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Understanding mechanisms of novel gene expression in polyploids

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Abstract

Polyploidy has long been recognized as a prominent force shaping the evolution of eukaryotes, especially flowering plants. New phenotypes often arise with polyploid formation and can contribute to the success of polyploids in nature or their selection for use in agriculture. Although the causes of novel variation in polyploids are not well understood, they could involve changes in gene expression through increased variation in dosage-regulated gene expression, altered regulatory interactions, and rapid genetic and epigenetic changes. New research approaches are being used to study these mechanisms and the results should provide a more complete understanding of polyploidy.

The increase in chromosome number and consequent genome redundancy created by polyploidy has long been recognized as a prominent feature in the evolution of flowering plants [1] and some animal species [2]. Many of our most important crop plants have the hallmarks of autopolyploidy (e.g. alfalfa and potato) or allopolyploidy (e.g. wheat, oat, cotton, coffee and canola) (Box 1). Other crops, such as maize [3], soybean [4] and cabbage [5], appear to have undergone polyploidization in their ancestry (paleopolyploids), although the evidence for this has been obscured by genomic rearrangements. Even the small genome of the model organism *Arabidopsis thaliana* appears to have undergone polyploidy in its history based on analysis of the full genome sequence [6]. Perhaps every plant species has gone through cycles of polyploidy during their evolution, although we can only recognize the most recent events [7].

The prominence of polyploidy in flowering plants implies that it has some adaptive significance. Polyploids often show novel phenotypes that are not present in their diploid progenitors or exceed the range of the contributing species (reviewed in Refs [8-10]). Some of these traits, such as increased drought tolerance, apomixis (asexual seed production), pest resistance, flowering time, organ size and biomass, could allow polyploids to enter new niches or enhance their chances of being selected for use in agriculture. The mechanisms by which

polyploidy contributes to novel variation are not well understood, but one long-held view is that duplicate genes have relaxed constraints on their function, and thus can diverge creating new phenotypes in polyploids (neofunctionalization). Most of the limited evidence supporting this concept consists of results from DNA sequence analyses pointing to the possibility of divergent selection of duplicate genes in ancient polyploids (reviewed in Ref. [7]). Examples of subfunctionalization (divergence in only part of a gene's function, such as tissue specificity [11]) have been found for ancient duplicated genes in maize [12,13].

Whereas functional divergence of duplicate genes might confer a selective advantage to polyploids over a long time period, it is unlikely to provide any immediate advantage to new polyploids. And yet polyploidy must sometimes confer an immediate advantage because rare polyploid events (estimated to be $\sim 10^{-5}$ among offspring of diploids [14]) can lead to successful species. Polyploidy does have immediate phenotypic effects, such as increased cell size and organ size, and sometimes greater vigor and biomass, and new phenotypic and molecular variation can arise shortly after polyploid formation (reviewed in Refs [7,10,15-19]). The appearance of these novel phenotypes in new polyploids probably involves changes in gene expression. In this article, we present ideas on how polyploidy could lead to novel forms of gene expression through increased variation in dosage-regulated gene expression, altered regulatory interactions, and rapid genetic and epigenetic changes. We also discuss new research approaches that will further elucidate these mechanisms.

Increased variation in dosage-regulated gene expression

Polyploidy has the general effect of increasing gene expression levels on a per cell basis in proportion to the gene dosage conferred by ploidy level, as was shown for most genes in a euploid series (monoploid, diploid, triploid and tetraploid) of maize [20]. In diploids, allele-dosage effects have been observed for many genes, including key regulatory genes of developmental processes, such as plant architecture (*tb1* in maize [21]), fruit size (*fw2.2* in tomato [22]) and flowering time (*FLC* in *Arabidopsis* [23]), and they appear to be a major contributor to the control of quantitative traits in general [24,25]. Allele-dosage effects are observed in heterozygous genotypes as intermediate gene expression levels and phenotypic effects, as compared with null/low-expressing alleles or high-expressing alleles in homozygous genotypes. Thus, for genes having allele-dosage effects, polyploidy increases the potential variation in expression levels (Fig. 1). This phenomenon might not expand the range of phenotypes (unless increasing the dosage of high- or low-expressing alleles confers even more extreme phenotypes), but it could lead to more intermediate phenotypic classes, some of which might have selective advantages. Both autopolyploids and allopolyploids could benefit from this effect, although the intermediate genotypic classes would become stabilized in self-pollinating disomic polyploids (Box 1, Fig. 1).

Box 1. Definitions, descriptions and characteristics of polyploids

Polyploidy has had a rich history of research, and various approaches have been used to study it. Some researchers have focused on the origins of polyploids, defining them as autopolyploids if they derive from the multiplication of a single genome or genomes of the same species, or as allopolyploids if they combined two or more genomes from distinct species. The discovery of colchicine's chromosome doubling effect [60] has led to the synthesis of many autopolyploids and allopolyploids. Other researchers have focused on the genetic or cytogenetic characteristics of polyploids, defining them as polysomic polyploids if the duplicated chromosomes are completely homologous and segregate from multivalents or random bivalents in meiosis, or as disomic polyploids if the duplicated chromosomes are only partially homologous (homoeologous) and segregate strictly from bivalents of homologous chromosomes. In general, autopolyploids have polysomic

inheritance and allopolyploids have disomic inheritance, but some polyploids (especially new allopolyploids [14]) exhibit both modes of inheritance and have been named segmental allopolyploids [61]. Regardless of these distinctions among polyploids, they all have some common features. One feature is high levels of gene duplication compared with their diploid parents. A second feature is high levels of heterozygosity. Autopolyploids maintain exceptionally high levels of heterozygosity compared to diploids due to outcrossing, polysomic inheritance and the potential for multiple alleles at a locus. In allopolyploids, a situation similar to heterozygosity is created between homoeologous loci from each of the diploid genomes and remains fixed due to disomic chromosome inheritance within each genome.

Variation in dosage-regulated gene expression has not been studied widely in polyploids, and as yet there are no concrete examples. However, it could explain some of the variation in flowering time of *Brassica* species through the expanded phenotypic effects of replicated *FLC* loci (Box 2). It could also explain, in part, the high levels of retention in duplicate gene function observed in long-established polyploids. Theory predicts that one copy of most duplicated genes should become silenced with a half-life of ~4 million years [11]. However, data from polyploid species point to much higher levels of duplicate gene preservation (reviewed in Ref. [7]). Lynch and Conery [26] suggest that this discrepancy might be reconciled if dosage requirements play an important role in the selective environment of gene duplicates. Variation in dosage-regulated gene expression created by polyploidy might be a key component of the selective environment for some duplicate genes, especially those that regulate important developmental processes in a dosage-dependent manner.

Could the potential for increased variation in expression of dosage-regulated genes also affect new polyploids? Obviously, this would require the presence of allelic variation, of which there are two possible sources. One source is through multiple polyploid events involving different diploid parents with different alleles. Several studies have provided evidence that polyploids can form multiple times in a small geographic area over a short time period (reviewed in Ref. [18]). A second source of new alleles is through mutations. As discussed below, new polyploids appear to be particularly prone to genetic and epigenetic changes that affect gene expression. These changes would most probably result in the loss or silencing of alleles, but in combination with existing alleles, they could create new variants through reduced levels of gene expression.

Altered regulatory networks

The expression of most genes is dependent on networks of regulators, such as transcription factors, that are organized into hierarchies [24,25]. The numbers of regulators in diploid networks is high, but in polyploids they can be expanded several fold (Fig. 2). The functioning of regulatory networks in polyploids will depend, in part, on how effectively regulators encoded by the contributing genomes interact. One can imagine that network functioning might be more severely altered in allopolyploids derived from divergent diploids than in those derived from genetically similar diploids. Autopolyploidy also could alter the functioning of these networks by potentially increasing the number of different interacting regulators through an increase in number of alleles (e.g. $A_1A_2A_3A_4$ in an autotetraploid versus A_1A_2 in a diploid), although the alteration might be much less than in allopolyploids because all alleles come from the same species. The overall effects on the expression of genes at the ends of regulatory cascades are difficult to predict because some regulators act as promoters and others as inhibitors of gene expression. In some cases, altered interactions could change the developmental timing or tissue specificity of gene expression.

Box 2. Polyploidy might increase the variation in expression and phenotypic effects of *FLC*

FLC is a single-copy gene with allelic and transgenic dosage effects on transcript levels and inhibition of flowering in *Arabidopsis thaliana*. Michaels and Amasino [23] have proposed that *FLC* acts through a rheostat-like mechanism to control flowering time in *Arabidopsis*. *Brassica rapa* is related to *A. thaliana*, but it has multiple copies of *FLC* probably due to polyploidy after it diverged from *A. thaliana*. For two *B. rapa* genes, early- and late-flowering alleles were shown to have additive effects both within and between loci [62]. Thus, a larger number of flowering times can be conferred by allelic variation at replicated *FLC* loci in *B. rapa* than at the single locus present in *A. thaliana*. In essence, polyploidy might have expanded the rheostat-like effect of *FLC* on flowering time.

Altered regulatory interactions could play a role in the increased vigor associated with polyploidy, and with heterosis in general. Heterosis, or hybrid vigor (the converse of inbreeding depression), is the phenomenon whereby individuals that are heterozygous display several characteristics that exceed even the better of the two more inbred parents. Heterosis undoubtedly has played a major role in plant evolution by impacting the types of breeding systems that occur [27], but also by conferring an advantage to polyploids. Both autopolyploids and allopolyploids have built-in mechanisms for maintaining high levels of heterozygosity (Box 1), and vigor is positively correlated with increasing heterozygosity or diversity of the contributing genomes in autotetraploid alfalfa [28], potato [29] and maize [30].

Various theories have been put forward to explain heterosis on the genetic level. One theory is that hybrids merely show complementation of different alleles from the two parents (i.e. genes from one parent compensate for the defects in the other). In terms of gene expression, the extreme view is that alleles would be strictly additive (at the midparent value) in the hybrid. The other extreme possibility is that regulatory interactions occur that alter target gene expression, which in turn affect the phenotype. In this case hybrids would exhibit gene expression that is not at the midparent value. Our initial results in maize favor the latter scenario for some genes. The experimental challenge is to define those changes in gene expression that are crucial to providing advantage to the heterozygote. Because heterotic characteristics are under complex genetic control and are seldom increased more than twofold, relatively minor changes of many genes might play an important role as opposed to more dramatic effects of a few. Heterosis might contribute to the molecular basis of polyploid characteristics; however, virtually nothing is known about gene expression in heterotic states at different ploidy levels.

Rapid genetic and epigenetic changes

Although established polyploids often exhibit a spectrum of variation not present in their presumed diploid progenitors, the potential contribution of genetic and epigenetic changes to this variation is difficult to study because the exact contributing genomes often are unknown and have evolved since polyploid formation. Fortunately, analogs of many natural polyploids can be resynthesized from current forms of diploid progenitors, and these materials allow exact comparisons. Recent studies with resynthesized polyploids have provided evidence that many genetic and epigenetic changes occur within a few generations after polyploid formation, and in some cases, they have been associated with altered gene expression and phenotypes.

Evidence for rapid genetic changes in polyploids has come from analysis of DNA fragments detected as restriction fragment length polymorphisms (RFLPs) and/or amplified fragment length polymorphisms (AFLPs) in resynthesized allopolyploids of *Brassica* species [31] and wheat [32-35]. These studies found loss and gain of many DNA fragments in the generations immediately following polyploid formation. Song *et al.* [31] listed several potential

mechanisms that could generate these changes, including homoeologous recombination and other types of DNA rearrangements, point mutations and gene conversion-like events. In another study, Axelsson *et al.* [36] found no evidence for genome changes in *Brassica juncea* based on mapping data from a natural \times resynthesized population. Clearly, additional research will clarify this discrepancy. Studies on genetic changes in resynthesized wheat polyploids have focused on the observation of DNA fragment loss, and the potential effect that deletions would have on stabilizing disomic chromosome pairing in new allopolyploids [33]. The evolutionary significance of this observation is supported by the higher frequency of fragment loss in resynthesized allopolyploids that are analogs of natural species than in those with no known natural counterpart. To date, the mechanism generating DNA fragment loss in wheat is not known, although it appears not to require meiosis since fragment loss occurs in some first-generation resynthesized allopolyploids.

The impact of rapid genetic changes on gene expression in polyploids is not well studied but can be anticipated if these changes involve coding sequences. Song *et al.* [31] observed changes in RFLPs using complementary DNAs (cDNAs) as probes, and although earlier wheat studies did not target coding sequences [33,34], a more recent study found that gene loss caused the disappearance of some transcripts in resynthesized wheat polyploids [35]. Deletions of gene sequences, or their potential replacement through gene conversions or nonreciprocal translocations, can remove only one gene homoeolog, but this could affect the expression levels of dosage-regulated genes or the function of regulatory networks, as well as the composition of enzymes and structural proteins in new polyploids.

Recent studies also have provided evidence for frequent epigenetic changes in new polyploids. Epigenetic changes do not involve alterations in DNA sequences, but affect gene expression through several interrelated modifications, including DNA methylation, histone modification and chromatin packaging [37,38]. One of the best-studied epigenetic phenomena in polyploids is nucleolar dominance: the transcriptional silencing of one set of progenitor rRNA genes in interspecific hybrids and allopolyploids [39]. In *Arabidopsis* and *Brassica* allopolyploids, the silenced genes were associated with high levels of DNA methylation and low levels of histone acetylation [40]. Changes in DNA methylation also have been found for other sequences of new allopolyploids of *Brassica* [31], wheat [32,34,35] and *Arabidopsis* [41]. In *Arabidopsis*, the overall levels of methylation were not different between parents and allopolyploids, but the allopolyploids showed sequence-specific changes and the same changes were observed in several independent allopolyploid lineages [41].

How polyploid formation triggers epigenetic changes is not known, but it might be related to the intergenome function of chromatin remodeling factors affecting DNA methylation, histone modification and/or chromatin packaging. In stable diploid species, chromatin remodeling factors presumably have evolved to act in a coordinated and consistent fashion. However, in new allopolyploids, imbalances in the interaction of factors encoded by the different genomes, as well as the dysfunction of factors encoded by one genome on chromatin of the other genome, could lead to altered gene expression (Fig. 3). Data comparing the resynthesized allopolyploid *Arabidopsis suecica*, its autotetraploid parents *A. arenosa* and *A. thaliana*, and diploid *A. thaliana* [41] suggest that epigenetic changes are triggered by the merger of two genomes, rather than by chromosome doubling *per se*. However, data on resynthesized wheat suggest that either process can trigger epigenetic changes [35]. The *A. suecica* study also found that some DNA sequences were more frequently altered by methylation than others. One hypothesis is that repeat elements in regions containing these sequences could be especially susceptible to altered chromatin remodeling mechanisms triggered by allopolyploid formation [15]. Such changes might also lead to transcriptional and transpositional activation of DNA transposons and retrotransposons, as was shown recently for the *Arabidopsis* *DECREASE IN DNA METHYLATION* mutant, *ddm1* [42-44]. Comai *et al.* [45] described the transcriptional

silencing and subsequent reactivation of a repeat element with similarity to transposable elements in resynthesized *A. suecica*, and some of the transcriptionally activated sequences in wheat were retrotransposons [35].

What is the evidence that epigenetic changes can alter the transcriptome and phenotype? Changes in DNA methylation, gene expression and phenotype were observed in resynthesized allopolyploids of *A. suecica*, and a direct connection was established between DNA methylation and gene silencing by the reactivation of some silenced genes after treating the allopolyploids with a demethylating agent [41]. This treatment also caused more new phenotypes to appear. Additionally, resynthesized allopolyploids of *A. suecica* and wheat expressed transcripts that were not detected in the parents [34,35,41]. Thus, epigenetic changes in new polyploids might lead to repression of gene expression or expression of sequences that were repressed in the diploid (derepression). These changes could affect phenotypes directly if they involve genes encoding enzymes or structural proteins; however, changes in the expression levels of regulatory genes could also affect phenotypes through increased variation in dosage-regulated gene expression or altered function of regulatory networks, as described above.

Changes in DNA methylation status are sometimes heritable [37], and thus could be manifested through many generations and have an impact on polyploid evolution. The analysis of natural *A. suecica* allopolyploids has provided evidence for stability of epigenetic silencing: some genes that were expressed in the progenitor species were silenced in *A. suecica* [46]. Even epigenetic changes that are not permanently stable could have an effect on polyploid evolution, especially if they affect reproduction or ecological adaptation. For example, resynthesized *Brassica napus* has been shown to quickly evolve heritable variation in flowering time [47]. Although this could be caused by epigenetic changes that are not stable over the long term, flowering time variation could help a new polyploid become reproductively isolated from its diploid progenitors and fill a new ecological niche. Selective forces could maintain epigenetically regulated variation until genetic mutations have established stable variation.

Research opportunities

Additional research to understand fully the mechanisms of altered gene expression (Fig. 4) and their impact on evolution should be carried out on several model polyploid systems, because the importance of different mechanisms could vary among species. For example, data on resynthesized allopolyploids of cotton show very little evidence for rapid genetic changes [48]. Most of the research to date has been on new polyploids of *Arabidopsis*, *Brassica*, cotton, maize and wheat, but investigations on additional taxa might be enlightening.

The ability simultaneously to survey expression levels of potentially all genes using DNA microarrays [49] has been used to study the effects of autopolyploidy on gene expression in yeast [50], and it provides a powerful new approach to study the effects of autopolyploidy and allopolyploidy in plants. Microarrays currently available for plants will be useful for studying overall changes in the expression of sets of homoeologous genes. Many of the changes are likely to be small (<2-fold) and to detect them will require rigorous experimental designs and analyses [51]. In the future, microarrays could be designed to interrogate every sequence in the genome, including sequences that are not predicted to be genes. Methods such as AFLP-cDNA [41,45,46] are useful for detecting homoeolog-specific expression, but they require *post hoc* sequencing to identify fragments of interest. Researchers of polyploidy would benefit from inexpensive methods that survey entire genomes, provide sequence information, and have the specificity to distinguish transcripts from different homoeologs.

Survey methods that detect every sequence in the genome and have homoeolog specificity also would be useful for future studies on rapid genetic changes. The experimental challenge is to

delineate the types and regions of genome rearrangements that occur in new polyploids. This research will help pinpoint mechanisms, such as translocations, gene conversions or short deletion events, and determine their relative importance. When combined with transcription profiles, we can learn how specific rearrangements affect global gene expression.

Surveys of epigenetic changes will provide more information if they also reveal the genomic location of the changes. Traditional methods (e.g. Southern blotting using methylation-revealing isoschizomers, or bisulfite sequencing) are effective for confirming the methylation state of selected genes, but are limited in throughput. Detection of methylation changes by AFLP [41] has greater throughput, but broad coverage and more information can be gained by microarray surveys using methylated and unmethylated DNA fractions as hybridization probes on gene or whole genome microarrays [52]. Changes in methylation status detected by this method can be immediately related to DNA sequence characteristics and to changes in gene expression detected using the same microarray platform.

DNA methylation is only one of the epigenetic marks that differentiate active and silent chromatin. Histone modifications, such as acetylation, phosphorylation, ubiquitination and methylation, have been associated recently with heterochromatic silencing [37,38], and might play a role in epigenetic changes in polyploids [40]. Chromatin immunoprecipitation can be used to identify the DNA sequences associated with particular histone modifications [53] and provides a powerful profiling tool when labeled as a target for microarrays. *Arabidopsis* mutants in chromatin remodeling genes could be profiled alongside polyploids to identify likely candidate mechanisms for gene-specific and homoeolog-specific silencing. Whole genome microarrays are superior to gene microarrays for this analysis because they include unannotated non-coding RNA, chromatin binding sites in intergenic regions, and methylated regulatory DNA sequences.

What other mechanisms might direct chromatin modification specifically toward duplicate genes and other sequences in polyploids? Gene-specific effects might be conferred by RNA silencing [54,55]. RNA silencing was observed with increasing dosage of a transgene in *Drosophila* [56], so a similar phenomenon might occur for some duplicated genes in polyploids. Alternatively, a case has been described of a transgene in *Arabidopsis* that is silenced in the diploid state, but activated at the triploid level [57]. Recently, a connection between histone modification and RNA silencing was found in *Schizosaccharomyces pombe* [58]. The relationship between polyploidy and RNA silencing could be explored by studying the expression profiles of diploids and polyploids having mutations in components of the RNA silencing machinery.

Integration of gene expression, DNA and histone modification, and chromatin remodeling information should cast light on the targets of polyploid transcriptome restructuring as well as on the mechanisms responsible for this action. For example, the tools described above can be used to determine if epigenetic changes target transposons by exploring the correlation between chromatin state and transcriptional activity of these elements. Methods such as transposon display [59] can be employed to investigate transposon movement. Once the targets are identified, the role of specific proteins in epigenetic regulation can be elucidated by studying the effect of mutations at loci encoding regulatory factors. In autopolyploids, recessive loss-of-function mutations will suffice, and resources are available for identifying these in diploid *Arabidopsis* (<http://www.Arabidopsis.org/links/insertion.html>). In allopolyploids, dominant negative alleles might be required to silence the homoeologs in both parental genomes, and these are also becoming available in diploid *Arabidopsis* and maize (<http://www.chromdb.org/>). Some genomic and transcriptomic responses to polyploidy might depend on the activity of certain regulatory factors, and these responses should not occur in mutant polyploids.

The detailed analysis of gene expression changes in many polyploids could be needed to uncover the dynamics of new variation and its impact on polyploid evolution. Ozkan *et al.* [33] analyzed the first few generations of 20 resynthesized species (analogs of natural forms) and 15 synthetic species (no natural analogs) of allopolyploids in the *Aegilops-Triticum* group. This large sample size gave more impact to their observation that the resynthesized species eliminated a specific set of sequences earlier than the synthetic species (i.e. this process could be important to the establishment of natural species). Analysis of many allopolyploids of a single species resynthesized from the same diploid parents also could prove useful. For example, changes in gene expression that occur immediately in all co-derived allopolyploids might indicate altered regulatory networks, whereas changes that occur in only a portion of the allopolyploids could indicate genetic and epigenetic changes. Understanding the timing and frequency of changes among new allopolyploids, and how their genomes compare with those of natural allopolyploids, will provide insight into the evolutionary impact of rapid genome changes in new polyploids.

Acknowledgements

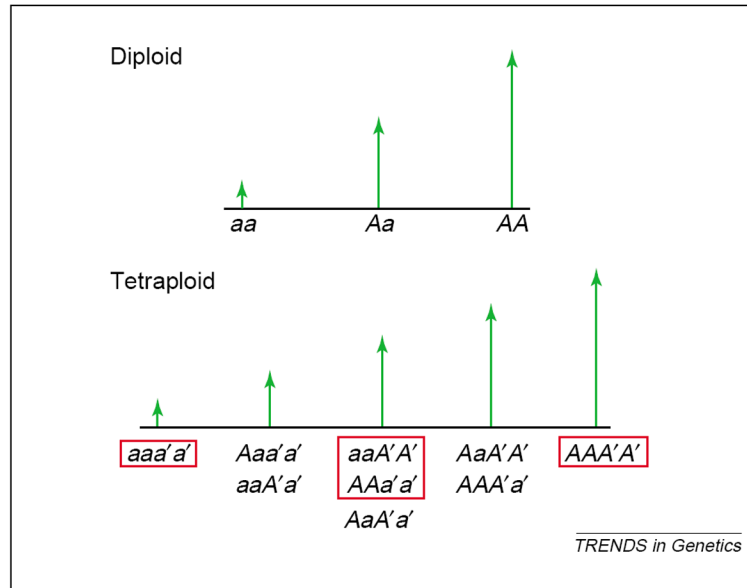
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**Fig 1.**

Increased variation of dosage-regulated gene effects in polyploids for one locus with two alleles (A and a) having additive interaction and dosage-dependent expression of allele A . Three genotypes at the diploid level expand to nine genotypes at the tetraploid level. The phenotypic effects and gene expression levels (shown by height of arrows) also expand from three for the diploid to five for the tetraploid. Genotypes in boxes would be fixed in a self-pollinating disomic tetraploid where each of two diploid genomes contributes two alleles A and a , and A' and a' .

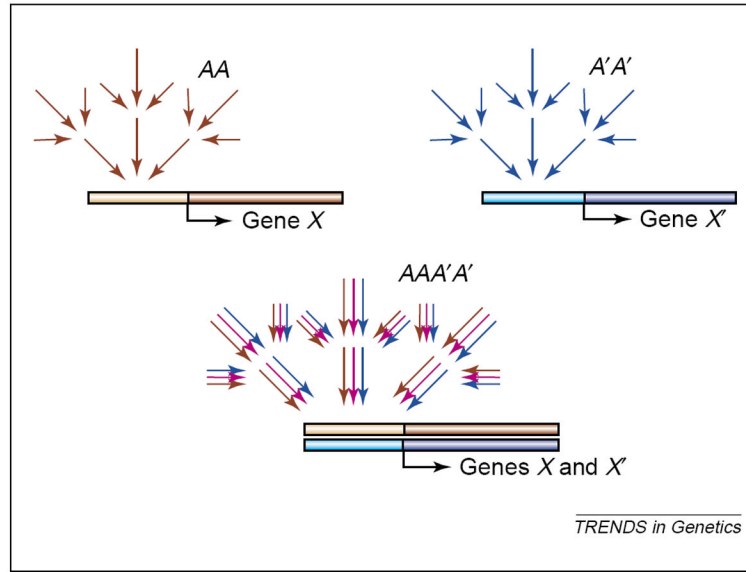


Fig 2. Increase in complexity of regulatory interactions with polyploidy. AA and A'A' represent two diploid genomes contributing to the autotetraploid or allotetraploid AAA'A'. Arrows represent trans-acting regulatory factors, each encoded by a separate gene, affecting expression of genes X and X' in a hierarchical fashion. The regulatory network is more complex in the tetraploid because all of the factors from genomes AA and A'A' interact to affect expression of X and X'. The complexity increases threefold for regulators that are dimeric proteins (homodimers represented by blue and red arrows, heterodimers represented by purple arrows).

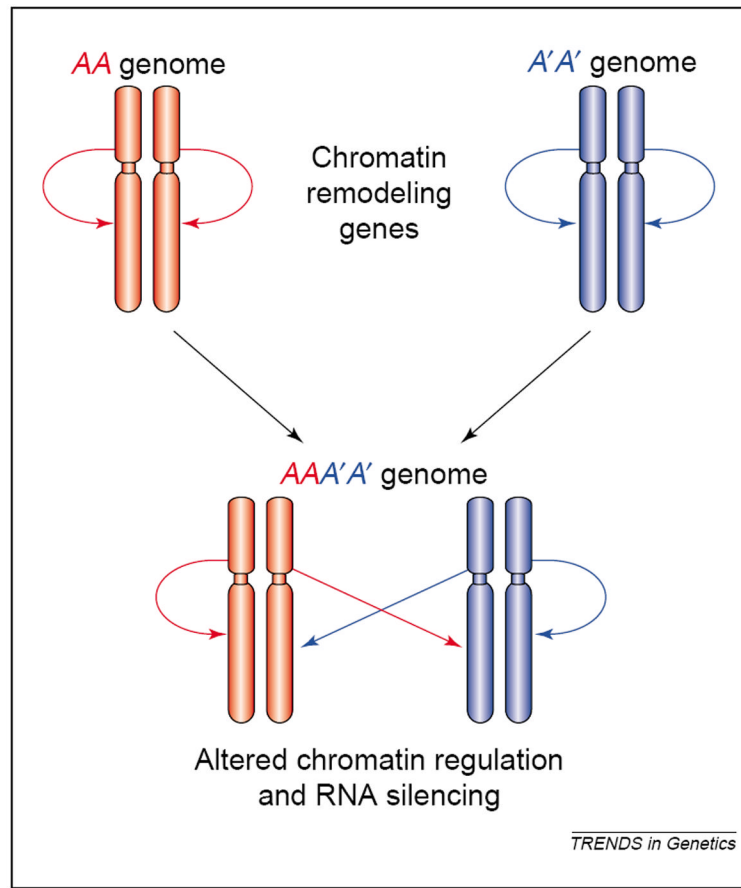


Fig 3. Simplified model for the potential effects of polyploidy on chromatin structure and gene expression. For diploid genomes AA and A'A', chromatin remodeling factors encoded by each genome affect chromatin only within that genome. For the tetraploid genome AA A'A', chromatin remodeling factors encoded by each genome can affect chromatin of both genomes. The intergenomic activity of these factors could cause novel forms of chromatin remodeling and epigenetic regulation in new polyploids. Interactions causing RNA silencing are also possible.

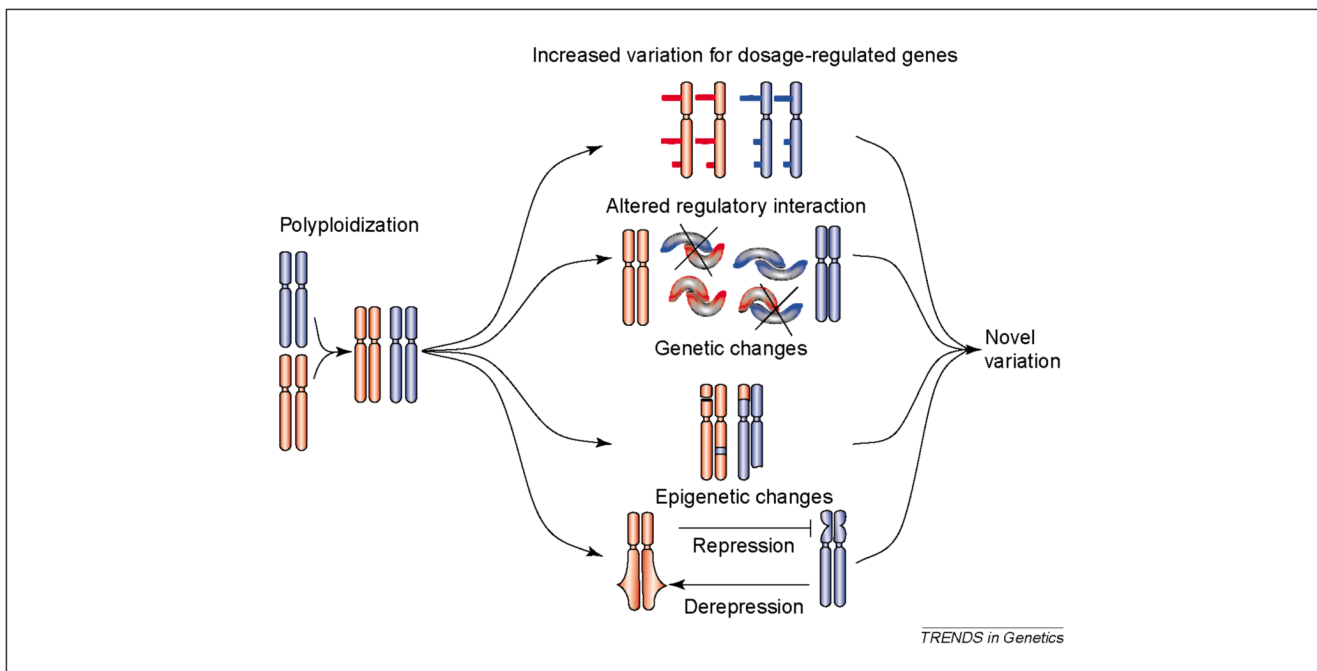


Fig 4. Potential causes of novel variation in polyploids. The merger of chromosomes from two diploid genomes (red and blue) into a tetraploid genome can cause (1) increased variation of dosage-regulated gene effects and expression (magnitudes of allelic effects and expression shown by size of blocks for three loci); (2) altered regulatory interactions (trans-acting regulatory factors shown as dimeric proteins, with heterodimers not functioning properly); (3) genetic changes affecting gene expression (e.g. insertions, deletions, translocations and gene conversions); and (4) epigenetic changes (repression or derepression of gene expression caused by genome interaction of chromatin modeling factors, which could also trigger movement of transposable elements).