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Allopolyploid-Induced Sequence Elimination

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ABSTRACT

Allopolyploids, which result from the combination of two or more copies of differentiated genomes, have undergone DNA sequence elimination in the early stage after their formation. Recent investigations have illuminated that sequence elimination is a nonrandom and reproducible event. It can be characterized by rapid, nonrandom, and reproducible event, which vary in different plant systems. Many studies have found that the eliminated sequences involve not only low-copy and non-coding DNA sequences but also high-copy and coding sequences, mostly from one of the parental genomes. Although the molecular mechanisms of sequence elimination are not very clear at present, many studies have indicated that it is not affected by the genotype of parental plants, by the cytoplasm, or by the ploidy level, and that it does not result from intergenomic recombination. However, our recent study indicated that sequence elimination might have some relationship with intergenomic recombination in the *Cucumis* group, complicating the mechanisms of the elimination event. It has been speculated that sequence elimination increases the differentiation of homoeologous chromosomes at the polyploid level, thereby providing the physical basis for rapid restoration of diploid-like chromosome pairing pattern in meiosis following polyploidization, and this may have contributed to the successful establishment of newly formed allopolyploids as a new species. Continued application of molecular genetic approaches to study novel genotypes or new allopolyploid species is needed to clarify the issue of allopolyploid-induced sequence elimination, moreover the genome evolution of allopolyploids.

Keywords: allopolyploid, DNA sequence elimination, diploidization, genome variation
Abbreviations: **AFLP**, amplified fragment length polymorphism; **CSSs**, chromosome specific sequences; **FISH**, fluorescence in situ hybridization; **GSSs**, genome specific sequences; **IGS**, intergenic spacers; **MSAP**, methylation-sensitive amplified polymorphism; **PMC**, pollen mother cell; **RFLP**, restriction fragment length polymorphism; **SE**, sequence elimination

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INTRODUCTION

Allopolyploid plants are hybrids that contain two or more copies of the genome from each parent. It has been estimated that ~50% to 70% of all angiosperm species are of polyploid origin (Stebbins 1971; Masterson 1994; Wendel 2000). As a consequence of the union of two genomes, abnormal phenotypes have been reported, involving sterility (Leitch and Bennett 1997); shape and color changes of leaf and flower (Schranz and Osborn 2000; Chen *et al.* 2002, 2003); novel homeotic phenotypes (Meyer 1970); and the global dominance of one parental phenotype (Heslop-Harrison 1990), but the causes of these phenotypes are largely unknown. McClintock (1984) described similar phenomena as 'genomic shock', which she defined as a preprogrammed response to an unusual challenge resulting in extensive restructuring of the genome. This 'response' may involve many aspects (for reviews, see Comai 2000; Wendel 2000), such as restructuring at the DNA and/or chromosome level (Wendel *et al*. 1995; Leitch and Bennett 1997), changes of gene expression systems (Scheid *et al*. 1996; Galitski *et al*. 1999; Comai *et al*. 2000), activation of retro-elements (Zhao *et al*. 1998; Fedoroff 2000), elimination of low and/or high copy DNA sequences (Feldman *et al*. 1997; Hanson *et al*. 1998; Liu and Wendel 2002; Lukens *et al*. 2006), among which sequence elimination (SE) was the most commonly studied in various species.

Recent molecular data indicate that SE is observed in the early generations of allopolyploid *Brassica* (Song *et al*. 1995), wheat (Zohary and Feldman 1962; Dvorak *et al*. 1990; Feldman *et al*. 1997; Liu *et al.* 1998a, 1998b; Comai *et al*. 2000; Ozkan *et al.* 2001; Shake *et al*. 2001; Feldman and Levy 2005) and *Arabidopsis* (Lee and Chen 2001; Madlung *et al*. 2002). For example, in *Brassica*, three genera with basic diploid genomes (A, B, C) were hybridized to form the allotetraploids. F_2 to F_5 generation of allotetraploids were analyzed using 89 nuclear DNA clones as probes, the results showed that the SE was a very common event from F_2 to F_5 generation (Song *et al.* 1995). An analysis of F_1 plants from crosses between *Aegilops sharonensis* and *Aegilops umbellate* showed that as much as 14% of the DNA sequences from one parent were eliminated in the

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synthetic (Shaked *et al*. 2001). Similarly, in synthesized allopolyploid triticale (*Triticum-Secale* hexaploids and octaploids), 38.4% of the *Secale* sequences were eliminated after 15 to 35 generations (Ma *et al.* 2004). These studies suggest that SE is one of the major factors associated with the process of allopolyploidization although the overall mechanisms involved are still elusive. Until now, when and how SE is initiated is not clear. Is SE due to the union of parental genomes or to the doubling of treatment? Does it occur in the F_1 hybrids or in subsequent generations? Which sequences are prone to be eliminated in the process of allopolyploidization? What is the mechanism underlying the SE? What is the potential contribution of SE to the evolution of polyploids? These questions make elimination events become complicated. Currently, many investigations suggested that SE might shift the pairing pattern in the raw allopolyploid from multivalent pairing to bivalent pairing, thus preventing recombination and yielding viable gametes. Therefore, benefit for the establishment of newly synthesized allopolyploids as successful species (Feldman *et al*. 1997; Liu *et al.*1998a, 1998b; Ozkan *et al*. 2001; Shake *et al*. 2001). It is not surprising that most empirical studies on allopolyploidy are focused on SE, which will be the focus of this review.

CHARACTERIZATION OF SE

Nonrandom and reproducible

In most cases of allopolyploidy reported thus far, SE pattern was nonrandom, namely, elimination of parental fragments from one parental genome and preservation of those of the other genome. The first evidence of rapid genomic changes in the evolution of allopolyploids was provided by Song *et al*. (1995), who used two reciprocal synthetic allopolyploids in *Brassica*, and by Southern hybridization analysis using 89 nuclear DNA clones, they reported that SE occurred in low-copy nuclear sequences in each of one to five selfed generations of a synthesized allopolyploid. In addition, most of the eliminated sequences were from the paternal genome in *B. rapa* × *B. nigra* (genome AABB), while this phenomenon was not observed in *B. rapa* × *B. napus* (genome AACC). Similarly, Ozkan *et al.* (2001) indicated that in *Aegilops sharonensis* × *Ae. umbellulata*, 14% of the loci from *Ae. sharonensis* were eliminated compared to only 0.5% from *Ae. umbellulata*. Noticeably, most of the eliminated sequences originated from the maternal genome in those studies. However, in our recent studies, by AFLP analysis with newly synthesized reciprocal F_1 hybrids (2n=2x=19) and allotetraploids (2n=4x=38) from S_1 to S_3 of selfed generations in *Cucumis* (Chen *et al*. 2007), the results showed that there were no significant differences between the parental fragment loss from the maternal and the paternal parents. Rather, it was found that the frequency of parental fragment loss of *C. sativus* (2n=14) was twice that detected in *C. hystrix* (2n=24). These results suggest that SE appears to be related to the relative ploidy level of the two parents, with elimination being more common from the parent that has fewer chromosome numbers. It seems almost certain that rapid, non-random SE may occur during polyploid formation or in the early stages of polyploid stabilization.

Previous reports in wheat indicated that in newly synthesized allopolyploids, rapid SE was a general, non-random event whose direction was determined by the genomic combination of the hybrid or the allopolyploid (Feldman *et al*. 1997; Liu *et al.* 1998a, 1998b; Ozkan *et al.* 2001). In allopolyploids of *Triticum* and *Aegilops*, Using several genome specific sequences (GSSs) and chromosome-specific sequences (CSSs) as probes, Feldman *et al.* (1997) and Liu *et al.* (1998a, 1998b) found that allopolyploid-induced SE was a common event in allopolyploid genomes, moreover, the results also indicated that whether the sequence would be eliminated depended on the existence of homoeologous sequences in the other parental genomes. Thus, only those sequences having the homoeologous in the other parental genome prone to be eliminated during the allopolyploidization. All these data indicated that the allopolyploid-induced SE was a general, non-random event.

Most of the allopolyploids used in the studies described above are re-synthesized allopolyploids. Many previous studies, such as in wheat, *Brassica* and *Arabidopsis*, the newly synthesized allopolyploid were compared to their natural counterpart allopolyploids, the results indicated that SE event occurred in the early generations of both natural allopolyploids and re-synthesized allopolyploids (Song *et al*. 1995; Feldman *et al*. 1997; Liu *et al.* 1998a, 1998b; Lee and Chen 2001; Shake *et al*. 2001; Madlung *et al*. 2002). For instance, In wheat group, the timing and patterns of SE in synthetic allopolyploids were often concordant with those of the natural allopolyploid species, which suggested that allopolyploid- induced SE was reproducible (Liu *et al.* 1998a, 1998b; Ozkan *et al.* 2001).

Timing

SE in allopolyploid is a rapid process, with most sequences eliminated from the first generation and completed in the early generations (usually in the second or third generation). A recent comparison, in the *Aegliops-Triticum* group, between the genomes of synthesized allopolyploids and those of their diploid progenitors showed that allopolyploid-induced SE occurred very early in the history of the nascent allopolyploid species. In *Aegilops* × *Triticum*, using 8 low-copy DNA sequences as probes to study the 35 F_1 interspecific hybrids and 22 synthesized allopolyploids, the results suggested that these sequences appeared in all of the diploid parental genomes, while they only appeared in one of the two genomes of allopolyploid genome as CSSs or GSSs (Ozkan *et al*. 2001). Meanwhile, they found that GSSs were eliminated from the F_1 hybrid, while CSSs were eliminated from the first generation of the alloploypoid, although, in both cases, the elimination process was completed in the second or third allopolyploid generation. In *Brassica*, Song *et al.* (1995) found that the process of SE began in F_1 hybrids, others in the first and second allopolyploid generations, and still others during subsequent generations. Similarly, Feldman *et al.* (1997) and Liu and Wendel (2002) reported that complete elimination of these sequences was achieved by the S_3 to S_6 generations in wheat synthetic allopolyploids.

These data show that SE is a rapid process that occurs as early as in the F_1 hybrid and then finished completely in the early generations of the allopolyploid. All these results combined with those of Rieseberg *et al.* (1995) indicate that the structure and constitution of the genome in a nascent allopolyploid becomes stable in the early generations after polyploid formation.

Eliminated sequences

Repetitive sequences are the major components of plant genomes, both of the tandem repeats (such as rDNA) and interspersed repeats prone to be concerted evolution, it is an established fact that the unbalanced exchange of chromosomes is the major factor of concerted evolution (Cronn *et al*. 1996). The result of unbalanced exchanges are: the sequence copied in one of the two chromosomes will be lost in the corresponding site of the homoeologous chromosomes (Dover 1982; Cronn *et al*. 1996). It is no doubt that the SE event occurs during concerted evolution. There are almost 50 species in *Gossypium*, among which 5 allotetraploids are formed by interspecific hybridization and polyploidization of the A and D genomes. Using FISH analysis, Hanson *et al.* (1998) found that the interspersed repetitive elements in the allopolyploid genome of *G. hrisutem* (AADD) had undergone concerted evolution during the polyploid process. Some of the repetitive elements in the D genome were substituted by those in the A genome, implying that the interspersed repeats were eliminated in the D

genome. In *Brassica*, in contrast, Waters and Schaal (1996) found no rDNA sequence to be eliminated from the genome of *B. juncea* (AABB), *B. carinata* (BBCC), and *B. napus* (AACC) allopolyploid. Obviously, SE events might occur in high-copy sequences, but the occurrence of SE is not the same among various species.

Many studies have indicated that the elimination events also occurred in low-copy, non-coding DNA sequences. Bread wheat is an allohexaploid (AABBDD) derived from the hybridization between *Triticum turgidum* (AABB) and a taxon similar to modern *Aegilops tauschii* (DD). Feldman *et al*. (1997) studied RFLP patterns in diploid and allopolyploid wheat using 16 low-copy, non-coding probes that were either chromosome-specific or were confined to several chromosomes within a single polyploid genome. Of these, 9 yielded a strong hybridization signal in all diploid genomes, suggesting that these sequences are relatively conserved and indicating that they were present in each of the progenitor genomes at the onset of polyploidization. Thus, the expectation was that each of these 9 sequences would be detected in both tetraploid and in all three hexaploid wheat genomes. However, the signal was detected in only one of the two genomes of allotetraploid (AABB). Obviously, the SE events occurred during the formation of AABB genome. Similar results were observed in the AABBDD genome; it could be proposed that the SE events occurred once again accompanying the formation of the AABBDD genome. In a follow-up study, Liu *et al*. (1998a) monitored RFLP fragment profiles in synthetic tetraploids, hexaploids, octoploids, and decaploids in *Triticum* and *Aegilops* using a similar set of probes as employed in the initial study. Consistent with the earlier results, rapid, non-random SE was observed from one or more genomes in every allopolyploid studied.

To address the question of whether the phenomenon of polyploidy-induced SE extends to coding sequences, Liu *et al*. (1998b) studied RFLP fragments in the same set of synthetic allopolyploids as in their first analysis, using coding sequences as probes that mapped to each of the 42 chromosome arms in hexaploid wheat. It was found that there were parental fragments disappearance and novel fragments appearance in the AABBDD genome. Therefore, the coding sequence was also eliminated during the allopolyploidization. But in their subsequent studies, they observed that sequence loss and gain was the result of DNA methylation instead of SE. The experiments in *Brassica* (Song *et al*. 1995) involved reciprocal synthetic allopolyploids between the diploids *B. rapa* and *B. nigra* and between *B. rapa* and *B. oleracea.* Thus, two different hybrids were generated in each of the two cytoplasms. The results showed that of 89 cDNA sequence probes, most showed rapid changes including loss of parental fragments and/or gain of novel fragments which did not appear in both parents, especially in the genome *B. rapa* × *B. nigra* (AABB) with most fragments having originated from the paternal genome, while a similar phenomenon was not observed in the AACC genome. It is important to point out that, in this study, the results observed by Song *et al.* (1995) might represent the whole genome of the allopolyploid, while Waters and Schaal (1996) only focused on the repetitive sequences, thus more investigations are necessary to clarify the elimination events in *Brassica.*

In our recent studies, using AFLP analysis, we found that most of the eliminated sequences were related to repetitive sequences, and that the coding sequences were also included in *Cucumis* allopolyploids (Chen *et al*. 2007). Moreover, using cDNA-AFLP analysis, the results indicated that about 3.37% of sequences changed and that 75% of them appeared to be lost after the formation of the polyploid. Our data indicated that the eliminated sequences in the *Cucumis* group involved both non-coding and coding sequences during allopolyploidization.

POSSIBLE MECHANIZMS OF SE

Song *et al.* (1995) observed that the frequency of changes was associated with divergence of the diploid parental genomes. Nearly twice as much change was detected in crosses involving the distant relatives *B. rapa* and *B. nigra* when compared to the more closely related species *B. rapa* and *B. oleracea*. In our recent study (Chen *et al*. 2007), using AFLP analysis, we found that *Cucumis* allopolyploid SE appeared to be related to the relative ploidy level of the two parents, with elimination being more common from the parent that had the lower chromosome number.

Plant growth and development entails a coordinated regulation of expression not only of nuclear genes but also of those in the chloroplast and mitochondrial genomes (Bogorad 1991; Leon *et al*. 1998). When polyploidization occurs, such that the nuclear genome becomes doubled but the organellar genomes do not, the stoichiometry between organellar genes and those in the nucleus is changed, potentially leading to regulatory disruptions or other sub-optimal physiological effects. These problems may be exacerbated by allopolyploidization where potential differences between two formally isolated but now merged nuclear genomes must become reconciled with each other and with one of the two sets of cytoplasmic genes.

In *Brassica*, in the RFLP study described above, no significant cytoplasmic effect was observed in reciprocal synthesized *B. rapa* × *B. oleracea* allopolyploids (A and C genomes), but an effect was detected in reciprocal synthetic *B. rapa* × *B. nigra* allopolyploids (A and B genomes). In particular, a biased loss of the B genome, and RFLP fragment was observed among nine F_5 individuals derived from an initial AB allopolyploid generated in the A cytoplasm. Song *et al.* (1995) indicated that the direction of fragment loss bias is the result of nuclear-cytoplasmic interactions in reciprocal allopolyploids whereas in other cases, this cytoplasmic effect on RFLPs has not been clearly observed. For example, in *Gossypium* (Brubaker *et al*. 1999), wheat (Liu *et al*. 1998a, 1998b), *Cucumis* (Chen *et al*. 2007) and other *Brassica* allopolyploids (Song *et al*. 1993, 1995). Therefore, it is likely that the cytoplasmic effect might be related to SE but it is not a major factor affecting elimination of the sequences surveyed in the present study.

An obvious and critical question arising from the results is whether the findings of SE from synthetic allopolyploids were produced by the methods, such as colchicine treatment, from which the allopolyploids were induced. Results from Feldman *et al.* (1997) and Liu *et al.* (1997, 1998a, 1998b) indicated that SE events occurred in three allopolyploids analogous to natural allopolyploids (i.e. three different combinations of *T. turgidum* ssp. *Durum* - *Ae. tauschii*) were obtained spontaneously as a result of the formation of unreduced gametes. Moreover, the allopolyploids *Ae. peregrine* - *Secale cereale*, *Ae. Kotschyi* - *S. cereale* and *Ae. ovata* - *S. cereale*, obtained through tissue culture by Barbara Wojciechowska (Institute of Plant Genetics, Polish Academy of Sciences, Posnan), exhibited elimination of CSSs and GSSs. Their results clearly showed that SE is induced by allopolyploidization and not by the method of chromosome doubling (e.g. colchicine treatment). Previous studies indicated that intergenomic recombination might be a major factor contributing to genomic changes in newly synthesized allopolyploids. Song *et al*. (1995) indicated that intergenomic recombination was the major factor of SE in *Brassica* allopolyploids; in addition, other possible examples of genome and chromosomal repatterning have been shown by comparative genome mapping (Helentjaris *et al*. 1988; Whitkusn *et al*. 1992). Genomic *in situ* hybridization has also provided evidence in support of intergenomic transfer of DNA in the allopolyploid *Milium montianum* (Bennett *et al*. 1992). It was found that the SE rate was similar in the F_1 hybrid and S_0 generation (obtained from spontaneous mutation) while the frequency of SE was doubled after the S_0 generation under-

going meiosis, and then remained the same in subsequent S_2 and S_3 generations. It was also observed that many multivalents were formed in the pollen mother cells (PMCs) of S_0 which showed that intergenomic recombination did occur in the S_0 generation. These results suggested that the intergenomic recombination might be related to SE in the *Cucumis* group (Chen *et al*. 2007).

In contrast, Feldman *et al.* (1997) and Liu *et al*. (1998a) claimed that SE of CSSs and GSSs in wheat was not caused by intergenomic recombination because one of the parental lines included in their study had Ph_1, a gene that suppressed homoeologous pairing at first meiotic metaphase. To address this claim, four different hybrids and two allopolyploids differing in their Ph_1 genotypes were developed and analyzed, the results indicated that similar elimination patterns, such as frequency and time of SE, were observed in these allopolyploids, suggesting that intergenomic recombination might not be a major factor affecting SE. This conclusion finds resonance with that of Axesson *et al*. (2000) who found no evidence for intergenomic recombination in synthetic allopolyploids of *Brassica*.

Voytas and Naylor (1998) suggested that retrotransposons might be responsible for genomic changes. Evidence for the activation of several kinds of retrotransposons in natural allopolyploids (Zhao *et al*. 1998; Hanson *et al*. 2000) and in newly formed allopolyploids (K. Kashkush, M. Feldman and A.A Levy, unpublished data) has been obtained. Moreover, in *Cucumis*, of 13 eliminated sequences in allopolyploids, four of them were similar to the retrotransposons of the wheat group, although it was difficult to ascribe the SE reported in our study related to the activity of retrotransposon. Therefore, these eliminated sequences might be related to the retrotransposon elements (Chen *et al*. 2007).

The work of Volkov *et al.* (1999) indicated that the evolution of intergenic spacers (IGS) between 18S-26S DNA had undergone three steps after the allopolyploid formation in *Nicotiana. tabacum.* First, nucleus competition, that is the two parental genomes compete with each other after allopolyploid formation, the rDNA of *N. sylvestris* that has a short IGS was controlled by the rDNA of *N. tomentosiformis*, which has a relatively longer IGS. Second, elimination and substitution of the IGS of *N. sylvestris* was eliminated step by step, and lastly, substituted by the rDNA of *N. tomentosiformis*. Third, in rDNA recombination, the introgressed rDNA sequences of *N. tomentosiformis* were copied and eliminated in various zones of *N. sylvestris* IGS, which promoted the genome of allopolyploid to become stable and evolve successfully.

In separate studies, a survey of approximately 22,000 genome loci from 9 sets of synthetic cotton allopolyploids by AFLP and MSAP finger printing suggested genomic stasis and lack of rapid DNA methylation changes at the symmetric CCGG site (Liu *et al*. 2001). Even in grasses, a recent study on a young natural allopolyploid species, *Spartina anglica*, did not reveal rapid genomic changes (Baumel *et al*. 2002). It should be pointed out that, all the species used in these studies were newly synthesized allopolyploids that did not exist in nature. Therefore, these allopolyploids may represent the newly formed allopolyploid which evolves unsuccessfully in nature. Further independent studies are needed to clarify the issue and particularly to address the mechanism of SE and its characterization.

It seems almost certain that several different mechanisms underlie the phenomenon of SE, there is no *a priori* reason, for example, to attribute SE in wheat to the same mechanism as RFLP fragment loss and gain in *Brassica*. Clearly, more research is needed using these and other experimental systems before we can achieve a fuller understanding of the prevalence and scope of the phenomena incorporated under the umbrella heading 'SE'. Nonetheless, it is still worth speculating about the responsible mechanisms.

EVOLUTIONARY SIGNIFICANCE

It has been proposed by Feldman *et al*. (1997) that the diploid-like meiotic behavior of polyploid wheat has been brought about by two independent systems that complement each other. The first system is based on the *Ph1* gene located on the long arm of chromosome 5B (Riley and Chapman 1958; Sears 1976), the main gene that suppresses homoeologous pairing in polyploid wheat. This gene presumably evolved at later evolutionary stages, and its effect is additive to the elimination system. The second system is based on nonrandom elimination of DNA sequences. The SE events occur soon after the formation of the allopolyploids, resulting in increased differentiation between homoeologous chromosomes. Indeed a positive linear relationship was found in S_1 to S_3 generations of the newly synthesized allopolyploids between the percentage of seed fertility and the percentage of elimination of CSSs and GSSs, and a negative relationship was found between multivalent per cell and the percentage of CSSs and GSSs eliminated (Ozkan 2000). These relationships, which were more obvious in natural than in non-natural combinations, indicated that elimination of CSSs and GSSs in early generations serves to reduce homoeologous pairing, improving homologous pairing, and enhance the fertility of newly synthesized amphiploids.

In the wheat group, it was suggested that SE, which enhanced the differentiation of the homoeologous chromosomes, provides the physical basis for the diploid-like meiotic behavior of the allopolyploid (Feldman *et al*. 1997). Shaked *et al.* (2001) and Chen *et al*. (2007) found similar findings in which they indicated that the resultant strict bivalent pairing prevents intergenomic recombination and brings about higher fertility and permanent heterosis between homoeoalleles, thus fostering the successful establishment of the newly formed allopolyploid species in nature.

Moreover, it was also reported that SE started earlier in allopolyploids whose genome constitution was analogous to natural allopolyploids as compared with allopolyploids that do not occur in nature (Song *et al*. 1995; Feldman *et al*. 1997; Liu *et al*. 1998a, 1998b; Comai 2000; Ozkan *et al*. 2001; Madlung *et al*. 2002). The difference in timing and rate of elimination was also detected among various GSSs and CSSs. This rapid genome adjustment has likely contributed to the successful establishment of newly formed allopolyploids as new species (Ma and Gustafson 2005). Obviously, SE was induced under the pressure of genome hybridization and polyploidization, thus it is assumed that SE facilitated the establishment of newly formed allopolyploids. Although, this needs more investigation with other species to confirm this issue, there is no doubt that genomic SE is of significance in the evolution of newly formed allopolyploids.

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