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Research review

Impact of transposable elements on the organization and function of allopolyploid genomes

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Summary

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Key words: allopolyploidy, diploidization, genome evolution, stress, transposable element, *Viridiplantae*.

Transposable elements (TEs) represent an important fraction of plant genomes and are likely to play a pivotal role in fuelling genome reorganization and functional changes following allopolyploidization. Various processes associated with allopolyploidy (i.e. genetic redundancy, bottlenecks during the formation of allopolyploids or genome shock following genome merging) may allow accumulation of TE insertions. Our objective in carrying out a survey of the literature and a comparative analysis across different allopolyploid systems is to shed light on the structural, epigenetic and functional modifications driven by TEs during allopolyploidization and subsequent diploidization. The available evidence indicates that TE proliferation in the short or the long term after allopolyploidization may be restricted to a few TEs, in specific polyploid systems. By contrast, data indicate major structural changes in the TE genome fraction immediately after allopolyploidization, mainly through losses of TE sequences as a result of recombination. Emerging evidence also suggests that TEs are targeted by substantial epigenetic changes, which may impact gene expression and genome stability. Furthermore, TEs may directly or indirectly support the evolution of new functionalities in allopolyploids during diploidization. All data stress allopolyploidization as a shock associated with drastic genome reorganization. Mechanisms controlling TEs during allopolyploidization as well as their impact on diploidization are discussed.

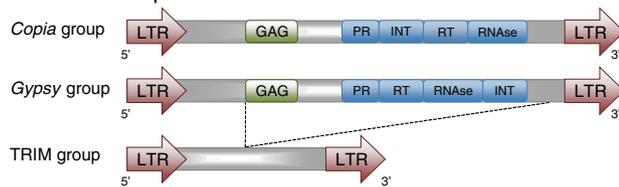
Introduction

Transposable elements (TEs) are stretches of DNA that move around the genome and can be categorized into two

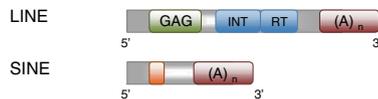
main classes: retrotransposons, which move via RNA intermediates, and DNA transposons, which move via DNA intermediates (Wicker *et al.*, 2007; Fig. 1). TE expression and mobility seem to respond to specific stimuli, such as

Class I transposable elements or retrotransposons

LTR retrotransposons



Non-LTR retrotransposons



Class II transposable elements or DNA transposons

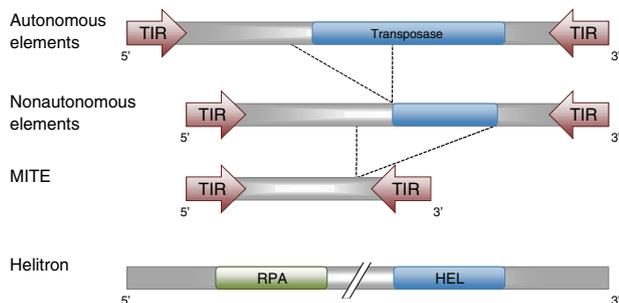


Fig. 1 Transposable elements (TEs) frequently observed in plant genomes. Class I TEs or retrotransposons move via RNA intermediates by a 'copy-and-paste' mechanism. Retrotransposons bordered by long terminal repeats (LTRs) are further divided into *Copia* and *Gypsy* according to the architecture of their coding sequence. Terminal-repeat retrotransposons in miniature (TRIMs) lack coding sequences and thus are nonautonomous. Non-LTR retrotransposons are divided into long and short interspersed nuclear elements (LINE and SINE, respectively). Class II TEs or DNA transposons move via DNA intermediates, by either a 'cut-and-paste' mechanism or replication of the DNA copy. Most transposons are bordered by terminal inverted repeats (TIRs) and are either autonomous or nonautonomous. Miniature inverted-repeat transposable elements (MITEs) represent a heterogeneous group of very short TEs. *Helitrons* are TEs that replicate via a rolling-circle mechanism. GAG, capsid protein; PR, protease; INT, integrase; RT, reverse transcriptase; RNase, ribonuclease H; RPA, replication protein A; HEL, helicase. See Wicker *et al.* (2007) for a comprehensive description.

stress conditions for plant long terminal repeat (LTR) retrotransposons (reviewed in Grandbastien *et al.*, 2005). Given that TEs represent a major fraction of eukaryotic genomes, especially in plants, they can be substrates for unequal and illegitimate recombination and account for a variety of mutations such as deletions, insertions, frameshifts, inversions, translocations and duplications (Lonnig & Saedler, 2002). Therefore, TEs contribute greatly to structural repatterning of genomes through transposition and/or recombination (Vitte & Panaud, 2005). TEs can also affect gene expression and function by inserting into genic regions (Lockton & Gaut, 2009). As expression of TE sequences is usually controlled by epigenetic host regulation, TE inser-

tions may indirectly participate in further regulation of gene expression (Slotkin & Martienssen, 2007). Given that TEs are highly mutagenic and silenced by overlapping epigenetic mechanisms, they are likely to play a pivotal role in fuelling genome reorganization and functional changes.

McClintock (1984) postulated that TE activation could play a significant role in genome changes under evolutionary challenges, such as interspecific crosses. Accordingly, allopolyploidization (i.e. the hybridization of divergent genomes associated with their doubling) is usually coupled with rapid structural and functional alterations of genomes (Leitch & Leitch, 2008), which seem to be especially dramatic in the repetitive component (Comai *et al.*, 2003). While most allopolyploid genomes show extensive and reproducible restructuring in the first generations following their formation (e.g. Lim *et al.*, 2007), some cases of near structural stasis have also been reported (e.g. Ainouche *et al.*, 2009). Polyploidy is certainly one of the predominant challenges encountered by flowering plants, but our understanding of the origin and genetic processes underlying their rapid genome repatterning is far from comprehensive and the impact of TEs deserves attention.

Polyploidization has generally been assumed to induce a burst of transposition (Matzke & Matzke, 1998; Comai, 2000). Indeed, several nonexclusive processes associated with allopolyploidy predicate the accumulation of TE insertions: genome doubling certainly relaxes purifying selection against deleterious TE insertions, which may result in apparent TE amplification in polyploids (the Redundancy hypothesis; Matzke & Matzke, 1998); polyploid formation usually involves an important reduction in effective population size, which may favor the fixation of neutral or moderately deleterious TE insertions through stochastic effects (the Bottleneck hypothesis; Lynch, 2007); hybridization between divergent genomes and polyploidization may represent a shock activating TEs and hence promoting transposition (the Genome Shock hypothesis; McClintock, 1984). While the Redundancy and Bottleneck hypotheses build on the evolutionary fate of inserted TEs, only the Genome Shock hypothesis assumes a polyploidy-induced change in TE activity *per se* (i.e. expression and transposition). However, if redundancy alone affects the observed rate of transposition, active TEs would show nearly continuous accumulation after polyploidization until full diploidization is reached. By contrast, a discrete burst of transposition is expected under the Bottleneck or Genome Shock hypothesis. A transient reduction in population size or shock-induced TE activation would indeed promote TE dynamics in the first generations after polyploidization, dynamics that would then be damped down as the population expanded. It should be possible to determine whether bursts of instability originate from bottlenecks or from genome shocks by considering different TE families, because a bottleneck is a demographic process influencing the whole

genome similarly (including most TE families), while a genome shock response is expected to affect specific TE families only.

In this paper, we review recent insights emerging from the analysis of different allopolyploid systems at different evolutionary time scales. More specifically, our aims here are: to evaluate patterns of TE dynamics after allopolyploidization to shed light on the processes underlying genome plasticity; and to explore the multiple mechanisms through which TE-induced genome reorganization may contribute to the evolution of allopolyploid genomes.

Short-term changes in the TE genome fractions of polyploids

Newly synthesized or recent (less than few hundred years old) allopolyploids allow one to examine immediate responses to allopolyploidy (short-term ‘revolutionary’ changes as defined by Feldman & Levy, 2009), and to distinguish the respective impacts of genome merging and genome doubling. Accordingly, allopolyploidy-induced genome reorganization associated with TEs can be quantified by comparing the organization of TE genome fractions in parental and nascent allopolyploid lineages using transposon display strategies. Transposon displays, such as sequence-specific amplified polymorphism (SSAP; Waugh *et al.*, 1997), simultaneously amplify multiple TE insertions throughout the genome, allowing one to visualize the gain and loss of fragments in hybrids and allopolyploids and to detect whether reorganization in TE fractions mainly affects

a particular parental subgenome (Fig. 2). Results from such investigations in various allopolyploid systems are reported in Table 1.

Transposition burst immediately after allopolyploidy?

Polyploid-specific SSAP fragments that are not present in the parental diploids (Fig. 2) are often considered as indicative of transposition. This assumption is not, however, always valid as new SSAP fragments may also result from molecular changes at insertion sites that modify the size of the amplification product, and assessment of the transpositional nature of new SSAP bands requires experimental validation (Petit *et al.*, 2010).

No evidence for transposition was reported in synthetic allopolyploids resulting from the doubling of a cross between *Arabidopsis thaliana* and *Arabidopsis lyrata* subsp. *petraea*, up to the third generation (Beaulieu *et al.*, 2009). Also, all TE insertions were found to be fixed in the recent allopolyploid *Arabidopsis suecica*, providing indirect evidence for the absence of a transposition burst after allopolyploidization (Hazzouri *et al.*, 2008). By contrast, limited evidence gathered from various polyploids of *A. thaliana* and *Arabidopsis arenosa* suggested transposition events involving *Sunfish* transposons (Madlung *et al.*, 2005).

Investigation of the 150-yr-old allopolyploid *Spartina anglica* yielded little evidence of transposition in natural populations. Comparisons of the SSAP profiles of the parental species (*Spartina alterniflora* and *Spartina maritima*), the F₁ homoploid hybrids (*Spartina* × *neyrautii* and *Spartina*

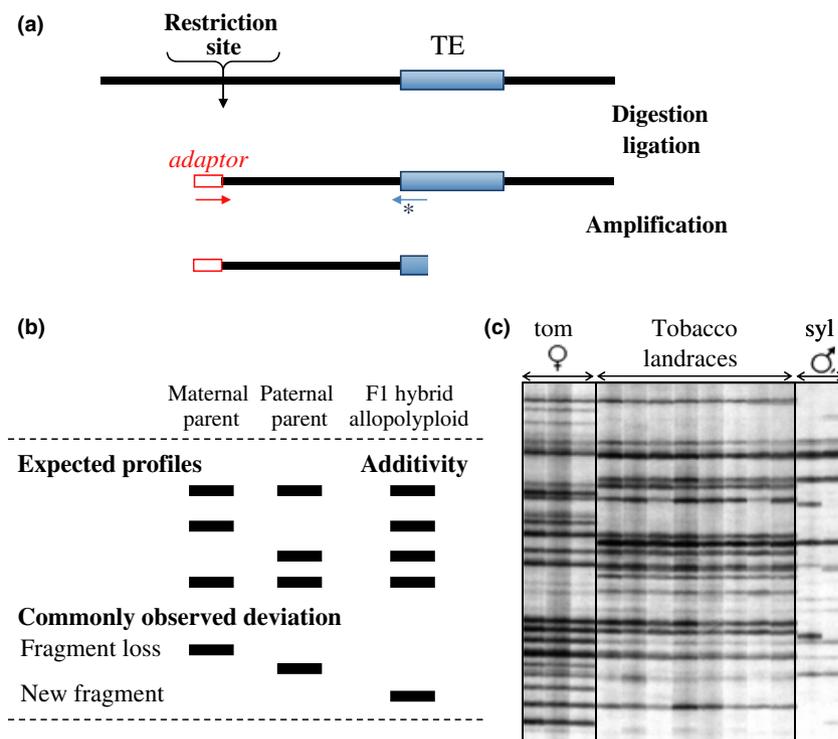


Fig. 2 Principle of the sequence-specific amplified polymorphism (SSAP) transposon display strategy. (a) Schematic representation of the SSAP procedure. This transposable element (TE)-anchored PCR strategy allows the simultaneous detection of labeled fragments containing the termini of inserted copies of a given TE and its flanking genomic region up to the nearest restriction site. (b) Schematic representation of commonly observed SSAP fragment patterns. F₁ hybrids and/or allopolyploids are expected to present profiles additive to those of their progenitor parents. Deviation from additivity corresponds to structural changes in the TE genome fraction. (c) Example of SSAP profiles with Tnt1 in the allopolyploid *Nicotiana tabacum* (various landraces) and its diploid progenitors *Nicotiana sylvestris* (syl) and *Nicotiana tomentosiformis* (tom) (Petit *et al.*, 2007).

Table 1 Current data on the reorganization of the transposable element (TE) genome fraction after allopolyploidy

Model species	TEs	Mobility	Loss of TE sequences	Transcriptional and epigenetic changes	References
Short-term reorganization					
<i>Arabidopsis thaliana</i> × <i>Arabidopsis arenosa</i>	<i>Sunfish</i>	ε	NE	Epigenetic changes and transcriptional activation	Madlung <i>et al.</i> (2005)
<i>Arabidopsis thaliana</i> × <i>Arabidopsis lyrata</i> (F1/S0, S1, S2)	CAC and AC-III	–	+ (P)	NE	Beaulieu <i>et al.</i> (2009)
<i>Spartina alterniflora</i> × <i>Spartina maritima</i> (F1)	Wis-like, <i>Cassandra</i> and <i>Ins2</i>	ε	++ (M)	++ (methylation levels)	Parisod <i>et al.</i> (2009)
<i>Spartina alterniflora</i> × <i>Spartina maritima</i> (S0 = <i>Spartina anglica</i>)	Wis-like, <i>Cassandra</i> and <i>Ins2</i>	ε	ε (M)	+ (methylation levels)	Parisod <i>et al.</i> (2009)
<i>Nicotiana sylvestris</i> × <i>Nicotiana tomentosiformis</i> (F1/S0)	Tnt1	–	–	NE	Pettit <i>et al.</i> (2010)
<i>Nicotiana sylvestris</i> × <i>Nicotiana tomentosiformis</i> (S4)	Tnt1	++	+++ (P)	NE	Pettit <i>et al.</i> (2010)
<i>Aegilops sharonensis</i> × <i>Triticum monococcum</i> (S1)	Wis 2-1A	–	NE	Transcriptional activation	Kashkush <i>et al.</i> (2003)
<i>Triticum turgidum</i> × <i>Aegilops tauschii</i> (S0, S1, S2)	Retrotransposons and CACTA	–	–	Epigenetic changes and transcriptional activation	Mestiri <i>et al.</i> (unpublished)
<i>Brassica rapa</i> × <i>Brassica oleracea</i>	<i>Athila</i> -like and MITEs	–	+	NE	V. Sarilar & K. Alix (unpublished)
Long-term reorganization					
<i>Nicotiana tabacum</i> (<i>Nicotiana sylvestris</i> × <i>Nicotiana tomentosiformis</i>)	Tnt1, Tnt2 and Tto1	++	+++ (P for Tnt2)	NE	Pettit <i>et al.</i> (2007)
<i>Brassica napus</i> , <i>Brassica carinata</i> , <i>Brassica juncea</i>	Retrotransposons	–	NE	NE	Alix & Heslop-Harrison (2004)
<i>Brassica napus</i>	CACTA <i>Bot1</i>	–	NE	NE	Alix <i>et al.</i> (2008)
<i>Gossypium hirsutum</i> (<i>Gossypium arboreum</i> × <i>Gossypium raimondii</i>)	Various TEs	ε	+++ (M)	NE	Zhao <i>et al.</i> (1998); Grover <i>et al.</i> (2007)
<i>Oryza minuta</i> (<i>Oryza punctata</i> × <i>Oryza officinalis</i>)	Various TEs	+	++ (P)	NE	Lu <i>et al.</i> (2009)
<i>Triticum aestivum</i> (<i>Triticum turgidum</i> × <i>Aegilops tauschii</i>)	Various TEs	+++	+++	NE	Chantret <i>et al.</i> (2005); Charles <i>et al.</i> (2008)

–, no evidence; ε, ≤ 5%; +, 5–10%; ++, 10–40%; +++, > 40%; NE, not evaluated.

P, paternal; M, maternal, referring to the parental origin of the TE sequences predominantly lost.

× *townsendii*) and the allopolyploid revealed only a few possible new TE insertions, suggesting that a transposition burst did not occur in the TE families investigated (Parisod *et al.*, 2009). In synthetic allopolyploid wheat (*Aegilops sharonensis* × *Triticum monococcum*), Kashkush *et al.* (2003) screened 1000 insertion sites of the retrotransposon *Wis* 2-1A but reported no evidence of polyploidy-induced transposition. Similarly, the organization of seven retrotransposons and one CACTA transposon in synthetic allohexaploid wheat (*Triticum aestivum*) revealed no evidence of *de novo* insertions in the first three generations (Mestiri *et al.*, unpublished).

In resynthesized allopolyploids of *Nicotiana tabacum* (*Nicotiana sylvestris* × *Nicotiana tomentosiformis*), amplification of the Tnt1 retrotransposon was detected (Petit *et al.*, 2010). Interestingly, TE proliferation was detected mostly for young and active Tnt1 elements, indicating that activation in response to allopolyploidy may be restricted to a few specific TE populations. However, there was evidence of transposition in neither newly synthesized F₁ homoploid hybrids nor artificially doubled lineages (S₀), but only in distinctive synthetic allopolyploids of the fourth generation (S₄), suggesting that TE activation may not be immediate and may require meiosis (S₀ to S₁) during which homeologous genomes may interact (Petit *et al.*, 2010).

In summary, few surveys have found evidence of a TE transposition burst immediately after allopolyploidization (Table 1), suggesting that triggering of TE mobility is not a common phenomenon and may be restricted to specific TE families in particular allopolyploids, and to mostly young active TE populations.

Structural changes in TE genome fractions immediately after allopolyploidy

Further rearrangements in TE sequences can be indicated by SSAP fragment losses. While such losses may be caused by excisions of DNA transposons, such restructuring is also frequently found with retrotransposons that do not excise, and the nature of genetic changes leading to fragment losses is often hard to determine. Fragment losses may be due to indels at TE insertion sites or rearrangements encompassing TE insertion sites (Petit *et al.*, 2010). Loss of fragments has been reported in most polyploids and is always more frequent than putative TE transpositions (Table 1).

In synthetic allohexaploid wheat, 186 insertions characterized in the progenitor genomes from various TEs showed additive patterns of amplification when checked for deletion by direct PCR, indicating no major short-term rearrangements (Mestiri *et al.*, unpublished). When different resynthesized *Brassica napus* allotetraploids are compared with their diploid progenitors (*Brassica rapa* and *Brassica oleracea*), work in progress indicates that genome reorganization seems to be dependent on the TE considered (V. Sarilar &

K. Alix, unpublished). A pericentromeric retrotransposon (*Athila*-like) generates mostly additive SSAP profiles, while an interstitial miniature inverted-repeat transposable element (MITE) yields up to 20% nonadditive SSAP fragments, notably in the more advanced generations after allopolyploidy. Transposition events were only rarely identified; most reorganization apparently includes other types of restructuring in the TE fraction.

In resynthesized *Nicotiana tabacum*, structural changes after polyploidization were predominantly losses of SSAP fragments of paternal origin (Petit *et al.*, 2010). By contrast, losses of SSAP fragments of maternal origin seem to predominate in *S. anglica* (Parisod *et al.*, 2009). In the latter case, changes in TE genome fractions mostly occurred after hybridization rather than genome doubling and differentially affected sequences near TEs (SSAP) as compared with random sequences (amplified fragment length polymorphism; AFLP), suggesting TE-specific genome reorganization associated with genome merging.

Thus, the first generations following polyploidization seem to be characterized by substantial structural rearrangements in the TE fraction, usually targeting chiefly one parental subgenome.

Epigenetic reorganization and transcriptional activation in the TE genome fraction of nascent allopolyploids

Drastic epigenetic and expression changes have been reported immediately after allopolyploidization, and epigenetic changes, such as changes of DNA methylation affecting the TE genome fraction, and their putative relationship to TE expression, are increasingly documented (Comai *et al.*, 2003; Josefsson *et al.*, 2006; Michalak, 2009). Increased expression of TEs was found in synthetic allopolyploid wheat (Kashkush *et al.*, 2002) and *Arabidopsis* (Madlung *et al.*, 2005), but did not seem to be regularly associated with increased transposition. However, epigenetic changes enhancing TE expression after polyploidization might have pervasive effects across the genome by rewiring gene expression networks. In wheat, alteration in gene expression (either activation or silencing) through transcriptional activation of retrotransposons was demonstrated (Kashkush *et al.*, 2003), showing that epigenetic changes in the vicinity of TE insertions also participate in reorganizing the functional genome after polyploidization.

The young allopolyploid *S. anglica* was recently investigated by SSAP using enzymes with differential sensitivity to DNA methylation (Parisod *et al.*, 2009). Methylation changes were significantly more frequent around TE insertions as compared with random sequences and predominantly affected the maternal subgenome. This reorganization mainly occurred just after hybridization, suggesting that TEs fuel epigenetic alterations at the merging

of diverged genomes. Whether methylation changes around TEs also affect gene expression remains an open question, but preliminary observations suggest that transcriptional changes following allopolyploidization may occur in the vicinity of TEs (H. Chelaifa & M. Ainouche, unpublished).

As a whole, epigenetic changes induced by polyploidy seem to widely and specifically affect TE genome fractions, suggesting connections with possible structural reorganization of the genome through TE activation. Furthermore, TEs also mediate epigenetic influences and may transfer them to neighboring sequences, generating heritable variation in gene expression (Slotkin & Martienssen, 2007).

Impact of TEs on the long-term reorganization of allopolyploid genomes

Long-term 'evolutionary' changes as defined by Feldman & Levy (2009) are usually studied in natural counterparts of synthetic hybrids or in related species resulting from allopolyploidy events occurring a few thousands to millions of years ago. Such comparative approaches in current species provide detailed information about the long-term dynamics of TEs during diploidization. However, adopting a genome-wide perspective within a well-defined phylogenetic framework is difficult, notably because the life-span of TE insertions is *c.* 3 Myr in most model systems (Vitte & Panaud, 2005), limiting the power of transposon display strategies. As a consequence, most studies have relied on the sequencing of orthologous loci in parental and derived allopolyploids, and our understanding of diploidization is presently limited to selected genomic regions. Nevertheless, this latter approach has the advantage of quantifying all existing TE insertions present in these regions and allowing accurate inferences about the nature of genetic changes.

Structural changes in the TE genome fraction of established allopolyploids

The combination of molecular cytogenetics and SSAP in various allopolyploid *Nicotiana* species highlighted the impact of repetitive sequences on the long-term diploidization of genomes (Lim *et al.*, 2007). Recent *Nicotiana* allopolyploid species (up to 200 kyr old) such as tobacco (*Nicotiana tabacum*) show partial restructuring in TE genome fractions, with gains and losses of SSAP fragments (Petit *et al.*, 2007). As in resynthesized allopolyploids, the proportion of new fragments in natural tobacco was found to be higher for young active retrotransposon populations, suggesting amplification events (Petit *et al.*, 2007). Furthermore, losses of SSAP fragments clearly are more frequent than possible transpositions. In older *Nicotiana* allopolyploids, such as *Nicotiana quadrivalvis* (*c.* 1 Myr old) and *Nicotiana nesophila* (*c.* 4.5 Myr old), evidence of considerable exchange of repetitive sequences between

subgenomes has been found (Lim *et al.*, 2007). Thus, a nearly complete turnover of intergenic regions occurred in less than 5 Myr in *Nicotiana* allopolyploids. Collectively, these studies demonstrate that the differential proliferation and removal of various repetitive sequences played an important role in the structural differentiation of homeologous genomes within a conserved karyotype structure.

By contrast, analysis of three diploid *Brassica* species (*Brassica rapa*, *Brassica oleracea* and *Brassica nigra*) and the three corresponding allopolyploids (*Brassica napus*, *Brassica carinata* and *Brassica juncea*) did not reveal any particular retrotransposon proliferation in the allopolyploids (Alix & Heslop-Harrison, 2004). In addition, Alix *et al.* (2008) characterized a *Brassica* C-genome-specific CACTA transposon (*Bot1*) that played a major role in genome divergence at the diploid level. Cytogenetic evidence revealed no spreading of *Bot1* to the other subgenome in the recent allotetraploid *B. napus*, suggesting that, in *Brassica*, polyploidization was not correlated to activation of TEs, or that homogenization of TE insertions had not yet occurred.

In cotton, molecular cytogenetic studies in *Gossypium arboreum*, *Gossypium raimondii* and the 1.2-Myr-old allopolyploid *Gossypium hirsutum* have demonstrated that different TEs spread from one to the other subgenome after allopolyploidization (Zhao *et al.*, 1998). Comparative analysis of the alcohol dehydrogenase A (*AdhA*) locus in the same species revealed breaks of microsynteny between the diploid genomes to be mostly attributable to insertions of *Gypsy* retrotransposons (Grover *et al.*, 2007). However, this study found no marked difference in TE activity across the ploidy levels in *Gossypium* (Table 1). Indeed, the dynamics at two loci (*AdhA* and cellulose synthase; *CesA*) revealed that structural changes in the allopolyploid were frequently small deletions in TE sequences (Grover *et al.*, 2008). Thus, diploid genomes were characterized by overall growth, while homeologous genomes in the polyploid showed an increased rate of deletion and a reduced rate of insertion (*i.e.* contraction). Lu *et al.* (2009) compared the *MONO-CULM1* region of the diploids *Oryza punctata* and *Oryza officinalis* with that of the *c.* 2-Myr-old allopolyploid *Oryza minuta*, and found restructuring of the TE fraction in the allopolyploid, including several specific insertions and losses of TE sequences. The fate of TE insertions seems dependent on the parental origin, as most of the TE insertions that were not fully transmitted to the polyploid were of paternal origin.

The genomes of hexaploid wheat diverged less than 3 Myr ago, and the sequencing of 10 genomic regions showed that they are *c.* 90% divergent as a result of differential proliferation of TE families (Charles *et al.*, 2008). After an ancestral proliferation of *Athila*-like retrotransposons, specific amplification of *Copia* and of *Gypsy* retrotransposons fuelled the divergence of genomes before

allopolyploidization. However, genome merging appears to have neither enhanced nor repressed transposition, which persisted at the same rate in diploid and allopolyploid species. By contrast, the different subgenomes of the hexaploid wheat presented extensive deletions in TE sequences as compared with their diploid progenitors, suggesting that TEs facilitate unequal or illegitimate recombination (Chantret *et al.*, 2005). Given that data gathered using transposon display provided similar evidence (Bento *et al.*, 2008), TEs are strongly suggested to mediate deletions and rearrangements in response to changes in ploidy levels.

In polyploid maize (*Zea mays*) (c. 10 Myr old), sequence comparison among diverged lineages revealed surprisingly variable arrangements of TE insertions in intergenic regions (Bruggmann *et al.*, 2006). Many TE insertions seem to have occurred after polyploidization, suggesting amplification. A recent study showed extensive recombination in the abundant CRM1 retrotransposon of maize, suggesting that the acquisition of advantageous mutations in TE sequences might account for their proliferation (Sharma *et al.*, 2008). The authors speculated that allopolyploidy might sustain inter-element recombination by bringing together TEs that evolved in different backgrounds and thus promote TE amplification. Studies in maize also revealed dramatic expansion and contraction of different portions of the genome under the influence of differential TE insertions/deletions and gene translocations, indicating that genome plasticity and turnover of intergenic regions might be largely controlled by TEs during diploidization.

Although genome downsizing after polyploidization appears to be a general trend, mechanisms underlying DNA sequence elimination remain largely unknown (Leitch & Bennett, 2004). Intrastrand recombination between retrotransposon LTRs would adequately remove substantial pieces of DNA and counteract genome expansion through transposition (Vitte & Panaud, 2005). However, illegitimate recombination, which only requires small homologous motifs, might be one of the dominant mechanisms for genome contraction after polyploidization. Although it mainly creates small deletions, comparative sequencing has revealed a multitude of truncated TE insertions and experimental evidence suggesting illegitimate recombination as a major process in genome restructuring during diploidization is accumulating (see Chantret *et al.*, 2005).

Long-term impact of TEs on the functioning of allopolyploid genomes

Recent studies illustrate the multiple mechanisms through which TEs regulate gene expression and function (Kashkush *et al.*, 2003; Bennetzen, 2005), and ultimately phenotypes. In addition, an indirect impact of TE-generated rearrangements on phenotypes has also been reported. For instance, unequal or illegitimate recombination implicating different

TEs has driven the recurrent deletion of the *Hardness* locus, which controls grain hardness in wheat, in different subgenomes of various polyploid wheat species (Chantret *et al.*, 2005). Given the high mutagenic nature of TEs, insertions may also allow duplicated genes to explore pioneering evolutionary trajectories and turn into beneficial innovation. A reasonable fraction of TE insertions might indeed affect regulatory sequences or coding portions of genes, some of which may be adaptive and account for the subfunctionalization or neofunctionalization of duplicates (Walsh, 2003). Although various mechanisms through which TEs might promote subfunctionalization or neofunctionalization have been demonstrated (Bennetzen, 2005), the extent to which TEs contribute to functional evolution according to this appealing model remains an open question in polyploids.

Impact of allopolyploidy on TE fractions and genome reorganization

The available evidence demonstrates considerable reorganization of the TE genome fraction after allopolyploidy. However, in contrast to the assumption that polyploidy induces spectacular bursts of transposition, only a small proportion of TEs showed increased insertional activity as compared with other types of structural changes in the TE genome fraction. Indeed, our survey of the literature suggests that allopolyploidization activates TE expression, but that transposition of particular TEs impacts the short- or the long-term evolution of allopolyploid genomes in only a few allopolyploid systems. By contrast, the studies we found in the literature mainly reported immediate loss of TE sequences and epigenetic repatterning in TE fractions after allopolyploidy. Furthermore, such reorganization usually seems targeted toward TE insertions from one subgenome. Discounting a significant role of either relaxed selection against TE insertions, as postulated by the Redundancy hypothesis, or evolutionary stochasticity, as assumed by the Bottleneck hypothesis, this pattern suggests that allopolyploidy is a major genome shock associated with drastic structural and epigenetic reorganization in the TE fraction, possibly to overcome incompatibilities unmasked by hybridization (McClintock, 1984; Comai *et al.*, 2003).

Immediate reorganization of TE sequences suggests that TEs play a central role during the early stage of allopolyploid evolution, and two nonexclusive hypotheses might explain the observed bursts of instability. On the one hand, prevailing restructuring of the TE fraction may simply reflect nontargeted DNA lesions resulting from failed chromosome pairing between homeologous chromosomes at the first meiosis of the hybrid structure, which could primarily reflect on TEs since they are major genome components. Additionally, epigenetic changes predominate around TE insertions compared with random sequences (e.g. Parisod *et al.*, 2009) and decreased methylation in response to stress

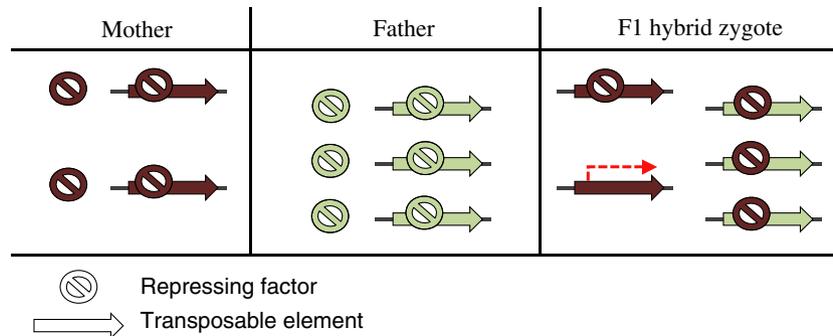


Fig. 3 Dosage-dependent induction model for the control of transposable elements (TEs) during allopolyploidization (after Josefsson *et al.*, 2006). Upon hybridization, maternally contributed repressors (e.g. small interfering RNA) are immediately available in the cytoplasm, but might be insufficient to saturate total TE insertions of both maternal and paternal origin. In cases where parental genomes have different TE loads, maternal and/or paternal TEs could escape silencing and become activated, resulting in deleterious transposition bursts. Thus, restructuring TE sequences might represent an additional source of TE repression.

has been associated with increased levels of recombination (Boyko *et al.*, 2007). Genome rearrangements in the TE fraction of nascent allopolyploids might thus result from temporal overlap between TE activation and the formation of DNA lesions. On the other hand, it is also tempting to speculate that a shock-induced burst of transposition would be deleterious and that viable polyploids would have circumvented proliferation by immediately repressing TEs. This could be achieved through both structural changes and reorganization of epigenetic marks in TE sequences. Indeed, overcoming incompatibilities related to parent-specific TE fractions and achieving proper development of hybrid zygotes requires balanced proportions of interacting components (Josefsson *et al.*, 2006; Michalak, 2009). For instance, insufficient quantities of repressing factors (such as small interfering RNAs) to saturate quiescent TEs could translate into TE activation and a burst of transposition (Fig. 3). As only the female parent immediately contributes to cytoplasmic TE repressing factors, it is possible that such incompatibilities may induce asymmetrical reorganization among the subgenomes. Such a dosage-dependent process could be accentuated in the triploid endosperm tissue (where the maternal genome contributes twice as much material as the paternal genome), which is crucial for seed viability. In this context, restructuring of TE sequences may add to epigenetic silencing in repressing excess TEs from transposition after allopolyploidization.

Although TEs are likely to be pivotal players during the diploidization process, little is actually known about their long-term impact on allopolyploid genomes. TEs may have played a major role during structural diploidization, as found in *Nicotiana* (Lim *et al.*, 2007) or maize (Bruggmann *et al.*, 2006), because extensive reorganization of the TE genome fraction as a result of recombination and, to a lesser extent, transposition is likely to break microsynteny and may increase the differentiation between homeologous genomes. TEs may also fuel functional diploidization by supporting various types of rearrangements with potential

implications for gene function and expression (Bennetzen, 2005).

To summarize, the effect of allopolyploidy on TE genome fractions may be more complex than generally assumed. As TEs are abundant and dispersed throughout genomes, they predispose a young allopolyploid genome to rapid shuffling, participating in natural genetic engineering and producing abundant raw material for adaptive evolution at a crucial moment. These effects of TEs warrant more detailed study in the future.

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