is more variable than any of the other two genomes (see Wendel, 2000). Being donated at a more recent date (Cox, 1998) the **D** genome in hexaploid wheat is expected to show less variation than the other genomes. However, the hybridization event leading to the formation of allotetraploid *T. turgidum* establishes an equal divergence time for the two other genomes, $\mathbf{A^u}$ and **B**. Provided that each genome, $\mathbf{A^u}$ and **B**, was donated only once (a hypothesis corroborated by the present study) a higher level of variation in the **B** genome implies a higher diversification rate of the **B** genome as compared to the $\mathbf{A^u}$ genome in polyploid wheat. This is supported by the present data as the number of character changes in the **B** genome of the polyploids exceeds the numbers of changes in the $\mathbf{A^u}$ genome by a factor four for the DMC1 sequences and by a factor two for the EF–G sequences.

Whether the rate difference is restricted to the polyploids or whether a rate difference is also a characteristic of the **B** and $\mathbf{A^u}$ genome donors is not clear from the present data as both donor species are represented only two randomly chosen specimens in our analyses. However, the actually observed higher numbers of character changes in both nuclear genes in the **B** genome diploids (EF–G: 5–6 changes; DMC1: 4 changes) compared to the $\mathbf{A^u}$ genome diploids (EF–G: 2 changes; DMC1: 0 changes) may suggest that a rate difference also exists between the diploids. Inclusion of more specimens from both species is required to test the hypothesis.

In addition to polyploid wheats, the study by Huang et al. (2002a) did include several accessions of *Ae. speltoides* but only one accession of *T. urartu*. Their Table 3 (Huang et al., 2002a: supporting information) shows substitution rates calculated for the ACC and PGK nuclear genes, with a more than 10-fold increase in the **B** genome compared to the $\mathbf{A^u}$ genome in both genes. However, the difference may be influenced by the unequal number of specimens of the two diploids, and the calculations do not allow for a distinction between rates in diploids and polyploids.

Whether the higher diversification rate of the **B** genome sequences compared to the $\mathbf{A^u}$ genome sequences is restricted to the polyploids or not, it is a likely reason for the hitherto greater difficulty of identifying the **B** genome donor compared to the $\mathbf{A^u}$ and **D** genome donors. How differential diversification rates are controlled or maintained in the polyploid species of wheat remains an open question.

Acknowledgments

This works was funded by the Danish National Sciences Research Council Grant No. 21-03-0511. We thank C. J. Humphries and an anonymous reviewer for valuable comments on the manuscript and we acknowledge the inestimable importance of the lab work assistance provided by C. Hansen and L. Knudsen.

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