Nonadditive Gene Expression in Polyploids

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Abstract

Allopolyploidy involves hybridization and duplication of divergent parental genomes and provides new avenues for gene expression. The expression levels of duplicated genes in polyploids can show deviation from parental additivity (the arithmetic average of the parental expression levels). Nonadditive expression has been widely observed in diverse polyploids and comprises at least three possible scenarios: (*a*) The total gene expression level in a polyploid is similar to that of one of its parents (expression-level dominance); (*b*) total gene expression is lower or higher than in both parents (transgressive expression); and (*c*) the relative contribution of the parental copies (homeologs) to the total gene expression is unequal (homeolog expression bias). Several factors may result in expression nonadditivity in polyploids, including maternal-paternal influence, gene dosage balance, *cis*- and/or *trans*-regulatory networks, and epigenetic regulation. As our understanding of nonadditive gene expression in polyploids remains limited, a new generation of investigators should explore additional phenomena (i.e., alternative splicing) and use other high-throughput "omics" technologies to measure the impact of nonadditive expression on phenotype, proteome, and metabolome.

INTRODUCTION

Homeologs: each pair of chromosomes or genes that are derived from two different parental species in an allopolyploid species

Subfunctionalization: partitioning of ancestral functions or expression patterns of duplicated genes so that both genes are retained following gene or genome duplication

Polyploidy, or whole-genome duplication (WGD), generally results in instant speciation, increasing biodiversity and providing new genetic material for evolution (e.g., 102, 103). New tetraploids are expected to have duplicate gene copies that share expression patterns and functions. However, polyploids experience the combined challenge and potential of having two or more genomes together in the same nucleus, and for survival, a polyploid individual must balance the potential benefits of extra heterozygosity and biochemical diversity with the cost of carrying and expressing multiple genomes. Through time, duplicate gene copies (homeologs) have several possible fates. Both genes may retain their original function, one copy may be silenced, or they may diversify in function or expression (e.g., 113, 131). One copy may be lost by deletion, or the copy number may be altered through homeologous exchanges. Duplicated genes may also interact via interlocus recombination, gene conversion, or concerted evolution. Furthermore, analyses of synthetic allopolyploids, crops, and *Arabidopsis* show that polyploidy can evoke rapid responses in both genome structure and gene expression, including concerted evolution of rDNA (48, 83, 94, 185), loss and restructuring of low-copy DNA (51, 57, 85, 100, 134, 135, 161, 167), activation of genes and retrotransposons (85, 86), gene silencing (35, 36, 41, 57, 99), epigenetic changes (104, 110, 112, 114, 132, 153), chromosomal rearrangement (88, 109, 138, 140, 141, 176), and organ-specific subfunctionalization of gene expression (1–3, 5). In this review, we focus on patterns of gene expression associated with polyploidy in plants, the factors that govern shifts in gene expression in polyploids, and the implications of altered gene expression for genome and organismal evolution.

Types of Polyploids

Two general types of polyploidy have long been recognized: those involving the multiplication of one chromosome set, autopolyploidy (auto = same), and those resulting from the merger of structurally different chromosome sets, allopolyploidy (allo = different) (89). This convention has long been employed (39, 46, 124) and is thought to represent a fundamental distinction between polyploid types (63, 168). However, nature has produced a continuum of polyploid types, many of which defy clear placement in either of these two groups (105, 168). Hence, there has been debate for more than 70 years as to the types of polyploids that should be recognized in nature and the proper definitions of autopolyploidy and allopolyploidy. For example, Garsmeur et al. (60) use a genomic approach and suggest that allopolyploids have genome dominance and biased fractionation, whereas autopolyploids do not have these features. However, it is easy to envision scenarios in which an allopolyploid would lack genome dominance.

Allopolyploids are derived from hybridization and chromosome doubling between two different species (usually congeners) and hence have two divergent parental genomes (*AABB* in an allotetraploid) (**Figure 1**). An allotetraploid therefore contains two of each pair of the counterpart chromosomes derived from two different species: These are called homeologous chromosomes, or homeologs (**Figure 1**).

Autopolyploids are formed by genome duplication within a species: This could involve doubling of one individual genotype or, more likely, hybridization between different plants/populations within a species and genome doubling (*AAAA*) (**Figure 1**). Autopolyploids exhibit little morphological or cytogenetic divergence from the diploid progenitor and harbor more than two copies of each chromosome (i.e., four in an autotetraploid); multivalents may therefore form during meiosis. Another consequence of autopolyploidy is polysomic inheritance, in which multiple alleles (i.e., more than two) of a given gene segregate randomly at meiosis and can be detected in the offspring.

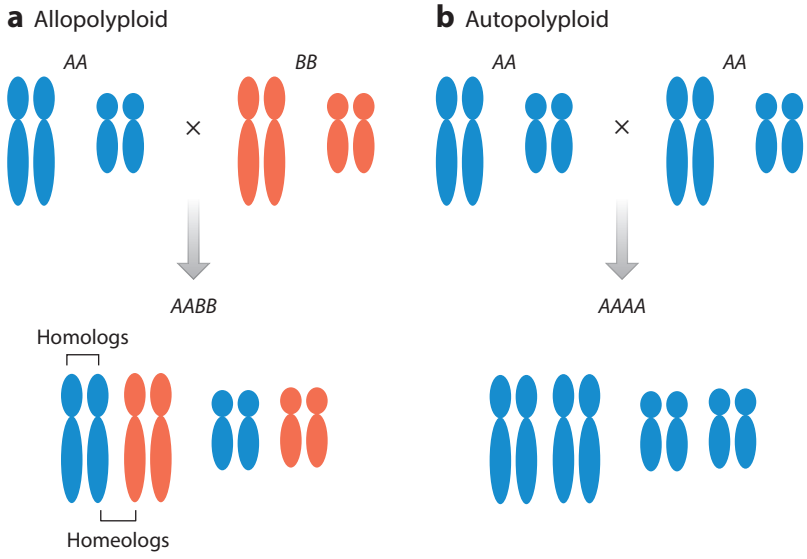


Figure 1

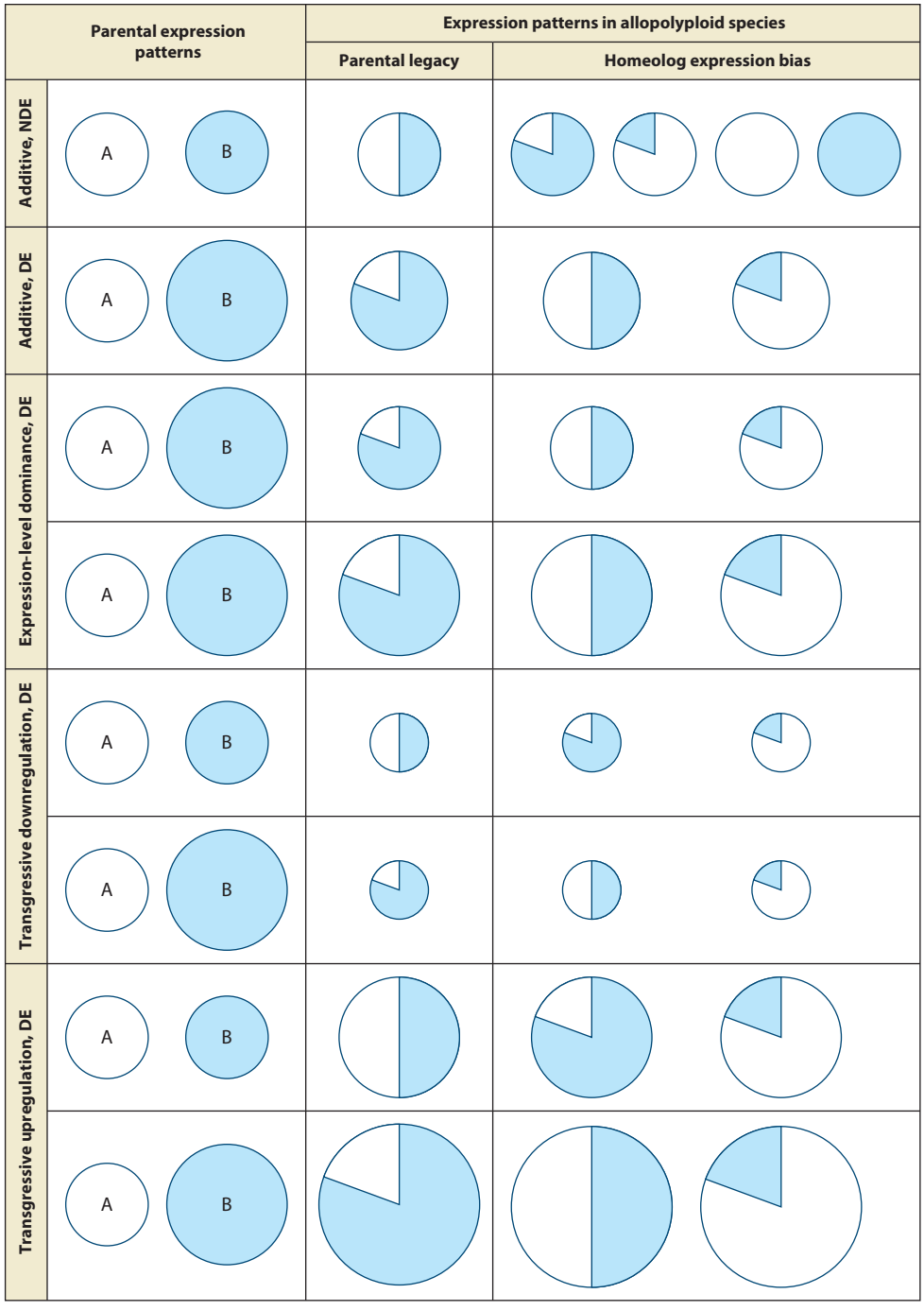
Two types of polyploids. (*a*) Allopolyploids are derived from hybridization between two related species and chromosome doubling. (*b*) Autopolyploids are formed by genome duplication involving a single species. Thus, an allotetraploid contains homologous and homeologous chromosomes, with the latter indicated by two of each pair of the corresponding chromosomes derived from the two different parental species.

Segmental allopolyploidy represents an intermediate condition between auto- and allopolyploidy (63, 168). The genotype of a segmental allotetraploid may be represented as $A_sA_sA_tA_t$ because the parental genomes are somewhat diverged.

On the basis of this background, discussion of nonadditive gene expression in polyploids requires the ability to differentiate the parental genomes. This ability does not apply to autopolyploids, and nonadditive gene expression is therefore a phenomenon applicable to the study of allopolyploid genomes in which the parental genomes are clearly distinct. One may also attempt to address nonadditive gene expression in segmental allopolyploids, but this may be problematic depending on how well-differentiated the parental genomes are in the study system.

Defining Nonadditive Expression Patterns in Polyploids

When two parental genomes are present in an allopolyploid nucleus, the expression levels between duplicated genes can show deviations from parental additivity (i.e., typically considered to be the arithmetic average of the expression levels of parental genes). Additivity can be referred to as expression conservation of parental genes, parental legacy, or the vertical transmission of preexisting expression patterns of parents (22). However, not all duplicated gene pairs exhibit additivity; such nonadditive expression pattern has been the focus of studies of polyploids with respect to relative expression of the respective parental copies (homeologs) or the total expression level of homeologs (**Figure 2**). The former can be considered as the preferential expression of one homeolog relative to the other and is often called transcriptome dominance (33), bias (54), nucleolar dominance (which refers to rRNA expression only) (36), genome dominance (60, 158), or gene dominance (188). However, recently the term homeolog expression bias was proposed to synthesize


Parental expression patterns
Expression patterns in allopolyploid species
Parental legacy
Homeolog expression bias
Additive, NDE
Additive, DE
Expression-level dominance, DE
Transgressive downregulation, DE
Transgressive upregulation, DE
A
B

these unequal expression patterns of homeologs in allopolyploids (64). Loss of expression of one homeolog could perhaps be considered the extreme case of this homeolog expression bias.

Considering the total expression of a duplicated gene pair in an allopolyploid compared with its parents, two nonadditive scenarios are possible. First, the overall expression level in the allopolyploid may be similar to that of one of its parents. This case was originally described in cotton as genomic expression dominance (145) and also genomic dominance (54), and as parental dominance in *Spartina anglica* (31). However, this phenomenon is derived from data at the transcriptomic level, so a more appropriate term is expression-level dominance (64). Alternatively, the overall expression level may be lower or higher than those of both parents (transgressive expression).

Nonadditive expression patterns may therefore derive from expression-level dominance, transgressive expression across all duplicated gene pairs, and homeolog expression bias at the level of individual homeologs (**Figure 2**). Although the latter reflects the preferential expression of one homeolog over the other, we must consider whether observed homeolog expression bias originates from differential expression between parental genes. For example, if one parental gene has a higher expression level than the other parental gene, and there is no regulatory change in the allopolyploid species, we expect differential expression between the two homeologs in the allopolyploid [**Figure 2**; additive, differentially expressed (DE)]. This does not mirror homeolog expression bias, but rather vertical transmission of preexisting patterns of the parents (i.e., parental legacy; see 22). Therefore, when considering homeolog expression bias, we must consider the initial parental gene expression patterns.

Investigating nonadditive expression on a genome-wide basis is problematic for most organisms. Because of the lack of a complete genome sequence for most species and the low sequence divergence between the parental genomes of many allopolyploids, only small-scale experiments have typically been conducted to assess homeolog expression bias. For example, only 13 genes were examined in coffee [*Coffea arabica* (42)] and 10 to 144 genes in *Tragopogon miscellus* and *Tragopogon mirus* (23, 92). Large-scale gene surveys of allopolyploids have been based on expressed sequence tags (ESTs) (1,318 duplicated gene pairs in cotton) (54), RNA-sequencing (RNA-Seq) technology and Sequenom [<2,000 transcripts in *Tragopogon* (20)], and RNA-Seq only [~25,000 in cotton (194); ~10,000 genes in coffee (43)]. The paucity of studies limits our understanding of how homeologs are regulated in allopolyploid species. Because of the advantages of RNA-Seq technology (see below), studies of homeolog expression bias can now be conducted without prior information on the complete (or nearly so) parental genomes. Technological advances have thus promoted easier investigation of homeolog-specific expression patterns in nonmodel allopolyploids.

Figure 2

Nonadditive expression patterns in allopolyploids. Considering the total expression level of homeologs, expression could be additive for either nondifferentially expressed (NDE) or differentially expressed (DE) genes between an allopolyploid and its diploid parents (*first* and *second rows*). Nonadditive expression can be classified into expression-level dominance, where the total gene expression of homeologs resembles one of the parents regardless of downregulation or upregulation (*third* and *fourth rows*), and transgressive expression, where genes are downregulated or upregulated relative to the diploid parents (*fifth*, *sixth*, *seventh*, and *eighth rows*). As for the relative expression of homeologs, two homeologs in an allopolyploid may inherit preexisting parental expression differences (parental legacy), or one homeolog may be preferentially expressed compared to the other one, which is known as homeolog expression bias. Homeolog loss has the effect of resulting in extreme homeolog expression bias, shown in the first row only, although the mechanisms of reduced expression are clearly different.

Several studies show expression-level dominance in allopolyploids formed in the past ~1 million years or less, including cotton (54, 145, 194), *Spartina anglica* (31), *Triticum aestivum* (26, 143), and coffee (12, 43). Expression-level dominance was originally described from global gene expression profiles in cotton as genomic expression dominance (145). All of these studies investigated expression-level dominance in a genome-wide manner, but all but two (43, 194) employed microarrays, an approach with limitations (see below). Using RNA-Seq (see below), three studies have attempted to elucidate how nonadditive expression patterns are related, especially the relationship between expression-level dominance and homeolog expression bias. Two studies showed no correlation between expression-level dominance and homeolog expression bias (43, 144), whereas the third study revealed that expression-level dominance might be caused by the upregulation or downregulation of the nondominant parental copy (194). Given that more studies using RNA-Seq will be conducted on additional allopolyploids (e.g., in *Tragopogon*, *Glycine*, *Senecio*, and *Spartina*), we may soon have a better view of how homeolog expression bias has affected global gene expression patterns.

Examining Expression in Polyploids

Most studies of expression nonadditivity in polyploids have used either microarrays or Sequenom, and more recently, transcriptome sequencing using RNA-Seq. Microarrays have been widely leveraged in global gene expression comparisons of allopolyploids and their diploid parents [e.g., in *Arabidopsis* (179), *Gossypium* (cotton) (54, 145), *Spartina* (31), *Senecio* (69, 70), *Triticum* (wheat) (26, 143), and *Coffea* (coffee) (12)]. These allopolyploids were globally differentiated from their progenitors with respect to overall gene expression levels. However, microarrays require prior sequence information for probe design, which limits global transcriptome profiling in nonmodel allopolyploids. Also, microarrays rely on hybridization between probes and target sequences, which is problematic in that microarrays may not be sufficiently sensitive to distinguish between homeologs.

Sequenom MassARRAY is a powerful tool for studying nonadditive expression in allopolyploids. It requires prior information on genome-specific single nucleotide polymorphisms (SNPs); genome-specific SNP variants are then amplified via PCR, and their relative homeolog transcript abundance quantitatively measured through mass spectrometry. Sequenom is very efficient and sensitive in detecting homeolog expression bias but is limited in the number of genes that can be examined because of the requirement of homeolog-specific SNPs. Sequenom has been applied to synthetic allotetraploid cotton (29) and *Gossypium hirsutum* (53) and *Tragopogon miscellus* (20, 21). These studies targeted only 11 to 70 duplicated gene pairs but included 18 to 24 tissues in cotton and 59 individuals from five natural populations in *T. miscellus*. Importantly, these studies showed how homeologs are temporally and spatially regulated relative to each other.

RNA-Seq has several advantages for gene expression profiling, including no need for a priori information on genome sequences, a wide range of quantification, and higher accuracy and reproducibility in measuring expression levels of homeolog-specific transcripts (181). Thus, RNA-Seq enables investigators to examine all nonadditive expression patterns at once and dissect the relationship between homeolog expression bias and gene-level nonadditive expression. Several transcriptome profiling studies have now been conducted, including analyses of *Glycine* (soybean) (79, 150), *Arabidopsis* (7), cotton (144, 194), coffee (43), and *Tragopogon* (M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results). Studies on cotton and coffee focused not only on global gene expression patterns in the allopolyploids relative to the parental species in a genome-wide manner but also on how homeolog expression bias is linked to expression-level dominance (43, 144, 194).

NONADDITIVE EXPRESSION IN ALLOPOLYPLOIDS: CASE STUDIES

Several allopolyploid systems have now been examined in detail for patterns of gene expression across much of the genome. Below we summarize expression data for some of the best-studied allopolyploid systems.

microRNA (miRNA): a small noncoding RNA molecule (approximately 22 nucleotides) that is involved in transcriptional and post-transcriptional regulation of gene expression

Small interfering RNA (siRNA): a double-stranded short RNA molecule (20∼25 nucleotides) that interferes with gene expression through RNA interference (RNAi); also known as short interfering RNA or silencing RNA

Arabidopsis suecica

Arabidopsis suecica ($2n = 26$) (Brassicaceae) originated 12,000 to 300,000 years ago, with *Arabidopsis thaliana* ($2n = 10$) and *Arabidopsis arenosa* ($2n = 16$) as the maternal and paternal parents, respectively. Morphologically, *A. suecica* is more similar to *A. arenosa* than to *A. thaliana*. Wang et al. (179), using microarrays, found that 5.2% (1,362) and 5.6% (1,469) of the genes displayed nonadditive expression in two synthetic allotetraploids; more than 65% of these nonadditively expressed genes are repressed (**Figure 3*a***). Most repressed genes (>94%) in the allotetraploids matched those genes that are expressed at higher levels in *A. thaliana* than *A. arenosa*. That is, allotetraploid plants show homeolog expression bias toward *A. arenosa*. *Trans*-effects might drive these homeolog expression patterns (162).

Chang et al. (27) used microarrays and RNA-Seq to investigate homeolog-specific retention and expression in *A. suecica*. Again, global expression favored *A. arenosa*: 3,458 genes preferentially expressed *A. thaliana* homeologs, whereas 4,150 expressed *A. arenosa* homeologs. Several hypotheses might explain this *A. arenosa* bias: (*a*) The *A. thaliana* subgenome is less capable of purging mildly deleterious mutations than the *A. arenosa* subgenome; (*b*) *A. arenosa* homeologs are a better fit for the environment in which *A. suecica* occurs; and (*c*) the *A. arenosa* transcription machinery is preferentially expressed in F_1 plants of *A. suecica*, and this initial pattern was then enhanced by homeolog-specific methylation (27).

Other research on *A. suecica* has focused on small RNAs, including microRNAs (miRNAs) (67, 90, 127, 175). In *A. suecica*, many small interfering RNAs (siRNAs) were nonadditively expressed; this nonadditive expression was related to tissue, growth stage, and developmental changes (67). Ng et al. (127) showed that the expression of *A. suecica* miR163 is biased toward *A. arenosa* rather than *A. thaliana*. These expression changes may reflect divergence between promoters in the two parents and the absence of putative *trans*-acting repressors in *A. thaliana*. In contrast, the miR172 loci from *A. arenosa* were repressed in the allotetraploid, whereas expression of *A. thaliana* loci was maintained after allopolyploidization (175). This nonadditive expression might be associated with the nonadditive expression of miR172 targets.

Brassica napus

Allotetraploid *Brassica napus* ($2n = 38$) (Brassicaceae) formed 5,000 to 10,000 years ago from *Brassica rapa* ($2n = 20$) and *Brassica oleracea* ($2n = 18$) (126); both parents are themselves ancient polyploids (137). Genetic changes caused by homeologous chromosome rearrangement are common in newly resynthesized *B. napus* (57, 167). Gaeta et al. (57) analyzed genetic, epigenetic, gene expression, and phenotypic changes in 50 independently resynthesized lines of *B. napus*. Nonadditive expression, measured by amplified fragment length polymorphism (AFLP)-cDNA and single-strand conformation polymorphism (SSCP)-cDNA, correlated with chromosomal rearrangements (57). Analysis of the newly synthesized polyploids (S_0 generation) revealed that genetic changes were rare and methylation changes frequent. Most of the S_0 methylation changes remained fixed in later-generation (S_5) progeny. Genetic changes were much more frequent in the S_5 generation, occurring in every line. Exchanges among homeologous chromosomes are a major mechanism for generating novel allele combinations and phenotypic variation (190). Fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) revealed

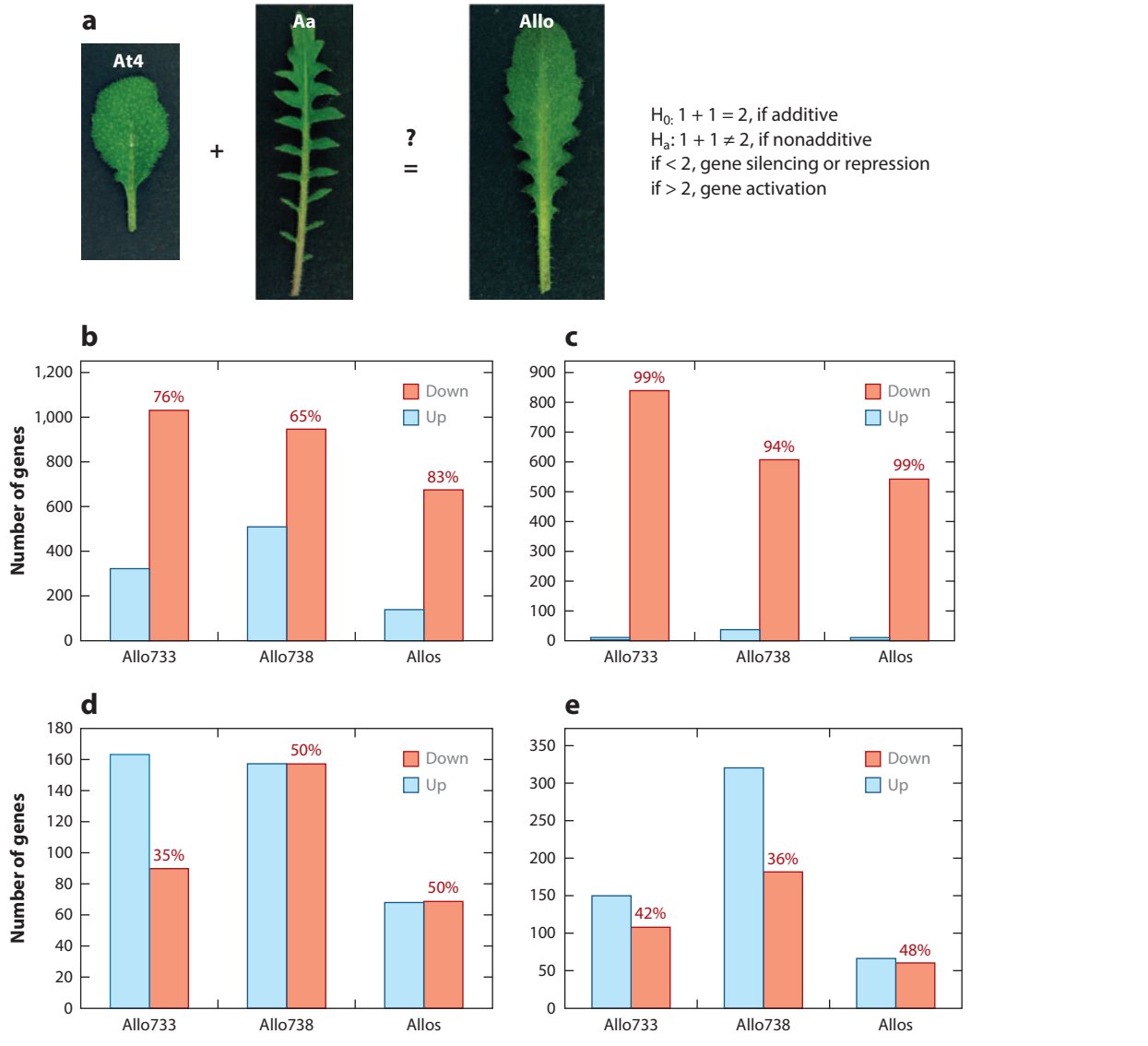


Figure 3

(*a*) Hypotheses for testing additive and nonadditive gene regulation in *Arabidopsis* allotetraploids. The null hypothesis (H_0) is that gene expression levels in an allotetraploid (Allo) are equal to the sum of the two progenitors, *Arabidopsis thaliana* autotetraploid (At4) and *Arabidopsis arenosa* (Aa). Typical seedling leaves in At4, Aa, and Allo are shown. Abbreviation: H_a, alternative hypothesis. Modified from Reference 33. (*b–e*) Downregulation of *A. thaliana* genes in the synthetic allotetraploids. (*b*) Distribution of nonadditively expressed genes detected in each allotetraploid (Allo733 or Allo738) or both allotetraploids (Allos). (*c*) The nonadditively expressed genes in each allotetraploid matched the genes that were highly expressed in the *A. thaliana* autotetraploid. (*d*) The nonadditively expressed genes in each allotetraploid matched the genes that were highly expressed in *A. arenosa*. (*e*) The nonadditively expressed genes matched the genes that were equally expressed in both parents. The percentages of downregulated genes are indicated above the columns in each histogram. Modified from Reference 179.

extensive chromosome rearrangements, aneuploidy, and homeologous chromosome compensation (190), similar to what has been reported in *T. miscellus* (38). Recent studies have been conducted in *B. napus* on alternative splicing (AS) (198) and the proteome (115), and current investigations on both resynthesized and natural *B. napus* are using a wide array of high-throughput "omics" approaches.

Coffea arabica

Coffea arabica (coffee; Rubiaceae) is an allotetraploid ($2n = 4x = 44$; ~50,000 years old) formed from diploids *Coffea eugenioides* and *Coffea canephora* (25, 97). The two diploid parents exhibit different ecological adaptations. As a result, most studies on coffee have involved investigation of environmental conditions. For example, how does growing temperature affect subgenome patterns of expression in allopolyploid coffee (12, 42, 43)? Genome-wide expression patterns of *C. arabica* relative to its parental species were investigated for ~15,000 duplicated gene pairs (12); evidence was obtained for the modulation of expression-level dominance in the allopolyploid based on growing conditions. Bardil et al. (12) showed that 67% of the genes examined showed nonadditive expression patterns in the allopolyploid, including expression-level dominance and transgressive expression (**Table 1**). However, the authors used microarrays and therefore could not examine homeolog expression bias. Combes et al. (42) surveyed the relative expression of homeologs of *C. arabica* using 13 genes; homeolog expression patterns were highly variable across plant organs and conditions.

Based on RNA-Seq data, 56,000 transcripts were assembled and quantitatively assessed; of these, ~10,000 duplicated gene pairs were investigated for homeolog expression bias and their influence on expression-level dominance on a genome-wide scale (43). This study revealed that the relative contributions of each subgenome to the transcriptome seemed to be altered by growing conditions, suggesting that the polyploid may tolerate a broader range of environmental conditions than do the diploid parents, although this appeared to be unrelated to differential use of homeologs.

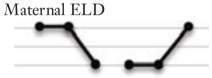
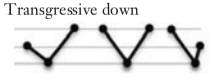
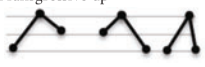
Glycine dolichocarpa

Coate et al. (40) used polysome profiling and RNA-Seq to quantify translational regulation of gene expression in the ~100,000-year-old allotetraploid *Glycine dolichocarpa* (Fabaceae), whose diploid parents are *Glycine tomentella* and *Glycine syndetika*. There was a slight homeolog bias toward *G. syndetika* and close agreement between the allopolyploid transcriptome and what Coate et al. term the translatome, but ~25% of the transcriptome is translationally regulated. Homeolog expression bias observed at the transcriptional level was largely preserved in the translatome. Coate et al. (40) found that translational regulation preferentially targets genes involved in transcription, translation, and photosynthesis, causing regional and possibly whole-chromosome shifts in expression bias between homeologs, and reduces transcriptional differences between the polyploid and its diploid progenitors. Translational regulation correlates positively with long-term retention of homeologs from a paleopolyploidy event, suggesting that it plays a significant role in polyploid evolution.

Gossypium hirsutum

Allopolyploid cotton, *Gossypium hirsutum* ($2n = 4x = 52$) (Malvaceae), formed ~1–2 Mya via hybridization between A-genome (similar to *Gossypium arboreum* and *Gossypium herbaceum*) and D-genome (much like *Gossypium raimondii*) progenitors. This polyploid event ultimately yielded five polyploid species (183). Studies on nonadditive expression have focused on homeolog

Table 1 Comparison of previous studies of genome-wide nonadditive expression patterns in allopolyploids[a]

Expression pattern in allopolyploids	Cotton leaf								Cotton petal				Coffee (22–26C)		Coffee (26–30C)		*Tragopogon* leaf			
	TX2094	%	Maxxa	%	TX2094	%	Maxxa	%	Maxxa	%	Maxxa	%	Java	%	Java	%	*Tragopogon mirus*	%	*Tragopogon miscellus*	%
Nondifferential expression	11,401	46.1	11,032	44.6	13,657	69.1	13,209	65.0	18,323	43.2	4,869	35.4	2,686	23.7	5,112	54.1	22,178	65.8	22,858	64.8
Additivity 	3,480	14.1	3,418	13.8	1,390	7.0	1,242	6.1	3,328	7.8	484	3.5	1,799	15.9	289	3.1	4,383	13.0	4,718	13.4
Maternal ELD 	3,525	14.2	3,946	15.9	1,519	7.7	1,885	9.3	7,940	18.7	1,365	9.9	1,234	10.9	1,859	19.7	3,348	9.9	3,435	9.7
Paternal ELD 	2,919	11.8	2,470	10.0	1,051	5.3	806	4.0	7,256	17.1	1,340	9.8	2,110	18.6	316	3.3	1,873	5.6	2,342	6.6
Transgressive down 	1,896	7.66	2,145	8.7	1,023	5.2	1,487	7.3	2,973	7.0	86	0.6	1,916	16.9	1,530	16.2	895	2.7	538	1.5
Transgressive up 	1,529	6.18	1,731	7.0	1,135	5.7	1,686	8.3	2,639	6.2	5,592	40.7	1,578	13.9	348	3.7	1,029	3.1	1,368	3.9
Total number of genes investigated	24,750		24,742		19,775		20,315		42,459		13,736		11,323		9,454		33,706		35,259	
Total nonadditive expression		39.9		41.6		23.9		28.9		49.0		61.0		60.4		42.9		21.2		21.8
Technology used (reference)	RNA-Seq (cotton 46A)				RNA-Seq (cotton D)				Microarray (cotton 46A)		RNA-Seq (cotton D)		Microarray				RNA-Seq			
Literature	194				Reanalysis of Reference 194				54		144		12				M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results			

[a]Red text shows biased expression-level dominance (ELD) toward one of the diploid parental species. In the line graphs, each dot represents the maternal parent, allopolyploid, and paternal parent, in order.

expression bias, including homeolog silencing, biased expression, and organ-specific expression differences, variously using a focused approach and a few (1, 2, 4, 53) to many (~1,400 genes) (29, 52, 54, 74) genes. All studies revealed differential homeolog expression in different organs (1, 2, 4, 53), developmental time points (29, 74), and evolutionary stages (29, 52).

Global transcriptome profiling was investigated with respect to expression-level dominance employing microarray [genome dominance (145) and genomic dominance (54)] and RNA-Seq techniques (144, 194). Rapp et al. (145) first introduced the concept of expression-level dominance in allopolyploids and showed that there was a bias in expression-level dominance toward one of the diploid parents. Flagel & Wendel (54) supported the presence of unbalanced expression-level dominance in petals and reported unbalanced homeolog expression (**Table 1**). Transgressive expression was greater in the five allopolyploid species than in a synthetic diploid F_1 hybrid, suggesting that long-term evolutionary processes might play a role in establishing transgressive expression (54). However, these two pioneering studies could not show how expression-level dominance and homeolog expression bias are related because of the limitations of microarrays.

Global expression-level dominance and homeolog expression bias were subsequently surveyed using RNA-Seq technology in leaf (194) and petal tissues (144). Yoo et al. (194) provided support for unbalanced expression-level dominance and homeolog expression bias; 40% of the genes investigated exhibited nonadditive expression in leaf tissue (**Table 1**). Furthermore, the degree of nonadditive expression, including transgressive and novel gene expression, increased over time. Expression-level dominance may be caused by upregulation or downregulation of the homeolog from the nondominant parent (194). However, another study using RNA-Seq (144) did not find unbalanced expression-level dominance or homeolog expression bias, indicating that nonadditive expression might be tissue-specific. They also reported more nonadditive expression (144) than did Flagel & Wendel (54), who investigated the same tissue using microarrays (e.g., 49% versus 61% of the genes examined in microarrays versus RNA-Seq, respectively, showed nonadditive expression; **Table 1**). The data suggest that use of different technologies, as well as different databases, might affect the degree or direction of nonadditive expression reported in an allopolyploid.

Senecio cambrensis

Senecio cambrensis (an allohexaploid; $2n = 60$) (Compositae) originated in the United Kingdom within the past 100 years following hybridization between diploid *Senecio squalidus* ($2n = 20$) and tetraploid *Senecio vulgaris* ($2n = 40$). Genome-wide expression patterns were examined using microarrays (68). Studies of resynthesized *S. cambrensis* revealed that hybridization initially results in genome-wide, nonadditive alterations to parental patterns of gene expression and DNA methylation; genome duplication then results in a secondary burst of transcriptional and epigenetic modification. In natural populations, different origins of the polyploid show genome-wide nonadditive patterns comparable to those seen in synthetics, so it appears that polyploid expression changes may be repeated across multiple origins. In synthetic *S. cambrensis*, phenotypic changes become apparent from the second to fifth generations, and, again, different origins of the polyploid and synthetics have similar patterns of change; evolutionary patterns are repeated.

Spartina anglica

Spartina anglica (Poaceae) is an allo-dodecaploid ($2n = 120$–124) that formed in the past 200 years from the hexaploid parents *Spartina alterniflora* ($2n = 60$) and *Spartina maritima* ($2n = 62$). The parents hybridized both in the United Kingdom and in France, yielding two hybrids (*Spartina* × *townsendii* in the United Kingdom and *Spartina* × *neyrautii* in France), but only the event in the United Kingdom yielded the allopolyploid *Spartina anglica*.

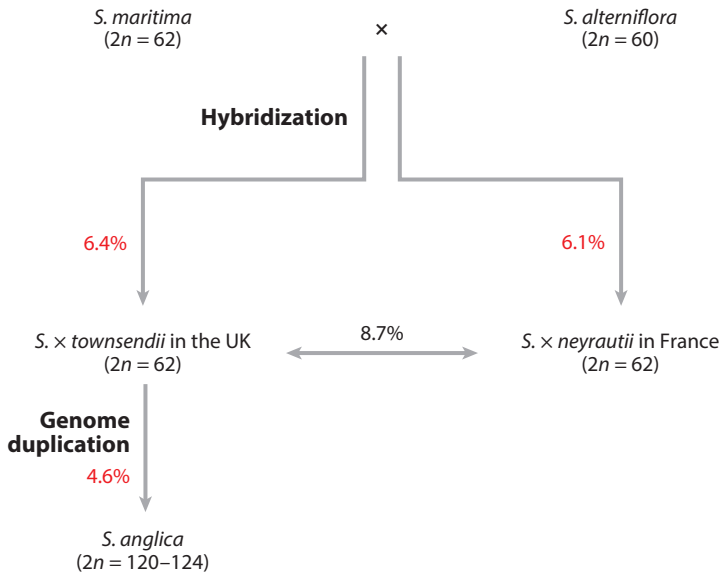


Figure 4

Nonadditive expression in allopolyploid *Spartina anglica*. Red text indicates transcriptomic changes, both parental expression-level dominance and transgressive expression, inferred using microarrays (30, 31). Modified from Reference 6.

Genome-wide patterns of expression change were examined in *Spartina* via microarrays (30, 31). *Spartina* is clonal, and populations of both hybrids are still living. The parental species *S. maritima* and *S. alterniflora* exhibited 1,247 differentially expressed genes (31), most of which were upregulated in *S. alterniflora*. Similar levels of nonadditive parental patterns of gene expression were observed in both of the natural hybrids *S.* × *townsendii* and *S.* × *neyrautii* (6.1% and 6.4% of the analyzed genes were nonadditive, respectively; **Figure 4**). Maternal (*S. alterniflora*) expression-level dominance and transgressively expressed genes were observed in both F_1 hybrids (31). However, maternal expression-level dominance was more pronounced in *S.* × *townsendii* than in *S.* × *neyrautii*, with ~8.7% of the genes differentially expressed between these two F_1 hybrids (**Figure 4**) (6). Hence, the two independent hybridizations yielded different consequences in terms of gene expression. There are also phenotypic differences between the two hybrids.

Genome duplication in *S. anglica* resulted in additional transcriptome changes (**Figure 4**) (6), with an attenuation of the maternal expression-level dominance observed in the F_1 hybrid as well as an increase in the number of transgressively overexpressed genes (31). Hence, both hybridization and genome duplication have been important, but with different effects on the transcriptome: Hybridization has had a greater impact on gene silencing than genome doubling per se.

Tragopogon

Tragopogon (Compositae) provides textbook examples of recent polyploidy (133, 163–165), with two recently (~80 years old; 40 generations in these biennials) and repeatedly formed natural allotetraploids (*T. mirus* and *T. miscellus*) and their diploid parents (*Tragopogon dubius*, *Tragopogon pratensis*, and *Tragopogon porrifolius*). In both allotetraploids, homeolog-specific patterns were examined using Sequenom (23), and expression-level dominance and transgressive expression patterns were investigated using RNA-Seq (**Table 1**) (M.J. Yoo, J. Koh, A.M. Morse, J.L.

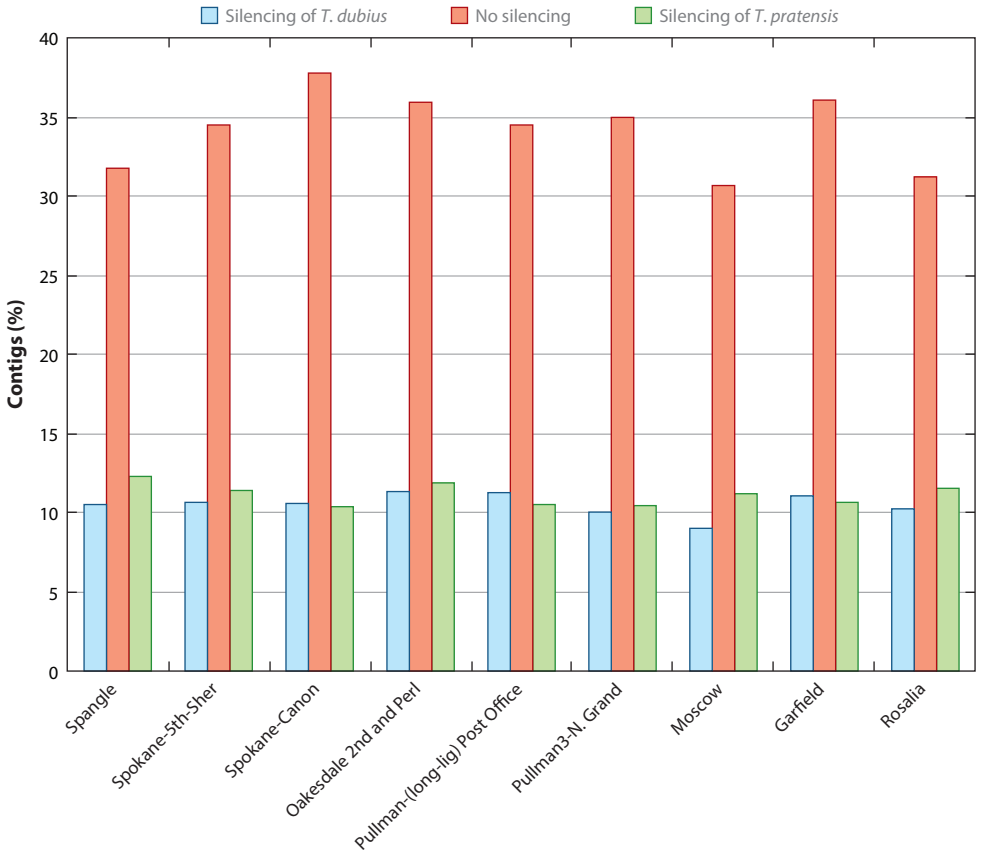


Figure 5

Evolution of genome-wide expression is repeated across natural populations of *Tragopogon miscellus*. Approximately 40–45% of all loci (contigs) show evidence of homeolog loss in *T. miscellus* (not shown); of the remaining 55–60% of all loci, most show equal expression of both parental homeologs, with nearly equal silencing of the two parental homeologs. Overall, approximately 35% of all loci express both parental homeologs, with about 10% of all loci showing silencing of the *Tragopogon dubius* homeolog and about 10% showing silencing of the *Tragopgon pratensis* homeolog. Likewise, populations of *Tragopogon mirus* show similar patterns of gene expression (not shown); however, in *T. mirus*, the *Tragopogon porrifolius* homeologs are silenced to a greater extent (~5–9% of all loci expressed) than the *T. dubius* homeologs (~10–12% of all loci expressed) (I.E. Jordon-Thaden, R.J.A. Buggs, L.F. Viccini, J. Tate, J. Combs, M. Chester, A.V.C. Silva, R. Sanford, S. Chamala, R. Davenport, B. Jordon-Thaden, W. Wu, C.T. Yeh, A. Hu, P.S. Schnable, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, unpublished results).

Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results). In *T. miscellus* the homeolog expression of one parent dominates, and changes occur immediately with hybridization, with a smaller impact of polyploidy per se (22). Significantly, evolution is repeated across natural populations and synthetic lines (**Figure 5**), with similar results for *T. mirus* (I.E. Jordon-Thaden, R.J.A. Buggs, L.F. Viccini, J. Tate, J. Combs, M. Chester, A.V.C. Silva, R. Sanford, S. Chamala, R. Davenport, B. Jordon-Thaden, W. Wu, C.T. Yeh, A. Hu, P.S. Schnable, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, unpublished results).

Genome-wide nonadditive expression patterns were investigated using de novo assembled transcriptomes via RNA-Seq (M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre,

W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results). As in cotton and coffee, *Tragopogon* allopolyploids exhibited unbalanced expression-level dominance (toward the maternal parent): Approximately 21–22% of transcripts investigated were nonadditively expressed in leaf tissue of the two recent allopolyploids (**Table 1**) (M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results), mirroring results for homeolog-specific analyses of *T. miscellus*. However, the degree of nonadditive expression was lower in these recent allopolyploids compared to cotton and coffee, both of which are much older, suggesting nonadditive expression may increase over time, via selection and modulation of regulatory networks (54).

Triticum aestivum

T. aestivum (wheat; Poaceae) is an allohexaploid ($2n = 42$) that arose ~8,000 years ago from cultivated allotetraploid *Triticum turgidum* ($2n = 28$, AABB genome) and diploid ($2n = 14$) goatgrass, *Aegilops tauschii* (D genome). On the basis of ESTs and SNPs, 11 of 90 genes examined initially exhibit homeolog silencing (122); six are from the A genome, two from the B genome, and three from the D genome. In a study of 236 single-copy genes using SSCPs, homeolog silencing was found in 27% of the genes in leaf tissue and 26% of the genes in roots (19). An SSCP-cDNA analysis showed that ~13% of 30 homeologs were differentially expressed in synthetic hexaploid *T. aestivum* relative to the parents of this synthetic line (*A. tauschii* and *T. turgidum*); microarray analysis showed that ~16% of the genes displayed nonadditive expression in synthetic hexaploid wheat (142), and 2.9% of nonadditively expressed genes exhibited transgressive expression, indicating that allopolyploidization per se results in rapid changes in homeolog and gene expression.

In an Affymetrix-based study, 34,000 parent-specific features were detected in wheat, 19% of which showed evidence of nonadditive expression (8). Among those nonadditive expression genes, a bias toward *T. turgidum* parental expression was detected in hexaploid wheat. This bias might be caused by divergent mutations in *cis*- and *trans*-acting regulatory elements. Two other studies also used Affymetrix wheat genome arrays (26, 143), but the synthesized hexaploid wheat lines differed from those used in Reference 8 (see above). Using different lines of the diploid parent, Chague et al. (26) found no global gene expression bias or dominance toward either of the progenitor genomes (S_0 and S_7 generations). However, Qi et al. (143) used different lines of the tetraploid parent and found that the transcriptomes of the synthetic allohexaploid lines (S_4 and S_5 generations) exhibited maternal expression-level dominance. The different results of these studies (26, 143) may reflect the use of different parental lines in producing the synthetic allohexaploid lines. The results for wheat also contrast with *Tragopogon* polyploids, where changes were consistent across different polyploid lines.

Based on RNA-Seq data (98), 650 of 2,356 genes on chromosomes 1 and 5 exhibited differential expression among the three parental homeologs; 55% of those genes showed predominant expression of one homeolog, whereas 45% of the genes were co-upregulated by the other two homeologs (98). However, no global bias in homeolog expression toward one parent was detected, although B-genome homeologs tended to contribute more to gene expression (98). Consistent with a previous study (19), Leach et al. (98) suggested that *T. aestivum* has undergone extensive functional diploidization through homeolog loss and silencing.

Zea mays

Zea mays ($2n = 20$) (maize; Poaceae) is functionally diploid, but a polyploid origin was proposed more than a century ago (96) and is supported by diverse data (149). Although its parental species

are unclear, the presence within maize of two subgenomes indicates ancient allotetraploidy (154, 182). The parental genomes diverged ~12 Mya, but the age of maize is much younger (~5 Mya) (18, 157, 170, 191).

The maize genome has undergone extensive fractionation, or loss of duplicate gene copies (156, 158, 188). However, rather than random losses of one parental homeolog or the other across the polyploid genome, authors argue for biased loss, resulting from differential expression of parental homeologs (see 157 in particular). The pattern of genome dominance (homeolog expression bias), i.e., the biased retention of one subgenome over the other in an allotetraploid, is hypothesized to arise through differential selection against loss of homeologs exhibiting differential expression. For example, loss of a homeolog that contributes more than its share to the RNA pool has a more severe effect on the overall phenotype than loss of a homeolog with lower expression. Consequently, selection will favor loss of lower-expressing homeologs. If the low-expressing homeologs all originate from one parental genome, there is biased gene expression (homeolog expression bias), with the hypothesized outcome being genome dominance following loss of the low-expressing subgenome. Despite evidence of high- and low-expressing homeologs, it is not completely clear that all high-expressing (or low-expressing) homeologs trace to the same parental genome because they are unknown, thus complicating the interpretation.

Repeated patterns of gene expression change. Recent as well as ongoing studies of several polyploid systems beg the question: Are there repeated patterns of gene expression change that are observed? That is, are aspects of evolution repeated following polyploidization? Based on the data now available (reviewed above), the answer appears to be yes and no, depending on the system investigated and how gene expression is measured. In recent natural *Tragopogon* allopolyploids as well as synthetic lines, patterns of gene expression as well as homeolog loss are repeated (163). A smaller data set using older approaches (microarrays) suggests repeated patterns of expression change in independently formed *Senecio cambrensis* lines (68). In natural and synthetic lines of *Brassica napus*, some patterns of expression dominance are correlated with patterns of chromosomal exchanges, whereas other changes in expression may be due to epigenetic or other phenomena (57). Significantly, in synthetic lines of wheat, patterns of expression-level dominance may vary based on the parental lines employed.

Hybridization versus genome doubling. Recent studies of several polyploid systems also permit us to evaluate the critical question: What is more important, hybridization or genome doubling? Based on the studies to date, it appears that both hybridization and genome doubling per se have important consequences in terms of subsequent gene expression. However, only a few studies have been conducted in such a way to permit these two processes to be disentangled, in part because F_1 hybrids are often sterile and short lived. The use of synthetics is crucial for examining hybridization effects relative to immediate genome-doubling effects. Some investigations indicate a particularly important role for hybridization as a major determinant of changes in expression, as in *Arabidopsis* (162), *B. napus* (58) cotton (29, 52), *Senecio* (68), *Spartina* (6), and *Tragopogon* (23, 163). However, in cotton (54, 194) the degree of nonadditive expression was higher in natural polyploid species compared to synthetic diploid and polyploid accessions, suggesting that nonadditive expression might be responsive to long-term evolutionary alteration. Therefore, not only the immediate impact of hybridization and genome doubling must be considered, but also subsequent evolutionary history.

UNDERLYING CAUSAL FACTORS FOR NONADDITIVE GENE EXPRESSION

Nonadditive gene expression in allopolyploids may be due to many controlling factors acting separately or in concert. Among these is parental legacy, or the extent to which differences in gene expression between duplicate copies in an allopolyploid are a legacy of expression differences inherited from the progenitor diploid species (22). This concept, developed decades ago (62), has implications for interpretation of gene expression data today; i.e., differential homeolog expression may not necessarily reflect a departure from parental patterns and should not be attributed to post-polyploidization shifts in expression without careful analysis of parental expression. Thus, observed expression patterns in polyploids may derive from the combined effects of parental legacy and other influences, such as those described below.

Maternal-Paternal Influences

Nonadditive expression may be expected to reflect maternal influences, as cytonuclear incompatibilities have long been known (28, 101, 186, 187). Because organellar genomes are typically maternally inherited in plants (with paternal inheritance of plastids in conifers a notable exception), greater compatibility is observed between the nuclear and maternal, rather than paternal, genomes, at least in angiosperms. Certainly, genes that encode proteins assembled from nuclear and plastid genome components must undergo coordinated expression, and this cytonuclear balance is observed for *rbcS* and *rbcL*, the nuclear and plastid genes responsible for the small and large subunits, respectively, of RUBISCO (61, 159). However, reports attributing large-scale expression differences to maternal effects are rare, most likely because so few data sets have been explored.

Although the degree and direction of nonadditive expression are variable depending on the parentage, tissues, and technology employed, most studies revealed unbalanced expression-level dominance toward the maternal parent, as in cotton (145, 194), coffee grown under hot conditions (12), *S. anglica* (31), *T. aestivum* (143), and *Tragopogon* (21, 23; I.E. Jordon-Thaden, R.J.A. Buggs, L.F. Viccini, J. Tate, J. Combs, M. Chester, A.V.C. Silva, R. Sanford, S. Chamala, R. Davenport, B. Jordon-Thaden, W. Wu, C.T. Yeh, A. Hu, P.S. Schnable, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, unpublished results; M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results). Interestingly, these studies examined gene expression in leaf tissue, which contains many chloroplasts, suggesting that nonadditive expression could be affected by maternal-paternal influence. However, there are exceptions: One synthetic line of cotton (145) and coffee grown under cold conditions (12) both showed unbalanced expression-level dominance toward the paternal parent. Further studies are required, involving more polyploid systems and considering temporal and spatial factors.

The Role of Gene Function

Just as genes encoding proteins with nuclear and organellar components may exhibit biased gene expression, so, too, may genes of different functional classes. Given the cytonuclear patterns described above, it may be that photosynthetic genes, which are composed of nuclear and plastid-encoded subunits, exhibit nonadditive patterns, although these would be due more to their origin than their function per se. However, other classes of genes may consistently exhibit nonrandom gene expression in polyploids. Again, studies that have specifically addressed this question are few, but data may be available to pursue the issue in more detail.

In *A. thaliana*, certain classes of genes, based on Gene Ontology (GO) categories, have been either consistently retained as duplicates or consistently returned to singleton status (reviewed in 56). If expression shifts represent a prerequisite for duplicate gene loss (see below), then we may posit that nonadditive expression characterizes early duplicates of genes encoding structural genes, such as ribosomal protein genes, proteasomal protein genes, and transcription factors, at least in *Arabidopsis* and perhaps its relatives (17, 160). Similar patterns of duplicate gene loss and retention are reported for rice, puffer fish, and yeast (139); species of Brassicaceae (123); and species of *Paramecium* (119), suggesting possible generalities for factors controlling which genes are retained and which are lost from polyploid genomes. However, alternative patterns of gene retention and loss are observed for ancient duplicates in Compositae (13, 21), in which structural genes are retained in duplicate and transcription factors are typically singletons. Preliminary evidence from *Tragopogon* suggests that these alternative patterns in Compositae may begin early in polyploidization. However, these results are based on a small number of genes in *Tragopogon*, a very young polyploid in which loss and expression changes are ongoing. The possible differences in patterns of retention in Compositae compared to other systems certainly require more thorough analysis.

Gene Dosage Balance Hypothesis

Nonadditive expression patterns observed in allopolyploids can be explained by the gene-balance hypothesis (**Figure 6**), which was formulated from classic observations and more recent studies of gene expression modulation in aneuploid and polyploid species (reviewed in 16). This hypothesis states that the stoichiometric differences among members of macromolecular complexes affect the function of the whole complex, eventually leading to phenotypic effects (16). In particular, it has been reported that organisms that experience genome fractionation (diploidization) after WGD (polyploidization) exhibit nonrandom distribution of genes that are lost during diploidization. That is, genes belonging to specific functional classes, such as ribosomal protein genes and transcription factors, were more often retained in duplicate (16, 56, 119, 123, 139).

This concept could be applied to nonadditive expression patterns observed in allopolyploids, specifically homeolog expression bias. Preferential expression of one homeolog can be explained by biased fractionation of subgenomes (**Figure 6*b***). This has been shown in several species, including *A. suecica* (27, 179), *Z. mays* (158), and *B. rapa* (37, 180, 188); the less-expressed parental genome or subgenome experienced more sequence deletion (60), resulting in biased homeolog expression in the allopolyploid (27, 179). However, only one example (*A. suecica*) involves a relatively recent allopolyploid, whereas the others are diploidized, ancient polyploids that experienced extensive fractionation. Thus, more studies are needed to link biased fractionation and homeolog expression bias via detailed analysis of more allopolyploid species, including paleo-, neo-, and synthetic polyploid species (188).

Transgressive expression can be viewed in terms of gene balance. If the expression level of one homeolog is higher than that of the other homeolog, that gene may not function properly because of the imbalance between two members of the gene pair if genes are under functional constraint. Somehow, two homeologs are regulated to be similarly expressed by upregulating or downregulating the counterpart homeolog (**Figure 6*c*,*d***), which can affect gene expression modulation in allopolyploids. However, investigation of the functional categories of these genes is needed to determine whether they belong to specific functions, such as transcription, and structures, such as the signal transduction pathway, the ribosome, and the proteasome, that are known to be under functional constraint.

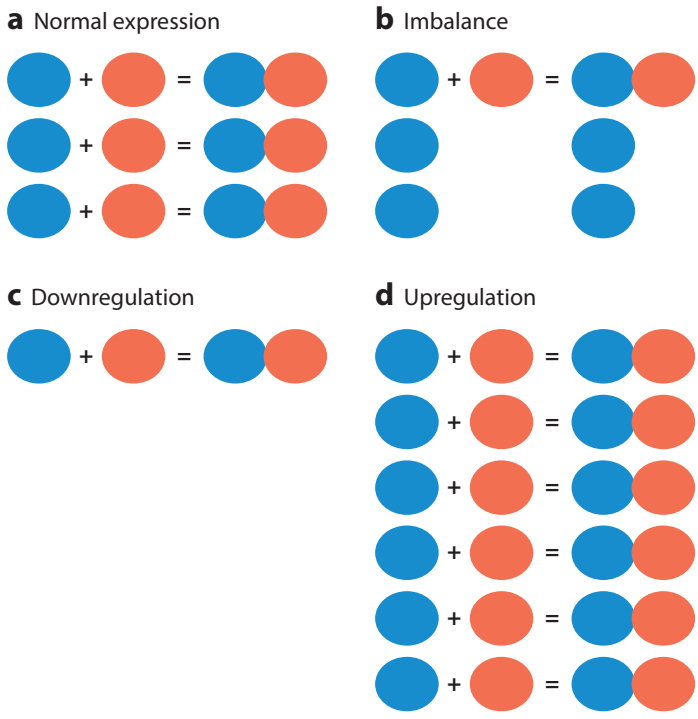


Figure 6

Gene balance hypothesis. Stoichiometry among members of macromolecular complexes is critical for the proper function of the whole complex; for example, (*a*) three dimers of Blue-Red are required for normal expression under the gene balance hypothesis. If these molecules are not constrained under gene dosage, they might be (*b*) relatively easily lost or (*c*) downregulated (transgressive-down), which leads to a decrease in the amount of the dimer. This dimer cannot function properly or find a new function. (*d*) The upregulation of both members (transgressive-up) can cause an increase in the dimer, possibly leading to a new function.

cis- and *trans*-Regulation

Among nonadditive gene expression patterns found in allopolyploids, homeolog expression bias can result from modulation of *cis*- and/or *trans*-regulatory elements. Genome merger combines two divergent regulatory systems and can therefore lead to different patterns of homeolog activation and repression. Two parental genomes that exhibit low sequence divergence, such as those found in coffee [1.35% in 60,000 coding nucleotides (25)], cotton (65, 66), and *Tragopogon* [1.45% in 50,766 coding nucleotides (M.J. Yoo, J. Koh, A.M. Morse, J.L. Boatwright, L.M. McIntyre, W.B. Barbazuk, D.E. Soltis, P.S. Soltis, S. Chen, unpublished results)], may enable cross talk between parental copies of *trans*-elements in resulting allopolyploids. Thus, it is possible that homeologs might be regulated by intertwined mechanisms of *cis*- and *trans*-elements that originated from both parents.

Only a few polyploids have been investigated for *cis*- and/or *trans*-regulatory factors and homeolog expression bias [e.g., cotton (29, 194), *A. suecica* (162), and coffee (43)]. Although *cis*-regulatory changes explain most homeolog expression differentiation in *Arabidopsis* [2,775 of 14,713 gene pairs (19%) (162)] and cotton [15 out of 30 genes (50%) (29)], a combined regulation of *cis*- and *trans*-elements explains the differential expression between homeologs in coffee [774 of 1,434 gene pairs (54%) (43)]. Interestingly, these two different polyploid species exhibit a similar

extent of nondifferential expression between homeologs, 68% and 69% in *A. suecica* and cotton, respectively (162, 194). However, these studies did not examine how *cis*- and/or *trans*-regulatory mechanisms affect overall gene expression levels, such as expression-level dominance and transgressive expression patterns. In addition, studies have so far investigated a relatively small number of genes, ranging from 30 (29) to ~3,000 genes (43). Therefore, a genome-wide approach on the effects of *cis*- and/or *trans*-regulatory elements needs to be conducted in more allopolyploid species.

Neofunctionalization: one member of a duplicate gene pair takes on a new function

Absolute dosage: more protein concentration (more gene product) is beneficial, so duplicated gene pairs are retained, opposing the loss of a duplicated copy

Relative dosage: stoichiometric balance is important for genes involved in networks or multisubunit protein complexes; thus, duplicated gene pairs are maintained

Regulation by Transposable Elements

McClintock (118) noted that interspecific hybridization can bring about genome shock, which results in activation of transposable elements (TEs) and genomic instability. The merged TEs affect gene expression modulation: specifically, nonadditive expression patterns. So far, there is little evidence for gene expression changes by TE transposition or deletion in polyploids. Kashkush et al. (86) showed that gene expression was altered by transcriptional activation of retrotransposons, indicating that epigenetic changes in the expression of TEs might play an important role in gene expression modulation in allopolyploids (136).

Recent studies of TEs in *A. thaliana* have shown that methylated TE insertions can be a cause of downregulation of nearby genes, implying TE methylation not only reduces TE activity but also leads to perturbed gene expression (72, 73). siRNA-targeted TEs are related to reduced gene expression in *A. thaliana* and *Arabidopsis lyrata*, the latter with two or three times more TEs than the former, as well as to gene expression differences between orthologs (73). Study of *B. rapa*, a diploidized paleopolyploid, also suggested that small RNA-mediated silencing of TEs can affect the regulation of nearby genes (188). These authors hypothesized that genes in allopolyploids can have novel expression balance between homeologs based on the coverage of parental transposons. Epigenetic regulation of parental TEs merged in one polyploid nucleus may therefore affect gene expression modulation in allopolyploids.

Frequent methylation around points of TE insertion was reported in allopolyploid *Spartina* (136), and massive methylation changes were detected in specific TEs in synthetic allopolyploid wheat (95), but these studies did not examine how methylated TEs affect gene expression around them. Further study is needed to assess how methylated TEs are associated with gene expression modulation in allopolyploids in a genome-wide manner.

INTEGRATING MODELS OF GENE RETENTION

How Does Nonadditive Gene Expression Relate to Gene Retention? A Case for Pluralism

As described above, nonadditive gene expression can be caused by a wide array of phenomena. One intriguing corollary to the gene balance hypothesis is that these phenomena can be genome wide and inherited through time, with nonadditive expression starting out as a bias in epigenetic silencing and leading to a bias in gene retention patterns (60, 125, 188). To address this scenario, we need to consider what forces might preserve duplicate genes, including neofunctionalization, subfunctionalization, and absolute dosage (selection to increase copy number of gene products) (45). Recently, another means of duplicate retention [relative dosage (selection on relative copy number of gene products)] has been recognized (15, 16, 56). Under relative dosage, genes that are in stoichiometric balance are maintained by selection to avoid the deleterious consequences of having dosage-sensitive genes out of balance. Relative dosage effects of aneuploidy and polyploidy

on phenotype have been known for almost a century, but only recently has it been hypothesized that the selection for stoichiometry is more widespread and may be linked by a unifying hypothesis: the kinetics of multisubunit protein complex formation (16, 177).

Many researchers currently attribute nonadditive gene expression to one of these models (neofunctionalization, subfunctionalization, absolute dosage, or relative dosage). But over time, duplicate genes may have had several of these phenomena acting to preserve them in duplicate, defying strict classification systems. An even more fundamental classification problem is that these models are often difficult to distinguish. For example, although the duplication, degeneration, and complementation (DDC) version of subfunctionalization is nonadaptive (55), the escape from adaptive conflict (EAC) version of subfunctionalization is adaptive (47). This is problematic, as adaptive sequence evolution is also used to classify models of neofunctionalization. In addition, sequence divergence is also compatible with gene balance hypotheses because new effective balances or dosages may evolve. Thus, there need not be only a single force acting on a duplicate gene pair. Bekaert et al. (15) suggested that several mechanisms of preserving duplicate genes could be at play, speculating that relative dosage may be important immediately after polyploidization, and other mechanisms such as subfunctionalization, neofunctionalization, and absolute dosage could be operating later in evolutionary time. Although some researchers continue to explain nonadditive gene expression primarily in terms of neofunctionalization and subfunctionalization (148, 150), we think it important to avoid this simple dichotomy to explain the mechanisms that retain duplicate genes. A more pluralistic framework not only considers additional phenomena (i.e., absolute and relative dosage) but also allows for more complex relationships and interactions of multiple mechanisms that may overlap or change over time (14–16, 44, 77, 117, 177).

Elucidating Mechanisms for Nonadditive Gene Expression Requires More Than Transcriptomes and Genomes: Emergence of Systems Biology

As reviewed above, transcriptomic (RNA-Seq) and genomic data are providing an abundance of evidence concerning nonadditive gene expression and gene loss in polyploids. One common result of these studies is that duplicate genes are often found to have one copy exhibiting higher expression than the other copy at the same tissue or time point or that one copy is expressed and the other copy is not expressed at all in the same tissue or time point, and this evidence is taken to implicate subfunctionalization as the cause (148, 150, 194). In our view, if there are no expression data on related species for inferring the ancestral state, extreme caution should be taken before concluding that neofunctionalization, subfunctionalization, absolute dosage, relative dosage, or some other mechanism is the sole cause of nonadditive gene expression. Although nonadditive gene expression changes from transcriptome data are often used as defining characteristics of subfunctionalization, there are alternative reasons as to why there may be tissue-specific or time-point-specific expression. For example, in diploids, variation in expression may be due to neutral divergence or variation among individuals within a population or across ecotypes. A parallel situation would be similar in paralogs in polyploids and therefore could be due to neutral divergence and not necessarily subfunctionalization, as has been recently claimed (e.g., 148, 150). Again, the divergence among paralogs for subfunctionalization is not inconsistent with dosage balance.

In the context of allopolyploidy, nonadditive gene expression has recently been hypothesized to be a genome-wide phenomenon in which one of the parental diploids is epigenetically dominant over the other diploid, and this sets the stage for longer-term patterns of gene loss, biased fractionation, and genome evolution (60, 125, 188). The hypothesized scenario links how biased epigenetic silencing in recent allopolyploids induces biased changes in gene expression to patterns

of biased gene retention. Additional evidence for this view may perhaps be emerging with possible associations with the evolution of conserved noncoding sequences.

As we now have multiple genomes all possessing the same polyploid event, it is possible to phylogenetically date both nonadditive gene expression and gene losses. Plant biologists can then begin using systems biology tools already being deployed in polyploid yeast and *Paramecium*, where dosage balance has also been found to play a role in duplicate gene retention.

In sum, neofunctionalization and subfunctionalization are often considered as the only two alternatives to consider for duplicate gene retention and nonadditive gene expression. However, the mechanisms for duplicate gene retention include an array of mechanisms that span various types of selection and neutral evolution. In addition to various mechanisms being difficult to distinguish, they may also overlap over time or even be complementary. For example, retention of duplicate genes right after a polyploid event may be due to relative dosage balance, which would allow for a longer period of retention for other forces such as absolute dosage, subfunctionalization, or neofunctionalization to occur (15, 120). This pluralistic framework is timely, given that data from genomes and transcriptomes alone are now being seen as insufficient for explaining nonadditive gene expression, and new systems biology methods and frameworks will allow for new types of investigations and novel explanations of nonadditive gene expression following polyploidy (44).

ALTERNATIVE SPLICING AND NONADDITIVE EXPRESSION

RNA AS occurs after a precursor mRNA (pre-mRNA) transcript forms from template DNA (11, 87, 146, 171). In this process, introns in the pre-mRNA are removed, and exons are reconnected in multiple ways (11, 146) (**Figure 7**). The frequency of AS varies greatly. In humans, >95% of genes are alternatively spliced, whereas in *A. thaliana*, 61% of intron-containing genes show AS. AS can influence gene expression on several levels: (*a*) AS creates multiple forms of mRNA from a single gene, which then create multiple types of protein isoforms; (*b*) studies in *Arabidopsis* (50, 84, 130) indicate that AS could influence mRNA stability through the nonsense-mediated decay pathway; and (*c*) studies in *Arabidopsis* and rice suggest that AS can modulate mRNA stability and translation through miRNA regulation (71, 121, 172, 192, 193).

Studies of AS in plants have progressed rapidly with the development of high-throughput approaches, such as RNA-Seq and large-scale microarrays. In *Arabidopsis*, AS frequency was first estimated using EST data as 1.2% of total intron-containing genes (199), whereas in a recent genome-wide analysis, this frequency increased to 61% (116). Plant species for which AS analyses are under way include *A. thaliana* (116), *Oryza sativa* (195), *Z. mays* (107), *Solanum tuberosum* (111), *Physcomitrella patens* (189), *T. aestivum* (174), *Brachypodium distachyon* (178), *B. napus* (198), *G. raimondii* (108), and *Glycine max* (152).

Although the important role of AS is now appreciated in eukaryotes (e.g., 146, 147, 171), few studies have analyzed the impact of either gene duplication or polyploidy on AS. Fractionation, neofunctionalization (131), and subfunctionalization (55, 78) are important processes that occur following polyploidy, but the impact of these on alternative transcript processing after WGD is unknown. Early studies suggest a negative correlation between AS and genome duplication and that alternatively spliced isoforms between gene duplicates may differ dramatically (80, 169, 173). More recent investigations suggest a more complex correlation between AS and gene duplication (32, 82, 151). AS frequency decreases significantly with the increase of family size, whereas among singletons and small families, AS frequency may increase. Consistent with the notion that duplication may affect AS potential, Zhang et al. (197) found that exonic splicing enhancers and silencers rapidly diverge after gene duplication, whereas Santos et al. (155) present evidence of isoform loss

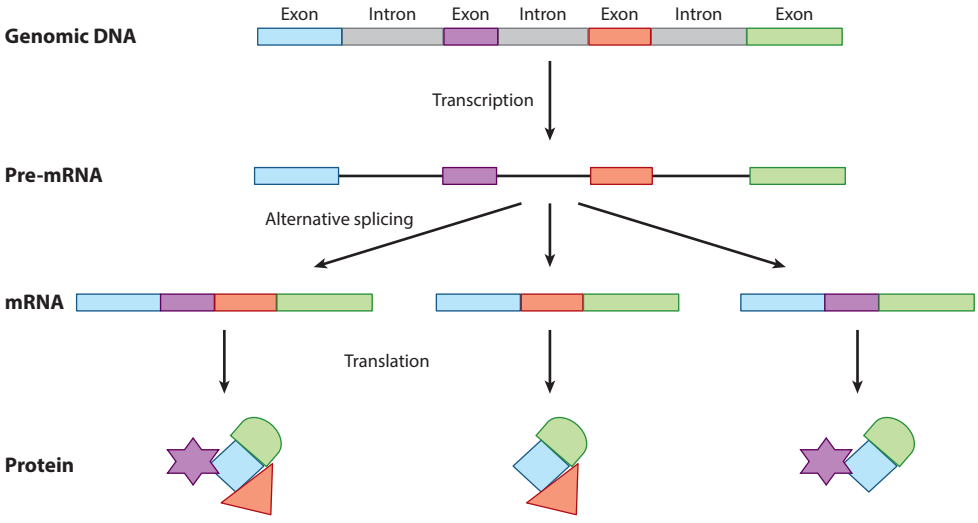


Figure 7

RNA alternative splicing process, from single gene to multiple types of protein, using exon skipping as an example. Colored and gray bars indicate exon and intron, respectively.

and neofunctionalization after duplication. Zhang et al. (196) provide evidence of divergence of AS patterns following gene and genome duplication in *Arabidopsis*; some of the differences occur in an organ- or stress-specific manner.

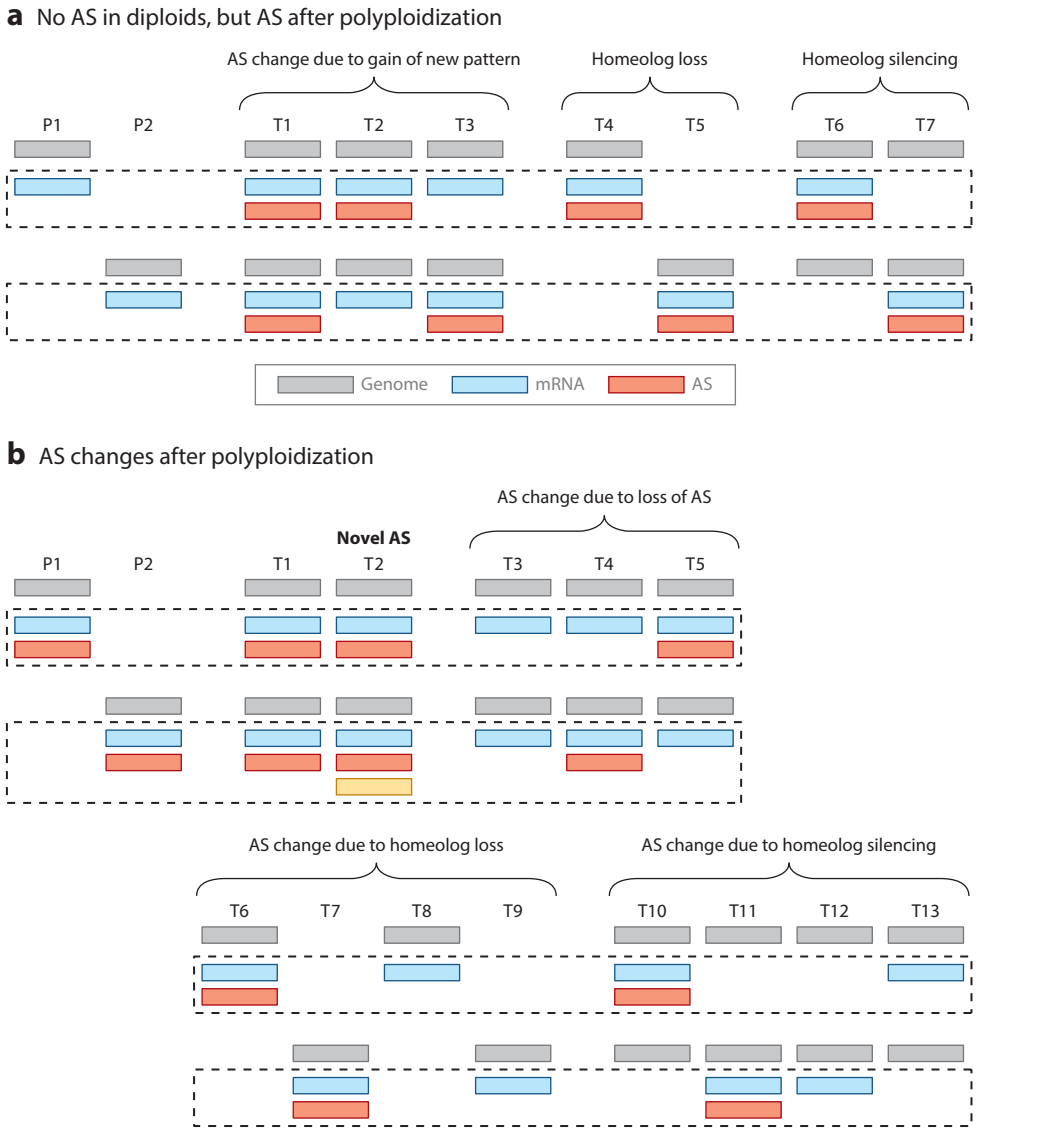
Allopolyploidization could inhibit the efficient splicing of the *DREB2* homolog *WDREB2* in hexaploid wheat (174). In *B. napus*, 16 of 82 AS events (20%) showed AS changes associated with polyploidy, indicating that AS patterns can change rapidly after genome doubling (198). Also, two independently synthesized tetraploid lines showed parallel loss of AS events after polyploidy, which indicated that some changes may be repeated after polyploidization (198).

More investigations of AS following polyploidy are clearly needed. A new set of major questions can now be posed: Given that AS increases proteomic flexibility, what occurs following

Figure 8

(*a*) No alternative splicing (AS) events are present in the diploids at a given gene, but a new AS isoform is present after polyploidization: gain of AS in homeologs from at least one parent. T1–T3: gain of AS in one or both homeologs without homeolog loss/silencing. T4–T5: AS pattern changes associated with homeolog loss; only one homeolog could be found in the genome, constitutive splicing (CS) mRNA products, and AS mRNA products. T6–T7: AS pattern changes associated with homeolog silencing; homeologs from both parents could be found in the genome, but only one homeolog could be found in CS mRNA products and AS mRNA products. (*b*) AS events were observed in both diploids at a given gene, and the AS pattern changes after polyploidization: gains or losses of AS in the homeologs from the different parents. T1: no change in homeolog AS patterns; homeologs from both parents could be found in CS mRNA products and in AS mRNA products. T2: gain of novel AS isoform; homeologs from both parents could be found in CS mRNA products and in AS mRNA products. T3–T5: loss of AS in one or both homeologs without homeolog loss/silencing; homeologs from both parents could be found in the genome and CS mRNA products, but only one homeolog could be found in AS mRNA products. T6–T9: AS pattern changes due to homeolog loss; only one homeolog could be found in the genome, CS mRNA products, and AS mRNA products. T10–T13: AS pattern changes due to homeolog silencing; homeologs from both parents could be found in the genome, but only one homeolog could be found in CS mRNA products and AS mRNA products.

polyploidy? Are both parental AS profiles maintained? Does one parent dominate? How much novel AS occurs? Considering the differing homeologs contributed by the diploid parents to an allotetraploid, several possible AS patterns might occur in an allopolyploid (**Figure 8**). If no AS event is detected in the diploid parents, new AS events could occur in one or both homeologs after polyploidization (**Figure 8*a***, T1–T7). If the diploid parents have AS events, the polyploid may retain, lose, or gain novel AS events in one or both homeologs (**Figure 8*b***, T1–T13). Homeolog loss/silencing after polyploidization could also change AS patterns, and the splicing transcripts caused by homeolog loss and homeolog silencing could be the same (e.g., T4 versus T6 in **Figure 8*a***; T6 versus T10 in **Figure 8*b***; transcripts are showed in dashed boxes in **Figure 8**).



FUTURE STUDIES

More Homeolog-Specific Data and More Taxa

Although numerous studies have provided many new insights into the genetic and genomic consequences of polyploidy, we still know very little about patterns of nonadditive gene expression. In large part, this is because appropriate data that distinguish the patterns of individual homeologs are available only for a few polyploid species. Furthermore, the data available represent only a few angiosperms: We need more data for angiosperms, and we have no data for nonangiosperm lineages. Given the importance of polyploidy in ferns and lycophytes, these lineages also require study. With new technologies, it should be possible to obtain homeolog-specific expression data for more polyploids, and not just for a few well-studied models, enabling the search for broader patterns.

Do Differences in Expression Translate to Phenotype (i.e., the Proteome)?

Many studies reveal that polyploid genomes undergo major chromosomal, genomic, and genetic changes (e.g., 6, 20, 21, 23, 34, 38, 49, 57, 68, 81, 184, 190). Despite great progress in clarifying the genomic and transcriptomic changes that accompany polyploidization, few studies have explicitly correlated these to changes in phenotype (but see 57). New "omics" technologies can now be used in a high-throughput manner to investigate phenotypic changes, including the proteome, metabolome, and important agronomic traits that can be measured in phenotyping facilities.

The impact of WGD on the proteome has been one of the first applications of these technologies (9, 10, 24, 59, 75, 76, 91, 93, 128). Given that the functional states of proteins directly affect molecular and biochemical events that determine phenotype, investigating how changes in genomes, gene expression profiles, and AS events relate to protein-level changes is essential for understanding more fully the molecular and evolutionary consequences of polyploidy, including molecular, biochemical, and physiological mechanisms that ultimately result in evolutionary change. However, the proteome is not necessarily a complete reflection of what is transcribed. A comparison of transcriptomes and proteomes revealed that ~62% of the transcript changes did not reflect differential protein abundance in resynthesized *B. napus* relative to its diploid progenitors (115). This discordance between transcriptome and proteome was also reported from other polyploids, including cotton (76) and synthetic *A. suecica* (128). However, recent studies suggest that an underestimation of mRNA and/or protein abundance may have contributed to the poor correlation between transcriptome and proteome (106, 129). Indeed, the cited studies examined the steady-state levels of RNA and protein. It is possible that the rates of synthesis of proteins might be the critical aspect for assembly of enzymatic and structural features of the cell that ultimately impact how WGD fractionates over time. Improved technology will soon help to better quantify the transcriptome versus proteome, providing new insights into the correlation between gene and protein expression.

What Is the Role of Alternative Splicing?

Studies of diverse eukaryotes reveal the enormous importance of AS in increasing protein flexibility. However, few data are available for polyploids: This is a crucial area of future research. Compared with singletons, a duplicated gene could result in a further increase in AS (32, 82), thus yielding additional genetic and protein flexibility. Extensive gene duplication via polyploidy could provide even greater opportunity for AS and protein diversity. Data on AS in polyploids

are limited; a single study has found slightly decreased AS in synthetic *B. napus* (198), but more studies are needed to determine the generality of this result.

During polyploidization, genomes from different species are merged to form one new genome. During this process, immediate changes in the abundance, composition, and activity of splicing factors could occur (11, 146, 171). Such changes and further changes to splicing sites could affect the presence and absence of AS in polyploids. Predicting the presence and absence of AS isoforms after polyploidization becomes complicated. First, AS could create new transcript isoforms and hence change the protein's amino acid sequence and domain arrangement. As a result, subcellular localization, stability, and function of the resulting protein may differ (11, 147). Second, AS can regulate transcript levels through the nonsense-mediated decay (NMD) pathway (146, 171). After AS, new premature termination codons could be introduced into transcript isoforms; these then trigger NMD pathway degradation. Third, AS could also modulate miRNA-mediated regulation of gene expression via retention or loss of miRNA target sites in some AS isoforms, and via the regulation of splicing of pri-miRNAs (primary transcripts of miRNAs) (146).

All of these consequences may affect the adaptation and evolution of a polyploid species in response to different environmental conditions (171). Although more work is needed to determine the frequency of AS events after polyploidization, current studies suggest that AS change is a nonrandom response to polyploidization (198). There may be some parental homeolog preferences, as well as tissue-specific and stress-specific AS patterns, in a polyploid plant. To investigate the role of AS after polyploidy, more quantitative and functional data are necessary. Currently, such data for a given AS isoform are not available for polyploid plants. One obvious reason for these limitations is the lack of well-annotated genome references for most polyploid plants, especially nonmodel species. By applying high-throughput sequencing and computational approaches, and perhaps a plant in vitro splicing system, AS may be more readily addressed in the near future. We have only scratched the surface in terms of the role of AS in gene expression following polyploidy.

Taking Complexity into Account

As reviewed, multiple factors are involved in nonadditive gene expression; these are not easily distinguishable because they may overlap, interact, shift, and vary temporally and spatially in regulating gene expression. This complexity challenges researchers to elucidate an explicit mechanism for nonadditive gene expression in allopolyploids—this represents one of the major avenues of future research needed. Therefore, a pluralistic view is very important for exploring nonadditive gene expression in which duplicate genes might have been preserved by interaction of multiple overlapping and shifting mechanisms over time.

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