Mol Genet Genomics
DOI 10.1007/s00438-010-0539-2

ORIGINAL PAPER

Molecular markers based on LTR retrotransposons *BARE-1* and *Jeli* uncover different strata of evolutionary relationships in diploid wheats

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Received: 4 December 2009 / Accepted: 29 March 2010
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Abstract Molecular markers based on retrotransposon insertions are widely used for various applications including phylogenetic analysis. Multiple cases were described where retrotransposon-based markers, namely sequence-specific amplification polymorphism (SSAP), were superior to other marker types in resolving the phylogenetic relationships due to their higher variability and informativeness. However, the patterns of evolutionary relationships revealed by SSAP may be dependent on the underlying retrotransposon activity in different periods of time. Hence, the proper choice of retrotransposon family is essential for obtaining significant results. We compared the phylogenetic trees for a diverse set of diploid A-genome wheat species (*Triticum boeoticum*, *T. urartu* and *T. monococcum*) based on two unrelated retrotransposon families, *BARE-1* and *Jeli*. *BARE-1* belongs to *Copia* class and has a uniform distribution between common wheat (*T. aestivum*) genomes of different origin (A, B and D), indicating similar activity in the respective diploid genome donors. *Gypsy*-class family *Jeli* was found by us to be an A-genome retrotransposon with >70% copies residing in A genome of hexaploid common wheat, suggesting a burst of transposition in the history of A-genome progenitors. The results indicate that a higher *Jeli* transpositional activity was associated with *T. urartu* versus *T. boeoticum* speciation, while *BARE-1* produced more polymorphic insertions during subsequent intraspecific diversification; as an outcome, each retrotransposon provides more informative markers at the corresponding level of phylogenetic relationships. We conclude that multiple retroelement families should be analyzed for an image of evolutionary relationships to be solid and comprehensive.

Keywords Retrotransposon · Wheat · Evolutionary genomics · Phylogeny · Molecular markers

Communicated by M.-A. Grandbastien.

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Introduction

Retrotransposons, mobile genetic elements with the ability to make copies of themselves via reverse transcription of RNA intermediate, are a major component of many plant genomes (Feschotte et al. 2002; Kumar and Bennetzen 1999). In grasses, retrotransposon-related sequences may account up to 70% of the total genome size (Wicker et al. 2009) or possibly more; their transpositional activity, susceptibility to deletions and the ability to alter the transcription of genes makes retroelements a powerful force driving the structural and functional evolution of plant genomes (Bennetzen 2000; Lisch 2009).

Most retrotransposons create novel copies of themselves randomly throughout the genome, although a preference for tRNA genes and other genes transcribed by RNA polymerase III was demonstrated experimentally for *Ty3* and, to a lesser extent, for *Ty1* in yeast (Chalker and Sandmeyer 1990; Ji et al. 1993); some clustering in gene-poor regions was also observed for plant LTR retrotransposons (Bertioli et al. 2009; Heslop-Harrison et al. 1997;

Published online: 21 April 2010

Messing et al. 2004). Each insertion is non-reversible, other than by means of random deletions via recombination. It can usually be assumed that an ancestral state (absence at a given site) is known for each inserted copy and that independent insertion events into the same site are unlikely to occur. Polymorphic retrotransposon insertions can therefore be a powerful marker system for evolutionary studies (Ellis et al. 1998; Kumar and Hirochika 2001; Lee et al. 1990; Pearce et al. 2000; Schulman 2007; Tam et al. 2005; Vershinin et al. 2003).

Among the analysis methods developed for revealing the polymorphic retrotransposon insertions, sequence-specific amplification polymorphism (SSAP) has a high multiplex ratio and flexibility in regard to copy number of retrotransposon families analyzed (Ellis et al. 1998; Jing et al. 2005; Waugh et al. 1997). This approach has been successfully used for genetic mapping, diversity and evolutionary studies in many plant species (Ellis et al. 1998; Lou and Chen 2007; Pearce et al. 2000; Queen et al. 2004; Sanz et al. 2007; Syed et al. 2005, 2006; Tam et al. 2005; Vukich et al. 2009; Waugh et al. 1997).

In evolutionary studies of complex taxa, like *Triticum*/*Aegilops* group of species, one of the limiting factors is the ability of molecular techniques to detect diversity at the required taxonomic level; in addition, the multiplex systems like amplified fragment length polymorphism (AFLP) provide random markers that are distributed evenly (although with possibly varying polymorphism levels) across all the chromosomes in allopolyploid species, complicating the comparisons between species with different ploidy levels (Brandolini et al. 2006). High polymorphism provided by retrotransposon insertions makes them a promising source of markers for phylogenetic studies in wheat (Gribbon et al. 1999). Novel data are also emerging on transposon families unevenly distributed among the A, B or D wheat genomes (Charles et al. 2008; Sabot et al. 2006); the use of such families for development of genome-targeted markers requires further investigation.

Sequence-based analysis in A and B genome of polyploid wheat species revealed multiple waves of retrotransposon amplification, and the age of major waves was different for two major long terminal repeat (LTR) retrotransposon classes, *Ty1*/*Copia* and *Ty3*/*Gypsy* (Charles et al. 2008). This fact may possibly affect the patterns of genetic polymorphism revealed by SSAP markers based on the two LTR retroelement classes, as was shown for *PDR1*, *Cyclops* and *Pis1* mobile elements in pea (Vershinin et al. 2003). Diversity patterns based on specific *Ty1*/*Copia* families can also be different, as described by Pearce et al. (2000). We performed a comparative analysis of two retrotransposon families, *BARE-1* (*Copia* class) and *Jeli* (*Gypsy* class) as potential sources of polymorphic SSAP markers for phylogenetic studies in wheat. First, we determined the genomic distribution of *Jeli* in hexaploid wheat and compared the results with those published earlier for *BARE-1* (Queen et al. 2004). Second, we performed a phylogenetic analysis in diploid A-genome wheat accessions with SSAP markers based on these two families of mobile elements, with special attention to botanical classifications of the species. Diploid wheat is a source of the A genome for all polyploid wheat species and has a complex taxonomic structure (reviewed by Barkworth and von Bothmer 2009). Although the group has been studied extensively, many evolutionary questions still emerge (Goncharov et al. 2007; Heun et al. 1997, 2008; Kilian et al. 2007; Mac Key 1989), making it useful to establish a comprehensive view on the genetic diversity that retrotransposon-based markers can provide.

Materials and methods

Plant material

The plant seeds used in this study were received from the Vavilov All-Russian Institute of Plant Industry (VIR; St. Petersburg, Russia) and from other collections indicated in Table 1. The nulli-tetrasomic lines of common wheat cv. Chinese Spring (monosomic-tetrasomic for chromosomes 2A and 4B) were obtained from Wheat Genetic and Genomic Resources Center (Kansas State University, USA). Identification of diploid wheat species was made in accordance to Dorofeev et al. (1979). Total DNA was extracted from individual plants by a version of the CTAB protocol (Torres et al. 1993) with minor modification.

SSAP protocol

For the multiplex generation of retrotransposon-derived markers, the general principle of SSAP was applied; however, instead of using the original SSAP procedure (Waugh et al. 1997), we generally followed the protocol for NBS-profiling (van der Linden et al. 2004) with some modifications. This technique utilizes the amino blocked adapter system designed to suppress the amplification of adapter-to-adapter fragments (Fischer et al. 1995). Thirty nanograms of total DNA were digested by 10 U of *Taq*$^{\alpha}$I (NEB) in 40 µl reaction volume for 3 h at 65°C, followed by addition of 10 µl ligation mix containing 2.5 Weiss units of T4 DNA ligase (Invitrogen), 5 mM ATP (Fermentas) and 50 pmol of double-stranded adapter [upper strand: 5′-ACTCGATTCTCAACCCGAAAGTATAGATCCCA; lower strand: 5′-PO_4-CGTGGGATCTATACTT-(C_6 linker)-NH_2]. Restriction and ligation were performed in 1× buffer containing 50 mM Tris–HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 100 mM NaCl and 1 mM DTT. After 6-h

Table 1 List of diploid wheat accessions used in the study

ID	Species	Subspecies, if known[a]	Genebank	GB acc.	Origin
BO1	*T. boeoticum*/*T.urartu*[b]	–	VIR	K-58502	Lebanon
BO2	*T. boeoticum*	ssp. *thaoudar*	Kyoto	KU8120	Iraq
BO3	*T. boeoticum*	–	VIR	K-25811	Armenia
BO4	*T. boeoticum*	ssp. *boeoticum*	VIR	K-14384	Turkey
BO5	*T. boeoticum*	–	VIR	K-28132	Armenia
BO6	*T. boeoticum*	–	VIR	K-28300	Azerbaijan
BO7	*T. boeoticum*	ssp. *thaoudar*	Kyoto	KU8136	Iraq
BO8	*T. boeoticum*	ssp. *boeoticum*	VIR	K-61600	Greece
BO9	*T. boeoticum*	–	ICARDA	IG116198	Turkey
BO10	*T. boeoticum*	ssp. *boeoticum*/ssp. *thaoudar*[b]	VIR	K-20741	Germany
BO11	*T. boeoticum*	ssp. *thaoudar*	Kyoto	KU8162	Iraq
BO12	*T. boeoticum*	ssp. *boeoticum*	VIR	K-18424	Ukraine
BO13	*T. boeoticum*	ssp. *boeoticum*	VIR	K-27148	Turkey
BO14	*T. boeoticum*	–	USDA	PI-427328	Iraq
BO15	*T. boeoticum*	ssp. *thaoudar*	VIR	K-40118	Iraq
BO17	*T. boeoticum*	ssp. *thaoudar*	Kyoto	KU8059	Iraq
BO18	*T. boeoticum*	–	VIR	K-40117	Iraq
BO19	*T. boeoticum*	ssp. *boeoticum*	VIR	K-33869a	Armenia
BO20	*T. boeoticum*	–	Austral.	19372	Iran
BO21	*T. boeoticum*	–	Austral.	19375	Iran
BO22	*T. boeoticum*	–	Austral.	19371	Iran
BO24	*T. boeoticum*	–	Austral.	19373	Iran
BO25	*T. boeoticum*	–	VIR	K-27147	Turkey
BO27	*T. boeoticum*	–	Austral.	19376	Iran
BO28	*T. boeoticum*	–	UC	G2511	Iran
BO29	*T. boeoticum*	–	UC	G2523	Iran
BO30	*T. boeoticum*	–	VIR	K-58508	Armenia
MO1	*T. monococcum*	–	VIR	K-18140	Azerbaijan
MO2	*T. monococcum*	–	VIR	K-20398	Germany
MO3	*T. monococcum*	–	VIR	K-20409	Spain
MO4	*T. monococcum*	–	VIR	K-62499	UK
SI1	*T. sinskajae*	n/a	VIR	K-48993	Turkey
UR1	*T. urartu*	n/a	USDA	PI-538736	Lebanon
UR2	*T. urartu*	n/a	VIR	K-33869b	Armenia
UR3	*T. urartu*	n/a	USDA	PI-428276	Lebanon
UR4	*T. urartu*	n/a	ICARDA	IG116196	Turkey
UR5	*T. urartu*	n/a	VIR	K-33871	Armenia
UR6	*T. urartu*	n/a	ICARDA	IG45298	Syria
UR11	*T. urartu*	n/a	USDA	PI-428309	Lebanon
UR12	*T. urartu*	n/a	USDA	PI-428305	Lebanon
UR30	*T. urartu*	n/a	USDA	PI-428257	Armenia
UR31	*T. urartu*	n/a	VIR	K-58497	Iran
UR34	*T. urartu*	n/a	USDA	PI-428181	Armenia
UR35	*T. urartu*	n/a	USDA	PI-554599	Turkey
UR36	*T. urartu*	n/a	USDA	PI-554600	Turkey
UR38	*T. urartu*	n/a	USDA	PI-538746	Lebanon
UR44	*T. urartu*	n/a	USDA	PI-428182	Armenia
UR47	*T. urartu*	n/a	USDA	PI-428196	Turkey

Table 1 continued

ID	Species	Subspecies, if known[a]	Genebank	GB acc.	Origin
UR50	*T. urartu*	n/a	USDA	PI-428200	Turkey

T. boeoticum = *Triticum boeoticum* Boiss.; *T. urartu* = *Triticum urartu* Thum. *ex* Gandil.; *T. monococcum* = *Triticum monococcum* L.; *T. sinskajae* = *Triticum sinskajae* A. Filat. et Kurk. (a natural naked mutant discovered within *T. monococcum* (Goncharov et al. 2007), it is not referred in the text separately); ssp. *boeoticum* = *Triticum boeoticum* Boiss. ssp. *boeoticum*; ssp. *thaoudar* = Triticum *boeoticum* ssp. *thaoudar* (Reut. ex Hausskn.) Grossh.

VIR All-Russian Vavilov Institute of Plant Industry (St. Petersburg, Russia); *Kyoto* Kyoto University (Kyoto, Japan); *USDA* the National Small Grain Collection (Aberdeen, Idaho, USA); *Austral* Australian Winter Cereals Collection (Tamworth, Australia); *ICARDA* International Center for Agricultural Research in the Dry Areas (Aleppo, Syria); *UC* University of California, California Agricultural Experiment Station (Riverside, California, USA); n/*a* not applicable

[a] The information on subspecies classification was obtained from genebank databases or collection curators

[b] Both species or subspecies were reported by the genebank collection for these heterogenic accessions

ligation at 37°C, the reactions were inactivated for 1 h at 65°C and diluted twice with 50 µl deionized water. The subsequent PCR amplification consisted of two steps. In a preamplification step, 5 µl of diluted ligation mix were added to the tubes containing 1 U TrueStart™ *Taq* DNA polymerase (Fermentas), 1× TrueStart™ buffer, 0.5 mM $MgCl_2$, 20 µM each dNTP and 5 pmol LTR primer in 20 µl total volume. The temperature profile included 5 min denaturation at 95°C, 30 cycles (30 s at 95°C, 1 min at 62°C, 2 min at 72°C) and final elongation at 72°C for 10 min. 15 µl of preamplification products were supplemented with 10 µl PCR mix containing 1 U of *Taq* DNA polymerase (Sileks M, Moscow), 1× PCR buffer with $(NH_4)_2SO_4$ (Sileks M), 25 pmol LTR primer, 25 pmol adapter primer (5′-GTTTACTCGATTCTCAACCCGAAAG), dNTPs to the final concentration 200 µM each, $MgCl_2$ to the final concentration 1.5 mM. The same program as above was used for amplification, with 35 cycles instead of 30. All the reactions were set up in MJ Research PTC-100 thermal cycler.

The LTR primer sequences we used are reverse-complementary to the first bases of retrotransposon 5′-LTRs. *BARE-1* primer sequence is 5′-CTAGGGCATAATTCCAACA, as published in (Queen et al. 2004); in wheat, this primer also anneals at the LTR of *Wis2-1A* retroelement. *Jeli* primer sequence is 5′-CCCTAGGAACATAGCTTCATCA, based on *Jeli* consensus sequence TREP3458 from the ITMI Triticeae Repeat Sequence Database (Wicker et al. 2002). In order to reduce the number of amplification products, 2–4 selective bases were added to the 3′ ends of LTR primers: ACG, ACTG, AGTC or AGCA for *BARE-1* primers; AG, CAA, CTG, GAC, CTC or GGA for *Jeli* primers.

2.5 µl of PCR reaction products were separated by sequencing PAGE in a 38 × 50 cm Bio-Rad SequiGen GT cell, according to manufacturer's instructions. DNA fragments were visualized by silver staining (Bassam and Gresshoff 2007). The specificity of SSAP reaction was confirmed by sequencing 17 random bands which were excised from *BARE-1* gel-separated products and reamplified in the same conditions as the 2nd PCR step described above. All of the bands contained the sequence complementary to LTR primer at their 3′ ends (Genbank accessions GQ409834-GQ409850).

Data analysis

SSAP band patterns were scored visually. The genetic distances between accessions were calculated from the binary presence/absence matrices using Dice formula (Dice 1945). Neighbor-Joining phylogenetic trees were constructed in SplitsTree 4.10 (Huson and Bryant 2006) with bootstrapping (5,000 replicates). For principal component analysis (PCA), PAST package was used (Hammer et al. 2001).

Results

Jeli genomic distribution in *Triticum aestivum* L

Individual *Jeli* insertions, represented by SSAP bands, were assigned to wheat chromosomes using a set of common wheat cv. Chinese Spring (CS) nulli-tetrasomic lines developed by Sears (1954). In total, 168 bands were scored as missing in individual lines compared to all other lines and control (Fig. 1; Table 2). The majority of insertions was assigned to A-genome chromosomes (71.4%). In B-genome chromosomes, the number of insertions was one-fourth as large (17.9%), and D genome contained only 10.7% of all scored markers. At least one insertion was found in most chromosomes of common wheat, the exceptions being the 5D chromosome, which can be attributed to chance, and the 2A and 4B chromosomes, apparently because of mono-tetrasomic karyotype of the corresponding plants (Sears 1954). In the A genome,

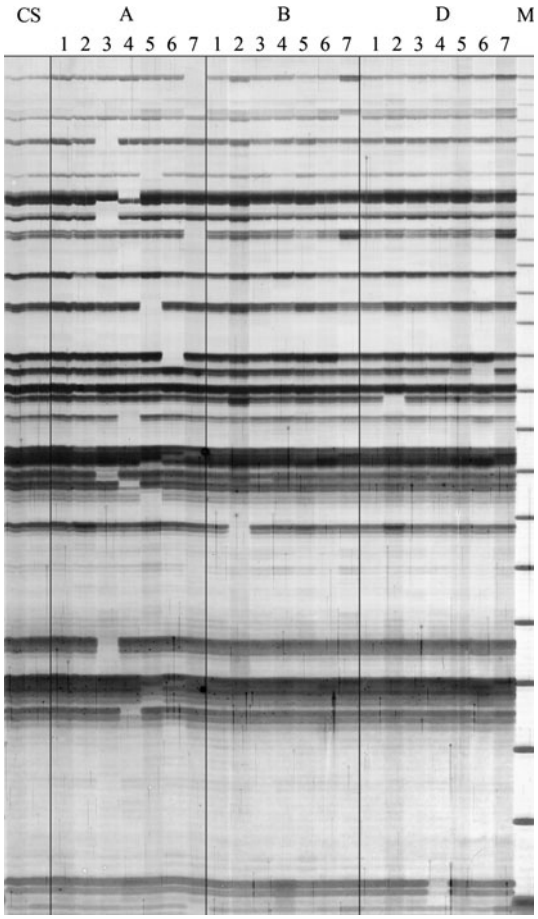

Fig. 1 SSAP markers generated using *Jeli* LTR primer (selective bases AG) for the control (Chinese Spring, CS) and nulli-tetrasomic lines of *T. aestivum* (the respective genome and chromosome is indicated above). M = 10 bp DNA ladder (Invitrogen)

chromosome 5A carried the most *Jeli* copies (27 insertions), while chromosome 3B had more than one-third of the insertions assigned to the B genome (11 out of 30). No novel bands were detected in aneuploid lines compared to the control. We also repeated the nulli-tetrasomic analysis for *BARE-1* using a limited primer set; the results were in agreement to the ones presented by Queen et al. (2004) (data not shown). When the published *BARE-1* frequency data was used as an expectation for the numbers of *Jeli* insertions in the A, B and D genomes, a chi-square test revealed significant difference between the genome-wide insertion distributions ($P = 3.1 \times 10^{-19}$). If the individual homeologous groups are considered, this difference is not so evident for chromosomes 1, 4 and 6 ($0.001 < P < 0.01$), but it is very significant for chromosomes 5 and 7 (P less than 10^{-49}; Table 2).

Therefore, it can be concluded that *Jeli* retroelement is predominantly abundant in the A genome of hexaploid wheat. Since no mechanism is known in polyploid wheats that can be responsible for *Gypsy*-class retrotransposon genome-specific target site preference on such a scale, a massive *Jeli* amplification burst must have occurred in a diploid progenitor of A genome after its divergence from the S or D genome of *Aegilops* lineages. This makes a *Jeli*-based marker system useful for tracing back events that happened in the evolution of diploid A-genome wheat species, concomitant with an increase in *Jeli* transpositional activity.

Diploid wheat diversity revealed by SSAP. Comparative analysis of diversity data generated from *BARE-1* and *Jeli* insertions

The A genome is found only within *Triticum* and is further subdivided to A^u and A^b, to reflect the origin of the genome—the wild diploid wheat species *T. urartu* Thum. ex Gandil. or *T. boeoticum* Boiss., respectively. In order to evaluate the pattern of polymorphism revealed by *Jeli*-based SSAP markers in diploid wheats, we analyzed the genetic diversity among 49 accessions representing the species with A^u genome (*Triticum urartu*) and A^b genome (*T. boeoticum*, *T. monococcum* L., *T. sinskajae* A. Filat. et Kurk.). In addition, we have also used a marker system based on *BARE-1*, a *Copia*-class LTR retroelement which

Table 2 *Jeli* bands mapped to individual chromosomes of wheat

Genome	Chromosome							Total
	1	2	3	4	5	6	7	
A	14	0[a]	23	20	27	15	21	120 (71.4%)
B	4	3	11	0[a]	5	1	6	30 (17.9%)
D	3	4	4	1	0	3	3	18 (10.7%)
Total	21	7	38	21	32	19	30	168
P (χ^2 test)[b]	3.6×10^{-3}	–	9.2×10^{-10}	1.1×10^{-3}	8.5×10^{-87}	6×10^{-3}	3.5×10^{-50}	3.1×10^{-19}

[a] No bands were mapped in these lines due to their mono-tetrasomic karyotype

[b] The normalized *BARE-1* distribution (Queen et al. 2004) was used as an expectation of the number of *Jeli* insertion sites in each chromosome. Chi-square test values were calculated for each homeologous group (except 2 for which *BARE-1* frequency data is incomplete), and for the total values per genome

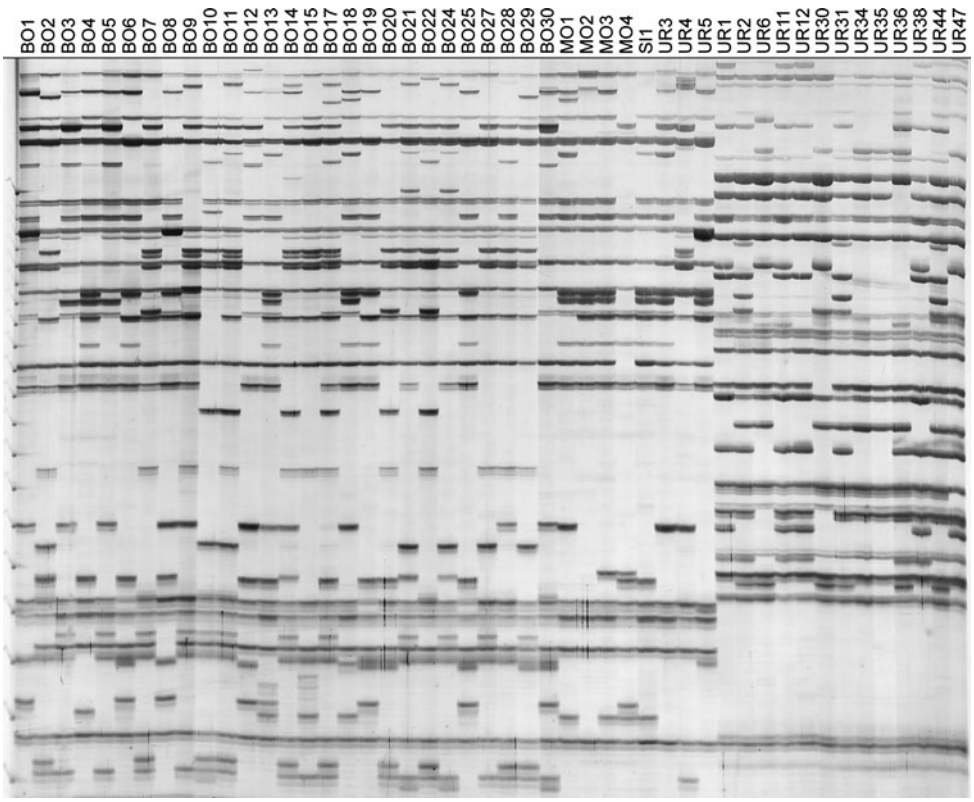

Fig. 2 SSAP markers generated using *BARE-1* LTR primer (selective bases AGCA) for the diploid wheat accessions

had already been successfully exploited for this purpose in studies on wheat, barley and some other *Triticeae* Dum. species (Gribbon et al. 1999; Queen et al. 2004; Waugh et al. 1997). In contrast to *Jeli*, *BARE-1* is known to have similar copy numbers in the A and B genomes of hexaploid wheat, with a reduced number in the D genome (Queen et al. 2004).

In total, 199 polymorphic bands were scored for *Jeli* and 248 polymorphic bands were scored for *BARE-1* (Fig. 2; Table 3). The resulting phylogenetic trees are shown in Fig. 3, along with the tree based on a combined data set (447 markers). As expected, the relationships between A^u- and A^b-genome species, comprising *T. urartu* and *T. boeoticum*/*T. monococcum*, respectively, were resolved very well. There were, however, three accessions designated in the collections as *T. urartu* (UR2, UR3 and UR4) that cluster with A^b-genome wheats. If we take into account the substantial difference in marker states discovered between the

Table 3 Statistical information for *BARE-1* and *Jeli* SSAP diversity data

	BARE-1	*Jeli*	Total
Number of polymorphic markers	248	199	447
within *T. boeoticum*/*T. monococcum*	155 (62.5%)	95 (47.7%)	250 (55.9%)
within *T. boeoticum* Group 1	95 (38.3%)	48 (24.1%)	143 (32.0%)
within *T. urartu*	73 (29.4%)	52 (26.1%)	125 (28.0%)
diagnostic for *T. boeoticum*/*T. monococcum*	9 (3.6%)	22 (11.1%)	31 (6.9%)
diagnostic for *T. urartu*	24 (9.7%)	34 (17.1%)	58 (13.0%)
Average PIC	0.280	0.310	0.293
within *T. boeoticum*/*T. monococcum*	0.265	0.297	0.277
within *T. boeoticum* Group 1	0.289	0.320	0.300
within *T. urartu*	0.339	0.347	0.343
'Presence' allele frequency	0.263	0.314	0.286
within *T. boeoticum*/*T. monococcum*	0.389	0.517	0.442
within *T. boeoticum* Group 1	0.515	0.700	0.591
within *T. urartu*	0.626	0.697	0.660

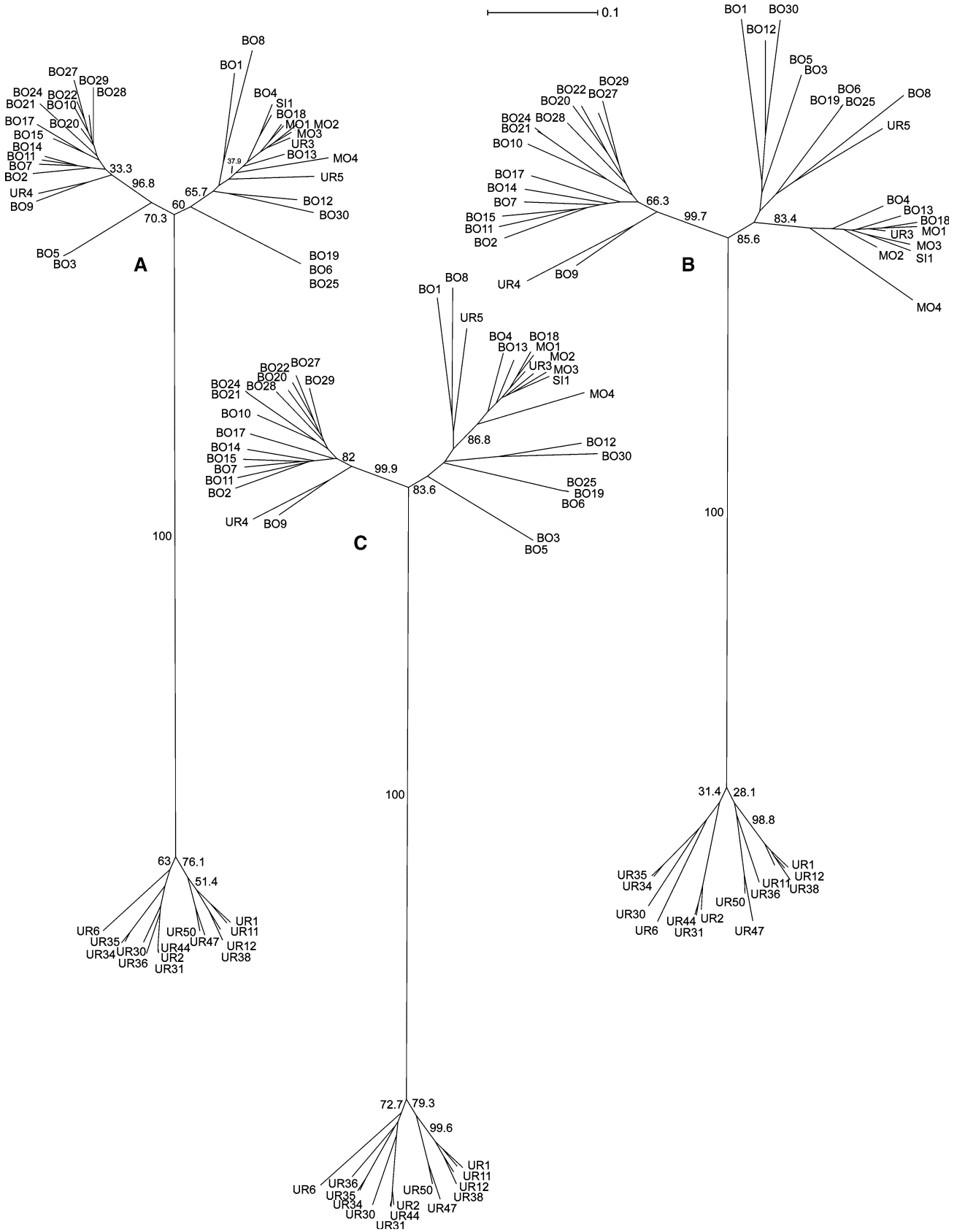

Fig. 3 NJ trees representing the genetic relationships between diploid wheat accessions, derived from SSAP marker data for *Jeli* (**a**), *BARE-1* (**b**) and the combined data set (**c**). The trees are shown in the same scale (dice distances, *top*)

two variants of A genome, these accessions are most likely to be misidentified *T. boeoticum*, assigned to different species due to their morphological similarity. The collection area for UR4 is similar to BO9 (Gaziantep, Turkey), and they were clustered closely together. Throughout the text we will treat these accessions as *T. boeoticum*, keeping in mind that botanical reidentification is necessary and refraining from making conclusions based on these accessions only. Another *T. urartu* accession (BO14; PI427328) was re-identified by us according to type characters and was assigned to *T. boeoticum* species. The gliadin pattern for BO14 published earlier also looks nontypical for *T. urartu* (Martín et al. 2008); interestingly, there have already been two changes in its taxon identification earlier.

T. urartu group is further subdivided into two major clusters, but the A^b-genome species have a more complex structure, explored further in the enlarged version of the combined tree (Fig. 4). A long branch with 99.9% bootstrap value separates a relatively compact cluster of 16 *T. boeoticum* accessions, which we designate Group 1; this group is subdivided further into smaller clusters with support values 65, 78 and 93%. These reflect the origin of accessions, Iran, Iraq and Turkey, respectively. The rest of accessions form a loose group which shows no evident correlations with the geographical data; however, a certain subgroup of diploid wheats within it demonstrates closer relationships and is separated from the rest of accessions by a branch with 87% support value; this cluster includes all cultivated *T. monococcum*/*T. sinskajae* accessions, as well as several representatives of wild *T. boeoticum*. The subgroup was designated Group 3 and the remaining accessions (which are not further subdivided into large clusters with 75% support or more) were assigned to Group 2.

The clear separation of *T. boeoticum* into two major clusters (Group 1 vs. Group 2 + 3) does not unambiguously correspond to the geographical distribution of collection points; on the other hand, it indicates the deep genetic differences that exist between the subspecies of *T. boeoticum* identified by morphological criteria. Indeed, when the known subspecies information for the accessions is considered, all the representatives of *T. boeoticum* ssp. *thaoudar* (Reut. ex Hausskn.) Grossh. appear within Group 1, while *T. boeoticum* ssp. *boeoticum* accessions fall into Groups 2 and 3 (Fig. 3).

For Group 1, five diagnostic bands were found which are present (or absent) in all representatives of this group, but nowhere else. Out of 447 polymorphic bands analyzed, 42 (9.4%) were found within Group 1 only. For Group 3, no diagnostic bands were found, and there were eight unique bands detected only in some representatives of this group. These data indicate that the degree of evolutionary distinctness and the evidence of possible isolation are much greater in the case of Group 1.

When the trees derived from *BARE-1* and *Jeli* data are compared, differences between them can be observed. First, *Jeli* reveals lower diversity, if compared to *BARE-1*, within the A^b-genome group and within *T. urartu* group, as can be seen from the shorter genetic distances between the

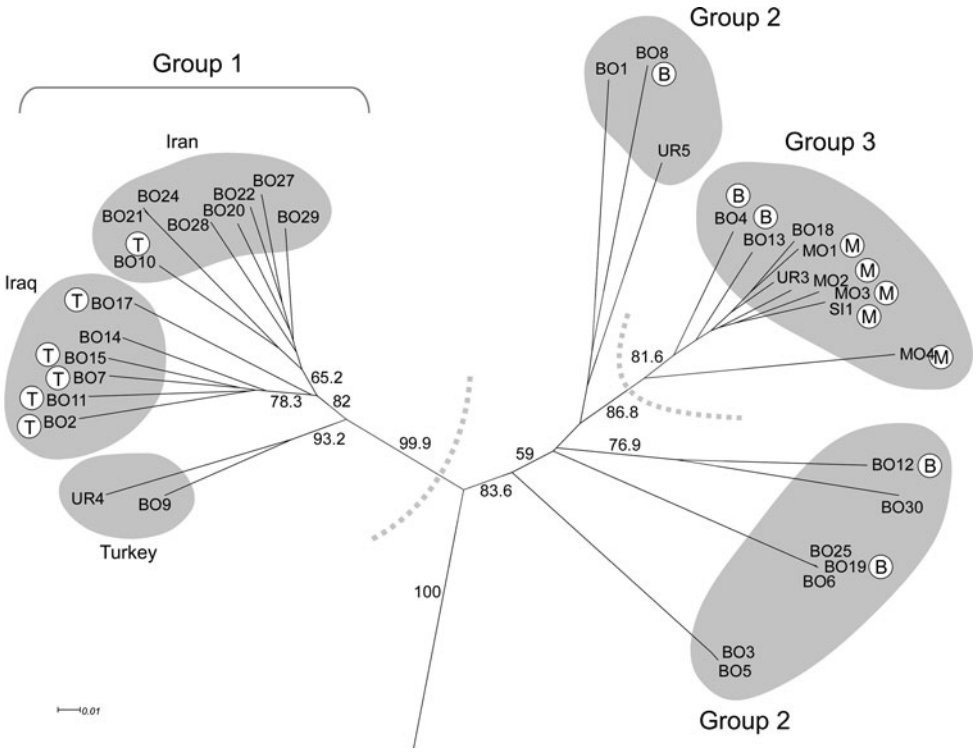

Fig. 4 NJ tree derived from combined SSAP data set as in Fig. 3c, enlarged

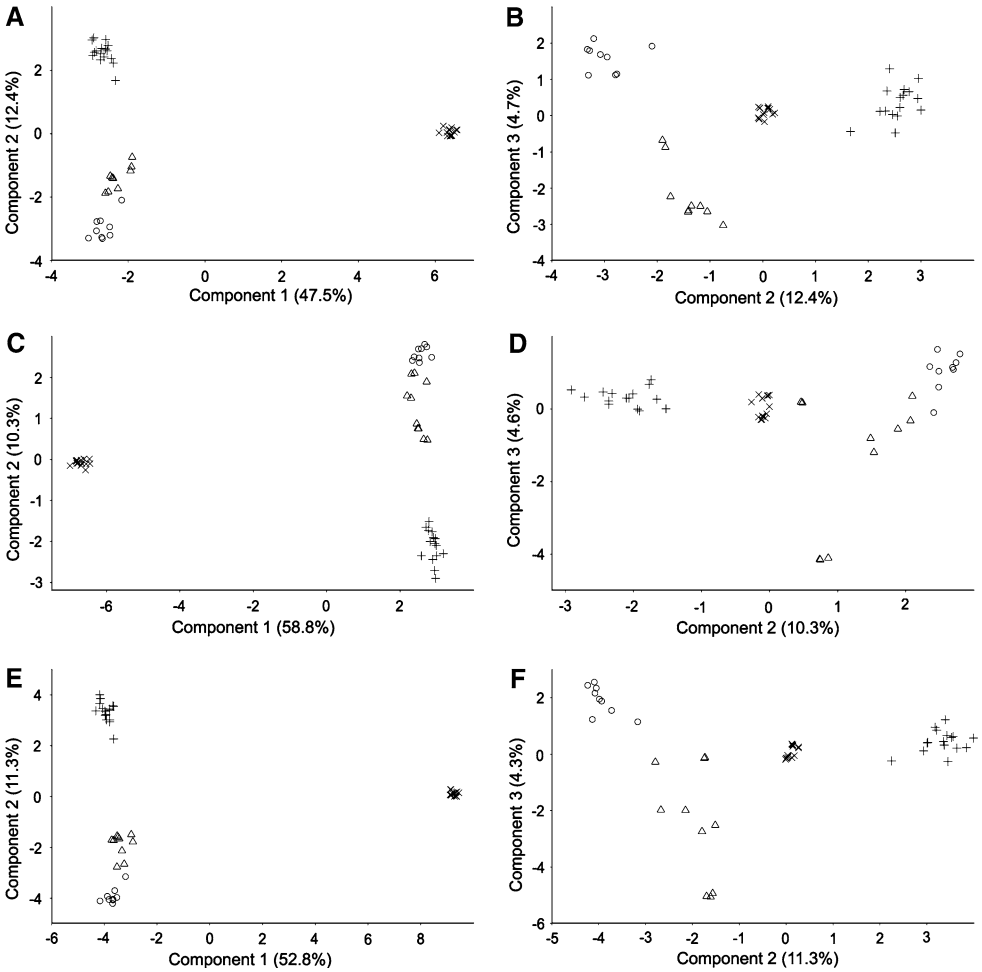

Fig. 5 Principal component analysis (PCA) of diploid wheat diversity data generated for *BARE-1* retrotransposon family (**a**, **b**), *Jeli* family (**c**, **d**) and the combined data set for both (**e**, **f**). Proportion of total variation explained by the first three components is shown. *Plus T. boeoticum* Group 1; *triangle T. boeoticum* Group 2; *Circle T. boeoticum/ T. monococcum* Group 3; *multi symbol T. urartu*

accessions; on the other hand, the branch separating these two major clusters is slightly longer in case of *Jeli*. Second, *Jeli* does not reveal Group 3 as a distinct cluster with high support; on the *BARE-1*-derived tree, this group clearly stands out. Third, several accessions have different positions on the trees derived from different transposon families. *T. boeoticum* BO3 and BO5 from Armenia appear close to Group 1 on *Jeli* tree, which is supported by 70.3% bootstrap value for the adjacent branch; in *BARE-1* and combined tree, these accessions occupy a position within Group 2. In *T. urartu* cluster, UR36 lies within one or another major subgroup, depending on which transposon family was analyzed. In general, however, the trees demonstrate consistency with each other and with a combined tree.

The principal component analysis (PCA) performed on *BARE-1*-derived, *Jeli*-derived and combined data sets reveal the same difference between *Jeli* and *BARE-1* diversity patterns: Group 3 is well separated from the rest of accessions only when the data for *BARE-1* is used (Fig. 5). Interestingly, each of the three major components explaining variation apparently contributes to each of the main splits observed in phylogenetic trees: between *T. urartu* and A^b-genome cluster (component 1); between Group 1 and Groups 2/3 (component 2); and between Group 2 and Group 3 (component 3). If the *T. urartu* accessions are omitted, the first principal component (PC1) calculated separately for *BARE-1* or *Jeli* explains the A^b-genome group diversity in a similar manner; the differences between the two marker sets become obvious in their PC2 versus PC2 plot (Fig. 6).

Statistical data obtained for both retrotransposon families are presented in Table 3. The tendencies mentioned above are also evident here: while *Jeli* demonstrates higher percentage of polymorphic bands distinguishing between *T. urartu* and *T. boeoticum*/*T. monococcum* groups, *BARE-1* reveals more polymorphism within those groups. It is especially evident in the case of *T. boeoticum* Group 1, where *BARE-1* is almost 60% more polymorphic. On the other hand, *Jeli* provided a total of 56 diagnostic markers which can unambiguously distinguish between A^u and A^b wheat genomes in our accession set, while for *BARE-1* this number only reaches 33 despite a higher total number of markers detected.

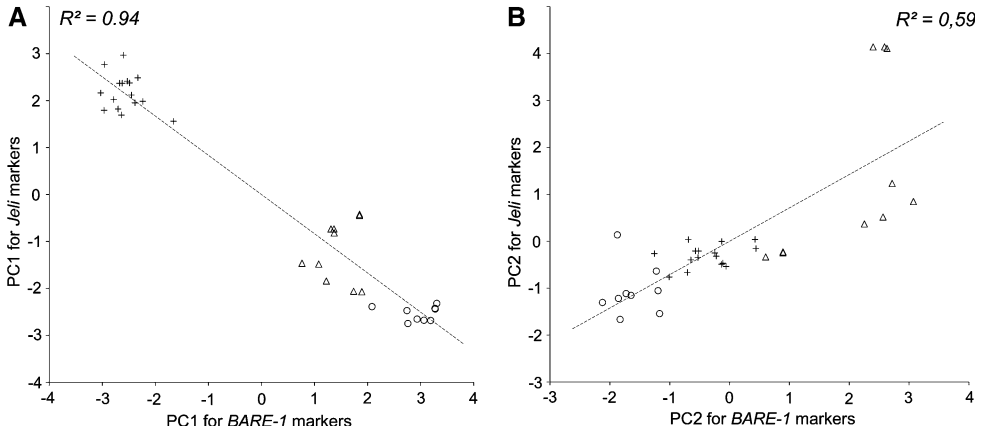

Fig. 6 The first (**a**) and the second (**b**) principal component (PC1, PC2) values calculated for *BARE-1* versus *Jeli* markers, plotted against each other, with *T. urartu* accessions omitted. *Plus T. boeoticum* Group 1; *triangle T. boeoticum* Group 2; *circle T. boeoticum*/*T. monococcum* Group 3

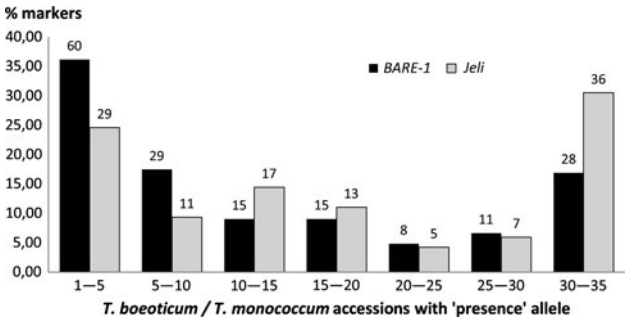

Fig. 7 The distribution of *BARE-1* and *Jeli* SSAP bands on the basis of their representation in *T. boeoticum*/*T. monococcum* accessions. For building the histogram, percent values were used; absolute values (number of SSAP bands in each bin) are indicated above

Polymorphic information content (PIC) values calculated for *Jeli* are also higher, as well as an average frequency of 'presence' allele for each *Jeli* band, compared to *BARE-1*. This is especially evident in case of A^b-genome group of accessions. The histogram in Fig. 7 reveals that 'rare' bands are more characteristic for *BARE-1*, while *Jeli* bands are more likely to be present in a large fraction of accessions or to be monomorphic within the A^b-genome group.

Discussion

Evolutionary dynamics of *BARE-1* and *Jeli* insertions and their utility in SSAP marker systems

The possibility of using retrotransposon-based markers for diversity analysis has been reported in many plant species, including wheat (Queen et al. 2004); at the same time, genomics-based studies demonstrate a wide diversity of retrotransposon structure and evolutionary history (Llorens et al. 2009; Wicker et al. 2007). It has been generally shown that SSAP markers served better than AFLP in diversity studies when a direct comparison was made (Ellis et al. 1998; Lou and Chen 2007; Syed et al. 2005; Tam et al. 2005). However, a question arises when a particular phylogenetic problem has to be resolved using SSAP approach: which retrotransposon family provides more informative markers for a given taxonomic scale? How will the parallel histories of plant species evolution and retrotransposon amplification bursts be reflected in the diversity data? In allopolyploids, the genome composition is more complicated because some transposon families may have a different copy number in the homeologous chromosomes, and the polymorphism data for their insertions is going to be biased accordingly. For example, a transposon family that has most copies in the D genome of hexaploid wheat (due to a recent amplification burst in the diploid progenitor of D genome) will likely generate markers that resolve the relationships between D genome of wheat and its *Aegilops* relatives very well, but is less useful for studying the origins of B and A genomes in hexaploid wheats; and vice versa. In theory, it means that a marker system based on a particular family of mobile genetic elements can be specifically targeted at analyzing certain evolutionary events, if there is at least some general information about where the members of this family reside and when the new insertions arose.

Unfortunately, there are often no solid statistics on sequence-based estimations of relative copy numbers and insertion ages. This is particularly true for the least abundant families for which it would be necessary to analyze tens of megabases in order for enough copies to be found (Charles et al. 2008; Wicker et al. 2009). While we can expect more sequences for common wheat in the near future, this is probably not true for its numerous wild relatives. On the other hand, chromosome substitution and addition lines can provide precise and virtually limitless information on retroelement copy numbers in the genomes that they represent, as long as the chromosomal identity is preserved between the parental lines and their derivatives.

In our study, using such an approach, we discovered that a high copy number retrotransposon family *Jeli* is a specific feature of the A genome of wheat, being 2.5 times more abundant there than in B and D genomes combined. While we have not explored *Jeli* polymorphism in hexaploid wheat accessions, this family seems to be a promising source of markers for studies where the A genome of polyploid species is concerned, for example, in genetic mapping, screening cultivars for diversity, evolutionary and phylogenetic applications.

Interestingly, *Jeli* was less convincing than *BARE-1* in resolving the relationships within diploid A-genome species, but more effective in detecting the differences between them. This can be explained by its amplification history: we can speculate that a massive *Jeli* transposition burst in A genome coincided with *T. urartu*/*T. boeoticum* speciation event, but subsequently (during the intraspecific diversification) the activity of the element was low. In contrast, *BARE-1* provided more information on the closely related *T. boeoticum*/*T. monococcum* accessions, which can mean that it retained constant activity and generated new copies throughout the recent events in einkorn evolution. These hypotheses are in accordance with allele frequency data (Table 3; Fig. 7). Interestingly, the PIC values are misleading, indicating that *Jeli* is more informative within each of the diploid wheat species than *BARE-1*. However, although an average PIC is higher for *Jeli* bands that are polymorphic within *T. boeoticum*, the effective number of polymorphic *Jeli* markers found within this group is lower (48 vs. 63% for *BARE-1*), resulting in weaker representation of intraspecific relationships. Considering the allele frequencies, the overall degrees of *Jeli* and *BARE-1* polymorphism within the A^b-genome species or between A^u- and A^b-genome clusters can be attributed to different rates of elements' transposition, but the differences in being able to distinguish Group 3 can be also due to a non-uniform localization of *BARE-1* and *Jeli* insertion sites within the genome, with *BARE-1* insertions linked more closely to the genomic regions that were evolving rapidly during Group 3 separation and *T. monococcum* domestication. Genome-wide mapping of retrotransposon insertions in diploid wheat will help to explore this option. In case of polyploid wheat species, however, it should always be important to consider the predominant location of *Jeli* insertions in the A genome as the major factor affecting the outcome of *Jeli*-based SSAP diversity studies.

Diploid wheat genetic diversity and the identification of taxon-specific molecular markers

According to our results, there are four entities comprising diploid *Triticum* that are more or less separate from each other: (1) *T. urartu* Thum. ex Gandil.; (2) *T. boeoticum* ssp. *thaoudar* (Reut. ex Hausskn.) Grossh.; (3) most representatives of *T. boeoticum* Boiss. ssp. *boeoticum*; (4) cultivated *T. monococcum* L. along with some *T. boeoticum* ssp. *boeoticum*. In total, 89 molecular markers were identified that are able to discriminate unambiguously between A^u and A^b genomes, at least within set of accessions studied (Table 3). We can speculate that speciation-associated amplification of mobile genetic elements coupled with evolutionary bottlenecks in the history of *T. urartu* and *T. boeoticum* could have led to fixation of multiple 'presence' alleles for novel retrotransposon insertions, which is why the proportion of species-specific SSAP markers is relatively high compared to the other marker types (Golovnina et al. 2007, 2009; Kilian et al. 2007; Yamane and Kawahara 2005). Interestingly, despite the recent efforts to investigate diploid wheat diversity, the problem of finding reliable diagnostic markers for A^u and A^b genomes has not been paid much attention, either due to lack of species-specific polymorphic states or because it was not among the aims of a study (Golovnina et al. 2007, 2009; Goncharov et al. 2009; Heun et al. 2008; Kilian et al. 2007; Sasanuma et al. 2002). The analysis of inheritance of morphological traits also failed to identify a species-specific character for the two wild diploid wheat species apart from the hairy leaf blade type (Dorofeev et al. 1979; Filatenko et al. 2002).

It is also clear that *T. boeoticum* ssp. *thaoudar* (Group 1) is more distinct and less related to other diploid wheat accessions than *T. monococcum* (Fig. 4). The discovery of five diagnostic SSAP bands for this group suggests that bottlenecks and isolation events played a role in its evolutionary history after the separation from Group 2/3. Our molecular data therefore support at least a subspecies status for ssp. *thaoudar*. Obviously, more well-characterized accessions may have to be analyzed if new taxonomical decisions are to be made. However, some of the existing options (Barkworth and von Bothmer 2009) may be favored over the others on the basis of our data: in the first place, ensuring that all accessions of *T. boeoticum* are identified to subspecies.

The close relationships found between *T. monococcum* and some *T. boeoticum* ssp. *boeoticum* accessions may be due to proximity of wild einkorn collection points to the site of *T. monococcum* domestication (Heun et al. 1997; Kilian et al. 2007); another possibility is that the above-mentioned *T. boeoticum* accessions apparently represent a 'feral' form of *T. boeoticum* ssp. *boeoticum*, originated from occasional crosses with *T. monococcum* when the latter was still being cultivated (Zohary and Hopf 1988).

It can be concluded that SSAP-based diversity data is in agreement with previous findings, providing high support values for major intraspecific clusters and giving insights into the evolution of retrotransposon families as well as

their hosts. If the diagnostic SSAP markers for A^u/A^b-genome groups and for *T. boeoticum* ssp. *thaoudar* are converted into high-throughput RBIP assays (Flavell et al. 1998) and their specificity is confirmed on a larger scale, the distinction between these taxonomic entities in genebanks will be further simplified and supported by molecular tools.

Conclusions

Supporting the idea that wheat retrotransposons may have significantly different distributions between homeologous chromosomes in polyploids, we have discovered that the *Gypsy*-class LTR retrotransposon family *Jeli* is substantially more amplified in the A genome of common wheat compared to B and D genomes. Although the accumulation of sequence data will likely lead to the discovery of similar cases, so far the *Jeli* family is unique in this regard.

Retrotransposon-based analysis revealed deep genetic differences between two major groups of A^b-genome wheat. These groups apparently correspond to the previously known two subspecies of *T. boeoticum*: ssp. *boeoticum* and ssp. *thaoudar*. The study has demonstrated that each of the two retrotransposon families is more suitable for analyzing diversity accumulated in different periods of diploid wheat evolution: while *Jeli* provides more data on the separation of A^u and A^b lineages, *BARE-1* markers do a better job in resolving the relationships between recently diverged *T. boeoticum*/*T. monococcum* subgroups. Hence, in order to obtain a comprehensive image of species' evolutionary relationships, it is necessary not to limit the study by analyzing only one particular family of retrotransposons, although it may be an efficient approach for focusing on particular closely related groups. Both marker systems described here can be used in various applications, including phylogenetic studies, genetic mapping, QTL analysis, species or cultivar identification. In addition, *Jeli*-based SSAP markers provide an opportunity to specifically target the A genome of wheat at higher levels of ploidy.

Acknowledgments The authors are grateful to Drs. O.P. Mitrofanova and O.A. Lyapunova (Vavilov Institute of Plant Industry, St. Petersburg, Russia), Dr. H. Bockelman (the National Small Grains Collection, Aberdeen, USA), Dr. T. Kawahara (Graduate School of Agriculture of Kyoto University, Kyoto, Japan), Dr. J. Valkoun (ICARDA, Aleppo, Syria) for providing the seed material used in the study. We would like to thank Dr. Elena Khlestkina (Institute of Cytology and Genetics of SB RAS, Novosibirsk, Russia) for providing DNA of common wheat cv. Chinese Spring aneuploid lines, and Dr. Ekaterina Badaeva (Vavilov Institute of General Genetics, Moscow, Russia) for helpful discussions. The work was supported by the subprogram "Gene Pools and Genetic Diversity" of the program "Biodiversity" of Russian Academy of Sciences, by a Young Scientist Support grant from Russian Academy of Sciences (F.K.) and by state contract no. 02.740.11.0281 from the Russian government.

Conflict of interest statement The authors declare that they have no conflict of interest.

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