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Retrotransposons Represent the Most Labile Fraction for Genomic Rearrangements in Polyploid Plant Species

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Arabidopsis · Genomic rearrangements · Polyploidy · Retrotransposons · Triticeae

Abstract
Understanding how increased genome size and diversity within polyploid genomes impacts plant evolution and breeding continues to be challenging. Although historical studies by McClintock suggested the importance of transposable elements mediated by polyploidisation on genomic changes, data from plant crosses remain scarce. Despite the absence of a conclusive proof regarding autonomous retrotransposon movement in synthetic allopolyploids, the transposition of retrotransposons and their ubiquitous dispersion in all plant species might explain the positive correlation between the genome size of plants and the prevalence of retrotransposons. Here, we address polyploidisation-mediated rearrangements of retrotransposon-associated sequences and discuss a tendency for a preferential restructuring of large ancestral genomes after polyploidisation. A comparative analysis of the frequency of modifications of retrotransposon-associated sequences in synthetic polyploids with marked differences in genome sizes is presented. Such analyses suggest the absence of a significant difference in the rates of rearrangements despite vast dissimilarities in the retrotransposon copy number between species, which emphasises the high plasticity of this genomic feature. See also the sister article focusing on animals by Arkhipova and Rodriguez in this themed issue. Copyright © 2013 S. Karger AG, Basel

Polyploidy results from the union of 2 or more genomes in the same nucleus and is the major mode of plant genome evolution that can be artificially recreated. In Angiospermae, 30–70% of plant species have a polyploid origin, and almost all present species are considered to be paleopolyploids [Wendel, 2000; Wolfe, 2001].

Polyploids are classified into auto- or allopolyploids according to their genomic origin and can exhibit different ploidy levels. Autopolyploids result from genome doubling within the same species, and allopolyploids are formed by the combination of 2 or more distinct but usually related genomes. A survey of the published literature revealed that autopolyploid formation is higher than that of allopolyploids, suggesting that autopolyploids are much more common than expected [Ramsey and Schemske, 1998], though the consequences of these evolutionary outcomes are still elusive [Parisod et al., 2010b]. Two prevailing models that can explain the natural emergence of allopolyploids are the '2-step' model, involving interspecific hybridisation followed by chromosome doubling of the F1 hybrid, and the 'one-step' model, which is based

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on fertilisation of unreduced gametes from different diploid species or direct interspecific hybridisation between distinct autotetraploid species [Chen and Ni, 2006]. Autopolyploids can arise by either model involving only one species; however, it is usually believed that most autoplolyploids resulted from fertilisation of unreduced gametes because spontaneous chromosome doubling is a rare event in nature [Chen and Ni, 2006].

As first suggested by McClintock [1984], the rise of both auto- and allopolyploids can induce a 'genomic shock', which is responsible for several genomic modifications that seem to be more pronounced in allopolyploids, reflecting their basic genome incompatibilities. The genetic and epigenetic modifications involved in the reorganisation of allopolyploid paternal genomes are extensively documented [Comai, 2000; Ma and Gustafson, 2005; Chen et al., 2008; Feldman and Levy, 2009; Jones and Hegarty, 2009], reaching from the number or organisation of chromosomes to genomic changes associated with genome downsizing and sequence modifications. In 1984, McClintock suggested that such widespread genomic changes can result from a higher activity of transposable elements (TEs). TEs constitute one of the main types of repetitive sequences, representing stretches of DNA that move throughout the genome and typically fall into 2 basic classes based on their transposition intermediate: RNA (retrotransposons or class I) and DNA (DNA transposons or class II). DNA transposons are present at low or moderate frequency in almost all eukaryotes, moving via DNA intermediates by either a 'cut-and-paste' mechanism or through DNA replication [Wicker et al., 2007]. Retrotransposons transpose via RNA intermediates without excising the original copy, are ubiquitously dispersed in all plant species and represent more than 50% of the genomic sequence in some cases [Kumar and Bennetzen, 1999; Lisch, 2013]. The retrotransposon 'copy-and-paste' mechanism of transposition introduces new copies of the original retroelement into the genome and may rapidly increase the frequency of repetitive elements in plant genomes [Schulman et al., 2004; Dvořák, 2009]. Such a tendency for an increase in retrotransposon copy number is counteracted by sequence deletions such as illegitimate retrotransposon recombination, which results in retrotransposon excision [Devos et al., 2002]. Thus, the ratio between TE insertions and deletions modulates the amount of repeated DNA in a genome and ultimately is largely responsible for the size of the genome [Dvořák, 2009].

Plant retrotransposon dynamics seems moreover to be influenced by different environmental conditions because it was demonstrated that various biotic and abiotic stresses, such as infection, cold, heat, hybridization, or generation of doubled haploids, increase their transcriptional activity [Bennetzen, 2000; Mansour, 2007]. Retrotransposons can, therefore, be a source of innumerous mutations, deletions, insertions, frameshifts, inversions, translocations, and duplications that play major roles in structural genomic changes [Kumar and Bennetzen, 1999; Lippman et al., 2004] and may affect gene functions through cis and/or trans gene regulation [Chen and Ni, 2006]. Although many studies review the importance of polyploidy or retrotransposons in plants, most consider them as separate issues, and few discuss the impact of polyploidisation on transposable element dynamics. In this article, we review the influence of polyploidisation on the retrotransposon copy number and genomic rearrangements associated with retrotransposon elements. The role of retrotransposons in genomic alterations in species with large and small genomes is further discussed based on new results from *Arabidopsis* allopolyploids.

Retrotransposon Classes and Abundance in Plant Genomes

Retrotransposons classify into 2 major categories: long terminal repeats (LTR) containing elements (LTR retrotransposons, the most numerous class of large retroelements) and non-LTR retrotransposons (fig. 1). LTR retrotransposons are further divided into *Copia* and *Gypsy* groups according to the degree of sequence similarity and coding sequence organisation. Both *Copia* and *Gypsy* retrotransposons are present throughout the plant kingdom and are normally found in high copy number – up to a few million copies in plants with large genomes [Kumar and Bennetzen, 1999]. LTR retrotransposons differ greatly in size, ranging from a few hundred base pairs to very large LTR retrotransposons, such as the 25-kb element *Ogre* in peas [Neumann et al., 2003]. The sequences flanking LTRs also vary from several hundred base pairs to more than 5 kb [Wicker et al., 2007] and do not encode any known proteins but contain promoters and terminators that regulate their transcriptional activity [Kumar and Bennetzen, 1999]. Large retrotransposon derivate elements with lengths exceeding 4 kb and terminal repeat retrotransposons in miniature with lengths smaller than 4 kb also belong to the LTR retrotransposon class but lack coding sequences which qualifies them as nonautonomous elements. Illegitimate LTR recombination results in solo-LTRs originating from retrotransposon excision and may

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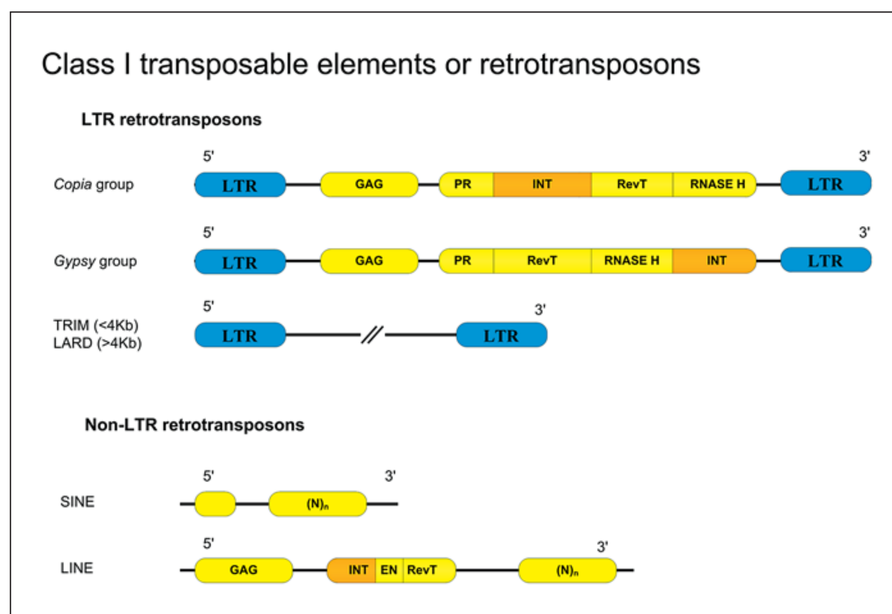

Fig. 1. Class I transposable elements or retrotransposons observed in plant genomes. Retrotransposons can be flanked by long terminal repeats (LTRs) and are further classified into *Copia* and *Gypsy* classes according to the architecture of their coding sequence organisation, composed of capsid protein (GAG), protease (PR), integrase (INT), reverse transcriptase (RevT), and RNase H (RNASE H) genes. Terminal-repeat retrotransposons in miniature (TRIMs) and large retrotransposon derivates (LARDs) lack coding sequences and, thus, are nonautonomous. Non-LTR retrotransposons are classified into short interspersed nuclear elements (SINEs), which contain a Pol III promoter and a 3′ variable region, and long interspersed nuclear elements (LINEs), which encode integrase, endonuclease, reverse transcriptase, and a variable region. EN = endonuclease; $(N)_n$ = variable region. Adapted from [Parisod et al., 2010a].

also occur between neighbouring retrotransposons of the same type [Devos et al., 2002]. Non-LTR retrotransposons are classified into short interspersed nuclear elements (SINE), which encompass 80–500 bp and are relatively rare in most plant genomes, and long interspersed nuclear elements (LINE), which can comprise several kilobases in length, possibly representing the most ancient class of retroelements [Wicker et al., 2007]. In fact, several studies suggest that initial LTR retrotransposons may have resulted from the incorporation of LTRs into LINEs [Bennetzen, 2000].

An extensive study of the Triticeae tribe, which encompasses species with large genomes, revealed that TEs represent up to 80% of those genomes and are comprised of retrotransposons, of which a larger fraction are *Gypsy*-like (corresponding approximately to 70% of the characterised TEs) and *Copia*-like retrotransposons (representing ~20%) as well as other less frequent classes [Middleton et al., 2013]. A positive correlation between plant genome size and retrotransposon frequency can easily be observed considering that in species with large genomes, such as *Triticum aestivum* (17,000 Mb), *Aegilops tauschii* (4,000 Mb) and *Zea mays* (2,665 Mb), retrotransposons represent ~63, ~53, and ~49% of these genomes, respectively [Bennett and Smith, 1976; Arumuganathan and Earle, 1991; Meyers et al., 2001; Li et al., 2004; Brenchley et al., 2012]. In contrast, in species with smaller genomes, such as *Brassica oleracea* (758 Mb), *Oryza sativa* (489 Mb) or *Arabidopsis thaliana* (125 Mb), retrotransposon elements only represent ~14, ~12 and ~5.6% of these genomes [Bennett and Smith, 1976; Olszewska and Osiecka, 1984; Arabidopsis Genome Initiative, 2000; Mao et al., 2000; Peterson-Burch et al., 2004; Zhang and Wessler, 2004].

It is plausible to believe that distinct retrotransposon families have descended from few ancient evolutionary lineages because a common origin was found for *Copia*-like retrotransposons present in wheat, rice and *Arabidopsis* [Wicker and Keller, 2007]. In addition to the existence of thousands of different retrotransposon families, it is quite interesting to note the dearth of families specific to only one species [Wicker and Keller, 2007] because almost all seem to be present in most genomes at least at a very low frequency [Middleton et al., 2013]. Through an in-depth study of 10 Triticeae taxa, Middleton et al. [2013] demonstrated that in each species, few families are very abundant and represent a large fraction of repetitive elements in large genomes. Similar disparity in the copy number of distinct LTR families was additionally observed in *B. rapa* [Wang et al., 2011]. Large sequencing projects in wheat additionally revealed that most LTR elements described are truncated [Choulet et al., 2010; Brenchley et al., 2012], and most likely originated from internal deletions, nested insertions and illegitimate recombination events resulting from the coexistence of TE families in certain chromosomal domains. Both in Triticeae species as well as in maize, most intergenic regions enriched in LTR retroelements are organised in complex nested insertions [SanMiguel et al., 1996;

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Dvořák, 2009]. Such nested arrangements of retrotransposons are rarely observed in *Arabidopsis* and *Brassica* species, which is in clear contrast to evidence from Triticeae [Arabidopsis Genome Initiative, 2000; Peterson-Burch et al., 2004; Alix et al., 2005; Wang et al., 2011].

Retrotransposons Are Involved in Polyploidisation-Associated Genomic Alterations

Autopolyploids are classified into 2 main types: 'typical' autopolyploids, characterised by multivalent pairing at meiosis and multisomic inheritance, and 'cytologically diploidised' autopolyploids, which exhibit almost exclusively homologous pairing at meiosis, although they have more than 2 genome copies [Eilam et al., 2010]. Cytologically diploidised autotetraploids display considerable genome downsizing immediately after autopolyploidisation, such as in the synthetic autopolyploid *Phlox drummondii,* where reductions of up to ~25% of the total parental DNA content in the third generation have been observed [Eilam et al., 2010]. In contrast, typical autopolyploids usually exhibit additive genome size values [Eilam et al., 2010]. Genomic studies of autopolyploids have been restricted to AFLP (amplified fragment length polymorphism) analyses, such as in *Arabidopsis*, where no significant changes were detected after autopolyploidisation [Ozkan et al., 2006]. The involvement of retrotransposons in autopolyploid genomic alterations has yet to be addressed, although activation of the *En/Spm*-like DNA transposon *(Sunfish)* was reported after investigating transposon instability by microarray analysis (and confirmed by a methyl-insensitive Southern blot) in *A. thaliana* autotetraploids [Madlung et al., 2005].

The impact of allopolyploidisation on transposable elements has been studied in few polyploid species, as reviewed by Parisod et al. [2010a], and the information regarding its influence on retrotransposons is even sparser. Despite reported changes in retrotransposon transcription, there is still a lack of evidence regarding autonomous retrotransposable element movement in synthetic allopolyploids [Kashkush et al., 2003]. For instance, in the allopolyploid *Aegilops sharonensis* × *T. monococcum*, the analysis of novel bands observed by cDNA-AFLP revealed the synthesis of new transcripts from adjacent sequences of *Wis*2-1A retrotransposon including antisense or sense genes, although no evidence of polyploidy-induced transposition was detected [Kashkush et al., 2003]. Although there is a lack of information regarding the effects of allopolyploidisation on retrotransposons, a study of the nonautonomous terminal repeat retrotransposons in miniature family *Veju* in allohexaploid wheat revealed a massive elimination (50%) of *Veju* LTRs in the first generation, followed by a burst in subsequent generations, which led to a marked increase in the *Veju* element copy number [Kraitshtein et al., 2010]. Transposition events were also reported for class II elements in allohexaploid wheat species [Yaakov et al., 2013]. Dot-blot analysis of natural wheat also indicated transposition bursts because the observed retrotransposon copy number was higher than the expected addition of retrotransposons in parental species. However, using the same methodology, no differences were detected in retrotransposon copy number immediately after polyploidisation in synthetic allopolyploids *A. speltoides* × *T. urartu, A. sharonensis* × *T. monococcum, A. speltoides* × *A. tauschii, and T. turgidum* × *A. tauschii* when compared with parental lines, suggesting that copy number variation occurs progressively after polyploidisation [Li et al., 2004].

IRAP (interretrotransposon amplified polymorphism) and REMAP (retrotransposon microsatellite amplified polymorphism) have also been important tools to evaluate the involvement of retrotransposon elements in allopolyploid genomic rearrangements. Using such markers in a comparative genomic analysis of the synthetic allopolyploid triticale (*T. aestivum* × *Secale cereale*) and the parental genomes, it was demonstrated that ~28% of retrotransposons and microsatellite-related sequences are rearranged in the polyploid [Bento et al., 2008]. Most rearrangements resulted from losses of parental bands, affecting both repetitive and coding sequences, and a minor frequency (4.2%) corresponded to novel bands. In the natural allopolyploid *Spartina anglica*, evidence of major changes in CpG methylation in the proximity of retrotransposon insertions were found by sequence-specific amplified polymorphism (SSAP) analysis; however, few new TE insertions were proposed among the retrotransposon families investigated (terminal-repeat retrotransposons in miniature, *Cassandra* and *Wis Copia*-like retrotransposons) [Parisod et al., 2009], although some fragment losses were detected immediately after hybridisation [Parisod et al., 2010a]. More recently, a study of *Athila*-like retrotransposons by SSAP in the synthetic allotetraploids *B. napus* revealed mostly additive profiles when compared with the diploid parents, and the characterisation of nonadditive SSAP bands indicated that genomic rearrangements had occurred rather than new transposition events [Sarilar et al., 2013]. Similar analyses revealed a proliferation of the *Tnt*1 retrotransposon after genome doubling in the synthetic *Nicotiana tabacum*. Structural

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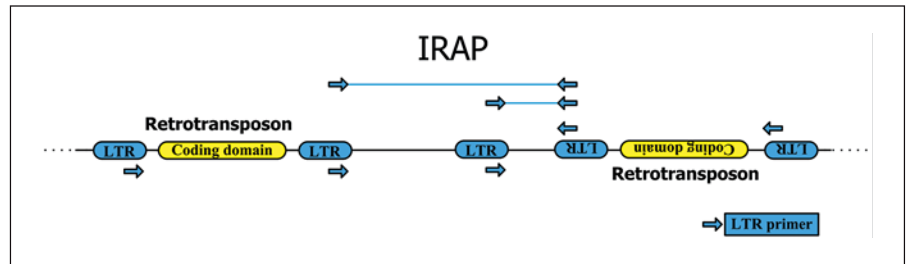

Fig. 2. Principles of IRAP (interretrotransposon amplified polymorphism). LTR primers (blue arrows) facing outward from the ends of LTRs will amplify intervening DNA from retrotransposons or solo-LTRs in opposite orientations. Retrotransposons or solo-LTRs in the same orientation will not result in amplification products. Adapted from Kalendar and Schulman [2006]. IRAP analysis allowed the characterisation of *Gypsy*-like *Athila*4-6 and *Tat*1 retrotransposon adjacent sequences (see online suppl. table 1, www.karger.com/doi/10.1159/000353308) in *Arabidopsis* polyploid lines.

changes affecting retrotransposons and adjacent sequences were also predominantly represented by losses of SSAP fragments of paternal origin, including indels or the complete loss of *Tnt*1 elements [Petit et al., 2010]. In contrast, in the natural allopolyploid *S. anglica,* losses of SSAP fragments from maternal origin seemed to predominate [Parisod et al., 2009]. Progenitor biases for rearrangements through IRAP and REMAP analyses were also observed in triticale, where the paternal rye genome is considerably more reorganised than the wheat genome [Bento et al., 2008]. A further in-depth analysis of Triticeae polyploids additionally revealed that the larger genome (comparing DNA content per haploid genome) is usually more affected independently of its maternal or paternal status [Bento et al., 2011]. Jiang et al. [2011] also corroborated this suggestion through an IRAP and REMAP study of the neosynthesised *Cucumis* allotetraploid, which revealed that 18% of the rearranged bands corresponded mainly to a loss from the larger parental genome.

Although it was previously demonstrated that the parental genome size is correlated with the frequency of rearrangements in its allopolyploid, no comparative analysis of genomic changes among allopolyploids with very different genome sizes has been performed. Genome sizes can vary as much as 1,000-fold between plant species from the small genome of *A. thaliana,* which is approximately 125 Mb [Arabidopsis Genome Initiative, 2000], to species with very large genomes, such as *T. aestivum* (17 Gb) [Brenchley et al., 2012] or *Fritillaria assyriaca* (123 Gb) [Bennett and Smith, 1976]; therefore, the relevance of genome size on the frequencies of polyploidisation-induced retrotransposon-related sequence rearrangements remains to be understood.

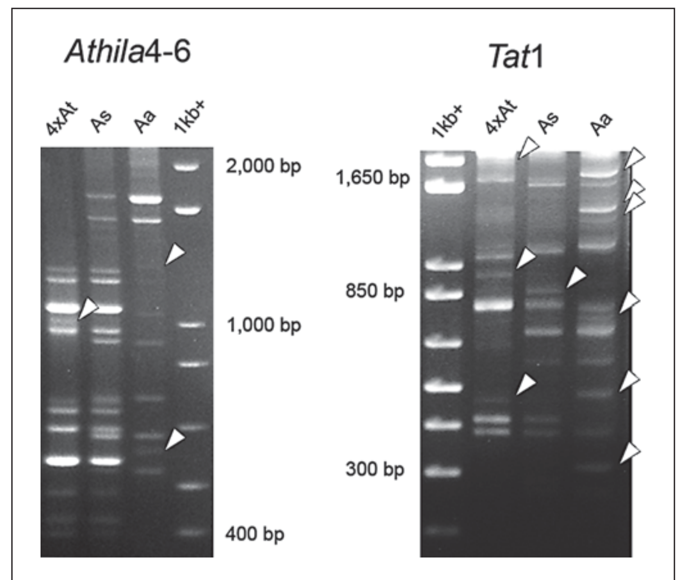

Fig. 3. IRAP banding profiles obtained with primers for *Athila*4-6 and *Tat*1 of *A. thaliana* LC612 2n = 4× = 20 (4×At), *A. suecica* synthetic allopolyploid 2n = 4× = 26 (As) and *A. arenosa* CARE-1 2n = 4× = 32 (Aa). Arrowheads indicate rearranged bands.

Frequency of Retrotransposon Rearrangements Is Independent of Allopolyploid Genome Sizes

Because the contribution of retrotransposons to rearrangements in allopolyploids with small genomes had not yet been evaluated, we used the IRAP methodology (fig. 2) to study a newly synthesised *A. suecica* line (2n = 4× = 26, F4 generation, NASC stock number N3899) produced by L. Comai's laboratory [Madlung et al., 2005]. The results obtained through a comparison of the profiles (fig. 3) of

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Table 1. IRAP analyses of the synthetic allopolyploid *A. suecica* and its parental lines

	IRAP		Total
	*Athila*4-6	*Tat*1	
A. thaliana tetraploid	12	8	20
A. arenosa	8	12	20
A. thaliana vs. *A. arenosa*			
Monomorphic bands[a]	0	2	2
Polymorphic bands[b]	20	16	36
A. suecica			
Expected[c]	20	18	38
Observed bands[d]	17	10	27
Rearranged bands[e]	3	10	13
Lost from *A. thaliana*	1	3	4
Lost from *A. arenosa*	2	6	8
Novel bands	0	1	1

[a] Common to both *A. suecica* progenitors; [b] observed in only one *A. suecica* progenitor; [c] sum of parental bands observed; [d] bands observed in the allopolyploid; [e] parental bands absent and novel bands only observed in the polyploid.

Table 2. Comparative analysis of AFLP and IRAP results in *A. suecica* and octoploid triticale (*Triticum aestivum* 'Chinese Spring' × Secale cereale 'Imperial') allopolyploids

	AFLP		IRAP	
	bands affected, n	total[a]	bands affected, n	total[a]
A. suecica	7 (2.3%)	308[b]	13 (33.3%)	39[d]
Triticale	830 (43.4%)	1,910[c]	15 (27.8%)	54[e]

[a] Total number of bands = expected bands corresponding to the sum of parental bands and allopolyploid novel bands not observed in any parental profile; [b] Madlung et al., 2005; [c] Ma et al., 2004; [d] in the present paper; [e] Bento et al., 2008.

Note: The χ^2 test was used to compare the results obtained for different species with the same technique and with different techniques for the same species.

the exact parental lines *A. thaliana* autotetraploid (LC612 line, 2n = 4× = 20, NASC stock number N3900) and *A. arenosa* (CARE-1 line, 2n = 4× = 32, NASC stock number N3901) were consistently reproduced in all PCR replicates from at least 3 distinct plants of each genotype (summarised in table 1). A high frequency (33%) of rearrangements (13 rearranged/39 total bands) was detected in the synthetic allopolyploid *A. suecica* comprising 92% parental band losses and only one novel band.

The frequency of allopolyploidisation-induced retrotransposon-associated genomic changes was further compared between *A. suecica* and triticale [Bento et al., 2008], based on IRAP analyses (table 2). Interestingly, regarding the large differences in retrotransposon copy number between these 2 allopolyploid model species, no significant difference (p = 0.62) was detected in the number of bands affected (33% in *A. suecica* and 28% in triticale). Thus, it is clear that retrotransposons represent a very labile portion of the genome, which is highly affected by polyploidisation yet is apparently independent of genome sizes. Such retrotransposon-associated genomic modifications are enigmatic; however, when comparing genomic rearrangement frequencies evaluated through AFLP, significant differences ($p = 6.58 \times 10^{-40}$) between both polyploids are detected; specifically, 43% of restructured bands were detected in triticale, and only 2.3% were detected in *A. suecica* [Ma et al., 2004; Madlung et al., 2005]. When comparing IRAP and AFLP, a significant difference in the frequency of rearrangements was observed in *A. suecica* ($p = 2.55 \times 10^{-12}$), but not in triticale (p = 0.14).

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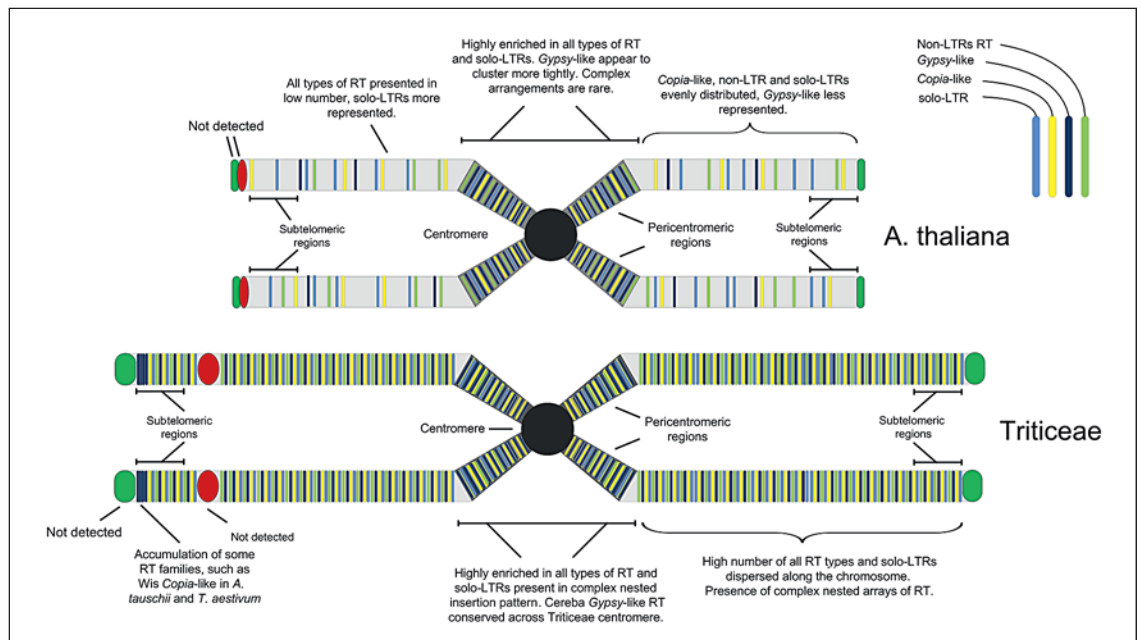

Fig. 4. Schematic representation of the distribution of retrotransposons (RT) in plant chromosomes. The upper part represents *A. thaliana*, and the lower part represents Triticeae species (telomeres in green, nucleolus organiser regions [NORs] in red). In *A. thaliana,* retrotransposons tend to be clustered within pericentromeric heterochromatin and are less represented across chromosome arms; *Gypsy*-like elements are especially abundant in pericentromeric regions. The presence of complex nested arrays of retrotransposon is rare. In Triticeae, retrotransposons may be clustered in heterochromatin domains but are also present in high copy number all over chromosome arms, frequently in complex nested insertions. Chromosomes are not drawn to scale [Li et al., 2004; Peterson-Burch et al., 2004; Dvořák, 2009].

Considering that AFLP detects changes in restriction fragments from random loci allowing analyses across the genome [Madlung et al., 2005], whereas IRAP assesses particularly labile repetitive sequences, such as retrotransposons [Kalendar and Schulman, 2006], our preliminary results suggest that domains rich in retrotransposon-related sequences are predominantly affected in *A. suecica,* whereas rearrangements in triticale appear to occur throughout the genome.

A plausible explanation for the discrepant rearrangement frequencies detected through AFLP and IRAP in triticale and *A. suecica* can be found by examining the differential retrotransposon chromosomal distribution in both species. The results of retrotransposon mapping through in situ hybridisation, which were later supported by large-scale sequencing initiatives, suggest that the retrotransposon distribution patterns clearly differ between species with large and small genomes (reviewed in fig. 4). The study of the chromosomal distribution of retrotransposons in *T. aestivum* through FISH (fluorescent in situ hybridisation) revealed that most *Gypsy*-like retrotransposon elements analysed, such as *Sabrina, Wham, Wilma, Nusif*, and *Fatima* as well as *Copia*-like retrotransposons *Angela* and *Wis*, are detected over the entire length of all chromosomes [Li et al., 2004]. Choulet et al. [2010] also confirmed this highly widespread distribution of retrotransposons in *T. aestivum*. In the small genome of *Arabidopsis*, most copies of *Gypsy*-like, *Copia*-like and non-LTR retrotransposons are preferentially clustered in pericentromeric heterochromatin. Nevertheless, *Copia*-like and non-LTR retrotransposons differ in their genomic organisation from *Gypsy*-like retrotransposons and are more loosely associated with pericentromeric regions, being widespread throughout chromosomes though in low copy number [Peterson-Burch et al., 2004]. A similar distribution of retrotransposons was also observed in closely related species such as *B. oleracea* and *B. rapa*, where most retroelements clustered in pericentromeric heterochromatin, yet coincided with a wide distribution of *Athila*-like *Gypsy* elements throughout chromosomes [Alix et al., 2005; Wang et al., 2011]. Thus, our comparative analysis suggests that retrotransposon elements might be similarly rearranged in

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model allopolyploid species with both large and small genomes, independent of their distinct abundance and distribution patterns. However, the understanding of the real causes for the discrepancy between AFLP and IRAP in detecting rearrangement frequencies in triticale and *A. suecica* requires further research to extensively characterise the restructured bands at the sequence level.

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