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ORIGINAL PAPER

# Copy number variation of transposable elements in *Triticum–Aegilops* genus suggests evolutionary and revolutionary dynamics following allopolyploidization

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**Abstract**
***Key message*** **Here, we report on copy number variation of transposable elements and on the genome-specific proliferation in wheat. In addition, we report on revolutionary and evolutionary dynamics of transposons.**
*Abstract* Wheat is a valuable model for understanding the involvement of transposable elements (TEs) in speciation as wheat species (*Triticum–Aegilops* group) have diverged from a common ancestor, have undergone two events of speciation through allopolyploidy, and contain a very high fraction of TEs. However, an unbiased genome-wide examination of TE variation among these species has not been conducted. Our research utilized quantitative real time PCR to assess the relative copy numbers of 16 TE families in various *Triticum* and *Aegilops* species. We found (1) high variation and genome-specificity of TEs in wheat species, suggesting they were active throughout the evolution of wheat, (2) neither *Ae. searsii* nor *Ae. speltoides* by themselves can be the only contributors of the B genome to wheat, and (3) nonadditive changes in TE quantities in polyploid wheat. This study indicates the apparent involvement of large TEs in creating genetic variation in revolutionary and evolutionary scales following allopolyploidization events, presumably assisting in the diploidization of homeologous chromosomes.

**Keywords** Transposable elements · Genome evolution · Copy number variation · Speciation · Wheat

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B. Yaakov and K. Meyer have contributed equally to this work.

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## Introduction

Transposable elements (TEs) are fragments of DNA that are able to "move" and proliferate within the host genome. TEs are commonly divided into two main groups: (1) Class I or retrotransposons, which replicate through a "copy and paste" mechanism; first, producing an RNA molecule from a promoter in a terminal repeat (LTR retrotransposons) or from an internal promoter (non-LTR retrotransposons), reverse transcribing the RNA, and integrating it into the host genomic DNA. (2) Class II or DNA transposons, which move via a "cut and paste" mechanism, producing double-strand breaks in the process. TEs are then subdivided into subclasses, orders, superfamilies, and families depending on their insertion mechanism, structure, and protein-coding similarities (Wicker et al. 2007).

TEs makeup a large fraction of most genomes. For example, they compose over 45 % of the human genome (Alexander et al. 2010), ~40 % of the rice genome (Tenaillon et al. 2010), ~80 % of the maize genome (Schnable et al. 2009), and ~90 % of the wheat genome (Charles et al. 2008). Class I non-LTR retrotransposons predominate in mammalian genomes, with class II making up less than 5 % of the TE fraction (Deininger and Batzer 2002). For example, ~30 % of the human genome is derived from just two non-LTR retrotransposon families, termed *LINE 1* (*L1*) and *SINE* (*Alu*) (Hancks et al. 2011; Lander et al. 2001). The yeast

*Saccharomyces cerevisiae* has only LTR retrotransposons, termed *Ty* elements (Bleykasten-Grosshans et al. 2011). Flowering plants, including monocots (e.g., grasses) and dicots (e.g., tomato and *Arabidopsis*), have a rich collection of both TE classes, with clear domination of class I LTR retrotransposons (Kumar and Bennetzen 1999; Tenaillon et al. 2010). The *Drosophila* genome also contains TEs from both classes, but mainly class I elements (Kaminker et al. 2002). In wheat (*Triticum–Aegilops* superfamily), there were several reports that showed copy number variation of TEs using limited number of BAC sequences (Charles et al. 2008; Sabot et al. 2005), or by analyzing specific TE families in several wheat accessions (Asakura et al. 2008; Tomita et al. 2008; Yaakov and Kashkush 2012). The variability of TE proliferation in *Triticum* and *Aegilops* revealed their usefulness as genetic markers (Queen et al. 2004). The variability of TE content in wheat might impact wheat evolution (Charles et al. 2008; Yaakov and Kashkush 2012).

In plants, transposons are usually silenced by epigenetic means through DNA methylation, chromatin modifications, and small RNAs (Cantu et al. 2010; Slotkin and Martienssen 2007). However, TEs may be reactivated in situations of cell stress, such as wide hybridization and polyploidization (Grandbastien et al. 2005; Lisch 2009; Mansour 2007). The activity of transposons may be one mechanism to create the genetic variability needed for speciation of reproductively isolated populations (Dubcovsky and Dvorak 2007; Hosid et al. 2012). The heritable variability brought about by TEs includes genetic changes resulting from transpositional activity or homologous (Xuan et al. 2012), nonhomologous and illegitimate recombination of TEs (Devos et al. 2002), and epigenetic changes affecting neighboring genes resulting from production of anti-sense RNA (Kashkush and Khasdan 2007; Puig et al. 2004) and changes in DNA methylation and chromatin modifications.

Allopolyploidy involves the hybridization of genetically distinct but related genomes and whole-genome duplication. Allopolyploidy is a common occurrence in plants, found in most economically significant crops such as cotton and wheat (Feldman and Levy 2005).

The evolutionary history of wheat involves two separate allopolyploidization events: the first includes wild wheat *T. urartu* ($2n = 2x = 14$, genome AA) and a species from section *Sitopsis* ($2n = 2x = 14$, genome BB) to produce *T. turgidum* ssp. *dicoccoides* ($2n = 4x = 28$, genome BBAA) and the second includes *T. turgidum* ssp. *dicoccoides* and *Ae. tauschii* ($2n = 2x = 14$, genome DD) to produce bread wheat *T. aestivum* ($2n = 6x = 42$, genome BBAADD).

For the study of mechanisms underlying the immediate consequences of polyploidy-induced genomic changes (revolutionary changes), wheat has been a useful model as its historical events of allopolyploidization can be mimicked in the lab, by hybridizing the known ancestors of wheat polyploids (Adams and Wendel 2005; Kashkush et al. 2003; Liu et al. 1998; Ma et al. 2004; Ozkan et al. 2001; Shaked et al. 2001; Yaakov and Kashkush 2011a, b). The impact of transposable elements on the genomic evolution of plants is not completely clear; however, the use of recently domesticated species, such as wheat, is essential for the study of rapidly changing genetic elements (Comai 2005). Previous studies have shown that newly formed polyploids undergo rapid and reproducible genomic and epigenomic changes (Chen 2007; Comai 2005; Feldman and Levy 2009). Furthermore, transcriptional activation of an LTR retrotransposon (*WIS2-1A*) was observed to occur as a result of polyploidization in wheat, as well as influenced adjacent gene products (Kashkush et al. 2003). Polyploidization in wheat also affected the methylation status (Kraitshtein et al. 2010; Parisod et al. 2009, 2010; Yaakov and Kashkush 2011a, b; Zhao et al. 2011), of associated siRNA (Kenan-Eichler et al. 2011) and rearrangements (Bento et al. 2008; Kraitshtein et al. 2010; Petit et al. 2010) of transposable elements.

In this study, we assessed in detail the genomic composition of 16 TE families, including LTR and non-LTR retrotransposons and DNA transposons, in 22 *Triticum* and *Aegilops* accessions, including diploid (AA, BB, and DD), tetraploid (BBAA), and hexaploid (BBAADD) species. We used our quantitative PCR protocol (Baruch and Kashkush 2012; Kraitshtein et al. 2010; Yaakov et al. 2013) to assess the relative copy number of each TE in all species in a relatively high resolution. The use of species that donated the AA, BB, and DD genomes to modern wheat, and the use of newly synthesized wheat allohexaploids, facilitated the detailed analysis of evolutionary and revolutionary (genetic and epigenetic changes that occur in the first generations of the nascent allopolyploid species) dynamics of TEs. The possible activity of TEs during the evolutionary history of wheat, and reactivation due to genomic stress resulting from the allopolyploidization process, is discussed.

## Materials and methods

### Plant material

In this study, we used 22 wheat accessions (see details in Supplemental Table 1): five accessions of *Aegilops searsii*; nine accessions of *Ae. speltoides*, *Ae. sharonensis*, *Ae. longissima;* two accessions of *Triticum urartu*, *Aegilops tauschii*, *Triticum turgidum* ssp. *dicoccoides*, *Triticum turgidum* ssp. *durum*, and *Triticum aestivum.* In addition, four generations (S1–S4) of newly formed wheat allohexaploid (genome BBAADD) and its parental lines, *Triticum*

*turgidum* ssp. *durum* (accession TTR19; genome BBAA) and *Aegilops tauschii* (accession TQ27; genome DD) were used. Note that we refer to all *Sitopsis* species as containing the B genome (equivalent to the S genome) to prevent confusion, because the genomic composition of the polyploid species are considered BBAA and BBAADD. The newly formed allohexaploid resembles natural hexaploid wheat (Ozkan et al. 2001). DNA was extracted from young leaves (4 weeks post germination) using the DNeasy plant kit (Qiagen).

### Quantitative PCR

We used qPCR assay (Baruch and Kashkush 2012; Kraitshtein et al. 2010; Yaakov et al. 2012; Yaakov et al. 2013) to relatively quantify 13 retrotransposons (including 11 LTR and 2 non-LTR families) and three DNA transposons (see details in Supplemental Table 2) from the *Triticeae* Repeat Sequence Database (http://wheat.pw.usda.gov/ITMI/Repeats/). We compared each reaction to amplification of the *VRN1* gene, as this gene is found in one copy in each wheat genome ($\Delta C_t = C_{tTarget} - C_{tVRN1}$) (Kraitshtein et al. 2010). The normalized quantities were then compared to the quantity in *Ae. tauschii* (accession TQ27), such that the relative quantity (RQ) in this sample was 1 [$\Delta\Delta C_t = \Delta C_{tSample} - \Delta C_{tTQ27}$; see (Kraitshtein et al. 2010)]. The efficiency of the PCR reactions ($E$), as determined by a standard curve through serial dilutions of mixed templates (Supplemental Fig. 1a), as well as the relative ploidy level of each sample ($P$; diploids = 1, tetraploids = 2, and hexaploids = 3), were taken into account. The calculations can be summarized in the equation: $RQ = P \times (2 \times E)^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). As validation, we ran the products of the PCR reaction using primers for *Fatima* (see Supplemental Table 2), in all 9 analyzed species and *Triticum monococcum* (accession TMB02, genome $A^mA^m$), on agarose gel, and observed amplification in all cases (Supplemental Fig. 1b). In addition, we ran all the samples again, using two different primer sets for *Angela-A* (*Copia* retrotransposon) and *Fatima* (*Gypsy* retrotransposon), and observed results very similar to the ones presented below.

## Results and discussion

### Copy number variation of TEs in *Triticum* and *Aegilops* species

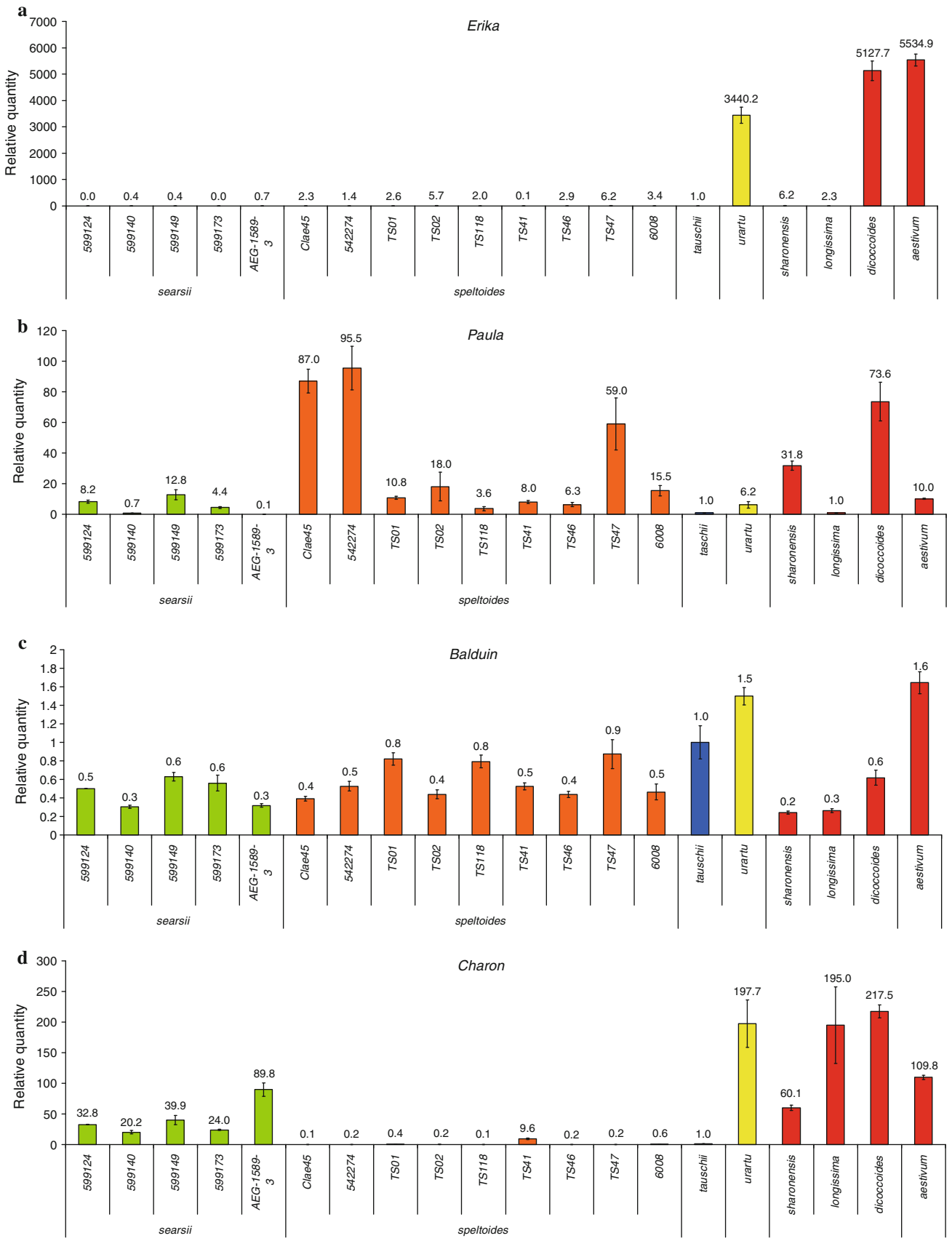
Complete analysis of the copy number variation (CNV) of TE content requires the availability of fully sequenced genomes. In the absence of a complete assembled sequence for wheat, various strategies have been used to estimate the copy number of TEs in wheat. For example, Sabot et al. (2005) and Charles et al. (2008) have analyzed a limited number of BAC sequences (representing ~0.3 % of the wheat genome) to estimate the copy number of TEs, while Asakura et al. (2008) have used Southern blot analysis to estimate the copy number of one *Mutator*-like TE family in various wheat species. The availability of whole-genome shotgun sequencing for hexaploid wheat using 454-pyrosequencing (Brenchley et al. 2012) allowed the analysis of the copy number of miniature TEs such as MITEs (Yaakov et al. 2013). The small size of MITEs in wheat (55–300 bp in length) facilitated the retrieval of intact MITE elements from the 454 databases (average sequence size of ~400 bp). However, retrieving long TEs, such as the ones used in this study from the 454 database is very challenging because of the huge redundancy of TE sequences and the inability to properly differentiate between TE sequences. In a very recent study, Senerchia et al. (2013) used low coverage 454-sequences of *Ae. cylindrica* and *Ae. geniculata* to analyze the abundance of several LTR retrotransposon families.

We have previously showed that the data produced by our qPCR assay is very efficient by analyzing rice TEs and comparing the relative quantity to the absolute copy numbers from the fully sequenced rice species (Baruch and Kashkush 2012). In addition, we have performed a similar analysis for MITEs in wheat (Yaakov et al. 2013).

In this study, we have assessed the relative quantity of six *Gypsy*, four *Copia* and one *Copia*-like LTR retrotransposon, two non-LTR retrotransposons, and three DNA transposons. It is very important to note that the primer pairs that were used for each TE family were designed from conserved sequences specific to each family (based on the analysis of multiple sequence alignment of several members of each family retrieved from the NCBI database). In addition, for some cases, we used different primer combinations to validate the results (see "Materials and methods").

#### *Relative quantity of Gypsy LTR retrotransposons*

The analysis of the relative quantities of six *Gypsy* families (Fig. 1a; Supplemental Fig. 2a–e) revealed that *Fatima* is very abundant in *Ae. searsii* and *Ae. speltoides,* while it is least abundant in *Ae. tauschii* and *T. urartu*. *Fatima* content in *Ae. searsii is* ~4,300 times its content in *Ae. tauschii* and ~93 times its content in *T. urartu.* Interestingly, the relative quantity of *Fatima* in tetraploid and hexaploid species was dramatically lower (~10 times less) than the one observed in *Ae. searsii*. The high abundance of *Fatima* in *Ae. speltoides* was also detected using FISH analysis (Salina et al. 2011). For each TE, we have calculated the


a
Erika
Relative quantity
7000
6000
5000
4000
3000
2000
1000
0
0.0 0.4 0.4 0.0 0.7 2.3 1.4 2.6 5.7 2.0 0.1 2.9 6.2 3.4 1.0 3440.2 6.2 2.3 5127.7 5534.9
599124 599140 599149 599173 AEG-1589-3 Clae45 542274 TS01 TS02 TS118 TS41 TS46 TS47 6008 tauschii urartu sharonensis longissima dicoccoides aestivum
searsii
speltoides
b
Paula
Relative quantity
120
100
80
60
40
20
0
8.2 0.7 12.8 4.4 0.1 87.0 95.5 10.8 18.0 3.6 8.0 6.3 59.0 15.5 1.0 6.2 31.8 1.0 73.6 10.0
599124 599140 599149 599173 AEG-1589-3 Clae45 542274 TS01 TS02 TS118 TS41 TS46 TS47 6008 taschii urartu sharonensis longissima dicoccoides aestivum
searsii
speltoides
c
Balduin
Relative quantity
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.5 0.3 0.6 0.6 0.3 0.4 0.5 0.8 0.4 0.8 0.5 0.4 0.9 0.5 1.0 1.5 0.2 0.3 0.6 1.6
599124 599140 599149 599173 AEG-1589-3 Clae45 542274 TS01 TS02 TS118 TS41 TS46 TS47 6008 tauschii urartu sharonensis longissima dicoccoides aestivum
searsii
speltoides
d
Charon
Relative quantity
300
250
200
150
100
50
0
32.8 20.2 39.9 24.0 89.8 0.1 0.2 0.4 0.2 0.1 9.6 0.2 0.2 0.6 1.0 197.7 60.1 195.0 217.5 109.8
599124 599140 599149 599173 AEG-1589-3 Clae45 542274 TS01 TS02 TS118 TS41 TS46 TS47 6008 tauschii urartu sharonensis longissima dicoccoides aestivum
searsii
speltoides

◀ **Fig. 1** Relative quantification (compared to *Ae. tauschii*, set as 1, see materials and methods) of four transposable elements in various wheat species. The elements presented are examples of **a** genome-specific proliferation (*Erika*), **b** *Ae. speltoides* as donator of the B genome (*Paula*) and nonadditive values in **c** tetraploid *T. turgidum ssp. dicoccoides* (*Balduin*), and **d** hexaploid *T. aestivum* (*Charon*) wheat. All relative quantities are normalized to *Ae. tauschii*. Standard deviation was calculated based on three technical replicates

coefficient of variation (CV) parameter, a statistical value that is based on the observed standard deviation in RQ of all species divided by the average RQ of all species, to reveal the level of variability of each TE, which in turn indicates the level of dynamics (activity) for each TE (Wright and Schoen 2000). In the *Gypsy* superfamily, *Fatima* together with *Erika* (specifically proliferated in the A genome, Fig. 1a) showed relatively higher CV values (1.56 and 1.66, respectively, Supplemental Table 3) in all species, indicating their relatively higher dynamics in wheat. In addition, *Sabrina* and *BAGY2* showed relatively lower CV values in all species (0.41 and 0.72, respectively), indicating their lower dynamics in wheat compared to other *Gypsy* TE families. Finally, the *Latidu* family showed specific proliferation in *Ae. sharonensis*: ~73 times its content in *Ae. tauschii* and *Ae. speltoides*, twice its content in *Ae. longissima*, and three times its content in *Ae. searsii* (Supplemental Fig. 2c).

*Relative quantity of Copia LTR retrotransposons*

The analysis of relative quantity of five *Copia* families (Supplemental Fig. 2f–j) revealed that *Angela*, *Barbara*, and *WIS-A* showed similar levels of variability among the wheat species (CVs of 0.41, 0.35, and 0.36, respectively, Supplemental Table 3). Interestingly, all three elements showed relatively higher content in one of the seven *Ae. speltoides* accessions, TS01 (Supplemental Fig. 2f–h). Among the *Copia* superfamily, *BARE1C* showed significantly higher variability (CV of 1.1, Supplemental Table 3) compared to the other three elements. Finally, the *Veju* element [*Copia*-like element, (Kraitshtein et al. 2010)], showed low variability (CV of 0.45) among species. In addition, *Ae. tauschii* (genome DD) showed significantly lower quantities compared to the A and B genomes, similar to what was estimated by Kraitshtein et al. (2010).

*Relative quantity of non-LTR retrotransposons*

The analysis of relative quantity of two non-LTR retrotransposon families (Fig. 1b; Supplemental Fig. 2l) revealed that both elements showed relatively high variability among species (CV of 1.32 for *Ramona* and 1.23 for *Paula*, Supplemental Table 3). The two elements also show greater quantities in the tetraploid *T. turgidum* ssp. *dicoccoides*, but *Paula* has much less relative quantity in the hexaploid *T. aestivum* compared with *Ramona*.

*Relative quantity of DNA transposons*

The analysis of relative quantity of three DNA element families (Fig. 1c, d; Supplemental Fig. 2k) revealed that *Balduin* had the lowest CV values, while *Rong* had the highest CV values (Supplemental Table 3), except the CV value for *Ae. speltoides* which were elevated due to a specific proliferation of *Charon* in one accession (TS41) from Israel (Fig. 1d; Supplemental Table 1). In addition, *Rong* had high relative quantities in *Ae. sharonensis* and a particular accession of *Ae. speltoides* (542274, Supplemental Table 1) from Adiyaman in Turkey, *Balduin* had high relative quantities in *T. urartu* and *T. aestivum*, and *Charon* had high relative quantities in *T. urartu*, *Ae. longissima*, and *T. turgidum* ssp. *dicoccoides*.

*Variation across all species*

The CV of all the species ranged from 0.354 (*Barbara*, a *Copia* family) to 1.661 (*Erika*, a *Gypsy* family), indicating that the former is the least active and the latter is the most active element during the evolutionary history of wheat (Supplemental Table 3). Furthermore, the *Gypsy* superfamily, except for *Sabrina*, seemed to have higher CV values than the *Copia* superfamily, except for *BARE1C*. Our data indicate that TE dynamics are specific to each TE. For example, while the CV values of the *Erika* and *Fatima* families from the *Gypsy* superfamily indicate that they are two of the most active elements, the CV value of the *Sabrina* family from the same superfamily showed that it is one of the least active elements. Thus, in contrast to what was reported by Sabot et al. (2005), our data indicate that one cannot draw a general conclusion regarding the comparison of TE activity among different superfamilies.

*Genome-specific proliferation*

We examined the genomic uniqueness of each element to certain genomes and observed that *Erika* (*Gypsy* retrotransposon; Fig. 1a) had proliferated in the A genome, as it appeared to a much greater extent in *T. urartu* (genome $A^uA^u$), *T. turgidum* ssp. *dicoccoides* (genome BBAA), and *T. aestivum* (genome BBAADD). *Fatima* (*Gypsy* retrotransposon), *Rong* (*PIF/Harbinger* DNA transposon), and *Paula* (non-LTR retrotransposon) had proliferated in the B genome, as they appeared in very low levels in *Ae. tauschii* (genome DD) and *T. urartu* (genome $A^uA^u$).

*Relative quantity among Ae. searsii and Ae. speltoides accessions: the candidate donors of the B genome to wheat*

We studied the relative quantities of TEs in five accessions of *Ae. searsii* and nine accessions of *Ae. speltoides*, as these are the two candidate species for the contribution of the B genome to form tetraploid wheat (*T. turgidum* ssp. *dicoccoides)*. The results showed elements with proliferation specific to each species (*t* test $p < 0.05$): *Latidu* (*Gypsy* retrotransposon; Supplemental Fig. 2c), *Sabrina* (*Gypsy* retrotransposon; Supplemental Fig. 2a), *BAGY2* (*Gypsy* retrotransposon; Supplemental Fig. 2d), and *Charon* (*Mutator* DNA transposon) were specific to *Ae. searsii* and *Erika* (albeit at low levels), *Angela-A* (*Copia* retrotransposon; Supplemental Fig. 2f), and *Ramona* (non-LTR retrotransposon; Supplemental Fig. 2l) were specific to *Ae. speltoides*. This data, together with the finding that all the tested elements are found in the polyploid species, indicate that we cannot favor either of the two species to be the donor of the B genome. As previously reported, the true donor of the B genome might be a common ancestor to the two species (Salse et al. 2008; Yaakov and Kashkush 2012).

Evolutionary-scale changes in TE quantity in polyploids

In order to assess TE dynamics following polyploidization events, we examined the changes from the expected RQ for the tetraploid and hexaploid species (*T. turgidum* ssp. *dicoccoides* and *T. aestivum*, respectively), which may be calculated by combining the RQ for *T. urartu* (genome AA) and any accession of *Ae. speltoides* or *Ae. searsii* (genome BB) for the tetraploid (genome BBAA) and *T. turgidum* ssp. *dicoccoides* (genome BBAA) and *Ae. tauschii* (genome DD) for the hexaploid (genome BBAADD). A deviation from the expected RQs in the tetraploid was counted only if no combination of accessions could add up to the observed RQs in the tetraploid. We observed a deviation from the expected RQs for the tetraploid *T. turgidum* ssp. *dicoccoides*, which showed higher than expected values for *Erika* (Fig. 1a; $p = 0.0002$) and lower than expected values for *Balduin* (*CACTA* DNA transposon; Fig. 1c; $p = 0.0001$). When comparing the additive values of the natural tetraploid *T. turgidum* ssp. *dicoccoides* (genome BBAA) and *Ae. tauschii* (genome DD) with the values of the natural hexaploid *T. aestivum* (genome BBAADD), the hexaploid *T. aestivum* showed higher than expected values for *Rong* ($p = 0.0001$) and lower than expected values for *Geneva* (*Gypsy* retrotransposon; $p = 0.0001$), *BARE1C* (*Copia* retrotransposon; Supplemental Fig. 2; $p = 0.0001$), *Charon* (Fig. 1d; $p = 0.0001$), and *Paula* ($p = 0.0001$). However, *Fatima* and *Latidu* also had higher than expected values in the hexaploid, but were just above statistical significance ($p = 0.061$ and 0.0563, respectively). Importantly, the only *Triticum*-specific element (*Erika*) showed a deviation from the expected values in the allotetraploid, which was also observed for *Triticum*-specific *Stowaway*-like MITEs (*Minos* and *Fortuna*) (Yaakov et al. 2013). Thus, the genome-specific elements may play a role in the differentiation of sub-genomes following polyploidization, via transcriptional, transpositional, or recombinational events evoked by epigenetic changes (Mirouze et al. 2012).

TE dynamics in the first generations of newly formed allohexaploid

Because the examination of relative TE quantity in natural polyploids results in an assessment of TE activity within long time scales [$\sim$10,000 years for the hexaploid and $\sim$0.5 million years for the tetraploid; see (Feldman and Levy 2005)], we decided to investigate the immediate effects of polyploidization on the relative quantity of each TE by performing the same qPCR analysis on a system of newly formed allohexaploid wheat (see "Materials and methods").

The relative copy number values in the newly formed polyploid offspring (S1–S4 generations) were then compared to the expected additive parental copy number (the sum of the copy numbers of both parents), as the polyploid harbors both parental genomes, and each generation of the polyploid was compared to the successive generation. Thus, for each transposon, a pattern of increase or decrease in relative copy number could be observed. For 6 of the 16 elements [*Fatima* (Fig. 2a; $p = 0.0041$), *Angela-A* (Supplemental Fig. 3a; $p = 0.0001$), *BARE1C* (Supplemental Fig. 3b; $p = 0.0142$), *Paula* (Supplemental Fig. 3c; $p = 0.0001$), *Rong* (Supplemental Fig. 3d; $p = 0.0004$), and *WIS-A* (Supplemental Fig. 3e; $p = 0.0001$)], a significant decrease in relative copy number (between the additive parental values and the first generation of the newly formed allohexaploid, S1) was seen. However, the subsequent generations showed different patterns of change between these elements, consisting mostly of decrease in relative copy number in different generations. Another large group (9 of 16 elements) showed a significant increase in relative copy number, followed mostly by a decrease in different times in later generations. These elements included: *Erika* (Fig. 2b; $p = 0.0001$), *Balduin* (Supplemental Fig. 3f; $p = 0.0001$), *Ramona* (Supplemental Fig. 3g; $p = 0.0109$), *Barbara* (Supplemental Fig. 3h; $p = 0.0001$), *Charon* (Supplemental Fig. 3i; $p = 0.0005$), *Veju* (Supplemental Fig. 3j; $p = 0.0001$), *Geneva* (Supplemental Fig. 3k; $p = 0.0001$), *BAGY2* (Supplemental Fig. 3l; $p = 0.0021$), and *Latidu* (Fig. 2c;

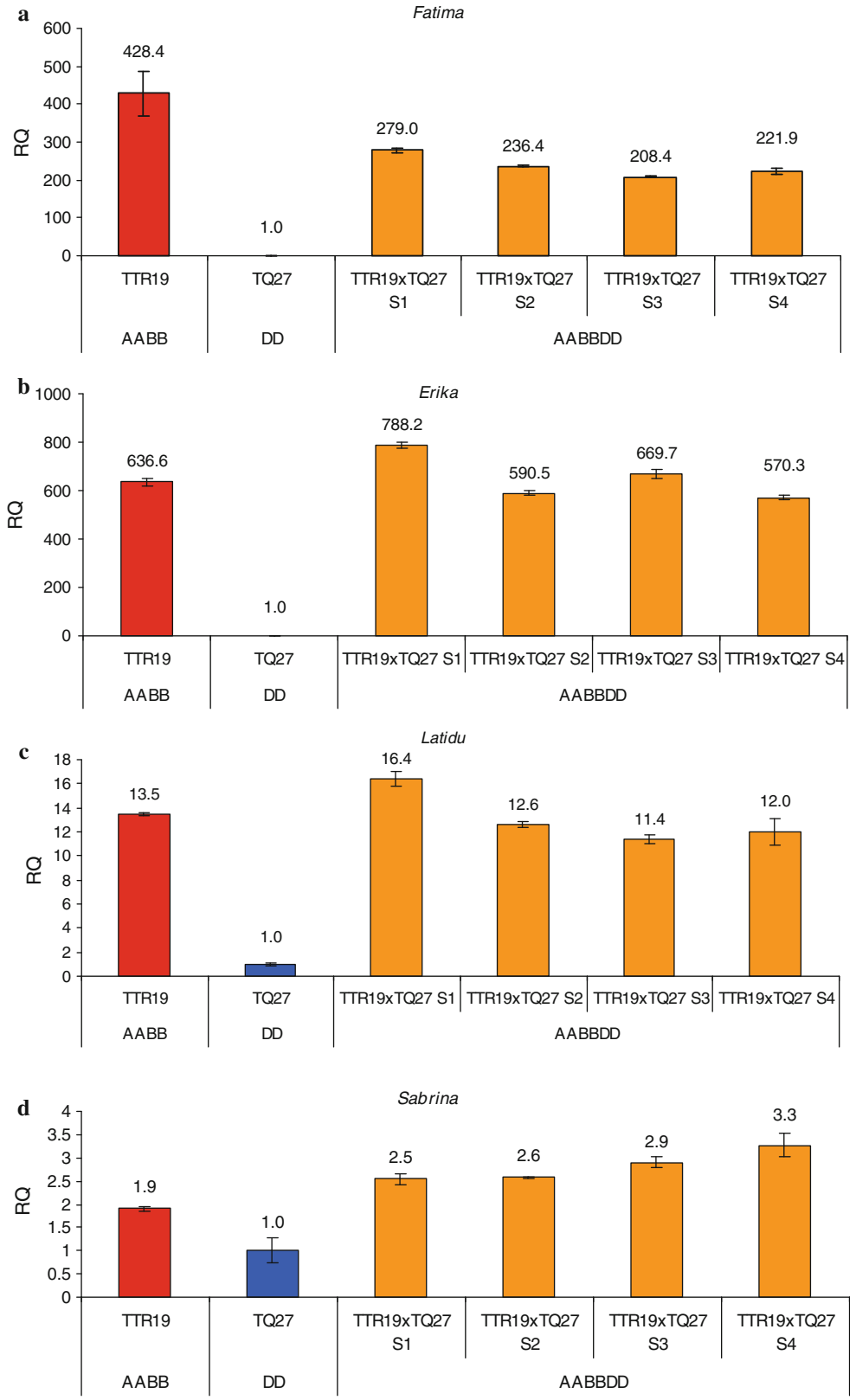



**Fig. 2** Relative quantification (RQ) of two parental species (*T. turgidum* ssp. *durum* and *Ae. tauschii*) and their newly formed polyploid offspring (S1–S4) for **a** *Fatima*, **b** *Erika*, **c** *Latidu*, and **d** *Sabrina*. The *numbers at the top of each bar* indicate the relative quantity of the element compared to *Ae. tauschii*. The *error bars* represent standard deviations from three technical replicates. The genome composition of each species is indicated at the *bottom*

$p = 0.0002$). Only one element (*Sabrina*; Fig. 2d) showed no change in its relative quantities in S1, but did show an 11.4 % increase between generations S2 and S3. These results indicate that the genomic changes that occur in transposable element sequences, following polyploidization, are unique to each family of transposable element. This is in agreement with our observation of the level of TE dynamics that was calculated based on their variability among the different species (see above), where the dynamics might dramatically vary from one TE family to another in the same superfamily.

Another interesting observation, a specific group of three *Gypsy* superfamily elements, which include *Erika* (Fig. 2b), *Geneva* (Supplemental Fig. 3k), and *BAGY2* (Supplemental Fig. 3l), showed a particular pattern of increased relative copy number between the expected additive parental values and the S1 generation, followed by a decrease between the S1 and S2, an increase between S2 and S3, and a decrease between S3 and S4. This phenomenon might indicate the unique dynamics of the *Gypsy* superfamily in wheat.

Finally, we have assessed the timing of TE dynamics and found that ~94 % of the elements showed a significant change in their quantity in S1 compared to the additive value of the parental lines, while ~75 % of the elements showed a significant change between S1 and S2, ~68 % of the elements showed a significant change between S2 and S3, and 65 % of the elements showed a significant change between S3 and S4.

Interestingly, we found that the *Charon*, *Rong*, *Paula*, *BARE1C*, *Fatima*, *Latidu*, and *Geneva* families showed significant changes, both in the natural hexaploid species (compared to its diploid and tetraploid parental species) and in the newly formed allohexaploid (compared to the TTR19 and TQ27 parental lines). This data suggests that these early changes in element copy numbers impact the subsequent evolution of each element in the genome.

In conclusion, in this study, we have performed a genome-wide analysis of the relative quantity of 16 large TE families representing different superfamilies from both TE classes in a large number of *Triticum* and *Aegilops* species, including accessions from the donors of the AA, BB, and DD genomes of polyploid wheat. In addition, the use of natural wheat allopolyploids and newly formed allopolyploids allowed us to assess the TE dynamics both at revolutionary and evolutionary scales. Furthermore, we have analyzed several accessions of the potential donors of the B genome (*Ae. searsii* and *Ae. speltoides*), which has not been performed before, allowing us to track the possible evolutionary trajectory of these wheat species.

Based on our observations, we have reached the following conclusions: (1) there exists copy number variation of TEs among *Triticum* and *Aegilops* species, which might be the result of different activity levels; (2) long elements were active in specific genomes during the evolutionary history of wheat, contributing to the diversification of diploid wheat species; (3) *Ae. speltoides* by itself cannot be the only contributor of the B genome to polyploid wheat; (4) elements which proliferate in specific genomes are, apparently, reactivated (or undergo rearrangements) following polyploidization and might play a role in the genetic differentiation of polyploid homeologous chromosomes; (5) the changes that occur following polyploidization events are unique to each TE family; and (6) early changes in TE copy numbers impact the subsequent genomic evolution of that element.

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