The Impact of Polyploidy on Grass Genome Evolution

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Polyploidy is an evolutionary process whereby two or more genomes are brought together into the same nucleus, usually by hybridization followed by chromosome doubling. As a result, the new polyploid is genetically isolated from its diploid progenitor(s) and a new species is formed. The importance of polyploidy was recognized early in the 20th century and for the past decades many studies have addressed the different categories of polyploids, their mode of formation, their cytogenetic behavior, the ecological implications, the impact of polyploidy on various population genetics aspects such as heterozygosity, mating mode, buffering of mutations, etc. (Stebbins, 1950, 1971). In the past few years, molecular and computational tools have provided new ways to probe the history of genomes, leading to the discovery that polyploidy is even more widespread than previously thought.

The once controversial proposal that evolution moves forward through whole genome duplication (Ohno, 1970) is gaining recognition thanks to sequence analysis that is more sensitive than the traditional methods (chromosome counting, analysis of meiotic chromosome pairing, and marker-based mapping) that have been used to assess polyploidy. Accumulating evidence shows that genome duplications occurred in the lineage of all vertebrates (Wolfe, 2001, and refs. therein), including humans (*Homo sapiens*), whose genome might have been shaped by two rounds of duplication, suggesting octopolyploidy. Recent studies provide strong indications that even yeast (*Saccharomyces cerevisiae*), with its compact genome, is in fact an ancient tetraploid (Wong et al., 2002). In plants, polyploidy was proposed to have occurred in the lineage of at least 70% of angiosperms (Masterson, 1994) and in 95% of pteridophytes (Grant, 1981). Moreover, the first two species whose genomes have been fully sequenced, Arabidopsis (Arabidopsis Genome Initiative, 2000) and rice (*Oryza sativa*; Goff et al., 2002), were chosen principally because of their small genome. Therefore, it came as a surprise when sequence analysis revealed that these particular species, considered as classical diploids, are apparently ancient polyploids (paleopolyploids). *Paleopolyploids* are ancient polyploids which have a disomic inheritance and whose progenitors cannot be identified by cytological tools or DNA markers. Paleopolyploidy is detected by rather sophisticated bioinformatics tools that reveal similarity and colinearity between genes which diverged tens of millions of years ago (see Gaut, 2001 as example). If these minigenome "diploids" are in fact paleopolyploids, then it is reasonable to assume that many more, if not all, higher plant species, considered as diploids because of their genetic and cytogenetic behavior, are ancient polyploids that underwent a process of extensive diploidization. Thus, polyploidy is one of the major processes that has driven and shaped the evolution of higher organisms.

The ubiquitous role of genome duplication in evolution is one of the important discoveries from the post-genomic era, explaining the renewed interest in polyploidy. Several reviews have been written recently on polyploidy in plants, covering various aspects such as the impact of polyploidy on speciation, genome structure, and gene expression (Leitch and Bennett, 1997; Comai, 2000; Soltis and Soltis, 2000; Wendel, 2000; Pikaard, 2001). This review will focus mainly on the effect of polyploidy on genome evolution in grasses—a family that has contributed some of the best models to the study of polyploidy. It will review the accumulating evidence of polyploidy as a trigger and/or facilitator for accelerated genome evolution in this family. Overall, we will try to learn from the grasses the causes for the formidable evolutionary success of polyploidy in nature.

GRASSES AS A MODEL SYSTEM TO STUDY POLYPLOIDY

The grass family, Poaceae (=Gramineae) is one of the largest families of flowering plants, including approximately 10,000 species classified into 600 to 700 genera that diverged from an ancestral progenitor 50 to 70 million years ago (mya; Kellogg, 2001; Huang et al., 2002). As discussed below, most, if not all, grasses are polyploids; all major types of polyploids, namely autopolyploids, segmental allopolyploids, and allopolyploids, can be found in this family.

Autopolyploids contain multiple sets (>2) of the same or similar genomes in their nucleus. They originated mostly from intraspecific crosses followed by chromosome doubling because of unreduced gametes and their chromosomes may form multivalents during meiosis and exhibit a multisomic inheritance. One advantage of autopolyploidy is the capacity to maintain high levels of heterozygosity, with multiple alleles per locus, or more rarely, to reach homozygosity with multiple dosage of a given allele. On the

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other hand, the formation of multivalents during meiosis is often associated with sterility. Therefore, it is not surprising that many autopolyploids are perennial species that also propagate vegetatively.

Segmental allopolyploids contain both homologous and homoeologous chromosomal segments and, therefore, exhibit both bivalents and multivalents during meiosis. As a result, they have a mixed disomic/polysomic inheritance.

Allopolyploids (or amphiploids) contain two or more diverged—homoeologous— genomes. They were derived from interspecific or intergeneric hybridization between species with diverged genomes. The resulting hybrid is sterile but fertility is obtained after chromosome doubling. Allopolyploids are characterized by bivalent pairing, full fertility, and disomic inheritance. Most allopolyploid grasses are annual species. Allopolyploidy enables the permanent fixing of heterozygosity between homoeoalleles, even in self-pollinating species.

Grass crops such as wheat (*Triticum* spp.), maize (*Zea mays*), and rice are well-studied genetic systems, each corresponding to a prototype polyploid (allopolyploid, segmental allopolyploid, and paleopolyploid, respectively); all types of synthetic polyploids can be readily made, enabling one to mimic and study the genetic and epigenetic events that occurred early on at the onset of polyploidy. All the above makes grasses one of the best systems to study polyploidy.

Most, if not all, grasses are polyploids: Based on the assumption that all genera and families, which have a basic chromosome number of x = 12, are derivatives of lines that underwent genome duplication at some time during their evolutionary history (Stebbins, 1971), and because all the primitive grass subfamilies, namely Anomochlooideae, Pharoideae, and Puelioideae (Grass Phylogeny Working Group, http://www.ftg.fiu.edu/grass/gpwg/) have a basic chromosome number of x = 12, this would imply that the ancestor of the grasses was itself a polyploid. In accordance, all the diploid Poaceae species would be paleopolyploids. The family also contains a large number of species (>60%), distributed in all the clades, that are classified as polyploids (Goldblatt, 1980), i.e. based on the above hypothesis, underwent an additional cycle of chromosome doubling (neopolyploids). In these species, the duplicated genomes did not diverge much from their "diploid" progenitors and chromosome number and cytological behavior are still indicative of genome duplication. Most of these neopolyploids (>65%) were derived from distant interspecific or intergeneric hybridizations, giving rise to new allopolyploid species and the remaining derived from intraspecific or close interspecific hybridizations giving rise to new autopolyploid cytotypes (Stebbins, 1971).

Prototype polyploid grasses are as follows. Bread wheat (*Triticum aestivum*) represents one of the best-characterized examples of evolution through allopolyploidy. It is an allohexaploid (genome BBAADD) containing $n = 21$ chromosomes that arose from hybridization, followed by chromosome doubling, between a tetraploid (genome BBAA, $n = 14$) and a diploid (genome DD, $n = 7$) species about 9,500 callibrated years ago (Feldman, 2001). Two of its three diploid progenitors have been identified: *Triticum urartu* (genome AA) and *Aegilops tauschii* (genome DD) (Feldman, 2001). The origin of the B genome remains elusive. The diploid species whose genome is the closest to the B genome is *Aegilops speltoides*. In a pioneering tour de force, Sears (1954) classified the 21 pairs of bread wheat chromosomes into seven homoeologous groups based on the ability of a tetrasome (four doses of a given chromosome) to compensate for the absence of each of the other two chromosomes of the same group. This work was the first evidence for syntenic relationships among the grass genomes. Despite their genetic relatedness, homoeologous wheat chromosomes do not pair under normal conditions. However, in the absence of the homoeologous pairing suppressor gene *Ph1*, pairing and recombination between homoeologs can occur (Sears, 1976). Suppressing inter-genomic pairing is important in maintaining the disomic inheritance and, as a consequence, the fertility of wheat, as well as facilitating permanent heterozygosity between homoeoalleles.

Maize is thought to be an ancient segmental allotetraploid (Gaut and Doebley, 1997). The evidence for this is based on chromosome number and molecular analysis: Maize has $n = 10$ chromosomes, compared with $n = 5$ for species in its tribe (Andropogoneae). Moreover, sequence analysis of 14 pairs of duplicated genes revealed two sets of gene pairs: those that diverged approximately 20 mya and those that diverged approximately 11 mya. A likely explanation for such data is a segmental allotetraploid origin, whereby some chromosomes did not recombine in the early stages of polyploidy and duplicated sequences on these chromosomal segments have the age of the common ancestor (approximately 20 mya), whereas on chromosomes that did recombine, the duplicated sequences have the age of the polyploid formation (approximately 11 mya). This analysis has been extended to a large number of loci (from the UMC98 map) using new computational approaches (Gaut, 2001). It reveals that up to 80% of the maize genome remains organized in colinear regions.

Rice is the best characterized paleopolyploid in the grasses (Goff et al., 2002): >2,000 duplicated cDNAs were plotted by chromosomal regions and the extent of genome duplications were defined. This analysis showed that, with the exception of a large segment shared between chromosomes 11 and 12, the duplicated segments were relatively small, suggesting that many translocations had occurred since the original genome duplication event. The rate of amino acid

substitution was used to estimate the time that duplicated genes had diverged. A peak of duplication events was found 40 to 50 mya, the putative time of whole genome duplication. Finally, we do not have enough molecular data for other "diploid grasses" but it is likely that sorghum (*Sorghum bicolor*) and barley (*Hordeum vulgare*), which have several duplicated loci, might also be paleopolyploids.

POLYPLOIDY IS AN ONGOING PROCESS IN THE GRASSES

Polyploidy is not only widespread in the grasses, but it is also an ongoing process (Fig. 1; Stebbins, 1950; Grant, 1981). *Spartina*, a genus from the Chloridoideae, provides one of the best cases where natural interspecific hybridization and chromosome doubling was "caught in the act," giving rise to a new invasive species. In the end of the 19th century, the appearance of a new sterile hybrid was reported and, subsequently, chromosome doubling occurred, giving rise to the allopolyploid *Spartina anglica* and to related aneuploids ($2n = 120$, 122, and 124). *S. anglica* is a fertile, vigorous plant that has rapidly invaded salt marshes and estuaries in England and France and is now found on several continents. An account of the evolutionary history of the *Spartina* species has been published recently (Baumel et al., 2002b). Although this species is a threat to local flora in many regions, it is a remarkable example for the increased fitness that can be gained as a result of allopolyploidy.

Another recent allopolyploidy event that had dramatic consequences for humankind is the formation of bread wheat (genome BBAADD), approximately 9,500 calibrated years ago. When domesticated tetraploid wheat (genome BBAA) was brought to western Iran, where *Ae. tauschii*, the diploid donor of the D genome grew, hybridization took place in a farmer's field and gave rise to bread wheat, an allohexaploid that does not exist in the wild. Wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*; genome BBAA), the progenitor of macaroni wheat (*Triticum turgidum* subsp. *durum*; genome BBAA), was presumably formed a few hundred thousand years ago, whereas *Triticum timopheevii* (genome GGAA), another allotetraploid wheat, appeared later, about 50,000 to 300,000 years ago (Mori et al., 1995; Huang et al., 2002).

Maize and rice were probably formed approximately 11 mya and approximately 40 to 50 mya ago, respectively, as discussed above. Altogether, these data indicate that polyploidy is an ongoing process that has been occurring millions of years ago and is still occurring now, giving rise to new species at a rapid rate.

The fact that polyploidy in the grasses is an ongoing process and is widespread suggests that it may also occur recurrently from independent events. Recent studies indicate that many polyploid taxa are of multiple origin (Soltis and Soltis, 2000, and refs. therein). Such an origin can widen the genetic basis of the polyploid species, promoting its adaptation to a variety of new habitats and increasing its capability to compete successfully with other species. However, the indications for multiple origins of several grass polyploids are not strongly substantiated. In the wheat (*Aegilops* spp.-*Triticum* spp.) group, the only allopolyploid species that might have originated from two independent events is *Ae. triuncialis* (genome UUCC), having one subspecies that contains the U cytoplasm and another that contains the C cytoplasm (Ogihara and Tsunewaki, 1988). Such a polymorphism, however, could also be derived from hybridization between the allopolyploid and the paternal diploid parent of the allopolyploid, now as a female, and introgression of the cytoplasm via backcrossing the triploid hybrid to the allopolyploid. Moreover, there is not sufficient evidence for multiple origin of tetraploid and hexaploid wheat. On the contrary, studies of chloroplast and mitochondrial DNA revealed little, if any, variation in their plastotypes (Miyashita et al., 1994), indicating a single origin of these allopolyploids. It is surprising that despite the sympatric distribution of the diploid parents of polyploid wheat, there exist only a few successful allopolyploidization events. Therefore, it is not clear to what extent multiple independent origins have been a major factor in the establishment of these polyploids.

POLYPLOIDY AS A REVOLUTIONARY EVENT

Evolution under polyploidy comprises the events that take place early on, after hybridization, chromosome doubling, and during the establishment of the new polyploid, and the events that take place on the

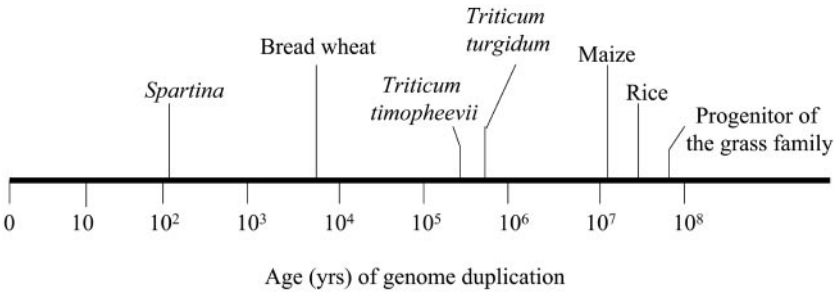

Figure 1. Polyploidy in grasses as an ongoing process. The estimated number of years (Age) since polyploidy occurred is shown for various grass species.

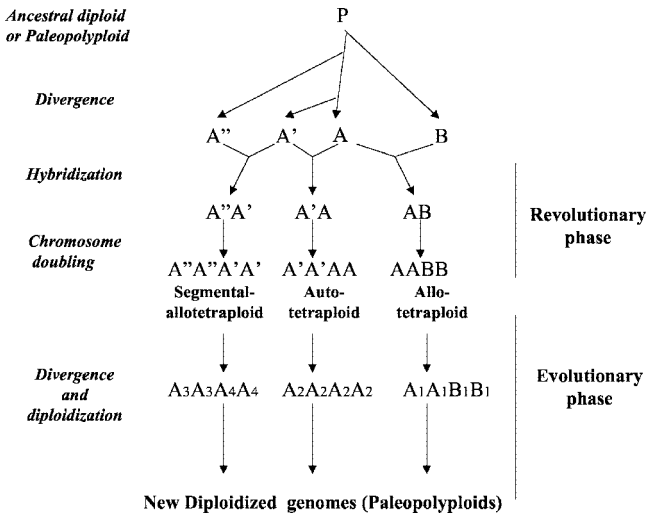

Figure 2. Evolution under polyploidy. Divergence from an ancestral species, which can be diploid or paleopolyploid, generates species with related genomes. In the example shown, genome A is very closely related to A′ and the chromosomes of A and A′ can still pair in an AA′ hybrid. A and A″ share partial similarity with some chromosomes that can pair and other chromosomes that are more diverged and do not pair. A and B are more distant and, therefore, their chromosome cannot pair and the hybrid AB is sterile. Upon chromosome doubling, hybrids AA′, AA″, and AB give rise to an autopolyploid, a segmental allopolyploid, or a genomic allopolyploid, respectively (see text for definitions). A series of genetic and epigenetic changes occur in the early phase of the hybrid and/or after chromosome doubling (the revolutionary phase). Later on (the evolutionary phase), the genomes of the polyploids keep diverging and the similarity to their diploid progenitors decreases but can still be detected by cytological analysis. Note that at this stage, lateral gene flow between species is possible in polyploids that share one genome. With further diploidization, the traces of the original genome duplication can be detected only by a thorough sequence analysis. By the end of this process, new diploid, or paleopolyploid, genomes are formed.

long term, on an evolutionary scale (Fig. 2). We discuss here the early phase of the polyploid's history.

Polyploidization is the most common and maybe unique process that can give rise to a new species in one step. What makes such a process an evolutionary success is particularly fascinating considering that genome doubling is probably a genomic shock. It involves the necessity of solving problems of gene dosage, dealing with increased genome size, orchestrating the replication of multiple and eventually different genomes, and ensuring pairing between homologs while repressing pairing between homoeologs. The ubiquity of polyploidy and the fact that it is an ongoing process may suggest that plants are preadapted to cope with challenges of polyploidy. The new polyploid has to be equipped with mechanisms enabling it to cope with the genomic stress of chromosome doubling, already in the early stages of its formation, so that it does not have reduced fitness and can compete with its parents as well as other species. We review here the various revolutionary genetic and epigenetic events that occur in newly formed polyploids, some of which may contribute to their evolutionary success.

Physical Divergence (Cytological Diploidization)

It was found recently that DNA elimination of low-copy DNA sequences occurs in the very early stages of polyploidy (Feldman et al., 1997). Further work on 35 different interspecific and intergeneric combinations of hybrids and amphiploids between species of *Aegilops* and *Triticum* showed that elimination occurs in a reproducible manner as soon as in the F_1 hybrid and/or in the first generation after chromosome doubling and may involve up to 15% of the genomic DNA (Ozkan et al., 2001; Shaked et al., 2001). This was demonstrated by different methods (DNA hybridization and AFLP), using DNA restriction with various enzymes, including methylation-sensitive and -insensitive isoschizomers and through mapping with aneuploid lines. Elimination was only from one genome in tetraploid and from two genomes in hexaploid; therefore, one consequence is to increase the physical divergence between homoeologous chromosomes. A possible result of this process is the suppression of inter-genomic recombination and, thus, increased fertility. The ability to eliminate DNA was positively correlated with seed fertility and negatively correlated with the observation of multivalent pairing (H. Ozkan, personal communication). Significant reduction in the copy number of *Spelt1*, a repetitive subtelomeric DNA sequence that represents 2% of the *Ae. speltoides* genome, was found in the first generation of amphiploids having *Ae. speltoides* as a diploid parent (Salina et al., 2000). Neither ploidy level nor the direction of the cross affected the pattern of changes in the newly formed amphiploids. Rearrangements in repetitive DNA, such as rDNA, was also observed in the early stages of allopolyploid formation (Sasakuma et al., 1993). The underlying mechanism of these changes is not known. Finally, and in agreement with the above molecular data, the total nDNA content of Triticale was lower than that expected for the combined genomes of its wheat and rye (*Secale cereale*) progenitors (Boyko et al., 1988). Similarly, a decrease in DNA content was found in the first generations of several wheat amphiploids (H. Ozkan, personal communication). Another physical change that may affect chromatin structure and, as a consequence, gene expression and chromosome pairing, is the alterations in cytosine methylation (demethylation or new methylation) that was found for 13% of the studied genomic loci (Shaked et al., 2001).

Changes in Gene Expression (Genetic Diploidization)

An important aspect of rapid adjustment to duplicated genomic dosage is through control of gene

expression. In wheat, inter-genomic suppression, as seen by disappearance of storage protein subunits, was observed immediately upon formation of a wheat allohexaploid (genome BBAADD; Galili and Feldman, 1984). Interestingly, suppression was reversible: The storage proteins reappeared upon extraction of the tetraploid BBAA genomes. Similarly, attempts to transfer resistance genes from tetraploid to hexaploid wheat failed because of a suppressor gene that was mapped to chromosome 7D (Kerber and Green, 1980). Another well-studied example of inter-genomic suppression is the silencing of rye ribosomal RNA genes in the presence of the wheat genome. Cytosine methylation is involved in this silencing as suggested by reactivation of the rye ribosomal RNA genes upon treatment with 5-aza-cytidine and by the use of methylation-sensitive/-insensitive isoschizomers (Houchins et al., 1997). In addition to inter-genomic suppression, dosage compensation for storage proteins was also observed in aneuploid series of wheat with doses of 0 to 6 for the storage protein-coding chromosome (Galili et al., 1986). Various types of dosage response can be expected in polyploids, as suggested by the work of Guo et al. (1996), who have analyzed the transcript level of 18 genes in a maize autopolyploidy series of 1x to 4x and found positive linear dosage, dosage compensation, and negative dosage. In a recent analysis of the wheat transcriptome of a newly synthesized wheat allotetraploid, Kashkush et al. (2002) found 60 transcripts (of 3,072 screened) that were present in one or both diploid progenitors and disappeared in the allotetraploid. Transcript disappearance was caused either by gene loss or gene silencing. Some, but not all, of the silenced genes were hypermethylated in the allotetraploid. In addition, activation of new transcripts was found in the allotetraploid, corresponding to retrotransposon-like sequences. All the above rapid and reproducible genetic and epigenetic responses to polyploidy emphasize the plasticity of grass genomes. It is tempting to speculate that such plasticity is exploited to facilitate the establishment of the new species.

POLYPLOIDY AS AN ACCELERATOR OF EVOLUTION

In addition to the above-mentioned events that occur at the onset of the formation of the polyploid, we discuss below how the polyploid background per se enables, in the long term, genomic changes that cannot be achieved at the diploid level and, thus, facilitates the acceleration of genome evolution. Stephens, Harlan, and Ohno (see review by Wendel, 2000 and refs. therein) have proposed that polyploidy buffers the mutation load and, thus, facilitates the acquisition of new functions. This proposal has not been tested systematically in an adequate experimental system, part of the difficulty being to study events on an evolutionary scale. Nevertheless, we present a series of evidence that supports this hypothesis.

Horizontal Transfers

First, some events, such as inter-genomic horizontal transfer of chromosomal segments, transposons, or genes between the constituent genomes, can occur almost exclusively in an allopolyploid background. For example, 18 inter-genomic translocations were found by genomic in situ hybridization in allohexaploid oat (*Avena sativa*; Chen and Armstrong, 1994). Inter-genomic translocations can be species specific, such as the 4AL/7BS translocation that probably occurred early in the emmer wheat lineage, whereas several different translocations have originated in *T. timopheevii*, another tetraploid wheat (Maestra and Naranjo, 1999). Invasion of the A genome by sequences from the B genome was detected in wild tetraploid wheat (genome BBAA) using genomic in situ hybridization (Belyayev et al., 2000). The time course of such invasion is not known. The underlying mechanism might be inter-genomic transposition, or gene conversion between homoeologs. Another unique feature of evolution through allopolyploidy is that it enables recombination between genomes that would not occur at the diploid state. It was shown in tetraploid *Aegilops* spp. that recombination can occur between homoeologous genomes (e.g. B and C) in hybrids between amphiploids that share one genome and differ in the other genome(s) (e.g. AABB and AACC; Zohary and Feldman, 1962). Such a recombination may lead to the production of recombinant genomes that cannot be formed at the diploid level (e.g. a BC diploid genome would be completely sterile in nature, whereas an AABC is only partially sterile, presumably because of the buffering effect of genome AA). The B genome of wheat is thought to be such a recombinant genome (Feldman, 2001).

In summary, the possibility of inter-genomic transfer through translocation, recombination, or transposition seems to be widespread in grasses and is a unique feature of allopolyploids that adds to their genome's plasticity and enables to create new genetic combinations that are beyond the addition of two genomes.

Accelerated Diploid-Like Evolutionary Processes?

Another interesting question is whether evolutionary processes that normally occur in diploids, such as microsatellite expansion, insertions, and point mutations, occur at a faster rate in polyploids compared with their diploid progenitors? In support of this hypothesis are theoretical considerations, such as a lack of selection against mutations because of genetic redundancy in the polyploid and experimental data showing resistance of polyploids to mutagens compared with their diploid progenitors (Sears, 1972). Nevertheless, despite these considerations, there is no direct evidence that diploid-like evolutionary processes are accelerated as a result of polyploidy.

Transposon-induced mutations could also contribute to genetic diploidization by knocking out redundant genes, e.g. the recent Wis-2 retroelement insertion in a bread wheat glutenin gene (Harberd et al., 1987); however, there is no evidence for a transposition burst as a result of polyploidy. Retrotransposon display in *Spartina* spp. has shown that only a few insertions have occurred since its formation (Baumel et al., 2002a). Similarly, the big bang of transposition in the maize genome is estimated to have occurred during approximately the past 6 mya ago, i.e. approximately 6 mya after maize polyploidization (SanMiguel et al., 1998). Finally, transcriptional activation of wheat retrotransposons was detected in a newly synthesized wheat polyploid (Kashkush et al., 2002); however, this transcriptional activation was not associated with a burst of transposition (K. Kashkush, M. Feldman, and A.A. Levy, unpublished data). Overall, there is a lack of direct experimental evidence for an acceleration of evolutionary processes such as point mutations, transposition, and microsatellite expansion as a result of polyploidy.

CONCLUDING REMARKS

What lessons have we learned from polyploidy in the grasses? Starting from the end, polyploidy is not an exotic feature of a few species but it is an evolutionary successful path "chosen" by most of the grasses from the beginning of their formation to the present. It has played a key role in the evolution of the three most important crops, wheat, maize and rice, each of which provides a unique model system to study allopolyploidy, segmental allopolyploidy, or paleopolyploidy, respectively. Then, what are the causes of its success?

One possibility might be the great plasticity offered by polyploidy, such as the variety of genetic systems, namely disomic and/or multisomic inheritance with the implications on allelic multiplicity, or fixation of inter-genomic heterozygosity. In addition to this plasticity, the polyploid condition enables creativity through inter-genomic reshuffling of chromosomal segments and genes in a way that could not happen in a diploid background. Moreover, an interesting possibility that should be tested is whether the polyploid background enables an acceleration of evolutionary processes that are also present in diploids, such as point mutations, transposition, or microsatellite instability, which may increase the variation on which natural selection can work.

A fascinating and yet not understood possible cause for evolutionary success is that polyploid species seem preadapted to chromosome doubling because they deal so efficiently with the rather dramatic event of polyploidy, something that most animal cells cannot do. First, we have to assume that there is a sensing process signaling that polyploidy has occurred, then there must be transduction of this signal, and finally there will be harmonization of gene expression on a genomic scale by global regulation mechanisms leading to silencing, dosage compensation, or, on the other hand, to gene activation. All we know about these mechanisms is that methylation is sometimes involved. We also do not know what the key player genes are whose silencing or activation may contribute to an increased fitness in the polyploid. Systems such as cytological diploidization are also immediately put in place to ensure disomic chromosome segregation in allopolyploids. We have observed immediate and reproducible elimination of DNA sequences, a phenomenon that increases the divergence between homoeologs and, thus, reduces their chances of pairing. This kind of response also suggests preadaptation. Here, too, the mechanism is unknown and we do not know how widespread such a phenomenon is in the grasses. Another unexplored field of research in polyploidy is the study of the orchestration of DNA replication and chromosome condensation of the two genomes that share the same nucleus. Finally, it will be of interest to find out why some genomic combinations are successful and others are not. Is it related to the ability to react to the shock of polyploidization, or to a positive combining ability between genomes (the equivalent of heterosis between species)?

Whatever it is that makes polyploid species successful, grasses have it. It will take more brains and new tools to discover what it is. Among the new tools needed to study polyploidy in grasses are new bioinformatic algorithms that should provide a clearer understanding concerning the detection and aging of duplications (e.g. Gaut, 2001; Wong et al., 2002). New microarrays with probes to distinguish between homoeologs and, thus, determine the types of loci that are affected by polyploidy are also needed. This should provide insight on whether an orchestrated or a random series of changes in gene expression occurs in response to polyploidy. This may also point out to the key genes involved in response to polyploidy. The sequence of the rice genome, together with an integrated network for comparative genome analysis in the grasses, will be an invaluable resource for this purpose. Mutants will also be needed to study the involvement of specific genes on the response of the genome to polyploidy. Finally, the toughest challenge will be to determine which of the genetic and epigenetic changes that occur as a result of polyploidy increase fitness or are merely selectively irrelevant mechanistic consequences.

Note Added in Proof

In an abstract submitted recently to the Plant and Animal Genome XI (San Diego 2003), M.D. Bennett, L. Hanson, and I.J. Leitch report on the DNA C-values of 450 Gramineae species for which there is information on ploidy. The mean 4C DNA amount for diploids is 15.26 pg (178 species), 25.51 pg for tetraploids (145 species), 36.67 pg for hexaploids (55 species), and 36.98 pg for octoploids (17 species). The increase in genome size is not in linear relationship with ploidy level but rather it is less than expected for

additivity. This suggests that DNA loss following polyploidy is widespread in the grass family.

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LITERATURE CITED

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408:** 796–815

Baumel A, Ainouche M, Kalendar R, Schulman AH (2002a) Retrotransposons and genomic stability in populations of the young allopolyploid species *Spartina anglica* C.E. Hubbard (Poaceae). Mol Biol Evol **19:** 1218–1227

Baumel A, Ainouche ML, Bayer RJ, Ainouche AK, Misset MT (2002b) Molecular phylogeny of hybridizing species from the genus *Spartina* Schreb. (Poaceae). Mol Phylogenet Evol **22:** 303–314

Belyayev A, Raskina O, Korol A, Nevo E (2000) Coevolution of A and B genomes in allotetraploid *Triticum dicoccoides*. Genome **43:** 1021–1026

Boyko EV, Badaev NS, Maximov NG, Zelenin AV (1988) Regularities of genome formation and organization in cereals: I. DNA quantitative changes in the process of allopolyploidization. Genetika **24:** 89–97

Chen QF, Armstrong K (1994) Genomic *in situ* hybridization in *Avena sativa*. Genome **37:** 607–612

Comai L (2000) Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol **43:** 387–399

Feldman M (2001) The origin of cultivated wheat. *In* A Bonjean, W Angus, eds, The World Wheat Book. Lavoisier Publishing, Paris

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM (1997) Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics **147:** 1381–1387

Galili G, Feldman M (1984) Intergenomic suppression of endosperm protein genes in common wheat. Can J Genet Cytol **26:** 651–656

Galili G, Levy AA, Feldman M (1986) Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*. Proc Natl Acad Sci USA **83:** 6524–6528

Gaut BS (2001) Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. Genome Res **11:** 55–66

Gaut BS, Doebley JF (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. Proc Natl Acad Sci USA **94:** 6809–6814

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H et al. (2002) A draft sequence of the rice genome (*Oryza sativa L. ssp. japonica*). Science **296:** 92–100

Goldblatt P (1980) Polyploidy in angiosperms: monocotyledons. *In* WH Lewis, ed, Polyploidy, Biological Relevance. Plenum Press, New York, pp 219–239

Grant V (1981) Plant Speciation. Columbia University Press, New York

Guo M, Davis D, Birchler JA (1996) Dosage effects on gene expression in a maize ploidy series. Genetics **142:** 1349–1355

Harberd NP, Flavell RB, Thompson RD (1987) Identification of transposon-like insertion in a Glu-1 allele of wheat. Mol Gen Genet **209:** 326–332

Houchins K, O'Dell M, Flavell RB, Gustafson JP (1997) Cytosine methylation and nucleolar dominance in cereal hybrids. Mol Gen Genet **255:** 294–301

Huang S, Sirikhachornkit A, Su X, Faris J, Gill B, Haselkorn R, Gornicki P (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polypoid wheat. Proc Natl Acad Sci USA **99:** 8133–8138

Kashkush K, Feldman M, Levy AA (2002) Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics **160:** 1651–1659

Kellogg EA (2001) Evolutionary history of the grasses. Plant Physiol **125:** 1198–1205

Kerber ER, Green GJ (1980) Suppression of stem rust resistance in hexaploid wheat cv Cantach by chromosome 7DL. Can J Bot **58:** 1347–1350

Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. Trends Plant Sci **2:** 470–476

Maestra R, Naranjo T (1999) Structural chromosome differentiation between *Triticum timopheevii* and *T. turgidum* and *T. aestivum*. Theor Appl Genet **98:** 744–750

Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science **264:** 421–423

Miyashita NT, Mori N, Tsunewaki K (1994) Molecular variation in chloroplast DNA regions in ancestral species of wheat. Genetics **137:** 883–889

Mori N, Liu YG, Tsunewaki K (1995) Wheat phylogeny determined by RFLP analysis of nuclear DNA: II. Wild tetraploid wheats. Theor Appl Genet **90:** 129–134

Ogihara Y, Tsunewaki K (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor Appl Genet **76:** 321–332

Ohno S (1970) Evolution by Gene Duplication. Springer, New York

Ozkan H, Levy AA, Feldman M (2001) Alloploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. Plant Cell **13:** 1735–1747

Pikaard CS (2001) Genomic change and gene silencing in polyploids. Trends Genet **17:** 675–677

Salina EA, Ozkan H, Feldman M, Shumny VK (2000) Subtelomeric repeat reorganization in synthesized wheat amphiploids. *In* N Kolchanov, D Furman, eds, Proceedings of the Conference on Biodiversity and Dynamics of Ecosystems in North Eurasia. Institute of Cytology and Genetics Press, Novosibrsk, Russia, pp 102–105

SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL (1998) The paleontology of intergene retrotransposons of maize. Nat Genet **20:** 43–45

Sasakuma T, Ogihara Y, Tsujimoto H (1993) Genome rearrangement of repetitive sequences in the processes of hybridization and amphiploidization in Triticenae. *In* ZS Li, ZY Yin, eds, Proceedings of the 8th International Wheat Genetics Symposium, Beijing, China. China Agricultural Scientech Press, Beijing, pp 563–566

Sears ER (1954) The aneuploids of common wheat. Res Bull Univ Missouri Agric Exp Stn **572:** 1–59

Sears ER (1972) The nature of mutation in hexaploid wheat. Symp Biol Hung **12:** 73–82

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet **10:** 31–51

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell **13:** 1749–1759

Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. Proc Natl Acad Sci USA **97:** 7051–7057

Stebbins GL (1950) Variation and Evolution in Plants. Columbia University Press, New York

Stebbins GL (1971) Chromosomal Evolution in Higher Plants. Edward Arnold (Publisher) Ltd., London

Wendel JF (2000) Genome evolution in polyploids. Plant Mol Biol **42:** 225–249

Wolfe KH (2001) Yesterday's polyploids and the mystery of diploidization. Nat Rev Genet **2:** 333–341

Wong S, Butler G, Wolfe KH (2002) Gene order evolution and paleopolyploidy in hemiascomycete yeasts. Proc Natl Acad Sci USA **99:** 9272–9277

Zohary D, Feldman M (1962) Hybridization between amphiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. Evolution **16:** 44–61