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An analytical framework to understand regulatory novelty accompanying allopolyploidization

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Abstract

Allopolyploidy is a prevalent process in plants, having important physiological, ecological, and evolutionary consequences. Massive, genome-wide transcriptomic rewiring in response to genomic merger and doubling has been demonstrated in many allopolyploid systems, encompassing a diversity of phenomena including homoeolog expression bias, genome dominance, expression-level dominance, and revamping of co-expression networks. Here we present an analytical framework to investigate expression change in allopolyploids as governed by distinct sets of intra- and inter-subgenome *cis*-*trans* relationships. The analytical framework devised is a novel extension of classic allele-specific expression analysis to incorporate and distinguish the separate effects of parental regulatory as well as expression differences in *cis* or *trans*, while providing the conceptual basis and tools to reconcile various patterns of regulatory novelty following hybridization and allopolyploidy.

Keywords: allopolyploidy, allele-specific expression (ASE), *cis* and *trans*, homoeolog expression bias, non-additive expression, expression-level dominance

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Polyploidy, or whole-genome duplication (WGD), is exceptionally common in plants, having important physiological, ecological and evolutionary consequences (Stebbins 1940; Levin 1983; Ramsey and Schemske 2002; Leitch and Leitch 2008; Van de Peer et al. 2009; Madlung 2013; Soltis et al. 2014; Van de Peer et al. 2017; Soltis and Soltis 2016). Two types of polyploidy have long been recognized, autopolyploidy, resulting from the multiplication of one progenitor chromosome set, and allopolyploidy, involving hybridization and duplication of divergent parental genomes, classically from different species (Wendel and Doyle 2005). Allopolyploidy in particular is thought to provide avenues for regulatory novelty and hence phenotypic innovation, as evidenced by myriad non-additive and non-Mendelian responses, including gene loss and silencing (Schnable et al. 2011; Freeling et al. 2012; Liu et al. 2014; Mirzaghaderi and Mason 2017; Koh et al. 2010; Szadkowski et al. 2010; Tate et al. 2009; Anssour et al. 2009; Buggs et al. 2009; Eilam et al. 2009), activation of transposable elements (Senerchia et al. 2015; Parisod et al. 2010; Kawakami et al. 2010), epigenetic modifications (Song et al. 2017; Jackson 2017; Madlung et al. 2002; Chen 2007; Salmon et al. 2005; Rapp and Wendel 2005; Bottley 2014; Fulnecek et al. 2009; Kovarik et al. 2008; Yu et al. 2010; Shcherban et al. 2008), and massive, genome-wide transcriptomic rewiring. The latter encompasses a diversity of phenomena, including biased expression of homoeologs on a genic (Yoo and Wendel 2014; Flagel et al. 2008; Akama et al. 2014; Combes et al. 2013; Wang et al. 2016) or even genomic ("genome dominance") scale (Edger et al. 2017; Yang et al. 2016; Zhang et al. 2015; Flagel and Wendel 2010; Schnable et al. 2011; Cheng et al. 2016; Garsmeur et al. 2014), the poorly understood phenomenon of "expression level dominance" (Rapp et al. 2009; Yoo et al. 2013; Grover et al. 2012; Zhang et al. 2016; Akhunova et al. 2010; Liu et al. 2014), and the modification of duplicated gene co-expression networks as reviewed in Gallagher et al. (2016).

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A hallmark of all of these phenomena is deviation from vertical transmission of preexisting patterns, or the "parental legacy", inherited from the two progenitors (Buggs et al. 2014). These deviations collectively represent regulatory novelty that either accompanied or evolved following genome merger and doubling.

Notwithstanding this progress with respect to the scope and scale of expression alteration accompanying allopolyploidization, there remains a need to further develop the conceptual foundation for understanding gene expression evolution in allopolyploids. Here we propose a new analytical framework based on consideration of the duplicated sets of *cis-trans* relationships that are formed via allopolyploidy, which create both intra- and inter-genomic possibilities. We extend to the polyploid level the classical allele-specific expression analysis (ASE) model in diploids, which aims to distinguish both parental and newly formed *cis*- and *trans*-regulatory divergence. Our model provides a conceptual basis and methods to reconcile various patterns of regulatory novelty following hybridization and allopolyploidy.

As illustrated in Fig. 1 (upper left panel), we use the cotton (*Gossypium* L.) allopolyploid system as an example. Allotetraploid ("AD genome") cottons originated ~1-2 million years ago from a hybridization event between two diploid species ("A" and "D") followed by whole-genome duplication (Wendel and Grover 2015; Wendel and Cronn 2003; Wendel et al. 2010). The descendants of the parental diploid species remain extant ("A2" and "D5"), from which a synthetic F1 hybrid was generated and has previously been used to disentangle expression changes due to hybridization from those arising later from polyploidy and subsequent evolution (Yoo et al. 2014; Flagel et al. 2008; Flagel and Wendel 2010). Within the synthetic F hybrid and

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natural tetraploid cottons, the expression of each pair of duplicated genes (homoeologs "At" and "Dt"; t denotes subgenome) is governed by four sets of *cis-trans* relationships, including two intra-subgenome interactions derived from each of the parental diploids, and two newly formed inter-subgenome interactions. Noting that sequence evolution and other forms of immediate genetic changes are not expected in a synthetic F1 hybrid, our analytic model allows new mutations to be introduced into both the *cis* and *trans* elements in natural allopolyploids, as a possible source of expression novelty.

Although *cis* and *trans* regulatory divergence between species has long been the focus of classic ASE analysis (Wittkopp et al. 2008), the effects of inter-subgenome *cis-trans* interactions in an inter-specific F1 are inherently neglected based on the assumption that *trans* factors act equally on the *cis* elements of both alleles (Fig. 1, right upper panel; see "if inter = intra"). That is, for diploids, orthologous genes that became two alleles of the same gene in a F1 hybrid experience the same *trans* environment as aggregated *trans* regulatory factors of different parental origins. Thus, allele-specific differences in F1 (in the form of allelic expression ratios At/Dt) that mirror interspecific difference between parents, must reflect differences that are linked in *cis* to the gene itself (i.e., mutations in the gene or its flanking regulatory sequences). Additional differences that are observed between orthologs in the parental species (A2/D5 $\neq$ 1), but not within the hybrid (At/Dt = 1), thereby correspond to the effects of *trans* divergence. The combinatorial effect of *cis* x *trans*, estimated as allele-specific differences in the hybrid that do not mirror the patterns observed between parents (i.e. At/Dt $\neq$ A2/D5) has been consistently observed in different hybrid and allopolyploid systems (Shi et al. 2012; Lemmon et al. 2014; Xu et al. 2014; Combes et al. 2015).

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Here we present a conceptual model that considers allelic divergence in these systems to be determined by all four possible sets of *cis-trans* relationships, regardless of whether inter- and intra- interactions control gene expression differently. If the combination of *trans* factors has the same effects for the two co-resident homoeologs, as assumed in ASE analysis, regulatory divergence in the F1 hybrid reflects parental divergence in *cis* only, *or* expression ratios will be equal to 1 due to the identical *trans* effects. If, however, a *trans* factor acts on the *cis* element of its own origin differently from the *cis* element of its homoeolog, unexpected regulatory patterns might be observed that are attributed to divergence between parental regulatory factors. So with respect to allopolyploids (and hybrids), the category of *cis* x *trans* is interpreted to reflect inter-subgenome interactions. Our analytical framework applies the same algebraic inference as classical ASE analysis, but the conceptual difference is nonetheless meaningful. Insight into actual parental divergence (by only *cis* and only *trans* effects) will be obscured if the relative contribution of *cis* and *trans* effects are mistakenly derived from the combinatorial category of *cis* x *trans*. For example, Lemmon et al. (2014) examined the contribution of *cis* regulatory divergence between maize and teosinte by measuring the proportion of F1 allelic divergence in parental divergence (equivalent to $\frac{\mathrm{At/Dt}}{\mathrm{A2/D5}}$ in the cotton example), which masked the fact that over 30% of regulatory divergence in F1 (At/Dt) is in fact attributed to novel interactions rather than only parental divergence in *cis*; instead, regulatory changes attributed to novel interactions (represented by *cis* x *trans*) should be excluded when examining interspecific divergence between parental species. Using instead our analytical framework, changes accompanying genome merger and doubling are conceptually distinguished from intra-genomic genic and regulatory evolution of *cis* or *trans* elements themselves.

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We illustrate this approach in Fig. 1 for synthetic F1 hybrid and natural allopolyploid cotton (upper left panel). The resulting regulatory categories and their relative magnitudes will provide insights into the temporal dimension of evolutionary change between duplicated genes and genomes, i.e., to determine whether significant changes (or even lack of changes) in regulatory divergence are primarily associated with the onset of genomic merger, or with subsequent genome doubling followed by other long-term evolutionary changes. In cotton leaf transcriptomes, 28 and 611 genes exhibited novel expression relative to parental diploids in synthetic F1 and natural allotetraploid cottons, respectively (see Fig. 1 upper left; Yoo et al, 2013); this and previous results (Flagel and Wendel 2010; Flagel et al. 2008) consistently suggest a larger overall effect of expression modulation associated with long-term allopolyploid evolution. One might, for example, test the hypothesis that hybridization introduces regulatory divergence between homoeologs of the 28 genes due to the differences in parental regulatory machineries, whereas subsequent (if evolution proceeded by this route (Ramsey and Schemske 1998; Mason and Pires 2015; Kreiner et al. 2017; Kolar et al. 2017) to allopolyploidy) change, following genome doubling and natural selection, may resolve or amplify some regulatory incompatibility (Sun et al. 2017) while permitting the evolutionary exploration of additional expression space, as for the 611 genes having novel expression. Because homoeolog expression patterns in natural allopolyploid species are further complicated by sequence evolution following polyploidy, it has been difficult to distinguish post-polyploidy effects from immediate changes. Our analytical framework allows the evolutionary genetic changes of parental *cis* and *trans* factors to be modelled and integrated into the analysis of expression evolution accompanying allopolyploidy, bringing us a step closer to understanding longer-term post-polyploidy effects.

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Although several key phenomena that characterize novel patterns of duplicated gene expression have been extensively studied in various plants hybrids and allopolyploids, such as additive and non-additive expression, expression-level dominance, genome dominance, transgressive expression and homoeolog expression bias, as reviewed (Yoo et al. 2014), interpreting these patterns across systems remains difficult due to terminological inconsistency (Grover et al. 2012), among other factors. Conceptual relationships among these different phenomena are not well understood, thereby impeding the synthesis required to uncover the underpinnings of duplicate gene expression evolution. The approach outlined here may facilitate an understanding of the interplay between the foregoing phenomena and how ancestral and newly formed *cis-trans* relationships govern expression evolution accompanying genome merger and doubling. As examples, we highlight four specific questions for which our conceptual framework may find utility:

(1) Is homoeolog expression bias in hybrids or allopolyploids primarily determined by ancestral regulatory divergence between parental species, or does it arise from novel inter-subgenome interactions? Homoeolog expression bias is defined as one of two duplicated genes (homoeologs) being expressed more than the other. As outlined in Fig. 1 (upper right and middle panels), genes displaying homoeolog expression bias are classified as being regulated by *cis* and/or inter-subgenome (*cis* x *trans*) effects. Associating the A-biased and D-biased homoeolog expression patterns with categorized regulatory effects, with respect to the ancestral and newly introduced *cis-trans* interactions, will provide insights into the primary mechanism (by *cis* only divergence or

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by inter-subgenome interactions), both for single pairs of homoeologs and at a genome-wide scale to determine the global balance of A- and D-biases.

(2) What are the *cis* and *trans* regulatory origins of expression level dominance and transgressive expression? Considering the *total* expression aggregated for a pair of homoeologs, expression level additivity is expected in two cases, i.e., if regulatory divergence is determined by only *cis* effects, or when common *trans* effects equilibrate, and hence average, expression of the two ancestral states (Fig. 1, bottom panel). Therefore, non-additive expression patterns, including expression-level dominance and transgressive expression levels, arise from novel inter-subgenomic interactions; thus all four sets of regulatory interactions are involved. That is, non-additive *total* expression patterns are determined by the joint effects of inter- and intra-subgenome interactions, which can lead to large variations in the patterns of non-additive expression, depending on the divergence of parental genomes, the age of allopolyploid events, and condition-specific regulatory interactions. Consistent with this expectation, the fraction of duplicated genes that exhibit non-additive expression varies largely among allopolyploid systems and populations, various tissue types, developmental stages and environmental conditions, ranging from less than 1% to 7% in different allohexaploid wheat species (Chelaifa et al. 2013; Chague et al. 2010), from 23 to 61% among variable cotton tissues (Rambani et al. 2014; Flagel and Wendel 2010; Yoo et al. 2013), and from 42% to 60% under two temperature conditions in coffee (Bardil et al. 2011).

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(3) How is the direction of expression level dominance determined by *cis* and *trans* regulation, and is it related to the direction of homoeolog expression bias? It has been suggested that expression-level dominance toward one parent is mainly caused by up- or down-regulation of the allele of the other parent (Shi et al. 2012; Yoo et al. 2013; Cox et al. 2014; Combes et al. 2015). Taking the A-dominant expression pattern as an example (Fig. 1, bottom panel; see "A-dominant" row), the joint effect of both inter-subgenome interactions and the intra D-genome regulation approximates the effect of intra A-genome regulation; if up- or down- regulation is mainly observed for the Dt homoeolog (as governed by intra D→D and inter A→D), inter-subgenome interactions need to show a stronger effect on Dt than At (inter A→D ≫ inter D→A), indicating that expression level dominance towards one genome (A-dominant) is determined by its predominant *trans* actions (stronger A than D *trans* factor) in inter-subgenome interactions. In other words, previous studies consistently suggest that expression level dominance is associated with the asymmetric effects between two types of inter-subgenome *cis*-*trans* interactions, and preferentially toward the genome acting dominantly in *trans*. Because inter-subgenome interactions can up- or down- regulate the target alleles, the direction of expression level dominance appears *not* to be associated with the direction of homoeolog bias.

(4) Finally, how are gene-to-gene networks rewired by genomic merger and doubling, in terms of the genome-wide collection of inter- and intra- subgenome interactions? As recently reviewed by Gallagher et al. (2016), co-expression network analysis in polyploids not only has the potential to facilitate a better understanding of the complex

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'omics' underpinnings of phenotypic and ecological traits, but also may provide novel insight into the interaction among duplicated genes and genomes. As demonstrated for bread wheat (Pfeifer et al. 2014) and cotton (Hu et al. 2016) development networks, biased expression of homoeologs and asymmetric recruitment of the parental sub-network structures (modules) may be associated with specific functions and phenotypic traits. Because these wheat and cotton studies are based on aggregated co-expression relationships of homoeologs, one future direction is to generate networks considering homoeolog expression separately, thereby allowing the direct evaluation of inter- and intra-subgenome interactions in the network context. Accordingly, examining the derived network topology will reveal the degree of network conservation of ancestral interactions and how the union of two diverged regulatory systems reshapes network architecture through gain and loss of intra- and inter-subgenome interactions, respectively (Conant 2010; Conant and Wolfe 2008; Conant and Wolfe 2006). Although co-expression relationships do not necessarily represent physical interactions between *cis* and *trans* regulatory elements, the gene-to-gene interconnections that are inferred based on the "guilt-by-association" principle will provide an unprecedented opportunity to understand genome-scale transcriptional responses to genome evolution, when co-analyzed with parental *cis*-*trans* divergence, inter-subgenome incompatibility, homoeolog bias and expression-level dominance, as proposed.

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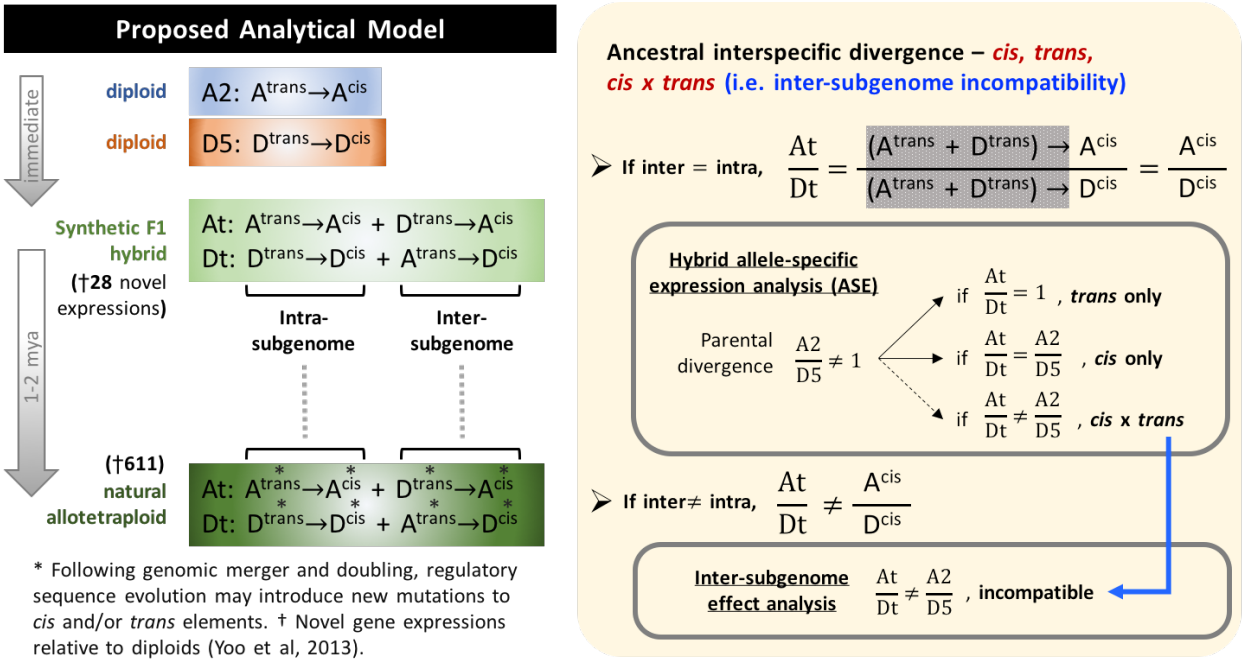

Partitioned homoeolog expression patterns – Homoeolog expression bias

Class	Hypothesis	Analytic model	Interpretation
At bias	At/Dt > 1	$A^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow A^{cis} > D^{trans}\rightarrow D^{cis} + A^{trans}\rightarrow D^{cis}$	Determined by Intra- (ancestral divergence) or inter- effects, or both.
Dt bias	At/Dt < 1	$A^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow A^{cis} < D^{trans}\rightarrow D^{cis} + A^{trans}\rightarrow D^{cis}$	

Aggregated homoeolog expression patterns – Additivity, dominance, and transgressive expression

Expression description	Measure	Model
Total expression (T)	T= At+Dt	$A^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow D^{cis} + A^{trans}\rightarrow D^{cis}$
Parental A-genome expression	A2	$2 \times (A^{trans}\rightarrow A^{cis})$
Parental D-genome expression	D5	$2 \times (D^{trans}\rightarrow D^{cis})$
Mid parental expression	(A2+D5)/2	$A^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow D^{cis}$

Factor of 2 is used to account for normalization across ploidy levels

Class	Hypothesis	Analytic model	Interpretation
Additivity	T = (A2+D5)/2	$D^{trans}\rightarrow A^{cis} + A^{trans}\rightarrow D^{cis} = 0$	inter- effects equal to 0
Transgressive expression	T > max(A2,D5) *or* T < min(A2,D5)	$D^{trans}\rightarrow A^{cis} + A^{trans}\rightarrow D^{cis} > 0$ or $D^{trans}\rightarrow A^{cis} + A^{trans}\rightarrow D^{cis} < 0$	inter- effects NOT equal to 0
A-dominant	T = A2	$A^{trans}\rightarrow A^{cis} = D^{trans}\rightarrow A^{cis} + D^{trans}\rightarrow D^{cis} + A^{trans}\rightarrow D^{cis}$	Asymmetric inter-effects have been consistently reported.
D-dominant	T = D5	$D^{trans}\rightarrow D^{cis} = A^{trans}\rightarrow D^{cis} + D^{trans}\rightarrow A^{cis} + A^{trans}\rightarrow A^{cis}$	

Figure 1. A proposed analytical model for understanding regulatory novelty accompanying hybridization and allopolyploidy, using *Gossypium* diploids A2 and D5, and their derived homoeologs At and Dt. Shown is an extension of the classic allelic-specific expression (ASE) analysis (upper right panel). Applications for understanding homoeolog expression bias (middle panel) and non-additive expression patterns (bottom panel) are illustrated.