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REVIEW

Genomic aspects of research involving polyploid plants

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Abstract Almost all extant plant species have doubled their genomes at least once in their evolutionary histories, resulting in polyploidy which provided a rich genomic resource for evolutionary processes. Moreover, superior polyploid clones have been developed during the process of crop domestication. Polyploid plants generated by evolutionary processes and/or crop domestication have been the intentional or serendipitous focus of research dealing with the dynamics and consequences of genome evolution. One of the new trends in genomics research is to create synthetic polyploid plants which provide materials for studying the initial genomic changes/responses immediately after polyploid formation. Polyploid plants are also used in functional genomics research to study gene expression in a complex genomic background. In this review, we summarize recent progress in genomics research involving ancient, young, and synthetic polyploid plants, with a focus on genome size evolution, genomic diversity, genomic rearrangement, genetic and epigenetic changes in duplicated genes, gene discovery, and comparative genomics. Implications on plant sciences including evolution, functional genomics, and plant breeding are presented. Polyploids will be a focus of genomic research in the future as rapid advances in DNA sequencing technology create unprecedented opportunities for discovering and monitoring genomic and transcriptomic changes. The accumulation of knowledge on polyploid formation, maintenance, and divergence at whole-genome and subgenome levels will not only help plant biologists understand how plants have evolved and diversified, but also assist plant breeders in designing new strategies for crop improvement.

Keywords Evolution · Genetics · Epigenetics

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Introduction

Polyploidy (genome doubling), discovered in 1907, is an important driver of eukaryotic evolution, evident in many animals, fungi, and plants (Grant 1981; Hovav et al. 2008; Wood et al. 2009). Almost all eukaryotes have had a history of ancient polyploidization events followed by diploidization and then repeated polyploidization (Birchler and Veitia 2010; Salmon and Ainouche 2010). Polyploid plants have been generated from evolutionary processes, crop domestication, and/or artificial synthesis via chemical or physical mutations. Traditionally, polyploid plants have been studied from the perspectives of crop domestication, e.g., creating crops with specific traits such as larger flowers and fruits. This type of research has been reviewed extensively in the

past several decades and thus will not be the focus of this review. Studies of polyploid plants across various genomic scales have occurred relatively more recently, particularly after the whole-genome sequence of *Arabidopsis* became available and it was discovered that this species has experienced at least two whole-genome duplication events (Blanc et al. 2003). Since then, sequencing of more and more plant genomes has revealed evidence of multiple whole-genome duplication events (Jaillon et al. 2007; Tuskan et al. 2006). To date, polyploid plants have been used in various aspects of genomics research including evolutionary genomics, functional genomics, and comparative genomics. In this review, we highlight recent research involving polyploid plants in a multidimensional context encompassing genomic origins (i.e., autopolyploids vs. allopolyploids), formation processes (i.e., natural vs. synthetic polyploids), and relative age of polyploid events (i.e., ancient vs. young vs. newly-formed polyploids). Given the extensive body of literature that exists on polyploid genomics, this review is not meant to be exhaustive. Please refer to some excellent review articles for more complete and detailed information related to genomics research in polyploid plants (Birchler and Veitia 2010; Chen 2010; Freeling 2009; Hegarty and Hiscock 2008; Hufton and Panopoulou 2009; Leitch and Leitch 2008; Parisod et al. 2010a, b; Salmon and Ainouche 2010; Soltis et al. 2009; Soltis and Soltis 2009; Van de Peer et al. 2009).

Classification and assessment of polyploids

Polyploid plants can be classified based on three different parameters: genomic origin, formation approach, and time after polyploid formation. Polyploid origin can be divided into autopolyploids, which are derived from a whole-genome duplication event of the same ancestral chromosome set, and allopolyploids, which are derived from a hybridization event of alternate parental genomes followed by genome duplication (Pignatta et al. 2010). Multiple pathways, as illustrated in Fig. 1 could lead to the formation of autopolyploids or allopolyploids. Chromosome doubling in polyploid formation involves two major mechanisms: somatic doubling and unreduced gamete (i.e., 2*n* gamete) formation (Bretagnolle and Thompson 1995; Carputo et al. 2003; Stuessy et al. 2004). Based on the time after polyploid formation, polyploids can be classified as ancient, young, and newly-formed polyploids. The "ancient" vs. "young" term is not rigidly defined in any absolute temporal basis, but are only relative to each comparison (Bennett 2004). Polyploids can also be divided into natural and synthetic polyploids. Natural polyploids result from spontaneous genome-doubling, whereas synthetic polyploids are the result of induced genome-doubling with or without prior genome-hybridization. Examples of different types of polyploid plants are listed in Table 1. Based on the sets of homologous chromosomes post-doubling, polyploids can be classified as triploid, tetraploid, hexaploid, octoploid, etc. However, this classification becomes challenging as many species with whole-genome sequences available indicate that they experienced multiple rounds of genome-doubling, meaning that they are ancient poplyploids (Bowers et al. 2003).

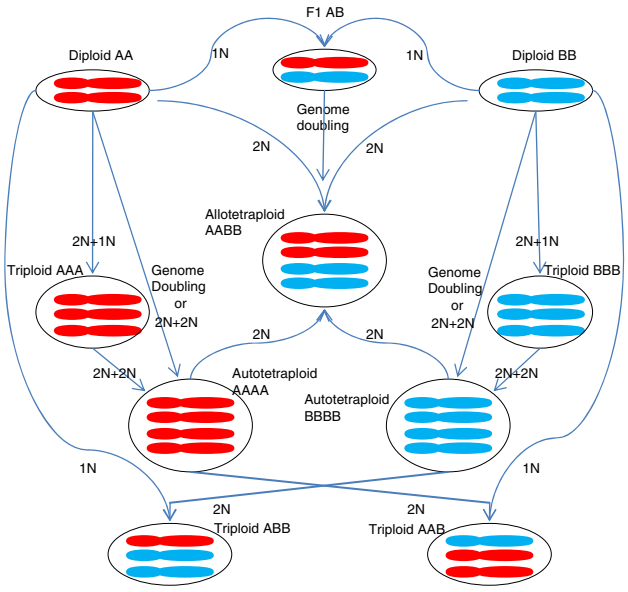

Fig. 1 Major pathways of polyploidization. Two parent genomes (in diploid) are represented by A and B, respectively. *N* is the gametic chromosome number

Ploidy level in plants is often estimated by measuring the C-value (amount of DNA in the unreplicated gametic nucleus) using flow cytometry (Clarindo et al. 2008; Dart et al. 2004; Eaton et al. 2004; Grundt et al. 2005; Halverson et al. 2008; Harbaugh 2008). The counting of chloroplast number in the epidermal guard cells of stomata can be used as an indirect ploidy indicator for the rapid screening of polyploids (Ewald et al. 2009; Ho et al. 1990). Measurement of the stomatal diameter with scanning electron microscopy has also been used to clarify the differences among polyploids (Shiga et al. 2009). Besnard et al. (2008) used flow cytometry to estimate genome content and six highly variable nuclear microsatellites to assess the presence of multiple alleles at co-dominant loci in *Olea europaea*. Their data provided strong evidence for polyploidy in the subspecies *cerasiformis* (tetraploid) and *maroccana* (hexaploid). Recently, Cousin et al. (2009) reported an efficient high-throughput flow cytometry method which involves placing young leaf samples in two 96-well plates, lysing the nuclei, filtering the samples, staining with propidium iodide, and then estimating ploidy using a BD FACS-Canto II flow cytometer. To determine the number

Table 1 A cross section of polyploid plants representing a range of origins, ages, and processes of formation

Age	Process	Origin	Example	Reference
Ancient	Natural	Autopolyploid or allopolyploid	*Vitis vinifera* ($2n = 38$)	Jaillon et al. (2007)
Young	Natural	Autopolyploid	*Helianthus decapetalus* ($2n = 4x = 68$)	Church and Spaulding (2009)
Young	Natural	Allopolyploid	*Tragopogon mirus* ($2n = 4x = 24$)	Soltis et al. (2004)
Young	Synthetic/induced	Allopolyploid	Triticale ($2n = 6x = 42$)	Chen (2010)
New	Synthetic/induced	Autopolyploid	*Arabidopsis thaliana* ($2n = 4x = 20$)	Santos et al. (2003)
New	Synthetic/induced	Allopolyploid	*Gossypium* ($2n = 4x = 52$)	Chaudhary et al. (2009)

and timing of ancient whole-genome duplication (or polyploidy) events in plant evolutionary history, Burleigh et al. (2009) developed an algorithm for the Episode Clustering problem, which, given a collection of rooted gene trees and a rooted species tree, seeks the minimum number of locations on the species tree of gene duplication events. Using this algorithm, they found evidence of large-scale gene duplication events in *Populus*, *Gossypium*, Poaceae, Asteraceae, Brassicaceae, Solanaceae, and Fabaceae that are consistent with previous genomic evidence.

Evolutionary genomics

Polyploid plants have been extensively used for genomics research in an evolutionary context. Figure 2 outlines most of the aspects in evolutionary genomics research involving polyploid plants. Here we discuss each of the aspects based on recent research progress.

Genome size evolution following polyploid formation

Genome expansion and contraction in polyploids is one of the important topics in polyploid genomics research. Intuitively, it is expected that polyploids have larger C-values than their diploid progenitors, increasing in direct proportion with ploidy (Leitch and Bennett 2004). However, all of the three possible fates (i.e., no change, contraction, expansion) of genome size evolution after ploidization have been reported. In some polyploid series, especially newly-formed polyploids, no change in genome size was observed (Leitch and Bennett 2004). Only a few reports showed genome expansion after polyploid formation. In recent research comparing the expected genome size of *Nicotiana* polyploids (based on summing the genome size of species identified as either a parent or most closely related to the diploid progenitors) with the observed genome size, five polyploids were shown to have an increase in genome size (Leitch et al. 2008). In contrast, a large-scale analysis combining available genome size data of 3,008 angiosperms revealed that the mean 1C DNA amount did not increase in direct proportion with ploidy and the mean DNA amount per basic genome (calculated by dividing the 2C value by ploidy) tended to decrease with increasing ploidy, suggesting that genome contraction is a widespread biological response to polyploidization, leading to diploidization of the polyploid genome (Leitch and Bennett 2004).

Mechanisms of genome expansion and contraction have been investigated. Grover et al. (2007) detected genomic contraction in a polyploid cotton (*Gossypium*) relative to its diploid progenitors. They hypothesized that this contraction

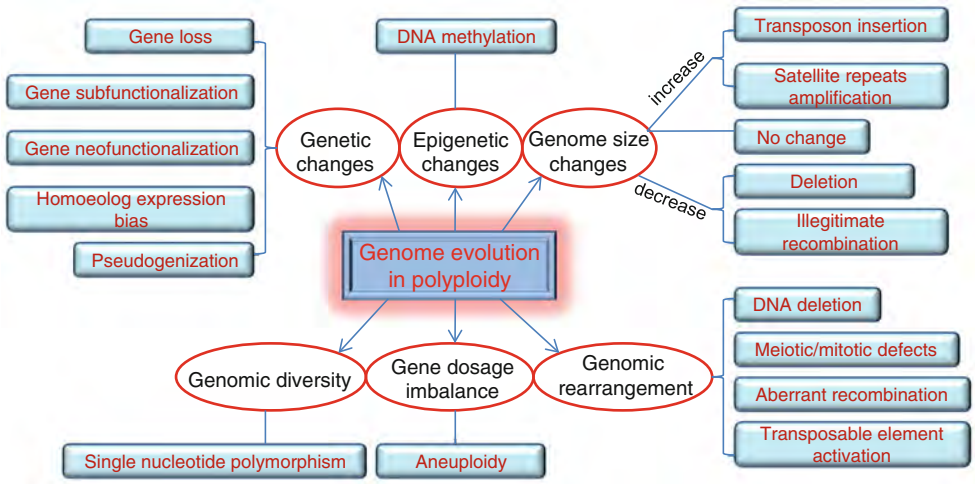

Fig. 2 Genomic evolution after polyploid formation

is due to an indel (insertion and deletion) bias leading to frequent and larger deletions and that increased illegitimate recombination may lead to a reduction in genome size. By examining the changes in the duplicated regions (homoeologous) in soybean following polyploidy, Innes et al. (2008) demonstrated that the largest contributor to genome expansion was retroelement content, with homoeolog 2 having expanded to threefold the size of homoeolog 1, suggesting that retrotransposon accumulation contributed to the genome expansion. A recent report suggested that amplifications of dispersed microsatellite repeats may play a role in genome size evolution in polyploids (Koukalova et al. 2010).

Genomic rearrangement in polyploid plants

Whole-genome duplication events have also been assocaited with genome rearrangement, including aberrant recombination, transposable element activation, meiotic/mitotic defects, and intron expansions and contractions (Hufton and Panopoulou 2009). Evidence currently available suggests that autopolyploids neither experience strong genome restructuring nor extensive reorganization during the first few generations following genome doubling, but that these processes may become more important in the longer term (Parisod et al. 2010a, b). However, in allopolyploids, genome changes (e.g., loss and/or gain of parental restriction sites, appearance of novel fragments) appear to occur in the early generations after polyploidization, as revealed in the F_2 to F_5 generations of synthetic *Brassica* polyploids after their formation (Song et al. 1995). Gao et al. (2007) sequenced and analyzed orthologous regions containing both gliadin and LMW-glutenin genes from the homoeologous A and B genomes of a tetraploid wheat to understand the evolutionary dynamics of the genome. Their comparative analysis of orthologous regions showed that gene movement contributed to the violation of microcolinearity between the A and B genomes, rapid sequence rearrangements, and differential insertion of repetitive DNA (Gao et al. 2007). In wheat (*Triticum aestivum*), genomic rearrangements (e.g., transposable element insertions, genomic deletions, duplications, and inversions) were shown to constitute the major differences when the same genomes (i.e., the A, B, or D genomes) were compared between species of different ploidy levels; and illegitimate DNA recombination, leading to various genomic rearrangements, constitutes one of the major evolutionary mechanisms in wheat species (Chantret et al. 2005). Recently, Wang et al. (2009) studied the evolution of the C-genome in *Oryza officinalis* complex, which contains successive polyploidization events forming CCDD tetraploids ca. 0.9 million years ago (Mya) and stepwise forming BBCC tetraploids between ca. 0.3–0.6 Mya, and identified inter-genomic translocations between B- and C-genomes in BBCC tetraploid, *O. punctata*.

Genomic diversity in polyploid plants

Single nucleotide polymorphisms (SNP) are common in plant genomes (Bundock et al. 2009) and have proven to be indispensable in association mapping and construction of high-density genetic maps (Akhunov et al. 2009). Polyploid genomes potentially hold ambiguity problems for most SNP assays. Akhunov et al. (2009) studied SNP in 91 homozygous polyploid wheat lines, including 53 tetraploid and 38 hexaploid lines using the Illumina GoldenGate assay. They demonstrated that about 89 and 84% of SNPs in tetraploid and hexaploid wheat, respectively, could be converted into unique genotyping assays. In the heterozygous polyploid sugarcane, a targeted SNP discovery approach, based on 454 sequencing technology, was developed by Bundock et al. (2009). They sequenced 307 polymerase chain reaction (PCR) amplicons (>59 kb of sequence) on a 454 Genome Sequencer FLX and generated 182,996 sequencing reads, with an average sequence depth of $\sim$300 and an average read length of 220 bases. Analysis of the 454 sequencing data revealed one SNP every 58 bases on average in one polymorphic sugarcane parent (Q165), demonstrating that PCR amplicon re-sequencing using the 454 system is a cost-effective approach for SNP discovery targeted to genes of interest in polyploid genomes (Bundock et al. 2009).

SNPs have also been discovered in transcriptome sequences of polyploid *Brassica napus* by Trick et al. (2009). In this research, $\sim$20 million expressed sequence tags (ESTs) were generated from each of two cultivars. 23,330–41,593 putative SNPs were detected between the cultivars, with the majority being indicative of transcriptional differences between the homoeologous genes from the two parental genomes. Salmon et al. (2010) used genomic resources in diploid and allopolyploid *Gossypium* to detect homoeologous SNPs in *G. arboreum* (A genome), *G. raimondii* (D genome), and *G. hirsutum* (AD genome). They estimated the proportion of contigs in *G. hirsutum* that have experienced nonreciprocal homoeologous exchanges since the origin of polyploid cotton 1–2 Mya to be between 1.8 and 1.9%. Furthermore, their phylogenetic analysis of six genes affected by homoeo-recombination in five *Gossypium* allopolyploids revealed that nonreciprocal homoeologous exchanges occurred throughout polyploid divergence.

Gene-level changes in polyploid plants

There are six possible changes in the protein coding region after gene duplication with combinations of retention (R),

degeneration (D), and neofunctionalization (N): RR, RD, RN, DD, NN, and ND (Yang et al. 2006). Current theories that address the fate of nuclear genes following duplication events (i.e., Gain of Function Hypothesis, Subfunctionalization Hypothesis, Increased Gene Dosage Hypothesis, Functional Buffering Model, and the Gene Balance Hypothesis) were recently reviewed by Edger and Pires (2009). Town et al. (2006) performed comparative analysis of 2.2 Mb triplicated *Brassica oleracea* genome segments, in which 177 conserved collinear genes were identified. From phylogenetic analysis using *Arabidopsis thaliana* as an outgroup containing a segmentally-duplicated region homologous with the triplicated *Brassica oleracea* genome segments, they found that 35% of the genes inferred to be present when genome triplication occurred in the *Brassica* lineage have been lost, most likely via deletion mechanisms. As genes were eliminated following polyploidization, the position of the retained genes was not random, that is, dosage-sensitive genes, including duplicates of transcription factors and members of signal transduction pathways, were significantly over-retained following whole-genome duplications, whereas these same functional gene categories exhibited lower retention rates following smaller scale duplications (e.g., local and tandem duplicates, segmental duplicates, aneuploidy; Birchler and Veitia 2010; Blanc and Wolfe 2004; Edger and Pires 2009). However, in a triplicated *Brassica oleracea* genome, genes encoding proteins involved in signal transduction or transcription were not found to be significantly more extensively retained than those encoding proteins classified with other functions (Town et al. 2006). Recurrent deletions of Puroindoline (Pin) genes at the grain *Hardness* (*Ha*) locus in four independent lineages of polyploid wheat were discovered by Li et al. (2008). They analyzed the *Ha* haplotype structure in 90 diploid and 300 polyploid accessions of wheat and demonstrated that Pin genes were conserved in all diploid species and deletion haplotypes were detected in all lineages of polyploid wheat. One example of pseudogenization following polyploid formation in a cotton allotetraploid containing two co-resident genomes (A_T and D_T) is that a mutation in the D_T copy of the integral membrane protein-encoding gene caused a premature stop codon to arise halfway through the coding region, resulting in a truncated protein (Grover et al. 2007).

Polyploidy through gene silencing has extensive effects on gene expression (Adams and Wendel 2005). Gene expression changes affecting newly-formed polyploid species may result from various, interconnected mechanisms, including (1) functional interactions between the homoeologous copies and between their products, (2) relaxed selective pressure on one of the parental copies leading to gene loss, pseudogenization, subfunctionalization or neofunctionalization, and (3) epigenetic changes that, in turn, affect gene expression (Salmon and Ainouche 2010). Subfunctionalization, in which the ancestral expression profile becomes partitioned among duplicated genes (termed homoeologs), appears to be a common phenomenon at the scale of organs in polyploidy (Hovav et al. 2008). Reciprocal silencing of duplicates in different organs has been observed, suggesting subfunctionalization and long-term retention of duplicates (Adams 2007). Subfunctionalization of genes duplicated by polyploidy in response to abiotic stress conditions was demonstrated in a study on homeologous-gene expression of the alcohol dehydrogenase gene in allopolyploid *Gossypium hirsutum* under cold, dark, and water submersion conditions (e.g., only one copy was expressed in hypocotyls during a water-submersion treatment and the other copy was expressed during cold stress; Liu and Adams 2007). Chaudhary et al. (2009) investigated relative expression levels of each homeolog for 63 gene pairs in 24 tissues in a naturally-occurring allopolyploid *Gossypium*, a synthetic allopolyploid of the same genomic composition, and models of the diploid progenitor species. Their data demonstrated that 40% of homeologs were transcriptionally biased in at least one stage of cotton development, with expression patterns consistent with subfunctionalization (7 cases) and neofunctionalization (15 cases), that genome merger per se had a large effect on the relative expression of homeologs, and that the majority of these alterations were caused by cis-regulatory divergence between the diploid progenitors. Recently, Buggs et al. (2010) studied the expression of homoeolog pairs in seven tissues of a natural allotetraploid *Tragopogon mirus* formed 40 generations ago and revealed reciprocal tissue-specific expression between homoeologs, indicating that subfunctionalization can arise rapidly in the early generations of natural allopolyploidy.

One unique feature of genetic changes in polyploids is gene expression bias at the genome level. Hovav et al. (2008) investigated the expression of thousands of pairs of homoeologs during the development of a single cell, using the seed trichomes ("cotton fiber") of allopolyploid (containing "A" and "D" genomes) *Gossypium* as a model, and demonstrated that $\sim$30% of the homoeologous gene expression was biased differentially toward the A or D genome, illustrating the functional partitioning of genomic contributions during cellular development. Similarly, 276 of the 461 genes sampled by microarray analysis were shown to have unequal expression levels biased toward the A- or D-genome of allotetraploid *Gossypium* (Udall et al. 2006). Furthermore, it was reported that homoeolog expression bias in allopolyploid cotton favored the allopolyploid D genome over the A genome (Flagel and Wendel 2010; Hovav et al. 2008). In natural allopolyploid *Spartina* formed via the merger of divergent genomes and genome duplication during the 19th century, Chelaifa et al.

(2010) found that deviation of gene expression from parental additivity occurred following allopolyploid formation and was accompanied by maternal expression dominance.

Most results concerning alterations in gene expression after polyploid formation have been obtained from studies in allopolyploids, in which changes to the nuclear environment are more profound than that in autopolyploids. Stupar et al. (2007) investigated the expression of ~9,000 genes in a potato (*Solanum phureja*) synthetic autopolyploid series including one monoploid (1*x*) clone, two diploid (2*x*) clones, and one tetraploid (4*x*) clone. They found that ~10.5 and 10.6% of the genes showed differential expression among different ploidy clones in the leaflet and the root tip, respectively. The majority of the differentially expressed genes in both tissue types displayed significant differences only between 1*x* and 2*x* genotypes ($1x < 2x \approx 4x$ and $1x > 2x \approx 4x$ patterns). Only 12 and 8% of the differentially expressed genes displayed completely ploidy-upregulated ($1x < 2x < 4x$) and ploidy-downregulated ($1x > 2x > 4x$), respectively. The molecular functions related to nucleic acid binding and structural molecule activity were enriched in the gene set that were differentially expressed in the leaflet tissue (Stupar et al. 2007). Pignatta et al. (2010) investigated the influence of autopolyploidization on gene expression in three independent lineages of autotetraploid *Arabidopsis thaliana* and their matched diploids. Their results obtained using two different approaches (microarray and enhancer trap) were contradictory: microarray analysis of ploidy-induced changes failed to provide a set of robustly-regulated genes, yet ploidy-induced changes could be easily demonstrated by the use of enhancer traps.

Almost all the gene expression data in polyploid plants have been obtained from transcriptome profiling. Recently, proteomics analysis was used to investigate gene expression in polyploid plants. Marmagne et al. (2010) applied comparative proteomics to early generations of resynthesized *Brassica napus* allotetraploids and demonstrated that 25 and 38% of the proteins expressed in the root and stem, respectively, displayed nonadditivity compared with the expected mid-parent value (MPV), whereas two-thirds of the genes encoding nonadditive proteins had additive transcript levels, indicating that most of the differential protein regulation was not explained by transcriptional changes.

Gene dosage imbalance in polyploid plants

Segmental aneuploidy (i.e., the relative excess or deficiency of specific chromosome regions) results in gene dosage imbalance and often causes severe phenotypic alterations in plants and animals (Makarevitch and Harris 2010). Triploid meiosis predominately produced aneuploid viable gametes in *Arabidopsis thaliana* and the chromosomal composition of the swarms produced by the triploid *A. thaliana* was strongly influenced by selection acting against specific gamete combinations with each of the five chromosome types responding differently to this selection, suggesting the presence of dosage-sensitive factor(s) critical for viability and encoded on different chromosomes (Henry et al. 2009). Recently, Makarevitch and Harris (2010) performed a detailed analysis of gene expression affected by aneuploidy in multiple maize tissues and found that many genes demonstrated qualitative changes in gene expression due to aneuploidy when the gene became ectopically expressed or completely silenced in aneuploids relative to wild-type plants. Their data suggested that quantitative changes in gene expression caused by variation in gene dosage progressed through tissue development and resulted in stable qualitative changes in gene expression patterns (Makarevitch and Harris 2010).

Epigenetic changes in polyploid plants

Some gene silencing events may be epigenetically induced during the allopolyploidization process (Adams et al. 2003). Mudge et al. (2009) reported efficient silencing of reporter transgenes coupled to known functional promoters in sugarcane, a polyploid crop species. The mechanism of this gene silencing was unknown. Recently, Khaitova et al. (2010) investigated expression of rRNA gene families in five pentaploid dogrose species (*Rosa canina*, *R. rubiginosa*, *R. dumalis*, *R. sherardii* and *R. caesia*) and in one tetraploid species (*R. mollis*). Their data showed that the families occurring on bivalent-forming chromosomes dominated rDNA expression in all dogrose species, suggesting that genes on bivalent genomes were stably expressed, whereas those on univalent genomes underwent variable levels of epigenetic silencing.

DNA methylation may play a role in evolutionary dynamics of duplicated genes (Yang et al. 2006). This epigenetic mechanism has the potential to affect plant phenotypes and respond to environmental and genomic stresses, such as hybridization and polyploidization (Verhoeven et al. 2010). As one of the interrelated processes leading to epigenetic regulation of gene expression, the DNA methylation status of newly formed species appears to be consistently affected following genome doubling (Salmon and Ainouche 2010). Rapid epigenetic changes were demonstrated in allopolyploid *Spartina anglica* which was formed by hybridization and genome duplication, with 30% of the parental methylation patterns altered in the allopolyploid through hybridization rather than genome doubling (Salmon et al. 2005). Liu et al. (2000) found that cytosine methylation changes were

abundant in both natural and synthetic polyploid wheat and that methylation changes already existed in the early generations (S-5, S-6 and S-7) of the synthetic hexaploid polyploid. The genetically identical offspring of asexual triploid F_1 dandelions plants showed modest levels of methylation variation, indicating that *de novo* methylation was triggered by the formation of triploids (Verhoeven et al. 2010). DNA methylation also affects the transposable element (TE) fraction in allopolyploid genome and methylation changes occur just after hybridization. Such changes were significantly more frequent around TE insertions compared with random sequences and predominantly affected the maternal subgenome, suggesting that TEs fuel epigenetic alteration at genome-merging (Parisod et al. 2010a, b).

Functional genomics

Although polyploid plants have not been used extensively in functional genomics research as in evolutionary genomics studies discussed in the previous section, some progress has been made through functional genomics research in polyploid plants regarding gene discovery and elucidation of the molecular mechanism underlying heterosis.

Gene discovery and functional analysis in polyploid plants

Quantitative trait loci (QTLs) are genomic regions associated with quantitative traits, such as yield, product quality or resistance to abiotic and biotic stresses (Yang et al. 2009). A total of 432 QTLs associated with fiber development was mapped by Rong et al. (2007) in one diploid and 10 tetraploid interspecific cotton plants. The two tetraploid subgenomes contained QTLs at largely non-homeologous locations, suggesting divergent selection acting on many corresponding genes before and/or after polyploid formation. They also found that crosses between closely-related genotypes differing by single-gene mutants yield profoundly different QTL positions, suggesting that fiber variation involved a complex network of interacting genes. Le Cunff et al. (2008) performed map-based cloning of a durable major rust resistance gene (*Bru1*) in sugarcane. They developed strategies to overcome marker ambiguity constraints associated with the highly polyploid genome in the successive steps of map-based cloning, including diploid/polyploid syntenic shuttle mapping with two model diploid species (sorghum and rice) and haplotype-specific chromosome walking. These strategies were successfully applied to develop a high-resolution map including the identification of closely-linked markers cosegregating with *Brul* (Le Cunff et al. 2008).

Flowering (i.e., the transition from the vegetative to the reproductive phase) is a common feature shared by angiosperm plant species whereas the opposite process, termed flower reversion (i.e., the reverse developmental steps in the transition from the reproductive to the vegetative phase), is a relatively uncommon phenomenon which can be induced by changes in photoperiod (Donnison and Francis 1994) and gene expression (Ampomah-Dwamena et al. 2002). In abnormal flowers of several wild-type accessions of natural allopolyploid *Arabidopsis suecica*, the expression levels of inflorescence maintenance genes AGAMOUS-LIKE-24 (AGL-24), APETALA1 (AP1), and SHORT VEGETATIVE PHASE (SVP) were reduced while mRNA levels of floral repressor SUPRESSOR OF CONSTANS1 (SOC1) were increased compared with normal flowers as a result of flower reversion in reponse to photoperiod changes (McCullough et al. 2010). This shed some light on the interplay of ploidy levels and the molecular mechanisms for floral reversion.

Small RNAs including microRNAs (miRNAs), siRNAs, and piwi-interacting RNAs (piRNAs), repress gene expression at the transcriptional or posttranscriptional levels and have critical functions in plant defense, growth, development, disease resistance, and stress responses (Zhu 2008). Lackey et al. (2010) produced dominant-negative transgenic *Arabidopsis* allotetraploid plants using RNA interference (RNAi) that downregulates the expression of miRNA biogenesis genes. Their results demonstrated that the miRNA biogenesis genes were effectively downregulated in the resynthesized allotetraploids containing redundant homoeologous genes that were difficult to manipulate via conventional mutation screens, indicating that RNAi was a useful approach to study the effects of gene expression on growth and developmental variation in polyploids.

Heterosis in polyploid plants

The abundance of polyploids indicates that the possession of multiple genomes confers an evolutionary advantage, probably through increased fitness afforded by fixed heterozygosity and because of the greater pool of genes and alleles available for selection (Hegarty and Hiscock 2007). Heterosis generally refers to superior levels of stature, growth, and/or fertility of the hybrid offspring compared to the parents (Chen 2010; Lippman and Zamir 2007). In allopolyploids, the positive interactions between homoeologous genes on alternate genomes appeared to be similar to the positive interactions between different alleles of one gene causing heterosis in heterozygous diploid genotypes. Moreover, the positive interactions (or heterosis) between homoeologous genes in allopolyploids could be fixed (Abel et al. 2005). The fixation of heterozygosity or

heterosis was reported in the polyploid grass *Cymbopogon martini* using an experimental strategy that facilitated fixation of heterozygosity in obligate asexual species (Lavania et al. 2010). The exact mechanisms of heterosis in polyploids remain unknown. However, with recent advances in genome and transcriptome sequencing, such mechanisms may be revealed in the near future.

Comparative genomics in polyploid plants

To unravel the complexities that polyploidy introduces into comparative genomics, Rong et al. (2005) explored in detail the comparative chromosome structural evolution of *Gossypium* and *Arabidopsis*. Based on genetic maps of extant diploid and tetraploid cotton, they inferred the approximate order of 3,016 loci spanning 2,324.7 cM in the genome of a hypothetical common ancestor of the A and D cotton genomes. The inferred cotton gene order corresponded more closely than the original maps to a similarly inferred ancestral gene order predating an independent paleopolyploidization (α) in *Arabidopsis* (Rong et al. 2005). Jannoo et al. (2007) investigated genome dynamics in polyploid sugarcane by analyzing two homoeologous sequences (97 and 126 kb) in a region that had been thoroughly studied in several cereals. Using comparative genomics approaches, their analysis showed that the two sugarcane homoeologous haplotypes displayed perfect colinearity as well as high gene structure conservation. Furthermore, the gene distribution in sugarcane showed high synteny and colinearity with sorghum and rice and partial colinearity with each of the two homoeologous maize segments, suggesting that the high ploidy in sugarcane did not induce genome rearrangement after the polyploidization. Shin et al. (2008) analyzed two pairs of lipoxygenase (Lx) regions generated by two rounds of polyploidy in *Glycine max* (Gm) and discovered differential evolutionary rates between *Glycine* and *Medicago truncatula* (Mt), with the median Ks values (synonymous substitution rates) of Mt–Mt and Gm-Gm paralogs determined to be 0.75 and 0.46, respectively, indicating that the molecular clock was slower in Gm than in Mt and that polyploidy may have impacted the rate of accumulated mutations.

Perspective and conclusion

Polyploid plants have been studied for decades and, more recently, been the focus of genomics research. Genomic changes after polyploid formation comprise a long, dynamic process that can be divided into three stages: (1) initial responses after genome doubling, (2) short-/mid-outcome, and (3) long-term outcome. Genomic studies in these three stages requires an investigation of alternate types of polyploid plants, as illustrated in Fig. 3. Genomics research using ancient polyploids, such as *Arabidopsis* with ancient tetraploid background (Thomas et al. 2006) and grapevine with ancient hexaploid origin (Jaillon et al. 2007), can provide insights into the long-term outcome of polyploid evolution. To understand the short-term evolutionary consequences in polyploidy, young polyploid lines generated from crop breeding, such as *Populus* (Tuskan et al. 2006) and wheat (Gao et al. 2007), may be appropriate plant materials. A new trend in genomics research has been to create synthetic polyploid plants in model species, such as cotton (Flagel and Wendel 2010), *Arabidopsis* (Beaulieu et al. 2009), and *Brassica* (Schranz and Osborn 2000). Using the newly-created polyploid plants, the researchers can investigate the initial genomic changes after polyploid formation. For genetic improvement in crop species, clonally-propagated polyploid plants may convey superior traits in commercial settings. In these cases, it may be desirable to maintain the genomic status of the selected polyploid clones or slow down the diploidization process. Genetic engineering is moving from the gene level to the whole chromosome level, with an artificial chromosome introduced for improvement in pathways or complex traits (Ananiev et al. 2009; Goyal et al. 2009). It will be increasingly important to understand the genomic consequences of "invasion" by a whole artificial chromosome. Studying evolutionary dynamics in newly-synthesized polyploids could increase our understanding of molecular mechanisms leading to stabilized allopolyploid plants, as demonstrated by a recent study on wheat allohexaploids (Mestiri et al. 2010). Previous studies using various polyploid plants have revealed a complex pattern of evolutionary dynamics and contributed to our knowledge about the relationship between genomic composition and gene function (Fig. 2). The ongoing advances in DNA sequencing technology offer unprecedented opportunities for monitoring genomic and transcriptomic changes in

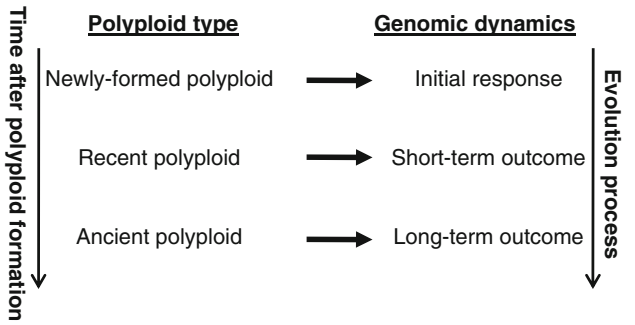

Fig. 3 Differential utilization of polyploid plants for studying evolutionary dynamics after polyploid formation

polyploid plants. More polyploid plants will likely be used for fundamental genomics research, consequently increasing our understanding of plant genomics as a whole. The increased knowledge on polyploid formation, maintenance, and post-duplication divergence at both whole-genome and subgenome levels will not only help plant biologists understand how plants have evolved and diversified, but also assist plant breeders in designing new strategies for crop improvement.

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