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Genetic and Epigenetic Aspects of Polyploid Evolution in Plants

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Abstract
Polyploidy, the condition of possessing more than 2 complete chromosome sets in the same nucleus, is frequent in nature and has implications for a species' prospects for evolution. Newly formed polyploids, so-called neopolyploids, undergo a wide spectrum of genomic changes upon genome merger and duplication. Here, we review recent literature describing genomic and transcriptomic changes along the pathway from neoallopolyploid formation to the stabilization of species and diversification at the allopolyploid level. We begin by reviewing pathways of polyploid formation and discuss the effects of genome doubling and hybridization on chromosome pairing. We then review our knowledge of epigenetic changes in allopolyploids, followed by a consideration of the effects of these structural genomic and epigenetic changes on the transcriptional activity of genes in allopolyploids. We discuss the effects of changes in gene expression in polyploids with respect to current evolutionary theory. Finally, we draw attention to the general question of the relationships between genomic and transcriptomic alteration and incipient diversification among sibling polyploid lines and populations.

Polyploidy is the condition whereby 2 or more complete sets of chromosomes co-occur in a nucleus. In plants, polyploidy arises either via autopolyploidy, in which genome duplication occurs within a species, resulting in multiple similar chromosome sets, or from allopolyploidy, involving the merger of 2 (or more) divergent chromosome sets [Stebbins, 1947; Grant, 1981; Lewis, 1980; Wendel and Doyle, 2005]. Cytogenetically, autopolyploids often differ from allopolyploids in the way their chromosomes pair during meiosis. Generally, in autopolyploids all homologous chromosomes can pair with each other, leading to bivalents (pairs) or multivalents (where more than 2 chromosomes are fully or partially aligned) during meiosis [Levin, 2002], although cases are known where strict bivalent pairing even occurs in autopolyploids [see discussion in Wendel and Doyle, 2005]. In allopolyploids, by contrast, chromosome pairing is mostly disomic (bivalent pairing) and occurs between the homologous, and not between the homoeologous chro-

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mosomes; the latter are the divergent chromosome sets originating from the different species making up the allopolyploid genome [Stebbins, 1947]. In addition to these 2 canonical forms of polyploidy, a continuum of cytotypic states is possible during meiosis. For example, there may be majority disomic homologous pairing in species where the homoeologous chromosomes are more diverged, but more frequent polysomic pairing, coupled with some homoeologous exchange, in other species that have greater homology between their homoeologous chromosomes. Such intermediate types of polyploids, classically referred to as segmental allopolyploids [Levin, 2002], are less stable and likely in a state of evolutionary flux until all chromosomes approach bivalent pairing [Coyne and Orr, 2004].

The potential for evolutionary change that arises from the multiplication of chromosomes, and in some cases divergent genomic material, in just a single generation has sparked interest in biologists for over a century. The question of the degree to which polyploidy is evolutionarily advantageous, as evidenced by increased fitness, has been experimentally tested in some cases, but definitive answers are still lacking [Madlung, 2013]. It is now well established that polyploidy has occurred frequently during angiosperm evolution, but it is still not clear whether polyploidy per se is responsible for enhancing diversification [Mayrose et al., 2011; Arrigo and Barker, 2012]. The addition of entire chromosomal complements constitutes a profound evolutionary event with long-term consequences vis-à-vis diversification because it creates a vastly increased genetic reservoir and combinatorial complexity upon which selection might act. Yet, in the short term, challenges may arise in neopolyploids during chromosome pairing and segregation, especially the formation of multivalents, which creates the prospect of unequal segregation of chromosomes into daughter cells. In addition, polyploidy has been shown to coincide with structural genomic changes that can involve whole chromosomes or chromosome segments. Chromosomal rearrangements can lead to genomic instability for generations to come and can potentially be either detrimental if the mutation decreases fitness, or it can be advantageous if the mutation leads to nonlethal novelty on which selection can act. In the long term, however, polyploidy clearly is a prominent process in plant evolution [Jiao et al., 2011] and thus it is clear that these stabilization challenges are in many cases evolutionarily overcome.

The genomics era has ushered in a multitude of new perspectives on polyploidy in plants, in the process providing clues to the evolutionary success of polyploidy in many lineages. In particular, instead of merely observing the challenges posed by cytogenetic and structural genomic changes, it is now clear that polyploidy has profound effects on gene transcription. Here, we review the literature on recent advances in our understanding of how wholesale genomic change during and after the formation of polyploids affects gene expression. We begin by describing genomic, cytogenetic and epigenetic changes that occur in polyploids, as these events likely are included among the causative mechanisms that underlie changes in gene expression. We then categorize the different types of frequently asked research questions surrounding the implications of transcriptional change in polyploids. Finally, we describe some specific effects of gene expression changes as they may pertain to the evolution of new functions of homoeologous genes. Throughout the article we pay special attention to one specific question: To what degree might transcriptional change in polyploids lead to diversification?

Generation of Polyploids

Since allopolyploids are duplicated hybrids combining divergent sets of chromosomes (2 different homoeologous groups), homoeologous chromosomes often differ enough from each other that multivalent formation is not as frequent during meiosis as it is in autopolyploids. In a survey of the literature, Ramsey and Schemske [2002] found that the occurrence of multivalents at meiosis in autopolyploids was significantly greater than the occurrence of multivalents in allopolyploids [Ramsey and Schemske, 2002]. However, the survey also clearly indicated that autopolyploids do not always form multivalents (multivalents were formed in only 28% of studies, number of studies = 93) and that allopolyploids are far from immune to multivalent inheritance (multivalents were reported in 8% of studies surveyed, number of studies = 78) [Ramsey and Schemske, 2002].

Multivalent pairing between homoeologs can thus occur and, therefore, increase the chances of homoeologous recombination in allopolyploids, which would lead to genetic exchange between regions of DNA that are similar, but not identical in sequence. If homologous or homoeologous chromosomes or chromosome segments in neoallopolyploids are involved in multivalent pairing, then this can also lead to unequal segregation and aneuploidy during meiosis. Therefore, mechanisms that reduce multivalent pairing should be selectively favored, since this would increase genomic integrity and successful meiosis. A re-

duction in multivalent pairing can be achieved through many different routes, including genic and structural mechanisms. Perhaps the most famous genic example is *Ph1* in wheat [Griffiths et al., 2006], which enforces strict homolog pairing in allotetraploid and allohexaploid *Triticum*. Recombination-induced DNA rearrangements also may reduce homology between homoeologous chromosome segments, thereby decreasing the ability of homoeologs to pair [Wang et al., 2009]. These various mechanisms of stabilizing pairing in allopolyploids seem essential for long-term establishment and diversification of new allopolyploid lineages.

To determine if recombination in general (and thus possibly also between homoeologs) is increased in allopolyploids relative to diploids, Pecinka et al. [2011] used an elegant screening system, in which 2 linked transgenes conferring differently colored fluorescence were used to assess the frequency of meiotic recombination. These authors assessed the recombination rates between the fluorescent markers in diploid, auto- and allotetraploids of *Arabidopsis* and reported a significant increase in meiotic recombination in both types of polyploids over recombination rates in diploids [Pecinka et al., 2011]. Although the molecular basis for this enhancement is not understood, these data suggest that both auto- and allopolyploids might benefit from a faster formation of novel recombinants, potentially endowing them with the ability to adapt to new or changing environments faster than diploids. A related mechanism for enhancing diversity in allopolyploids is through meiotic or mitotic non-reciprocal homoeologous recombination at the genic level. Extending the initial discovery of this type of inter-homoeolog exchange in *Gossypium* (cotton) allopolyploids, Salmon et al. [2010] and Flagel et al. [2012] used global analyses of transcriptomes to show that this type of 'gene conversion' characterizes perhaps 5% of all genes, that it is an ongoing process, and that it can generate novel or chimeric genes found in neither progenitor diploid. If these results from *Arabidopsis* and *Gossypium* are generalized, one can envision how allopolyploids have a greater likelihood for multivalent pairing, homoeologous exchanges and genomic rearrangements, all of which generate diversity by novel mechanisms and at a faster rate, relative to diploid relatives.

In the context of a comparative approach between plant and animal polyploids, it is of interest to note that most of the research in plant polyploids has been done in monoecious plants lacking sex chromosomes, thus making a direct comparison to species with heteromorphic sex chromosomes difficult. It has, however, been noted that sex-specific differences in cross-over frequency occur in monoecious plants without sex chromosomes [Giraut et al., 2011]. Theoretical considerations have led to the prediction that dioecy due to heteromorphic sex chromosomes in lower plants is likely a short-lived evolutionary stage that potentially can revert to monoecy via allopolyploidy [Gorelik, 2005].

Structural Genome Changes during Polyploidization

When McClintock [1984] coined the term 'genomic shock', which she proposed is a response to hybridization and other stressful events, she predicted that structural changes would arise in the genome, such as the formation of 'translocations, inversions, deficiencies, duplications, and the like'. Over the past 2 decades, genomic, epigenetic and cytogenetic analyses have repeatedly validated McClintock's predictions in many allopolyploid systems [for some recent reviews see Hufton and Panopoulou, 2009; Jackson and Chen, 2010; Mayfield et al., 2011; De Smet and Van de Peer, 2012; Heslop-Harrison, 2012; Soltis and Soltis, 2012]. Specifically, polyploidization has been associated with sequence loss [Shaked et al., 2001; Ozkan et al., 2003; Han et al., 2005; Tate et al., 2009], changes in epigenetic marks, such as cytosine methylation [Shaked et al., 2001; Madlung et al., 2005; Lavania et al., 2012], the activation of transposons [Kashkush et al., 2003; Madlung et al., 2005; Chen et al., 2008; Kraitshtein et al., 2010; Petit et al., 2010; Hegarty et al., 2011], and recombination between homoeologous chromosomes [Gaeta and Pires, 2010; Salmon et al., 2010; Szadkowski et al., 2010].

One set of studies that nicely illustrates the effect of polyploidy on structural genome change comes from *Brassica. B. napus* is an allotetraploid that originated from allopolyploidization between *B. oleracea* and *B. rapa*. Ancient hexaploidization in ancestral *Brassiceae* species, between 7.9 and 14.6 mya, has been suggested as the reason for large regions of homology within the modern-day *Brassica* species [Lysak et al., 2005]. These homologies make duplicated chromosomes in *Brassica* more likely to undergo homoeologous exchanges in modern allopolyploids. Using RFLP and simple sequence repeat markers in some 50 sibling lines of resynthesized neoallopolyploids in *B. napus,* chromosomal exchanges were indeed reported to occur frequently and to coincide with novel phenotypic variation [Pires et al., 2004; Gaeta et al., 2007]. Phenotypic analysis of sibling lines from this pedigree showed a wide range of flowering times. Closer analysis

of the chromosomal constitution in the earliest and the latest flowering lines at the genetic loci for the floral repressor *FLOWERING LOCUS C (FLC)* indicated that different types of chromosomal rearrangements in the various allopolyploid sibling lines led to the observed phenotypic variation and also coincided with variation in *FLC* transcript levels [Pires et al., 2004]. This study, therefore, suggests a mechanism by which genomic change in response to hybridization and genome duplication leads to changes in gene expression and phenotype, thus providing a possible connection between allopolyploidization and incipient reproductive isolation. A subsequent study using the same plant pedigree investigated chromosomal rearrangements in the same population with fluorescent in situ hybridization (FISH). Comparing the synthetic S1, S5 and S10 generations, Xiong et al. [2011] found evidence for many of the types of chromosomal changes predicted previously by McClintock [1984], including intergenomic rearrangements and deletions as well as aneuploidy. Interestingly, the authors noted that loss of fitness and chromosome changes were correlated, and that the fittest of the lines investigated exhibited additive (euploid) chromosome numbers. They further observed what appeared to be selection against individuals with chromosomal changes that upset the tetraploid chromosome balance by more than one copy [Xiong et al., 2011]. These studies suggest that allopolyploidization in *Brassica* leads to rapid and massive genomic restructuring, providing novel diversity for selection to act upon. It is noteworthy, however, that much of the genetic novelty in these lines also led to a loss of fitness.

A pair of recent cytogenetic studies in natural populations of the recently formed allopolyploids, *Tragopogon micellus* and *T. mirus*, [Lim et al., 2008; Chester et al., 2012] revealed genomic changes on a scale similar to that observed in resynthesized *B. napus*. Both allopolyploid species, *T. micellus* and *T. mirus*, have formed multiple times independently in the northwestern United States, since their initial formation approximately 100 years ago. FISH and genomic in situ hybridization experiments in both allopolyploid species of *Tragopogon* showed extensive chromosomal translocations and other structural chromosomal irregularities as well as aneuploidy in the allopolyploids [Lim et al., 2008; Chester et al., 2012].

Compared to the very recent allopolyploids in *Tragopogon*, *Arabidopsis suecica* is an older allopolyploid species. One of its strengths as a model for studying allopolyploidy in natural populations is that it arose only once in a hybridization between *A. thaliana* and *A. arenosa* [Mummenhoff and Hurka, 1995; O'Kane et al., 1996]. Its single origin has been estimated to have occurred between 12,000 and 300,000 years ago [Jakobsson et al., 2006], and this both shows the rarity of successful polyploidization in this species and allows genetic data interpretation without the uncertainty of allelic variation from multiple parents during recurrent allopolyploidization events. *A. suecica* can thus be used as an example of an allopolyploid where all observed diversity had to arise post-allopolyploidization. Molecular events during allopolyploidization in *A. suecica* have been studied in some detail, but the majority of research in this species has focused on resynthesized plants [for a recent review see Ng et al., 2012]. Natural *A. suecica* displays phenotypic variability between geographically separate accessions [Madlung et al., 2012], but genetic variation between populations is low [Lind-Halldén et al., 2002; Säll et al., 2003; Jakobsson et al., 2006, 2007; Hazzouri et al., 2008]. To assess the degree of variation in natural *A. suecica*, Wright et al. [2009] used FISH analysis and reported that some natural accessions of *A. suecica* displayed considerable somatic chromosome number variation in mitotically dividing cells, leading to aneuploid mosaics and suggesting a mechanism for the generation of variation in offspring. These plants are, however, phenotypically mostly stable, and fertility and offspring viability are not affected by the somatic aneuploidy [Wright et al., 2009]. Thus, it is unclear whether this perpetual pool of potentially novel cytotypic variation plays any role in adaptation or diversification of this species. To further analyze the potential role of somatic aneuploidy on increased variation in *Arabidopsis* neoallopolyploids, Matsushita et al. [2012] produced a synthetic allohexaploid pedigree by crossing a diploid *A. thaliana* and the tetraploid *A. suecica* [Matsushita et al., 2012]. The resulting mostly sterile triploid hybrid produced a pedigree of hexaploid siblings by spontaneous whole genome duplication and subsequent single seed descent. Results showed that the incidence of somatic aneuploidy in the allohexaploids was greatly increased over that in the tetraploids. Reported correlation between phenotypic variation and diversification of novel majority, mosaic cytotypes in this population suggests that somatic aneuploidy might, at least in this synthetic population, be a factor shaping evolution and providing novel phenotypes on which selection might act [Matsushita et al., 2012]. Mitotic aneuploidy had previously been reported in the hexaploid *Senecio cambrensis* [Ingram and Noltie, 1995], suggesting that increased levels of polyploidy can not only lead to higher tolerance of aneuploidy, but also to somatic mosaicism. Higher level polyploids can generally tolerate aneuploidy better than can diploids

[Coyne and Orr, 2004]. One example of a genus with high and variable levels of both polyploidy and aneuploidy is sugarcane, where, for example, the species *Saccharum spontaneum* has chromosome numbers ranging from 40 to 128 [Panje and Babu, 1960].

In summary, cytogenetic studies in resynthesized *B. napus* [Gaeta et al., 2007; Xiong et al., 2011], natural recent *T. micellus* and *T. mirus* [Lim et al., 2008; Chester et al., 2012], and in the established allopolyploid *A. suecica* [Wright et al., 2009; Matsushita et al., 2012] suggest that genomic instability can occur immediately after allopolyploidization, but can also persist for a long time even in fit, natural allopolyploid populations. The studies further show that structural genomic change and aneuploidy can be tolerated to a certain degree but after reaching a given threshold likely lead to extinction rather than continued evolution [Xiong et al., 2011; Matsushita et al., 2012]. It remains to be seen if this form of cytogenetic variability between individuals can act as a reservoir for diversity upon which selection might act or if it is a form of genomic instability that is tolerated within a certain range but selected against if the instabilities become detrimental.

Epigenetic Changes in Polyploids

Epigenetic variation refers to changes in phenotype or molecular behavior of a gene without an underlying change in the DNA sequence [Rapp and Wendel, 2005]. The most frequently studied epigenetic marks are cytosine methylation and histone modifications. DNA sequence contexts that often vary in their methylation state in plants include CG, CHG and CHH [Cokus et al., 2008], where H can be any nucleotide except for G, while those in mammals most frequently occur at CG sites [Saze et al., 2012]. Variation in DNA methylation between parental species and allopolyploids has been reported in *Arabidopsis* [Madlung et al., 2002, 2005; Beaulieu et al., 2009], wheat [Shaked et al., 2001; Zhao et al., 2011], *Brassica* [Lukens et al., 2006; Xu et al., 2009; Książczyk et al., 2011], dandelion [Verhoeven et al., 2010], and others. Interestingly, changes in methlyation, however, did not occur during allopolyploidization in cotton [Liu et al., 2001]. Changes in methylation have also been reported in response to autopolyploidization in the grass *Cymbopogon* [Lavania et al., 2012].

In many of the species mentioned above, methylation changes were accompanied by genome rearrangements and changes in transcriptional activity [e.g. Kashkush et al., 2002; Madlung et al., 2005; Gaeta et al., 2007]. With respect to the question of the degree to which polyploidization induces diversity, an interesting case study is the behavior of the recent allopolyploid *Spartina anglica.* In genomic studies using inter simple sequence repeat analysis, *S. anglica* showed little genetic variation between populations [Baumel et al., 2001], while displaying considerable phenotypic variability. This grass species evolved towards the end of the 19th century in southern England via the allopolyploidization of the European species *S. maritima* and the American species *S. alterniflora* [Ainouche et al., 2004]. The power of this system for the study of allopolyploidy stems from the fact that all 3 *Spartina* species and the infertile hybrid are rhizomatous and perennial, thus allowing the analysis of material in the same location where the hybridization and allopolyploidization is thought to have first occurred [Ainouche et al., 2004]. In *S. anglica* there is also no evidence of major genomic reshuffling after allopolyploidization in wild populations via retrotransposon activation [Baumel et al., 2002]. Despite the lack of observed genomic variability, morphological analysis of *S. anglica* showed significant variability between individuals and populations. Most of the phenotypic variation in these populations has been attributed to environmentally related plasticity, rather than genetic diversity [Thompson et al., 1991a–c]. The authors of these studies speculated that variability within a clonal population could be due to age-related somatic variation, explaining the decline in vigor and the phenotypic variability between individuals [Thompson et al., 1991a]. More recently, using methylation sensitive amplified polymorphism analysis, variability within the DNA cytosine methylome was reported between the allopolyploid, homoploid hybrid and its parent species [Salmon et al., 2005]. In a follow-up study, Parisod et al. [2009] used methylation-sensitive transposon display to test variability in CG methylation near transposons. Using the same plant material, these authors reported methylation changes upon hybridization and to a lesser extent with genome duplication, particularly near transposable elements [Parisod et al., 2009]. This pair of studies suggests that epigenetic variability, rather than genetic variability, could explain the phenotypic variation in *Spartina* allopolyploids. In the context of this example of a plant that propagates through rhizomes, it may be of interest to revisit the classical view that one reason why polyploidy is more frequent in plants than animals – and within the plants more frequent in perennials than annuals [Stebbins, 1971] – could be the fact that infertile organisms with a longer life span may either eventually propagate

through asexual means or simply have more opportunity to undergo spontaneous genome doubling later in life to restore sexual fertility [Jackson, 1976].

Histone modifications and histone variants have been shown to have large effects on gene transcriptional activity in plants [Deal and Henikoff, 2011]. Allopolyploidy in natural and resynthesized *A. suecica* coincides with changes in histone methylation at H3K4me2 sites and histone dimethylation and -acetylation at H3K9 sites and is correlated with effects on transcriptional activity [Wang et al., 2006a; Ni et al., 2009]. Wang et al. [2006a] showed that increased expression of the floral repressor *FLC* in *Arabidopsis* allopolyploids relative to its progenitor species is correlated with *FLC*-activating histone modifications and corresponding late flowering of the polyploid compared to its progenitors. Ni et al. [2009] showed that resynthesized *A. suecica* allopolyploids also show variations in histone modifications in the promoter regions of several circadian clock-related genes. These changes appeared to affect transcription of the effected genes, leading eventually to greater accumulation of starch and sugars in the allopolyploids, possibly explaining the greater biomass of these plants relative to *A. thaliana* and *A. arenosa* [Ni et al., 2009]. A recent study in *Arabidopsis* used a set of diploids and autotetraploids carrying a hygromycin resistance transgene to uncover what they termed polyploidy-associated transcriptional gene silencing (paTGS) [Baubec et al., 2010]. In this system, a stably inherited transgene was silenced only in tetraploids. Using a genetic screen, Baubec et al. [2010] found that paTGS was mediated by 2 genes: *DECREASE IN DNA METHYLATION 1 (DDM1)* and *HOMOLOGOUS GENE SILENCING 1 (HOG1)*. Together the 2 genes kept not only the hygromycin resistance gene silent in the tetraploids, but it appeared that additional sequences were also under this silencing control [Baubec et al., 2010]. The exact mechanisms that prevent this double lock from shutting down gene expression in diploids but enabling it in polyploids is, however, not clear.

Given that polyploidization leads to alterations in epigenetic control of gene expression, it is tempting to speculate that some portion of the success of polyploid lineages arises from epigenetically mediated phenomena. An additional twist on this theme comes from *Gossypium*, in which it has been shown that genes duplicated by polyploidy may be epigenetically silenced at the time of polyploid formation [Adams et al., 2003; Adams and Wendel, 2005]. Because alternative homoeologs may be affected in different organs or tissues, such that one copy is silenced in one tissue whereas the other homoeolog is silenced in a second tissue, epigenetic suppression may act to 'preserve' both duplicates; that is, to the extent that some gene expression is required or at least selectively favored in both tissues, both homoeologs will be epigenetically protected from mutational decay. Played out on a genome-wide scale, this process may epigenetically preserve thousands of genes for later evaluation by natural selection, as genes become epigenetically released under varying environments.

Effects of Polyploidy on Gene Expression

The previous sections dealt with structural and epigenetic changes in the polyploid compared to its diploid progenitors, which led to indications that these changes mediate altered gene expression. Over the last 2 decades, numerous studies have assessed transcriptional changes mostly in allopolyploids but also in some autopolyploids. Guo et al. [1996] measured transcript levels of 18 genes in a series of mono-, di-, tri-, and tetraploid maize plants and found various types of gene expression patterns, ranging from a proportional increase in expression with gene dosage to decreased levels per genome when compared to the diploid [Guo et al., 1996]. By contrast, expression of individual genes in an aneuploid dosage series had shown cases of dosage compensation, where the overall level of expression does not increase with dosage [Guo and Birchler, 1994]. Transcriptional studies in polyploids have evolved quickly from northern blotting to genomic methods, including cDNA-AFLP [Lee and Chen, 2001; Madlung et al., 2002; Tate et al., 2006; Koh et al., 2010], microarray technology [Hegarty et al., 2006; Wang et al., 2006b; Flagel et al., 2008; Gaeta et al., 2009; Flagel and Wendel, 2010] and transcriptome analyses using RNA-seq [Yoo et al., 2013]. While microarray analysis was an improvement in throughput over previous methods, it requires a priori sequence knowledge of the genome to be tested. Another important disadvantage of microarrays, especially for the analysis of allopolyploids, is that the technology has greater difficulty in distinguishing between homoeologs if they cross-hybridize to the same target spot on the array. RNA-seq circumvents these shortcomings since it uses sequence information that can distinguish between SNPs, allowing more precision in the analysis of the allelic or homoeolog-specific contribution to the total transcriptome for any duplicate gene pair.

Because gene 'expression' typically is more meaningful considered at the protein than the transcript level, one might ask to what extent results from transcriptome surveys will parallel those obtained from protein data, if such

data were readily obtained. In this respect, there are few proteomic studies of polyploidy to date [Albertin et al., 2006, 2007, 2009; Marmagne et al., 2010; Hu et al., 2011; Koh et al., 2012; Ng et al., 2012]. These early data show relatively weak correlation [Marmagne et al., 2010; Koh et al., 2012; Ng et al., 2012], raising the question of the significance of transcriptional change for protein-level responses (and ultimately, phenotype). Two recent studies in yeast and mouse, however, specifically investigated the correlation between protein abundance and mRNA levels and reported that while overall correlation between transcripts and proteins was only modest [Foss et al., 2007; Ghazalpour et al., 2011], proteins with varying levels between samples were statistically also more likely to vary in the corresponding transcript levels [Foss et al., 2007]. Additional research in this area is necessary to develop a fuller appreciation of the importance of these considerations.

For now, it is clear that analysis of the transcriptomes of polyploids serves multiple purposes. First, this research facilitates understanding of the direct or indirect outcomes of genome duplication and/or hybridization on gene expression, for any gene of interest and on a genome-wide scale, thus setting the stage for analyzing the effects of these changes on phenotypes and diversification. Second, this avenue of investigation leads to an enhanced mechanistic understanding of the genomic, genetic and epigenetic interactions that arise in polyploid plants relative to their diploid progenitors. Here, we categorize aspects of transcriptome analysis that have been of particular interest in plants into 7 major questions, as illustrated in figures 1 and 2:

(1) What is the frequency with which total expression of a given homoeolog pair in a polyploid is additive, with respect to the expression of that gene in the parents? In cases where one of the 2 parent species seems to determine the total expression of both homoeologs in the allopolyploid, the term describing this state is 'expression level dominance' (ELD) [Grover et al., 2012; Yoo et al., 2013] (fig. 1A). The degree of ELD has been the focus of investigations in species such as *Arabidopsis*, cotton, *Spartina*, wheat, and coffee [Wang et al., 2006b; Rapp et al., 2009; Chagué et al., 2010; Chelaifa et al., 2010; Flagel and Wendel, 2010; Bardil et al., 2011].

(2) To what degree is the expression of a gene skewed towards the contribution of one parent over that of the other parent in an allopolyploid ('homoeolog expression bias'; Grover et al. [2012])? Here the focus lies on the question of whether or not the 2 homoeologous copies of a gene are expressed equally in the allopolyploid (fig. 1B). In contrast to ELD, where total expression in the allopolyploid is compared to expression of the same gene in the 2 parents, here the interest is simply on the relative contribution of the 2 homoeologs to the transcript pool for that gene pair. Homoeolog expression bias has been reported in diverse species, including cotton, *Tragopogon*, *Brassica*, *Arabidopsis*, wheat, and coffee [Lee and Chen, 2001; Bottley et al., 2006; Flagel et al., 2008; Chaudhary et al., 2009; Buggs et al., 2011; Dong and Adams, 2011; Combes et al., 2012].

(3) What is the distribution of total gene expression levels in the allopolyploid compared to those of its parental species? This question focuses on the combined gene expression levels of all homoeologs of a given gene in the allopolyploid and compares it to levels in the parental species. Figure 1C shows the different types of possible changes in expression level for a gene from a polyploid compared to its parents [Rapp et al., 2009; Flagel and Wendel, 2010; Bardil et al., 2011].

(4) Are specific genes or transposons activated or repressed in the polyploid that could be of adaptive value to the new allopolyploid species? From a functional point of view, those genes upregulated beyond the expression level in their parental species (transgressive upregulation) or silenced in the polyploid while being expressed in the parents have received special attention. Such genes can be most easily envisioned to have an immediate impact on the polyploid's physiology and possibly lead to adaptation of the neoallopolyploid to its environment [Adams et al., 2003, 2004; Wang et al., 2004, 2006a, b; Ni et al., 2009; Buggs et al., 2011]. Such transgressive states (as exemplified for entire individuals in fig. 1C) can also lead to tissue-specific expression variation (fig. 1D).

(5) Is gene expression in polyploids regulated post-transcriptionally, for example via the use of different alternatively spliced variants, which create different proteins from the same mRNA by differential exon use during RNA maturation and translation (fig. 1E)? Examples of variability in splice product transcription were reported in allopolyploids of *Arabidopsis* [Madlung et al., 2005] and *Brassica* [Zhou et al., 2011] as well as in genes duplicated by ancient whole genome duplication in *A. thaliana* [Zhang et al., 2011] and autotetraploid *Capsella bursa-pastoris* [Slotte et al., 2009].

(6) Do changing environmental conditions affect gene expression differently in polyploids compared to diploids (fig. 1F)? Recent studies have shown that abiotic stress can result in the differential expression of homoeologs to the overall transcriptome of allopolyploid *Gossypium* [Bardil et al., 2011; Dong and Adams, 2011].

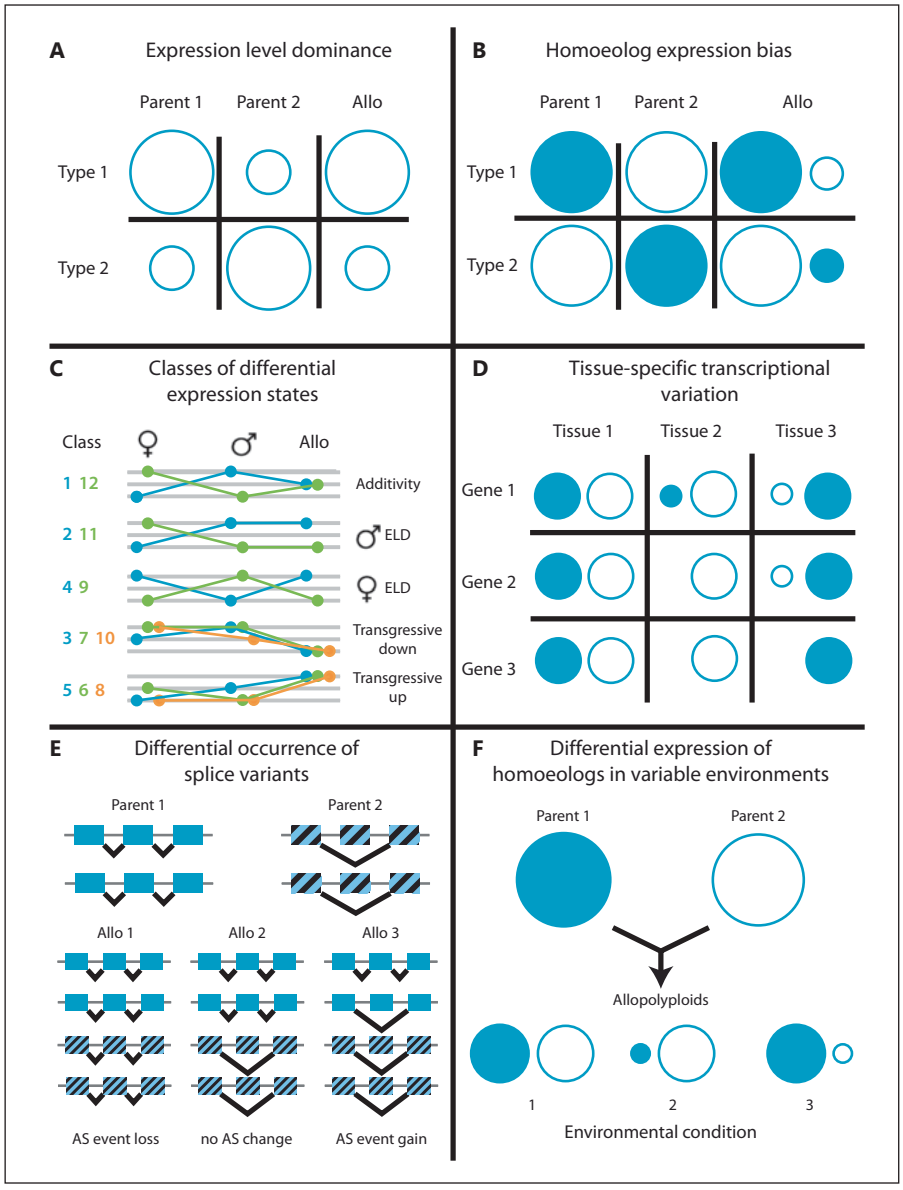

Fig. 1. Transcriptional responses to polyploidy. Various types of transcriptional alteration, relative to diploid progenitors, commonly arise in allopolyploids, of which several are depicted. **A** In cases where genes (type 1) are upregulated or downregulated in one parental diploid (parent 1) relative to the other (parent 2), the total expression of the 2 homoeologs may 'mirror' one of the 2 parents (in this case, parent 1); this condition is referred to as expression level dominance. **B** Homoeolog expression bias is evident when the proportion of the expression of 2 homoeologs in an allopolyploid is unequal. **C** There are 12 classes of differential expression among 2 diploids and an allopolyploid [Rapp and Wendel, 2009; Yoo et al., 2013]; partitioning gene expression into these categories may reveal the phenomenon of ELD. Categories include additivity (classes 1, 12), ELD either toward the paternal parent (classes 2, 11), or maternal (classes 4, 9) parent, and transgressive down- (classes 3, 7, 10) or upregulation (classes 5, 6, 8) in the allopolyploid relative to its 2 parents. **D** Transcript accumulation in allopolyploids can be variable among tissues, as illustrated for gene 1 in 3 different tissues, silencing for gene 2 in tissue 2, and reciprocal transcriptional silencing for gene 3 in tissues 2 and 3, where silencing of one homoeolog occurs in one tissue and reciprocally for the other homoeolog in a different tissue. **E** Splice variants of homoeologs can change between parental and allopolyploid generations and can, furthermore, differ between sibling lines of the same cross, leading to diversification among the neoallopolyploid offspring. In the example shown, parent 1 uses all 3 exons in its mature transcript, while the corresponding gene in parent 2 uses a different splice variant. In 3 different sibling lines of the allopolyploid, alternative splice versions (AS) of the homoeologs can be gained in the polyploid, lost in the polyploid or become additive. **F** Differential responses of allopolyploids to stress may be a special case of homoeolog expression bias described in **B**. Here, homoeologs can be expressed at different levels under different environmental (or abiotic stress) conditions. Examples of these phenomena are discussed in the text.

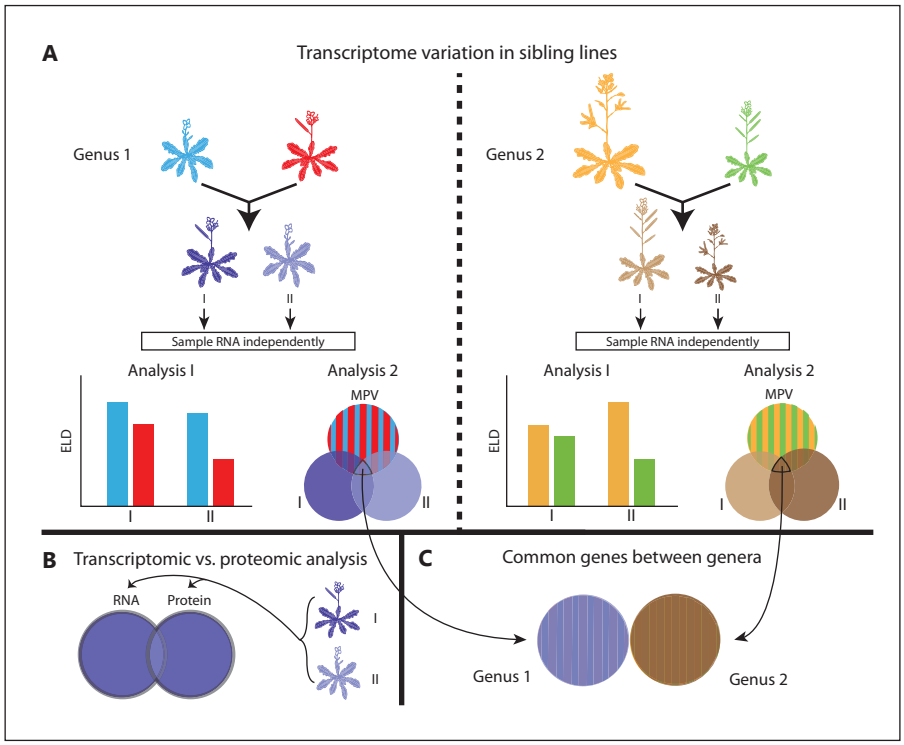

Fig. 2. Transcriptome variation in polyploids. Many analyses have revealed variability in the transcriptional responses to allopolyploidy. **A** Variation among independent sibling lines in their response to polyploidy. The figure shows 2 examples of 2 parents in one genus, each undergoing allopolyploidy. RNA analysis has shown similar responses with respect to ELD for any given species combination. For example, all allopolyploid crosses of *Arabidopsis thaliana* and *A. arenosa* have shown repression of the *thaliana* genome (and phenotype). ELD trending predictably in the same direction is depicted (Analysis 1). Comparison of transcriptional responses at the individual gene level (Analysis 2), using microarrays, RNAseq or other methods, has shown that individual responses for the expression of a specific gene (when compared to its mid-parent expression level) may be similar (predictable responses) or different (stochastic response) in 2 separate neoallopolyploids. **B** When comparing transcriptome and proteome responses to allopolyploidy, there may be some overlap between significantly affected genes. **C** Genes that are responsive to polyploidy in one genus (or study system) may not necessarily overlap extensively (or at all) with those from a different genus (or study system). Examples of these phenomena are discussed in the text.

(7) To what extent are the responses and genomic consequences to independent polyploidization events predictable or stochastic between sibling lines? Researchers have addressed this question by comparing separate lines of independent polyploids [Comai et al., 2000; Madlung et al., 2002, 2005, 2012; Wang et al., 2004, 2006b; Gaeta et al., 2007; Koh et al., 2010; Buggs et al., 2011]. This question is illustrated in figure 2.

Many recent reviews have addressed the foregoing questions [Chen, 2010; Cifuentes et al., 2010; Gaeta and Pires, 2010; Parisod et al., 2010a, b; Mayfield et al., 2011]. One aspect that has received comparatively less attention is the question of whether or not variation in transcription between individuals represents heritable and potentially significant variation, or if instead offspring of the same parental crosses have similar transcriptomic profiles. Recent work has addressed this question in *Tragopogon, Brassica* and *Arabidopsis.*

One of the best known models for recent (within the last ~100 years) and recurrent natural allopolyploid speciation is represented by the allotetraploids *T. mirus* and *T. micellus*, which formed repeatedly and often still are found in sympatry with their respective parents *(T. pratensis* and *T. dubius* for *T. micellus,* and *T. porrifolius* and *T. dubius* for *T. mirus)* in the northwestern United States [Ownbey, 1950; Soltis et al., 2004]. Given their biennial growth habit, these populations are estimated to have gone through ~40 generations since initial allopolyploid formation [Soltis et al., 2004]. Using cDNA-AFLPs and CAPS analysis, Tate et al. [2006, 2009] showed in 2 com-

plementary studies that transcriptional variation exists between different individuals from separate populations of the allopolyploid *T. miscellus*. Buggs et al. [2010] compared the homoeologous-specific transcription of 13 genes from 7 different tissues in 10 natural individuals of *T. mirus* and its parent species, *T. dubius* and *T. porrifolius*. The authors reported numerous differences in the transcription patterns among individuals in *T. mirus*, including variation in gene silencing [Buggs et al., 2010]. A similar study investigating a larger number of genes and individuals in an allopolyploid population of *T. miscellus* confirmed the variation in the transcriptional activity of homoeologous gene pairs between individuals in natural allopolyploids [Buggs et al., 2011]. Complementary to the studies by Buggs et al. [2011], Koh et al. [2012] investigated changes in protein expression of synthetic or natural polyploid *T. mirus* as well as diploid hybrids of the parents of *T. mirus*. These authors reported that 68 (14%) of the identified proteins were differentially expressed relative to additive expectations in the polyploids, while the other proteins were additive between the sum of the parents and the offspring [Koh et al., 2012]. Compared to a cDNA-AFLP based transcriptomic study using similar plant material [Koh et al., 2010], the authors found only a few genes for which transcript changes corresponded to protein changes, although it needs to be noted that the sample size of genes for comparison between these 2 studies was small. Collectively the data from these experiments in *Tragopogon* suggest, as the authors of these studies point out, that gene silencing can occur in a tissue-specific and individual-specific manner after only a few generations and possibly lead to subfunctionalization [Buggs et al., 2010, 2011] and thus potentially to physiological or developmental differences. If these differences in gene activity have adaptive value, then it is possible that each individual could follow a different evolutionary trajectory. This evolutionary potential might not be realized as long as the individual plants occur sympatrically or in the same ecological niche. It is tempting to speculate that dispersion of these plants into habitats with different environmental conditions might exert divergent selection on the individuals and over time lead to separation into distinct lineages or, eventually, into distinct species.

Variation in transcriptional activity has also been measured in allopolyploid *A. suecica*-like plants. Transcriptomic analysis in 2 sibling lines from the same synthetic cross between parental *A. thaliana* and *A. arenosa* showed between 5.2 and 5.6% divergence in gene activity from the midparent value (MPV) [Wang et al., 2006b], which is the averaged value that would be expected if gene expression in the parents was additive in the allopolyploid. This study represented an example of ELD (fig. 1A), since most of the genes repressed in the allopolyploids were those of the maternal parent *A. thaliana*. The same direction of dominance was found in both lineages of allopolyploids. But despite the relatively similar values in divergence from MPV, the study found that only between 56 and 60% of those genes differentially expressed between allopolyploids compared to the MPV were shared between the 2 allopolyploid lines. These data suggest that allopolyploidization in *Arabidopsis* can lead to stochastic changes for a large portion of the genes while other genes might be regulated in a concerted and predictable way between different sibling lines. The notion of variability among neoallopolyploids on the transcriptome level was also corroborated by a similar study in resynthesized *A. suecica* allopolyploids using cDNA-AFLP analysis [Wang et al., 2004], which showed variation in the transcription of several allopolyploid sibling lines compared to their parent species.

The third case study addressing transcriptome variation in neoallopolyploids comes from *Brassica*. Using cDNA-AFLP analysis of ~50 allopolyploid *B. napus* lines that were resynthesized from *B. oleracea* and *B. rapa*, Gaeta et al. [2007] reported variation in fragment size, indicating changes in gene expression, in 4% of all cDNA-AFLP fragments between the S0 sibling lines. Total fragment losses between siblings in the S0 generation were low (0.2%), but 5 generations later, such losses had increased to 3%. Gene expression changes (as measured by cDNA-AFLP fragment changes) in the S5 generation were correlated with losses or rearrangements of DNA fragments. The same study reported widespread variation in phenotype between sibling lines [Gaeta et al., 2007]. Using the same plant material, Gaeta et al. [2009] chose 3 lines, which were analyzed further by microarrays. The authors reported that only a few genes were differently regulated in all 3 allopolyploids, compared to their progenitor species. The analysis also showed variation in the expression of up- or downregulated genes between these 3 sibling lines, as compared to their progenitor species, suggesting stochastic transcriptional changes between siblings in resynthesized *B. napus* [Gaeta et al., 2007, 2009]. When comparing genes that were non-additively altered by polyploidy in resynthesized *B. napus* and *A. suecica* [Wang et al., 2006b; Gaeta et al., 2009], Gaeta et al. [2009] found only 8 genes in common between the 2 species. Of these 8 genes, only 3 were regulated in the same direction (up or down) in both species, suggesting that any correlation was due to chance.

Taking the studies in *Tragopogon, Brassica* and *Arabidopsis* described in this section together, there is agreement between the 3 species that at least some of the transcriptional changes accompanying hybridization and polyploidy are stochastic. At this time, no genes have been universally identified as particularly responsive to genome merger and doubling, though it is not unreasonable to posit that such genes might exist (e.g. those important to nuclear volume or replication). The absence of such universally regulated genes might suggest that allopolyploidization is a process that differs between species and to some degree between individuals of the same species. Stochastic events during allopolyploidization increase variation between siblings and provide more variable raw material on which selection can act during the subsequent evolution of the allopolyploid. Figure 2 illustrates the amount of novel transcriptional variation that arises during allopolyploidy and depicts areas of hypothesized stochastic versus concerted changes.

Above, we noted the possibilities of divergent fates of duplicated genes arising from polyploidy with respect to gene expression, including tissue-specific and population-specific genetic or epigenetic silencing. One of the interesting implications of the observation of reciprocal silencing or loss of gene duplicates is that over time, or even instantly in the case of wide allopolyploidy, 2 homoeologs may acquire slightly different function. The loss of one homoeolog in one population and the reciprocal loss of the other homoeolog in a second population may lead to reproductive isolation [Werth and Windham, 1991]. For example, if in an allopolyploid mutant alleles (lower case a, a') of 2 homoeologs A and A' evolve in separate populations, such that the 2 populations genetically are AAa'a' and aaA'A', selfed progeny of their hybrid (AaA'a') will be less fit because of the deleterious or partially deleterious recovery of double mutant homozygotes (aaa'a'). If the loss of different homoeologs in separate populations – known as the process of divergent resolution [Lynch and Conery, 2000] – is allowed to play out on a genome-wide scale, one can readily envision how this process may lead to reproductive isolation [Lynch and Conery, 2000; Taylor et al., 2001], thus connecting homoeolog behavior to a mechanism for allopolyploid diversification. Although this process has not convincingly been demonstrated for allopolyploid plants, we suspect that it soon will be. Examples of divergent fates of duplicated genes with evolutionary consequences exist in diploids, where, in accordance with the Bateson-Dobzhansky-Muller model, hybrid incompatibility can arise due to mutations in separate loci of related species [Orr, 1996]. Specific examples include the alternative silencing of ancestrally duplicated genes leading to reproductive isolation, as shown for duplicated (and reciprocally lost) copies of *DOPPELGANGER (DPL)* genes in the rice subspecies *O. sativa* subsp. *japonica and O. sativa* subsp. *indica* [Mizuta et al., 2010], and for duplicated and reciprocally lost copies of a histidinol-phosphate aminotransferase genes in different accessions of *A. thaliana* [Bikard et al., 2009].

Effect of Polyploidy on Small RNAs

Most of the recent transcriptional profiling experiments using microarrays have focused on protein-coding genes of various species, leaving open the question to what degree the observed changes might be influenced by the involvement of small RNA species.

Before discussing what is currently known about the role that small RNAs play in polyploids, it may be helpful to review briefly the different types of small RNAs. Plants contain a variety of small RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and trans-acting small interfering RNAs (ta-siRNAs). Additionally, animals also contain so-called piwi-interacting small RNAs (pi-RNAs). Small RNAs are processed from precursor transcripts by conserved mechanisms, and the final RNA products affect developmental pathways, responses to environmental stimuli (including pathogens) and genomic stability via the maintenance of heterochromatin [Chen, 2009]. miRNAs belong to a class of molecules that are 20–30 nucleotides in length [Ghildiyal and Zamore, 2009] and that generally repress genes involved in developmental aspects, either by translational inhibition or by degradation of the target mRNA. Most miRNAs are processed by DICER and DICER-LIKE endonucleases [Ghildiyal and Zamore, 2009], which process the precursor RNA into short nucleotide fragments. These fragments are then bound by ARGONAUTE proteins to produce the RNA-induced silencing complex (RISC). RISC is guided by the bound RNA fragments to complementary targets. By contrast, ta-siRNAs are generated by cleavage of precursor RNAs transcribed from ta-siRNA loci (TAS) [Ghildiyal and Zamore, 2009]. These cleavage products are amplified by an RNA-dependent RNA polymerase and subsequently processed into 21-nt long fragments [Peragine et al., 2004].

The applicability of McClintock's [1984] predictions about genomic shock in response to genome merger has been discussed in many studies and reviews. Osborn et al.

[2003] suggested that in addition to genetic changes, epigenetic changes might be the cause of novel variation in allopolyploids. Madlung and Comai [2004], and Martienssen [2010] suggested that in intraspecific crosses, miRNAs or siRNAs could lead to gene silencing of conspecific transposon-derived transcripts. These same miRNAs might, however, not be effective against a diverged transposon sequence from a different species in an interspecific cross or allopolyploid, leading to activation of the transposon and subsequent genomic shock in the hybrid [Madlung and Comai, 2004; Martienssen, 2010].

Only recently have the first studies been published that tested these hypotheses. Ha et al. [2009] showed that siRNAs associated with repeats or transposons in *A. suecica* varied significantly between the 2 progenitor species *A. thaliana* and *A. arenosa.* It is of particular interest that while siRNA expression was relatively stable in allopolyploids, the expression patterns of miRNAs and ta-siRNAs varied significantly between the allopolyploid and the parental species. These findings may indicate that genome integrity is largely preserved by the compatibility of siRNAs from the 2 parental genomes in the allopolyploid, allowing the continued silencing of transposon and repeat-associated sequences. Phenotypic change, and possibly evolutionary innovation in the allopolyploids, on the other hand, might be facilitated by the change in miRNA and ta-siRNA expression patterns in the allopolyploid. The authors of this study also reported ELD for miRNAs of one parent *(A. arenosa)*, consistent with previous findings in this study system, which had shown that rRNA and protein-coding genes from *A. thaliana* were also more often repressed in the allopolyploid than genes of *A. arenosa* origin. Differences in miRNA accumulation between early synthetic allopolyploids and the established natural allopolyploid of the same parentage *(A. suecica)* may indicate that small RNAs are evolutionarily labile. However, since the exact parents of the natural allopolyploid *A. suecica* cannot be sampled, it is not clear if the variation in small RNA expression reported [Ha et al., 2009] arose over time or was already present in the original parents of *A. suecica.*

Specific miRNAs have been implicated in phenotypic effects resulting from expression changes in allopolyploids compared to their parents. Ng et al. [2011] showed that differential regulation of MIR163 in the *A. suecica*-like resynthesized allopolyploid leads to changes in target transcript accumulation. These target genes of MIR163 are involved in the synthesis of secondary metabolites that play a role in insect resistance in *A. thaliana* [Ng et al., 2011]. Differential expression of these miRNAs in allopolyploids may thus lead to adaptive changes that could play a role in the evolution of the new allopolyploid. To address the question what causes the observed variation in the accumulation of miRNAs in the allopolyploids, Ha et al. [2009] conducted expression analysis of small RNA biogenesis genes, including *ARGONAUTE (AGO)* and *DICER-LIKE (DCL)* genes. Their data showed non-additive expression between parents and allopolyploids for some small RNA biogenesis genes in some tissues, but did not exhibit consistent covariation with the accumulation of small RNAs [Ha et al., 2009], although downregulation of *AGO1* and *DCL1* genes in RNAi dominant negative mutants of resynthesized *A. suecica*-like plants had yielded viable plants with consistently reduced levels of miRNAs [Lackey et al., 2010]. Together these data suggest a possibly more complex regulation of small RNA metabolism in allopolyploids than in diploids.

In contrast to the findings in the *Arabidopsis* system, a recent study comparing small RNA expression in diploid, triploid and hexaploid wheat found that the level of expressed siRNAs corresponding to transposons decreased with increasing ploidy level [Kenan-Eichler et al., 2012], suggesting that in this plant material hybridization and/or polyploidization potentially leads to destabilization of the genome [Kenan-Eichler et al., 2012]. The authors also reported that the abundance of miRNAs in the wheat material was mostly additive (MPV), but with several notable exceptions, where expression in the allopolyploid was significantly greater or smaller than in the parent species. Interestingly, one of these miRNAs upregulated in the polyploid was miRNA 168, which targets *AGO1*. Reduction in AGO1 has been shown to reduce formation of siRNAs [Vaucheret, 2008], possibly explaining the observation of lower levels of siRNAs in the wheat allopolyploids [Kenan-Eichler et al., 2012]. Comparing the studies in wheat and *Arabidopsis*, it is of interest that the allopolyploid wheat populations were highly unfit, while the *Arabidopsis* polyploids used by Ha et al. [2009] were vigorous. It is thus possible that small RNAs play a role as a gatekeeper for polyploid or hybrid evolution.

Conclusions and Outlook

In this article, we have reviewed current knowledge of gene expression changes associated with polyploidy. Transcription in polyploids is affected by a multitude of genetic, cytogenetic and epigenetic changes that are themselves induced by genome merger and/or duplication. Multiple studies in diverse experimental systems

from throughout the flowering plants demonstrate that gene expression alteration and various forms of non-additivity in allopolyploids are ubiquitous, and although there are fewer studies in autopolyploids, it is clear that this process too leads to genome-wide transcriptional rewiring. To what degree changes in RNA levels lead to altered protein abundance is beginning to be elucidated, setting the stage for understanding how duplicated genes lead to altered protein quantities and function as well as metabolic flux through pathways. This research should enhance our appreciation of how gene expression changes in polyploids lead to changes in physiology, morphology or other phenotypes, furthering insight into the general question of the role of polyploidy in diversification.

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