Viewpoint

Cis–trans controls and regulatory novelty accompanying allopolyploidization

Summary

Allopolyploidy is a prevalent process in plants, having important physiological, ecological and evolutionary consequences. Transcriptomic responses to genomic merger and doubling have 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. Notwithstanding the foregoing, there remains a need to develop a conceptual framework that will stimulate a deeper understanding of these diverse phenomena and their mechanistic interrelationships. Here we introduce considerations relevant to this framework with a focus on *cis–trans* interactions among duplicated genes and alleles in hybrids and allopolyploids. By extending classic allele-specific expression analysis to the allopolyploid level, we distinguish the distinct effects of progenitor regulatory interactions from the novel intergenomic interactions that arise from genome merger and allopolyploidization. This perspective informs experiments designed to reveal the molecular genetic basis of gene regulatory control, and will facilitate the disentangling of genetic from epigenetic and higher-order effects that impact gene expression. Finally, we suggest that the extended *cis–trans* model may help conceptually unify several presently disparate hallmarks of allopolyploid evolution, including genome-wide expression dominance and biased fractionation, and lead to a new level of understanding of phenotypic novelty accompanying polyploidy.

Polyploidy , or whole-genome duplication (WGD), is exceptionally common in plants, having important physiological, ecological and evolutionary consequences (Stebbins, 1940; Levin, 1983; Ramsey & Schemske, 2002; Leitch & Leitch, 2008; Van de Peer *et al.*, 2009, 2017; Madlung, 2013; Soltis *et al.*, 2014; Soltis & 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 & Doyle, 2005). Allopolyploidy in particular is thought to provide avenues for regulatory novelty and hence phenotypic innovation, as evidenced by myriad nonadditive and non-Mendelian responses, including gene loss and silencing (Anssour *et al.*, 2009; Buggs *et al.*, 2009; Eilam *et al.*, 2009; Tate *et al.*, 2009; Koh *et al.*, 2010; Szadkowski *et al.*, 2010; Schnable *et al.*, 2011; Freeling *et al.*, 2012; Liu *et al.*, 2014; Mirzaghaderi & Mason, 2017), activation of transposable elements (Kawakami *et al.*, 2010; Parisod *et al.*, 2010; Senerchia *et al.*, 2015), epigenetic modifications (Madlung *et al.*, 2002; Rapp & Wendel, 2005; Salmon *et al.*, 2005; Chen, 2007; Kovarik *et al.*, 2008; Shcherban *et al.*, 2008; Fulnecek *et al.*, 2009; Yu *et al.*, 2010; Zhao *et al.*, 2011; Bottley, 2014; Jackson, 2017; Song *et al.*, 2017; Wang *et al.*, 2017) and massive, genome-wide transcriptomic responses. The last of these encompasses a diversity of phenomena (Box 1), including biased expression of homoeologs on a genic (Flagel *et al.*, 2008; Combes *et al.*, 2013; Akama *et al.*, 2014; Yoo & Wendel, 2014; Wang *et al.*, 2016; Wu *et al.*, 2018) or even genomic ('genome dominance') scale (Flagel & Wendel, 2010; Schnable *et al.*, 2011; Garsmeur *et al.*, 2014; Zhang *et al.*, 2015; Yang *et al.*, 2016; Edger *et al.*, 2017), the poorly understood phenomenon of 'expression level dominance' (Rapp *et al.*, 2009; Akhunova *et al.*, 2010; Grover *et al.*, 2012; Yoo *et al.*, 2013; Liu *et al.*, 2014; Zhang *et al.*, 2016) and the modification of duplicated gene co-expression networks (Pfeifer *et al.*, 2014; Gallagher *et al.*, 2016; Hu *et al.*, 2016; Li *et al.*, 2016; Takahagi *et al.*, 2018). A hallmark of these phenomena is deviation from vertical transmission of pre-existing 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 in our understanding of expression alteration accompanying allopolyploidization, there remains a need to develop a conceptual framework that encompasses at least the rudimentary aspects of gene regulatory control. Here we introduce considerations relevant to this framework with a focus on regulatory divergence between parental species, and the implications of this divergence for subsequent changes at the allopolyploid level (or autopolyploids formed from divergent genotypes). This has long been a focus at the diploid level, where regulatory divergence has been formalized in classical allele-specific expression (ASE) analysis (Wittkopp *et al.*, 2004). However, how interactions among duplicated genes and alleles affect gene expression in hybrid and allopolyploid species remain largely unexplored.

As illustrated in Fig. 1(a), we use the cotton (*Gossypium* L.) allopolyploid system as an example, as it is illustrative of many of the model systems used today in studies of polyploidy. 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 & Cronn, 2003; Wendel *et al.*, 2010; Wendel & Grover, 2015). The descendants of the parental diploid species remain extant ('A2' and 'D5'), from which a synthetic F_1 hybrid was generated; this has been used to disentangle expression changes due to hybridization

Box 1 Definition of key phrases used.

Cis- and *trans*- regulation: Gene transcription requires *trans*-acting factors, such as transcription factors (TFs), to operate through sequence-specific DNA binding to their cognate *cis*-acting elements in the vicinity of a gene. A key step in enhancing our understanding of changes in gene expression is to decompose causal factors into *cis*- and *trans*-regulatory components.

Homoeolog expression bias: A common phenomenon whereby there is unequal expression of the two (or more) duplicated copies (= homoeologs) of any given gene in a polyploid, in one or more tissues. Biased homoeolog expression may be evaluated at a genic level, or overall for the two (or more) subgenomes in a polyploid. When homoeolog expression is preferentially biased towards one subgenome, the overall direction of homoeolog expression bias becomes 'unbalanced' (Grover *et al.*, 2012).

Additive and nonadditive expression: A condition of allopolyploid gene expression, referring to the *total* expression of all homoeologous copies, relative to the arithmetic average of the expression levels in parental diploids. Additivity refers to the conservation of averaged parental expression, while nonadditive expression describes various categories of deviation from the parental average, such as expression-level dominance and transgressive expression.

Expression-level dominance: A category of nonadditive expression in an allopolyploid, where the *total* homoeologous expression of a given duplicate gene pair is statistically equal to only one of the diploid parents; for this gene pair, the latter diploid is referred to as the 'dominant' parent. At a genome-wide scale, if the majority of gene pairs in an allopolyploid share the same 'dominant' parent, the allopolyploid is considered to exhibit 'genome-wide expression-level dominance' (Grover *et al.*, 2012). This term is often conflated with the terminologically similar but conceptually rather different concept of 'genome dominance', which describes an observation of biased genomic fractionation following allopolyploid formation (Cheng *et al.*, 2018; Wendel *et al.*, 2018), often associated with biased homoeolog expression.

Transgressive expression: Another category of nonadditive expression in an allopolyploid, where the *total* homoeologous expression of a given gene pair is statistically higher or lower than that of both diploid parents. The former and latter conditions are termed transgressive up-regulation and transgressive down-regulation, respectively.

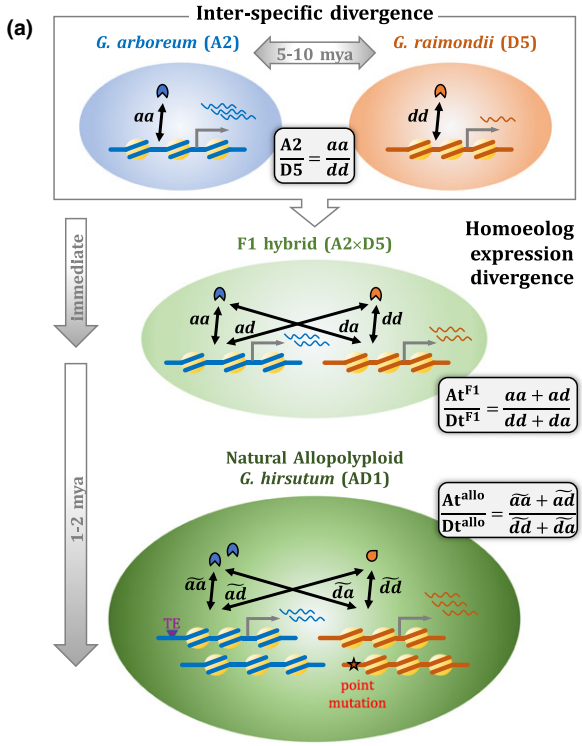
Genome dominance: A phenomenon describing nonequivalence of two (or more) subgenomes with respect to the overall level of gene loss following allopolyploid formation (Cheng *et al.*, 2018; Wendel *et al.*, 2018). The less highly fractionated subgenome is said to be 'dominant', and genes in this subgenome are more likely to have higher gene expression levels than their homoeologs in the more highly fractionated genome.

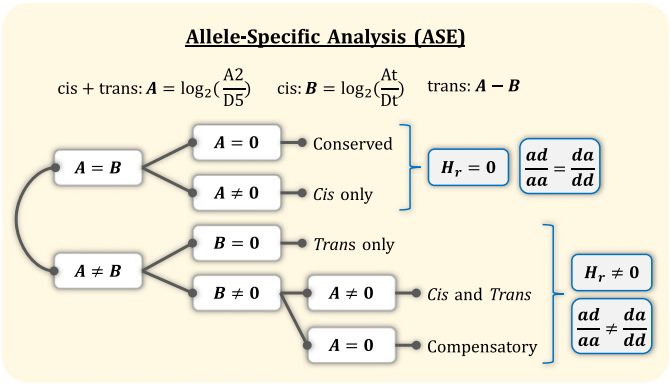
from those arising later from polyploidy and subsequent evolution (Flagel *et al.*, 2008; Flagel & Wendel, 2010; Yoo *et al.*, 2014). For the synthetic F_1 hybrid and natural tetraploid cottons, the expression of each pair of duplicated genes (homoeologs 'At' and 'Dt', with 't' denoting the particular genome in the tetraploid) is governed by four sets of *cis*–*trans* relationships, including two intra-subgenome interactions derived from each of the parental diploids (*aa* and *dd*), and two newly formed inter-subgenome interactions (*ad* and *da*; the first letter indicates genome origin of the *cis* elements, and the second letter indicates *trans* origin).

According to the ASE model (Wittkopp *et al.*, 2004), regulatory divergence acting only in *cis* between the parental diploids will be mirrored as allele-specific expression in the hybrid (At/Dt = A2/D5, where At, Dt, A2 and D5 refer to expression levels for those

Fig. 1 Extended analytical framework for understanding regulatory novelty accompanying hybridization and allopolyploidy, using the cotton (*Gossypium* L.) allopolyploid system as an example. (a) Between the parental diploid species *G. arboreum* (A2) and *G. raimondii* (D5), differential gene expression and/or chromatin accessibility are determined by the divergence of corresponding intra-genome *cis*–*trans* interactions *aa* and *dd*, respectively. Following hybridization, At and Dt homoeolog divergence is governed by two more sets of newly formed inter-subgenome interactions *ad* and *da* (the first letter indicates *cis* origin, and the second letter indicates *trans* origin). In natural allopolyploids, stoichiometric changes accompanying sequence evolution (e.g. transposable element (TE) insertion and point mutation) between parental diploids, and subsequent genome doubling, may further alter *cis*–*trans* interactions (denoted $\widetilde{aa}$, $\widetilde{ad}$, $\widetilde{dd}$ and $\widetilde{da}$). mya, million years ago. (b) Schematic diagram of classic allele-specific expression analysis (ASE). Allelic expression divergence, *B*, in F_1 hybrids provides a readout of relative *cis*-acting activity in a common *trans* environment, whereas expression differences, *A*, between parental species are attributed to both *cis*- and *trans*-acting variation; five regulatory patterns may be distinguished: conserved, *cis* only, *trans* only, *cis* and *trans*, and compensatory. Corresponding interpretations based on hybridization impact H_r (see Eqn 1 and text) and relative inter- vs intra- *cis*–*trans* interactions (*ad*/*aa* vs *da*/*dd*) are noted in the blue boxes. (c) Percentages of parental divergence, homoeolog expression bias and hybridization impact in various plant systems. For example, 31.7% of orthologous genes are differentially expressed between *Arabidopsis thaliana* and *A. arenosa*, and 23.9% of their homoeologs are differentially expressed in their F_1 hybrid; a significant impact of hybridization was inferred for 12.8% of genes when $H_r \neq 0$ based on Eqn 1 (see text). In the last column, the histogram of nonzero H_r is shown for cotton, representing the asymmetric distribution of relative effects of inter- vs intra-subgenome interactions. That is, more homoeolog pairs exhibit a stronger relative effect on At (*ad*/*aa* > *da*/*dd* on the right) than that on Dt (*ad*/*aa* < *da*/*dd* on the left). (d) The process of gene transcription can be summarized by a two-step kinetic model: first, the establishment of chromatin accessibility, and second, transcription factor (TF) binding to accessible regulatory sites to activate transcription. Estimating the impact of allopolyploidization (P_r) for different molecular traits enables hypothesis testing for 'genome dominance'. If the parental conditions in TE adjacency and epigenetic accessibility ($P_r^{epi} \cong 0$) are predominantly inherited to explain the extent and direction of homoeolog expression bias, the TE model (Steige & Slotte, 2016; Bird *et al.*, 2018; Cheng *et al.*, 2018; Wendel *et al.*, 2018) is supported. Not exclusively, if the parental divergence in TF affinity is inherited ($P_r^{TF} \cong 0$) and correlates with homoeolog expression levels, the euchromatin/TF model (Bottani *et al.*, 2018) is supported. (e) Aggregated expression patterns are categorized by the contrasting total homoeolog expression to parental and mid-parental expression levels, which can be interpreted as *cis*–*trans* regulatory interactions.

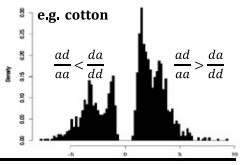
genic copies; Fig. 1b). Any deviations from the parental divergence (i.e. At/Dt ≠ A2/D5) can be assigned to the influence of *trans* variation, either acting only in *trans* (At/Dt = 1, because the common *trans* environment overrides differences in *cis*-regulation between homoeologous copies) or by variants acting both in *cis* and in *trans* (At/Dt ≠ 1). The latter combinatorial effect may also be invisible before interspecific hybridization (A2/D5 = 1 and At/Dt ≠ 1), as *cis* and *trans* variants may be compensatory. Such 'compensatory' patterns have been suggested to result from stabilizing selection in order to conserve gene expression levels





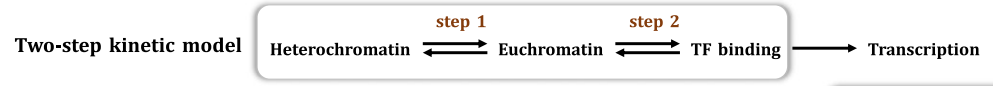
(c)

Species*	Parental divergence ($A \neq 0$)	Homoeolog bias ($B \neq 0$)	Hybridization impact ($H_r \neq 0$)	Histogram of H_r
arabidopsis	31.7%	23.9%	12.8%	
rice	37.7%	28.0%	29.7%	
maize	60.8%	51.1%	50.8%	
coffee	51.4%	33.0%	35.9%	
cotton	10.1%	8.7%	3.7%	

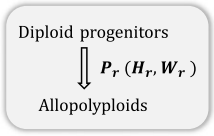


* Data extracted from arabidopsis (Shi *et al.*, 2012), rice (Xu *et al.*, 2014), maize (Lemmon *et al.*, 2014), coffee (Combes *et al.*, 2015), and cotton (Yoo *et al.*, 2013).

(d)



Molecular traits	TE distribution, epigenetic accessibility, etc.	TF affinity
"Genome dominance" hypothesis testing	**TE model:** $P_r^{epi} \cong 0$	**Euchromatin/TF model:** $P_r^{TF} \cong 0$



(e)

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

Expression description	Measure	Interpreted by regulatory interactions
Total expression (T)	T= At+Dt	(*aa* + *ad* + *dd* + *da*)/2
Parental A-genome expression	A2	2*aa*
Parental D-genome expression	D5	2*dd*
Mid-parental expression (M)	(A2+D5)/2	*aa* + *dd*

Class	Hypothesis	Interpreted by regulatory interactions	
Additivity	T = M	*ad* + *da* = *aa* + *dd*	inter- equals to intra- effects
Transgressive expression	T > max(A2,D5) *or* T < min(A2,D5)	*ad* + *da* > *aa* + *dd*, *or* *ad* + *da* < *aa* + *dd*	inter- NOT equal to intra-effects
A-dominant	T = A2	*aa* + *ad* + *dd* + *da* = 4*aa* & *aa* ≠ *dd*	Asymmetric inter-effects have been consistently reported.
D-dominant	T = D5	*aa* + *ad* + *dd* + *da* = 4*dd* & *aa* ≠ *dd*	

during divergence among diploids (Tirosh *et al.*, 2009; Shi *et al.*, 2012). These compensatory stabilizing regulatory processes may, however, give rise to immediate expression novelty following genomic merger, where different *cis* and *trans* factors contributed by two divergent diploids become united in a common nucleus. Although these regulatory patterns have been explored in various plant systems (Springer & Stupar, 2007; Chaudhary *et al.*, 2009; Shi *et al.*, 2012; Bell *et al.*, 2013; Lemmon *et al.*, 2014; Xu *et al.*, 2014; Combes *et al.*, 2015; He *et al.*, 2016), we point out here that the classic ASE model fails to adequately parse the various forms of *cis–trans* interactions that are created by allopolyploidy. As such, advancing our understanding of the molecular and regulatory basis of phenotypic innovations that emerge following allopolyploidization requires this model to be extended.

The classic ASE model masks individual, distinct effects of *cis–trans* relationships

The key assumption of the classic ASE model is that *trans*-acting factors create an environment common to all *cis*-regulatory elements. In the case of allopolyploidy, there are two such suites of *trans*-acting factors at the time of allotetraploid formation. At present, it is unknown how these divergent suites of newly homoeologous factors interact in a common nucleus; one can imagine any number of possibilities for such interactions, ranging from near-redundancy to a variety of forms of oppositional or compensatory regulatory influence. Even for *cis–trans* relationships that were relatively stable during diploid divergence, it may be inappropriate to simply assume additive inheritance in a polyploid nucleus, because parental species do not necessarily share the same regulatory circuits even when their expression outputs are equivalent (Tsong *et al.*, 2006). According to the Hill equation (Chu *et al.*, 2009; Bost & Veitia, 2014), the binding of a transcription factor (TF) to DNA exhibits a nonlinear relationship with the effective concentration of a TF, which is further dependent on the affinity and cooperativity of TF binding (modeled by dissociation constant K and Hill coefficient n, respectively). Upon hybridization, the concentrations of homoeologous TFs may be different from parental values (e.g. parental values of 2 and 4 nM may become homoeologous values of 1 and 2 nM, respectively), the binding affinity to the DNA substrates of the same parental origin may differ from that to the DNA substrates of different parental origin, and the kinetics of either TF binding may be affected by the presence of the other homoeologous copy. Any of these potential changes might lead to a range of transcriptional responses around parental levels, given how each individual set of TF-to-DNA acts. Further complicating these kinds of predictions are the many physical and cell biological properties and parameters that are changed by polyploidy, including cellular and nuclear volumes and other spatial relationships, each of which may alter biochemical kinetics. An additional complexity, not incorporated in classic ASE analysis nor in our proposed framework, is the inherent nonlinearity in gene expression resulting from higher order interactions among genes, TFs and other biochemical phenomena that affect gene expression output (Wright, 1934; Kacser & Burns, 1981; Becskei & Serrano, 2000; Rao *et al.*, 2002; Mangan & Alon, 2003; Fraser *et al.*, 2004; Payne & Wagner, 2014).

Notwithstanding these additional complexities, let us focus specifically on how *trans* regulators of different origins might act on their self-genome and cross-genome targets. For instance, expression of the At homoeolog is determined by its own *cis* elements interacting with both the A- and the D-genome *trans* factors (represented by $aa + ad$), while expression in the diploid parent is attributed to only the *cis–trans* relationships native to the A-genome diploid (aa). Thus, the difference between homoeolog-specific expression (At/Dt) and parental expression divergence (A2/D5) can be modeled as:

$$H_r = \log_2\left(\frac{At}{Dt}\right) - \log_2\left(\frac{A2}{D5}\right) = \log_2\left(\frac{aa+ad}{dd+da}\right) - \log_2\left(\frac{aa}{dd}\right) = \log_2\left(\frac{1+\frac{ad}{aa}}{1+\frac{da}{dd}}\right) \quad \text{Eqn 1}$$

where H_r represents the impact of hybridization on relative homoeolog expression, opposite to how the *trans* effect is estimated in classic ASE analysis (i.e. $trans = \log_2(\frac{A2}{D5}) - \log_2(\frac{At}{Dt})$, as illustrated in Fig. 1(b); thus, $trans = -H_r$). This acknowledges that hybridization inherently affects homoeolog-specific expression in *trans*, dependent on the relative effects of inter- vs intra-subgenome interactions.

Although the foregoing algebraic inference is not substantially different from that of classic ASE analysis, the perspective is nonetheless meaningful. Not only is the impact of hybridization, H_r, conceptually distinguished from how *cis* and *trans* variants contribute to parental divergence, but Eqn 1 also presents a method to quantify how inter-subgenome interactions differentially regulate each homoeolog relative to intra-subgenome interactions (*ad*/*aa* vs *da*/*dd*). As summarized in Fig. 1(c), the magnitude of significant hybridization impact (when $H_r \neq 0$, 4th column from left) is expected to vary across plant systems, which appears to correlate with the amount of expression divergence between parental species (2nd column). A histogram of nonzero H_r, as exemplified for cotton (Yoo *et al.*, 2013), is indicative of asymmetrical regulation by cross-genome interactions; that is, inter-subgenome interactions have a stronger relative effect on one genome than the other, in this case the At rather than the Dt subgenome. This realization focuses attention on inter-subgenome interactions, which are most relevant to gene expression alteration accompanying hybridization *per se*.

Additional modeling and other molecular tools are needed to extend classic ASE analysis to the allopolyploid level

In comparison with the *trans* action of hybridization *per se*, how genome *doubling* alters homoeolog gene expression is complicated by multiple issues of scaling and stoichiometry. With the increase of DNA content accompanying allopolyploidy, imperfect proportionalities and nonlinear relationships with cellular and nuclear

volumes set in motion a cascade of stoichiometric imbalances (among, for example, transcriptional machineries and TFs), which collectively alter gene expression (Doyle & Coate, 2019). Because the physiochemical responses of individual homoeologs vary from gene to gene, it is not yet possible to systematically predict how stoichiometric imbalances triggered by genome merger and doubling will impact regulatory interactions. It does appear, however, that the range of homoeolog-specific expression is increased, as reported in cotton (Yoo *et al.*, 2013), wheat (Wang *et al.*, 2016) and rice (Xu *et al.*, 2014; Sun *et al.*, 2017).

In a *cis–trans* framework, the effects of genome doubling on homoeolog expression, independent of those accompanying hybridization (Eqn 1), may be modeled by contrasting homoeolog-specific expression between the allopolyploid and the corresponding F_1 hybrid, when the latter is available. In this simplified model, only the dosage of *cis* and *trans* factors is doubled in the allopolyploid, whereas the combination of *cis–trans* relationships remains the same as in the F_1 hybrid. The impact of genome doubling W_r is as follows:

$$\begin{aligned} W_r &= \log_2\left(\frac{\mathrm{At}^{\mathrm{allo}}}{\mathrm{Dt}^{\mathrm{allo}}}\right) - \log_2\left(\frac{\mathrm{At}^{\mathrm{F1}}}{\mathrm{Dt}^{\mathrm{F1}}}\right) \\ &= \log_2\left(\frac{\widetilde{aa}+\widetilde{ad}}{\widetilde{dd}+\widetilde{da}}\right) - \log_2\left(\frac{aa+ad}{dd+da}\right) \\ &= \log_2\left(\frac{\widetilde{aa}+\widetilde{ad}}{aa+ad}\right) - \log_2\left(\frac{\widetilde{dd}+\widetilde{da}}{dd+da}\right) \end{aligned} \qquad \text{Eqn 2}$$

where the *cis–trans* interactions in allopolyploids are denoted with tildes, that is, $\widetilde{aa}$, $\widetilde{ad}$, $\widetilde{dd}$ and $\widetilde{da}$. Thus, the emergence of polyploid-specific patterns ($W_r \neq 0$) depends on the alteration of any or all of these *cis–trans* interactions, whereas the problem of how to determine the causal interaction(s) remains inevident from expression data alone. Understanding these interactions requires databases of TF-DNA binding parameters and modeling tools (see review by Teif (2015)), a largely unexplored but promising future direction. On the other hand, the direction and magnitude of W_r, in comparison with that of H_r, provides a mechanistic interpretation for expression novelty that is *not* attributed to the addition of inter-subgenome interactions.

The same notion applies to the overall effect of allopolyploidization, P_r:

$$\begin{aligned} P_r &= \log_2\left(\frac{\mathrm{At}^{\mathrm{allo}}}{\mathrm{Dt}^{\mathrm{allo}}}\right) - \log_2\left(\frac{\mathrm{A2}}{\mathrm{D5}}\right) \\ &= \log_2\left(\frac{\widetilde{aa}+\widetilde{ad}}{\widetilde{dd}+\widetilde{da}}\right) - \log_2\left(\frac{aa}{dd}\right) \\ &= \log_2\left(\frac{\widetilde{aa}+\widetilde{ad}}{aa}\right) - \log_2\left(\frac{\widetilde{dd}+\widetilde{da}}{dd}\right) \end{aligned} \qquad \text{Eqn 3}$$

which ensues from the full spectrum of genetic changes, stoichiometric responses, dosage effects and epigenetic remodeling. How these changes collectively affect regulatory interactions is relevant to several of the principal generalizations about gene expression in allopolyploids. For example, under what circumstances do these interactions preferentially shift homoeolog expression ratios towards one progenitor or the other (e.g. more $\frac{\mathrm{At}^{\mathrm{allo}}}{\mathrm{Dt}^{\mathrm{allo}}} > 1$ than $\frac{\mathrm{At}^{\mathrm{allo}}}{\mathrm{Dt}^{\mathrm{allo}}} < 1$)? In other words, how might altered *cis–trans* interactions in allopolyploids account for 'genome dominance' (Schnable *et al.*, 2011)? Similarly, how might this perspective shed light on the observation of preferential or biased transcription of one of the co-resident genomes in an allopolyploid ('unbalanced homoeolog expression bias' at the genomic scale; Grover *et al.*, 2012)?

One possible insight is offered by (Bottani *et al.*, 2018), who demonstrated how regulatory variation in TF binding and chromatin state can propagate to the level of differential expression between homoeologs. At the single-gene level, when homoeologs are regulated by a common set of TFs, parental differences in binding site affinity to TFs (modeled by dissociation constant K of the Hill equation), rather than in TF expression levels, were shown to be a key driver of differential transcriptional response. Bottani *et al.* (2018) presented a two-step model to interrogate the causal mechanisms of expression bias (Fig. 1d). Given that TF binding first requires the chromatin region to be accessible, and considering the existence of nonfunctional TF binding sites (Spivakov, 2014), the authors suggest that the parental genome with larger euchromatic content is likely to display higher functional binding affinity, in order to override the higher number of accessible but nonfunctional binding sites. Thus, following genomic merger and doubling, genes that harbor binding sites with high affinity become preferentially expressed, hence becoming the 'dominant' subgenome in allopolyploids. Despite the support offered by this mathematical model and simulation (Bottani *et al.*, 2018), the underlying biology remains largely unexplored. Do homoeologous chromatin states mainly reflect the *cis* divergence of parental euchromatic contents, or are *trans* effects on chromatin important? How do hybridization (H_r) and polyploidization (P_r) affect TF binding, and how does this correlate with both the upstream chromatin context and the downstream effects on gene expression? By dissecting the overall regulatory repertoire of gene expression into these separate *cis* and *trans* components, we can gain insight into the temporal and causal relationships of genetic and epigenetic variation in hybrids and allopolyploids.

The foregoing questions provide a scaffolding for a promising experimental agenda, one that focuses molecular biological tools using the perspective of the modified *cis–trans* framework presented here. One such example was recently shown for F_1 hybrids in mice (Wong *et al.*, 2017), in which the *cis* and *trans* contributions to TF binding occupancy and H3K4me3 enrichment were studied using ChIP-seq; the integration of these data sets revealed the interplay and coordination of multiple layers of regulatory changes. In plants, a spectrum of technologies is available to interrogate TF binding to promoters (Landt *et al.*, 2012; Weirauch *et al.*, 2014; Bartlett *et al.*, 2017; Jin *et al.*, 2017) and, similarly, a range of chromatin assays (Celniker *et al.*, 2009; Zentner & Henikoff, 2012; Lane *et al.*, 2014; Jiang, 2015; Lu *et al.*, 2017) permit the assessment of the relative accessibility of homoeologs and orthologs to the transcriptional machinery. A recent example is from maize, where chromatin states were connected with biased fractionation following an ancient polyploid event (Renny-Byfield *et al.*, 2017).

We speculate that the integration of chromatin interrogation technologies with expression data, using the conceptual partitioning described here, will facilitate a deeper understanding of duplicate gene behavior in hybrids and polyploids.

The extended *cis–trans* framework and expression patterns in allopolyploids

It is worth noting that the euchromatin/TF model by Bottani *et al.* (2018) is, to some extent, congruent with the prevailing explanation for biased homoeolog expression and biased genome fractionation, which is framed in terms of the 'genomic legacy' of transposable element (TE) differences contributed by the two diploid parents (Steige & Slotte, 2016; Wendel *et al.*, 2018). This explanation, hereafter referred to as the TE model, has emerged in recent years from the accumulating literature on chromatin modification, TE content and small RNA biology (Diez *et al.*, 2014; Springer *et al.*, 2016; Yang *et al.*, 2016; Renny-Byfield *et al.*, 2017; Zhang *et al.*, 2017). Phrased simply, the different parental TE loads and their relative distribution between sub-genomes lead to differentiated epigenetic controls (e.g. small RNA populations and preferential recruitment of epigenetic modifiers) on homoeolog expression. As a consequence, the homoeolog physically closer to epigenetically silenced TEs is more likely to be repressed via localized heterochromatinization, and even lost in the longer term (hence, 'biased fractionation'; see recent reviews (Bird *et al.*, 2018; Cheng *et al.*, 2018; Wendel *et al.*, 2018)).

A key difference between these models, which also makes them complementary to each other, is that the euchromatin/TF model is dependent on parental differences in TF affinities and euchromatin content, whereas the TE model mainly considers differences in chromatin accessibility and gene expression as mediated by parental TE adjacency (Fig. 1d). What the two models share is the requirement of inheritance of differentiated parental conditions, one being TF affinity while the other is TE adjacency. By analogy to studying the impact of allopolyploidy on homoeolog expression ratios (P_r), as defined above (Eqn 3), the effects of inheritance of these parental states can be evaluated, with superscripts denoting the partitioning of mechanistic effects, $P_r^{TF} \cong 0$ for the measure of TF affinity, and $P_r^{epi} \cong 0$ for TE adjacency and/or epigenetic accessibility. In reality, both scenarios are likely to be intertwined in natural situations, and may even be in conflict with each other. For example, two homoeologs may differ in terms of regulator TF affinity (for whatever reason), but the homoeolog with stronger TF binding may still be expressed at a lower level due to a nearby TE insertion. On the other hand, two homoeologs that differ in promoter accessibility may still be equally expressed, if stronger TF affinity is newly gained for the less accessible homoeolog, or the less accessible promoter has gained more functional binding sites since allopolyploidy. Clearly, a co-examination of both scenarios is most likely to uncover the determinative mechanisms for homoeolog expression divergence.

In addition to homoeolog-specific expression patterns of expression bias and genome dominance, other novel patterns of aggregated homoeolog expression have been studied (Box 1), such as additive and nonadditive expression, expression-level dominance, and transgressive expression, as reviewed by Yoo *et al.* (2014). Interpreting these patterns across systems remains challenging due to terminological inconsistency (Grover *et al.*, 2012; Yoo *et al.*, 2014) as well as other factors. Perhaps more germane is the point that conceptual and mechanistic 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 such an understanding, by focusing attention on the interplay between genomic legacy features such as TE adjacency and chromatin state, biophysical interactions such as TF binding efficiency, and how these ancestral as well as newly formed *cis–trans* relationships govern expression evolution accompanying genome merger and doubling. As examples, we highlight two broad questions for which the conceptual framework presented here may find utility.

To what extent do homoeolog expression bias and nonadditivity reflect novel, *cis*/*trans* interactions?

Homoeolog expression bias is when one of two duplicated genes (homoeologs) is expressed more than the other; that is, $\log_2\left(\frac{At}{Dt}\right) \neq 0$. As modeled in Fig. 1(a), four sets of inter- and intra-subgenome interactions are determinative, and even the parental sets may have been altered following genomic merger and doubling. The amount of homoeolog expression bias that resembles parental divergence is relatively consistent among plant species (<20%), whereas the amount of expression bias attributed to cross-genome interactions and other types of alterations is more variable (1.4–37.8%); these estimates were extracted from studies of widely diverged plants – arabidopsis (Shi *et al.*, 2012), cotton (Yoo *et al.*, 2013), maize (Lemmon *et al.*, 2014), rice (Xu *et al.*, 2014) and coffee (Combes *et al.*, 2015). Similarly, to test for expression additivity, it is common to compare *total* expression for a pair of homoeologs ($T = At + Dt$) to the average of parental expression values ($M = \frac{A_2+D_5}{2}$). Because current methods such as RNA-seq rely on per-transcriptome normalization to compare expression level across samples, there is an underlying assumption of equal *transcriptome size*. This assumption, however, probably does not hold in most cases (Coate & Doyle, 2010, 2015; Visger *et al.*, 2017; Doyle & Coate, 2019), due to the multiple stoichiometric and volumetric cascades that affect gene expression following hybridization and doubling. As shown in Fig. 1(e), additive expression patterns are determined by equal effects of the *total* inter-subgenome interactions and the *total* intra-subgenome interactions. which has no direct equivalence with any ASE category (Fig. 1b). Nonadditive expression patterns, including expression-level dominance and transgressive expression levels, arise from all four sets of regulatory interactions, these reflecting complex nonlinear biochemical and biophysical interactions. This may help to explain the large variation in nonadditive expression patterns, ranging from < 1% to 7% in different allohexaploid wheat species (Chague *et al.*, 2010; Chelaifa *et al.*, 2013), from 23% to 61% among variable cotton tissues (Flagel & Wendel, 2010; Yoo *et al.*, 2013; Rambani *et al.*, 2014), and from 42% to 60% under two temperature conditions in coffee (Bardil *et al.*, 2011). Teasing

apart the mechanistic basis of these novel *cis–trans* interactions poses an interesting research challenge for future studies.

How is the direction of expression level dominance determined by *cis* and *trans* regulation?

It has been suggested that expression-level dominance toward one parent is mainly caused by up- or down-regulation of the homoeolog of the 'less dominant' parent (Shi *et al.*, 2012; Yoo *et al.*, 2013; Cox *et al.*, 2014; Combes *et al.*, 2015). Taking the A-genome dominant expression pattern as an example (Fig. 1e, see 'A-dominant' row), the total expression of homoeologs is equal to the parental A-genome expression, which can be interpreted as regulatory interactions $aa + ad + dd + da = 4aa$. If the 'less dominant' Dt homoeolog had been up- or down-regulated, as previously observed, to approach an A-like expression (i.e. $dd + da = 2aa$), the equation requires the effects of inter- and intra-subgenome interactions of At to be equal to each other (i.e. $ad = aa$). This implies that At expression is mainly determined by its *cis* element regardless of the origin of *trans* factors, while at the same time Dt expression is under strong influence of the At *trans* factors. Thus, expression level dominance is likely to be associated with divergent *trans* factors between diploid progenitors, and the progenitor with stronger, more influential *trans* factors will become dominant with respect to *total* gene expression. In this context, it will be interesting to explore whether candidate *trans* factors such as TFs are differentiated between homoeologs in terms of concentrations and affinities. It will also be interesting to evaluate whether the strong *cis* effect of the dominant homoeolog is caused by binding motifs or by chromatin accessibility. Because inter-subgenome interactions can up- or down-regulate target homoeologs, the direction of expression level dominance appears *not* to be associated with the direction of homoeolog bias; it will be interesting to parse the underlying mechanisms of this distinction.

Beyond the gene-centric characterization of expression changes, another relevant and pressing question concerns how gene-to-gene networks are reshaped 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 'omics' underpinnings of phenotypic and ecological traits, but also may provide novel insight into interactions among duplicated genes and genomes. Given that previous work in allopolyploids (e.g. wheat (Pfeifer *et al.*, 2014) and cotton (Hu *et al.*, 2016)) was mainly 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 topological dynamics in terms of gain and loss of intra- and inter-subgenome relationships (Conant & Wolfe, 2006, 2008; Conant, 2010). 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 provide an alternative and parallel approach for understanding the impact of genomic merger and doubling, under the same analytical framework used for genes outside of a network context. Future analyses of gene networks could include integration with parental *cis–trans* divergence, novel cross-genome interactions, and various expression-level phenomena, together with other epigenetic and physiochemical datasets.

In conclusion, the opportunity to advance our understanding of transcriptome dynamics in hybrids and allopolyploids is being enabled by the maturation of multiple 'omics' technologies and conceptual advances, the latter including a focus on the mechanistic underpinnings of intergenomic *cis–trans* interactions, as explicated here. It is likely that these perspectives and approaches will yield new insight into the origin of physiological and phenotypic responses to hybridization and polyploidy, and thereby to the evolutionary process in general.

Acknowledgements

We thank Corrinne E. Grover and Justin Conover for helpful discussion and comments on the manuscript. We are also grateful to Jeff Doyle for his insightful comments on an earlier draft, which prompted us to revise and improve the manuscript. Research in the Wendel laboratory has been funded by the US NSF Plant Genome Research Program and by Cotton Incorporated, whose support we gratefully acknowledge.

ORCID

Guanjing Hu http://orcid.org/0000-0001-8552-7394
Jonathan F. Wendel http://orcid.org/0000-0003-2258-5081

Guanjing Hu and Jonathan F. Wendel*

Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA
(*Author for correspondence: tel +1 515 294 7172; email jfw@iastate.edu)

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Key words: allele-specific expression (ASE), allopolyploidy, *cis* and *trans*, expression-level dominance, homoeolog expression bias, nonadditive expression.

Received, 2 August 2018; accepted, 30 September 2018.