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RESEARCH ARTICLE

Cross-kingdom transcriptomic trends in the evolution of hybrid gene expression

Anna H. Behling[1] | David J. Winter[1] | Austen R. D. Ganley[2] | Murray P. Cox[1]

[1]School of Natural Sciences, Massey University, Palmerston North, New Zealand

[2]School of Biological Sciences, University of Auckland, Auckland, New Zealand

Correspondence
Murray P. Cox, School of Natural Sciences, Massey University, Palmerston North, New Zealand.
Email: m.p.cox@massey.ac.nz

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Abstract

Hybridization is a route to speciation that occurs widely across the eukaryote tree of life. The success of allopolyploids (hybrid species with increased ploidy) and homoploid hybrids (with unchanged ploidy) is well documented. However, their formation and establishment is not straightforward, with a suite of near-instantaneous and longer term biological repercussions faced by the new species. Central to these challenges is the rewiring of gene regulatory networks following the merger of distinct genomes inherited from both parental species. Research on the evolution of hybrid gene expression has largely involved studies on a single hybrid species or a few gene families. Here, we present the first standardized transcriptome-wide study exploring the fates of genes following hybridization across three kingdoms: animals, plants and fungi. Within each kingdom, we pair an allopolyploid system with a closely related homoploid hybrid to decouple the influence of increased ploidy from genome merger. Genome merger, not changes in ploidy, has the greatest effect on posthybridization expression patterns across all study systems. Strikingly, we find that differentially expressed genes in parent species preferentially switch to more similar expression in hybrids across all kingdoms, likely as a consequence of regulatory *trans*-acting cross-talk within the hybrid nucleus. We also highlight the prevalence of gene loss or silencing among extremely differentially expressed genes in hybrid species across all kingdoms. These shared patterns suggest that the evolutionary process of hybridization leads to common high-level expression outcomes, regardless of the particular species or kingdom.

KEYWORDS
comparative analysis, cotton, fish, genomics, molecular evolution, plants, theory, yeast

1 | INTRODUCTION

Hybrid species arise from successful interbreeding between two distinct parental species. Hybridization is ubiquitous across the major eukaryotic kingdoms (Bertioli et al., 2019; Campbell et al., 2017; Edger et al., 2017; Pereira et al., 2014; Schedina et al., 2014; Stukenbrock et al., 2012), despite numerous pre- and post-zygotic reproductive isolating barriers that typically prevent the production of viable offspring from genetically divergent species. Hybridization may occur with (allopolyploidization) or without (homoploid

hybridization) a concomitant increase in ploidy level. Both outcomes involve the merger of genomes from each parental species, causing major disruptions at every level of an organism's cellular biology: genomic (McClintock, 1984; Qin et al., 2016), transcriptomic (Cox et al., 2014; Depotter et al., 2021; McElroy et al., 2017; Yoo et al., 2013), proteomic (Holá et al., 2017; Koh et al., 2012; Ueno et al., 2019) and metabolomic (Jung et al., 2021; Zhang et al., 2019). However, successful adaptation to these challenges can confer new advantages on the hybrid, due to intergenomic heterosis (hybrid vigour) and enhanced genomic redundancy (Adams & Wendel, 2005; Baranwal et al., 2012; Fujimoto et al., 2018; Gu et al., 2003). Therefore, despite their initially improbable persistence, some hybrid species can even outcompete their parental species in the existing environment, or colonize transgressive niches unoccupied by either parent (Kim et al., 2008; Li et al., 2014; Mallet, 2007).

The effects of hybridization on gene expression can be inferred by comparing the expression of orthologs (copies of a gene in each parent) with their corresponding homeologs (parentally derived copies of a gene in the hybrid). Altered patterns of gene expression may be expected following hybridization, including compensatory *cis*- and *trans*-regulatory changes (Fraser, 2019; Landry et al., 2005, 2007; Wittkopp et al., 2004; Zhang & Emerson, 2019). The prevalence of hybrid species has led to considerable interest in the evolution of their regulatory systems. Previous investigations into the evolution of hybrid gene expression have largely focused on single hybrid species (Coate et al., 2012, 2014; Combes et al., 2013; Depotter et al., 2021; Han et al., 2017; Hovhannisyan et al., 2020; Koh et al., 2010; Kryvokhyzha et al., 2019; Matos et al., 2011; McElroy et al., 2017; Schedina et al., 2014; Tronchoni et al., 2017; Wu et al., 2016; Zhang et al., 2015, 2017, 2018). This research has furthered our knowledge of these specific species, but the different approaches and analytical frameworks employed by these studies preclude direct comparisons across different hybrid systems, thus limiting generalizations about hybridism and gene expression more broadly. Moreover, many studies have also focused only on a small number of gene families (Cui et al., 2020; Fulneček et al., 2009; Gong et al., 2014; Wen et al., 2019). Consequently, comparative whole-transcriptome analyses of multiple hybrid systems are rare (Schraiber et al., 2013), although we previously showed that a fungal allopolyploid and a plant allopolyploid share similarities in broad classes of homeolog expression (Cox et al., 2014). Still, to our knowledge, no systematic study of gene expression in multiple hybrid systems across kingdoms and ploidy levels has been undertaken.

Here, we perform a comparative analysis of the transcriptome-wide impact of hybridization on gene expression across three major eukaryotic kingdoms: plants, animals and fungi. We pair representative allopolyploid and homoploid hybrid systems from the same kingdom to investigate the relative effects of hybridization versus an increase in ploidy level on gene expression. To perform transcriptomic comparisons across species with markedly different gene complements, we employ a structured and generalized 'fates of genes' expression framework, rather than bespoke approaches focusing on species-specific genes in each individual system. The benefit of this generalized framework is that it can be applied to hybrid species from any kingdom as long as suitable data are available. We compare homeologs (in the hybrid) and orthologs (in the parents) to show that most genes with expression differences between the parental species have more similar expression in the hybrid species. Our results also support the growing view that hybridization has a greater effect on gene expression than an increase in ploidy. Finally, we highlight the prevalence of gene loss or silencing among extremely differentially expressed genes in hybrids. By transcending the individual species level, these generalizations reveal shared, species-independent patterns of gene expression outcomes following hybridization, regardless of species or kingdom.

2 | METHODS

2.1 | Species chosen for analysis

A broad survey of the literature was performed to identify allopolyploid and homoploid hybrid systems with available RNA-seq and corresponding genomic data (see File S1 for details). Surprisingly few data sets are available. The suitability of systems was assessed using a number of predefined obligatory and preferential criteria:

- Non-normalized RNA-seq data are available for each parent and the hybrid species, extracted from the same tissue, or from cells grown in the same medium (obligatory).
- A genome sequence or gene models are available for at least one of the parental species (obligatory).
- The systems contain naturally occurring, rather than synthetic, allopolyploid or homoploid hybrid species (preferential).
- The systems have minimal phylogenetic distance between the allopolyploid and homoploid hybrids (preferential).
- RNA-seq data with at least two biological replicates are available for each constituent species (preferential).
- Both parental species are extant, as opposed to being close relatives of extinct parents (preferential).

Hybrid systems were required to meet all obligatory criteria to be considered for analysis. For preferential criteria, it was important to minimize phylogenetic distance between the allopolyploid and homoploid hybrid to limit any potential taxon-specific differences observed in gene expression patterns. Data sets with two or more biological replicates were given priority, although they were surprisingly uncommon, particularly for animal systems. We also favoured data sets with longer read lengths, but this was largely dictated by the technologies in use when available data sets were generated.

2.2 | Acquisition and curation of gene sequences

To determine the level of sequence duplication within gene sets and standardize between species, each gene set was submitted to CD-HIT

v4.8.1 (Fu et al., 2012), with the user-defined similarity threshold set to 0.95. The resulting representative sequences from each gene cluster were resubmitted to CD-HIT to confirm a 1:1 gene sequence to cluster ratio.

2.3 | RNA-seq data processing

HYLITE (hybrid lineage transcriptome explorer) v2.0.2 (Duchemin et al., 2015), which has comparable mapping accuracy to related software packages (Hu et al., 2021; Kuo et al., 2020), was used to generate read count matrices for differential expression analysis. HYLITE was chosen over other programs for its ability to perform 'on-the-fly' homeolog SNP detection and read assignment, enabling the analysis of more commonly studied and data-rich hybrid systems such as *Gossypium* and *Saccharomyces*, as well as less common and much more data-poor systems such as *Squalius* and *Epichloë*. Single-nucleotide polymorphisms within the RNA-seq reads, indicative of parental origin, were identified by HYLITE, and subsequently used to classify reads in the hybrid to the parental subtranscriptomes. If a read contained only hybrid-specific and/or masked SNPs, HYLITE classified the read as 'unknown'. If a read could be assigned to either parent but contained no parentally diagnostic SNPs, it was classified as 'uninformative'. RNA-seq read sequences and the CD-HIT-reduced reference gene sequences were provided to HYLITE as input data, in addition to a 'protocol file' that defined the species, their parent–hybrid relationships, the biological replicates and corresponding expression data files. HYLITE used the protocol file to map the RNA-seq reads to the gene sequences with Bowtie 2 v2.3.4.1 (Langmead & Salzberg, 2012). To be consistent with the single-end read sets that were only available for many species, if paired-end reads were available, only the forward reads were mapped.

2.4 | Validation of read count data

Extensive automated and manual validation checks of read count data were performed. Code and example files are available online (https://github.com/annabehling/multi_bowtie).

2.5 | Differential expression analysis

HYLITE read count matrices were processed with hyliter (https://github.com/dwinter/hyliter) and provided as input to EDGER v3.36.0 (Chen et al., 2016; McCarthy et al., 2012; Robinson et al., 2010) for differential expression analysis. Parental read count totals included the HYLITE '+N' columns (these are reads with clear parental assignment that also contain one or more hybrid-specific SNPs). Two separate differential expression analyses were performed on each data set. First, expression was compared between orthologs in the parental species, then the expression of homeologs of these same genes was compared in the hybrid.

2.6 | Gene classification

Differential expression was defined as a fold change >2, with a p value <0.05 after adjusting for multiple testing. Gene expression with an adjusted nonsignificant p value was defined as equal expression. We excluded genes with no reads for both homeologs or both orthologs. Then, to characterize the fates of gene expression due to allopolyploid and homoploid hybridization, the results from the two differential expression analyses were combined into five gene expression categories (Figure 1). A subset of 'extremely differentially expressed' genes was subsequently defined as having a fold change >50 in either the parental or hybrid differential expression analysis.

3 | RESULTS

3.1 | Study systems

To compare gene expression in hybrid systems from different kingdoms, we sought data sets with RNA-seq data available for both the hybrids and their parental species. Following an extensive systematic search of the literature, we identified very few systems from each kingdom that met the two obligatory selection criteria (see File S1), and after consideration of the preferential selection criteria, we subsequently selected six systems for analysis in this study: one allopolyploid and one homoploid hybrid from each of animals, plants and fungi (Table 1). Most often, candidate systems did not meet the selection criteria due to an absence of suitable RNA-seq data, or hybrid and parental data produced under different experimental conditions (File S1). For animals and plants, we were able to acquire RNA-seq and genomic data for intragenus pairings of allopolyploid

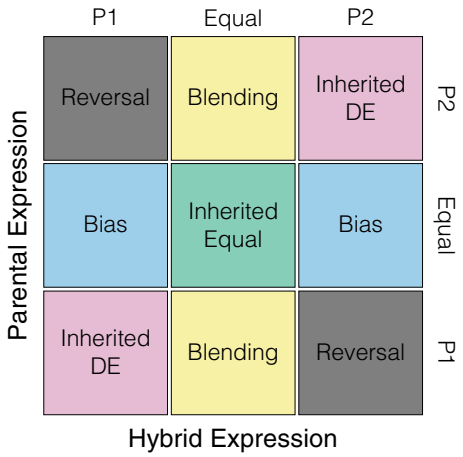

FIGURE 1 Fates of hybrid gene expression relative to parental expression. Following ortholog–ortholog (parental species) and homeolog–homeolog (hybrid species) differential expression analysis, each gene is defined as differentially expressed towards arbitrarily defined parent 1 (P1) or parent 2 (P2), or equally expressed. These nine hybrid expression outcomes can be grouped into five classes of hybrid gene expression, as indicated by the coloured boxes. DE, Differential expression

TABLE 1 Allopolyploid and homoploid hybrid study systems used for the cross-kingdom analysis

	Allopolyploid	Homoploid hybrid
Fungi	*Epichloë canadensis*	*Saccharomyces cerevisiae* × *paradoxus*
Plants	*Gossypium hirsutum* TX2094	*G. arboreum* × *raimondii*
Animals	*Squalius alburnoides* (PAA)	*Sq. alburnoides* (PA)

and homoploid hybrids, but obtained only intraphylum allopolyploid and homoploid hybrids for fungi (File S2).

3.2 | Classifying hybrid gene expression patterns

The animal, plant and fungal species used in this study self-evidently vary greatly in their ploidy level, number of genes and lifestyles. Thus, we required a systematic and generalized framework to enable the inference of cross-kingdom patterns of gene expression. HYLITE (Duchemin et al., 2015) was used to assign reads to homeologs using diagnostic SNP information to distinguish the different parental copies. As per the HYLITE protocol, sequencing reads were mapped using one of the parental gene sets from each representative system as a reference (File S2). We were able to align 0.6–15.6 million reads per biological replicate (Table S1), with the wide range of mapped reads reflecting the variation in size between the raw data sets. After excluding genes with very low coverage across all samples, we were able to analyse between 3576 and 9578 genes per system (Table S2).

Next, to perform differential expression analyses, we adapted an approach used previously in Yoo et al. (2013) and Cox et al. (2014), based on the integration of parental (ortholog–ortholog) and hybrid (homeolog–homeolog) differential expression analyses. Expression between each ortholog or homeolog pair is either differential (adjusted p value <0.05, fold change >2; towards one of the parental gene copies) or approximately equal, generating nine possible expression scenarios following the integration of the ortholog and homeolog results. These nine expression scenarios can then be grouped into the following five expression categories (Figure 1):

- *Inherited equal*: equal expression between parental orthologs remains equal between the hybrid homeologs.
- *Inherited differential expression*: parental differential expression is inherited in the hybrid.
- *Blending*: parental differential expression changes to equal expression in the hybrid.
- *Bias*: equal parental expression changes to differential expression in the hybrid.
- *Reversal*: differential expression occurs in both the parents and the hybrid, but the direction of expression bias is reversed in the hybrid.

3.3 | Parental expression differences tend to be lost in hybrids

We first tested our six systems for evidence of subgenome dominance; an observation sometimes seen among hybrid species where, following the merger of the two diverged parental subgenomes, there is unequal expression of parental contributions at a genome-wide level (Bird et al., 2021; Edger et al., 2017; Ren et al., 2019; Renny-Byfield et al., 2015). If subgenome dominance were present among our data sets, we would expect to see a substantial unidirectional parental bias in expression fold changes. However, we did not identify any evidence of this: the median hybrid $\log_2$ fold change in expression ranged from −0.3 to 0.2 (Table S3). Full data for the distribution of parental and hybrid $\log_2$ fold change in expression for each system can be found in Figures S1–S6.

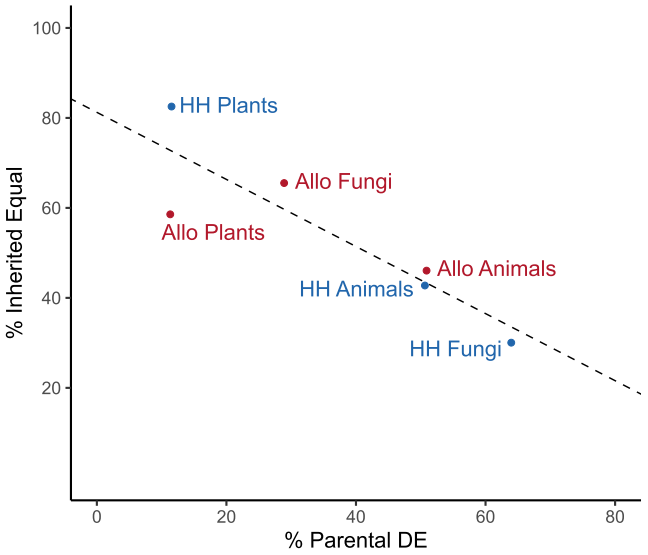

FIGURE 2 Inherited equal expression in hybrid species is dependent on the proportion of differential expression in the parent species. The percentage of differentially expressed parental orthologs plotted against the percentage of hybrid genes in the inherited equal category gives an inverse linear correlation across the study systems. The percentage of differentially expressed orthologs and homeologs was calculated relative to the total number of genes in the final data sets. Allo, Allopolyploid; HH, Homoploid hybrid; DE, Differential expression

Our six representative systems vary substantially in the proportion of genes with differential expression between the parents (ranging from 11.3% to 64.0%). This result has important ramifications, as the proportion of genes that are differentially expressed in the parental species, a statistic that is not related to hybridization at all, strongly influences the number of genes that can fall into each of the five expression categories. For example, high levels of parental differential expression necessarily renders a smaller proportion of genes available for the inherited equal category. Indeed, as expected, we find a large and significant inverse correlation between the level of parental differential expression and the proportion

of genes in the inherited equal category (adj. $r^2 = 0.74$, $p = 0.017$) (Figure 2). Therefore, for each system we examine the expression outcomes ('fates') of genes separately for genes with equal expression in the parental species, and those with differential expression for either parent, rather than simply comparing gene counts across the expression categories.

We allocated all genes in each parent–hybrid system to the five expression categories, and then used this information to investigate what happens to the expression pattern (equal or differential) for each parental ortholog pair after it was inherited as a homeolog in the hybrid (Figure 3). We found that the majority of genes with expression differences between the parents lose this differential expression in the hybrid transcriptome (blending). On average across the systems, 77% of differentially expressed parental genes (58%–93%) were blended, while on average only 14% of genes with equal expression (6%–34%) between the parents gained an expression bias in the hybrid. This outcome was statistically significant for all systems. The net result of this is a striking trend whereby genes typically show more equal expression between the two homeologs in the hybrid than between the two orthologs in the parents. This trend does not appear to be influenced by the degree of divergence in parental gene expression. Indeed, even for the plant allopolyploid system where the absolute number of genes becoming biased is greatest, the majority of differentially expressed genes are blended, so the proportion of differentially expressed genes that experience blending is still larger than the proportion of equally expressed genes gaining a bias (Figure 3).

One possible trivial explanation for the preponderance of blending is that it reflects a greater ability to detect a statistically significant difference in expression in the parents versus the hybrids, as the read count per ortholog/homeolog is half in a hybrid species compared to the parents (assuming the same number of reads for each species). If this artefact explained our result, we would expect blended genes to predominantly be genes with borderline expression differences in the parental species. To test this hypothesis, we divided all genes with parental differential expression into deciles based on their level of differential expression in the parents and calculated the proportion of blended genes for each decile. We found no trend in the proportion of blended genes across deciles, except for a predominant drop in blending in the tenth decile, which is the most highly differentially expressed set of genes (Figure 4). Thus, we conclude that the propensity to blend expression in hybrids is likely a biological phenomenon rather than a statistical artefact.

The proportion of genes with blended expression appeared to drop in the tenth decile of parental differential expression across the representative systems (Figure 4). We wondered if the lack of blending in this decile was the result of genes with very high levels of differential expression in the parent species being particularly recalcitrant to blending. To test this, we examined the fates of genes with an extreme (greater than 50-fold) difference in ortholog expression. While the numbers of these genes was too few to analyse in two of the hybrid systems (allopolyploid and homoploid hybrid plants), half of the hybrid systems that could be analysed showed little reduction in blending (Figure S7). Thus, it remains unclear what is driving the reduction in the proportion of blending among genes in the highest decile of parental differential expression.

3.4 | Gene loss or silencing is prevalent among extremely differentially expressed homeologs

The examination of parental extreme differential expression led us to wonder whether extreme differential expression in the parental species might be associated with extreme differential expression in the hybrid. Interestingly, we find no particular relationship between parental and hybrid extreme differential expression (Table S4), with 0–29% of extremely differentially expressed parental genes maintaining extreme differential expression in the hybrid across all six systems. Rather, we found that extreme differential expression is far more common in hybrids (i.e. between homeologs) than in the parents (Table S5). Hybridization can cause the loss or silencing of hybrid gene copies (Buggs et al., 2009, 2010; Cox et al., 2014; Feldman et al., 2012; Lashermes et al., 2016; Nasrallah et al., 2007), so we looked to see if gene loss/silencing might explain this preponderance of extremely differentially expressed homeologs by determining how many of these homeologs have no reads mapped to one gene copy. In the reversal, inherited differential expression and bias categories, a high percentage of extremely differentially expressed homeologs have no reads mapped to one of the copies (40%–96% of reversal genes; 29%–100% of inherited differential expression genes; 50%–96% of bias genes; Figure 5; Files S3–S5). In contrast, genes with no reads mapped to one gene copy comprise only small proportions (0.2%–9.3%) of the total gene sets, suggesting that gene loss or silencing is particularly overrepresented among extremely differentially expressed genes.

3.5 | Increased ploidy is not a major influence on altered hybrid gene expression patterns

Current literature suggests that hybridization, rather than the increase in ploidy level, has a greater impact on post-allopolyploidization gene expression patterns (Albertin et al., 2006; Chelaifa et al., 2010; Hegarty et al., 2005, 2006; Jung et al., 2015; Li et al., 2018; Wang et al., 2006). Our results are consistent with this view, as there are no clear differences in gene expression patterns between the allopolyploid and homoploid hybrid systems (Figures 2–5). To more systematically test whether allopolyploid and homoploid hybrid expression patterns might be distinguishable, we paired each kingdom's representative homoploid hybrid and allopolyploid systems to allow the comparison of hybrid gene expression patterns with and without an increase in ploidy level. We then performed hierarchical clustering of normalized expression category count data associated with genes that have changed gene expression category in the hybrid (the blending, bias and reversal categories). Count data were normalized by

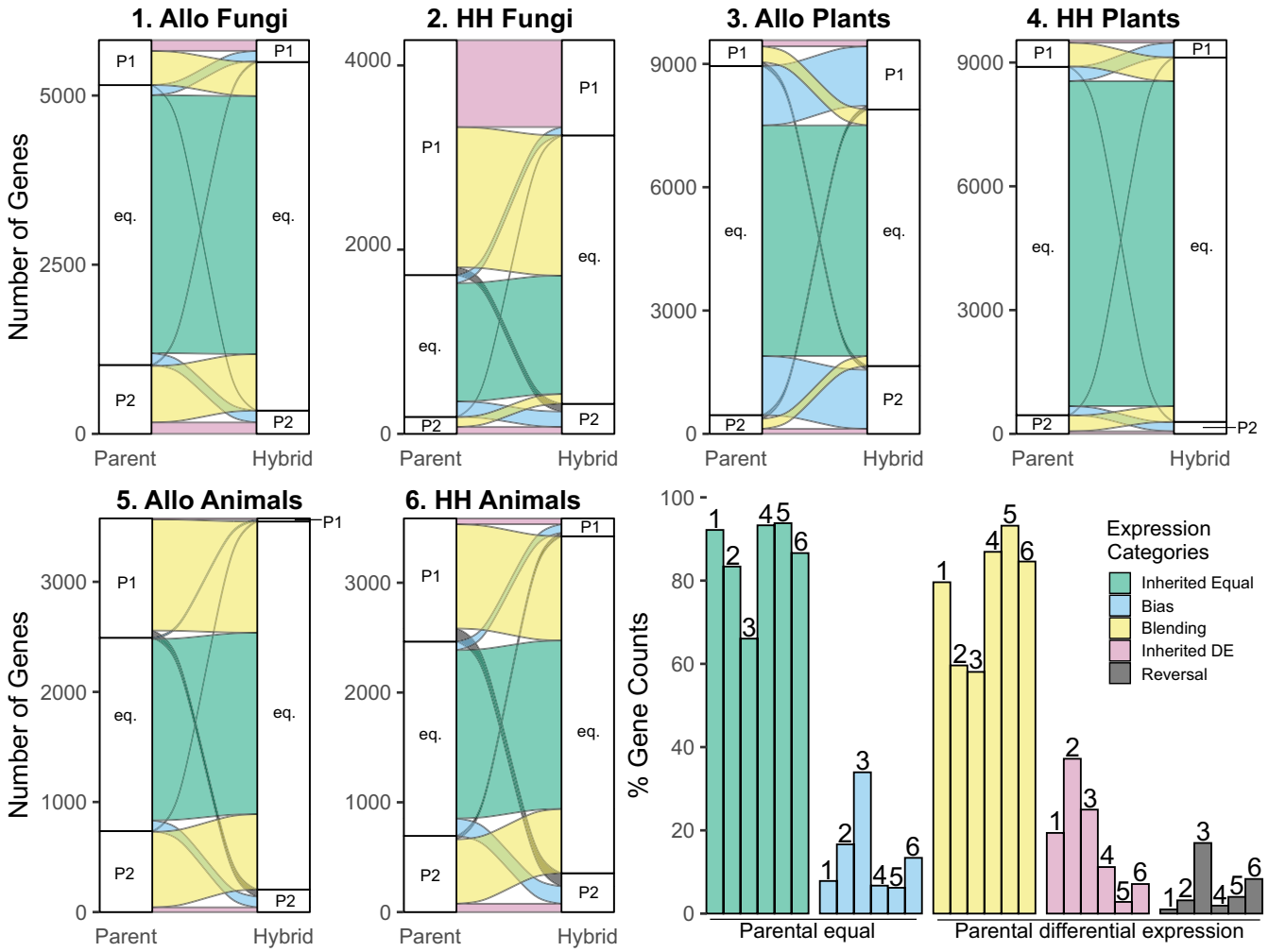

FIGURE 3 Genes with differential expression in parental species are more likely to have similar expression levels in hybrid species. Alluvial plots: The different fates of gene expression due to hybridization are shown as alluvial plots 1–6. The heights of the white boxes represent the absolute numbers of genes with differential expression in either direction (parent one – P1 or parent two – P2; fold change >2, adjusted $p < 0.05$) or equal expression (eq). Note that the number of genes differs across the systems. Bar chart: The bar chart (bottom right) shows the relative proportions of equally expressed genes that are inherited or gain an expression bias, and the relative proportions of differentially expressed genes that are inherited, blended or reversed. Differentially expressed parental genes are more likely to be blended in the hybrid (yellow) than inherit differential expression (pink), while equally expressed parental genes are more likely to be inherited as equal expression (green) than gain an expression bias in the hybrid (blue). Expression reversals (grey) are rare. Within each colour group, the bars are ordered to correspond with the alluvial plots, as indicated by the numbers above the bars. Allo, Allopolyploid; HH, Homoploid hybrid; DE, Differential expression

the total number of genes in these three categories of interest. If ploidy increase were the main driver of allopolyploid gene expression patterns, we would expect the allopolyploids and homoploid hybrids each to cluster together. This was not observed in the data (Figure 6), suggesting that hybridization is the greater influence on allopolyploid gene expression patterns.

4 | DISCUSSION

Hybrid studies to date have largely focused on single hybrid species. While these studies have greatly improved knowledge about each particular system, their bespoke approaches impede the synthesis of data into broad generalizations about hybrid gene expression. Here, we have employed a standardized framework to make comparisons between study systems across three eukaryote kingdoms: animals, plants and fungi. We performed separate differential expression analyses of orthologs and homeologs that, when integrated, enabled classification of the expression of each gene into one of five categories. We found that the proportion of genes with inherited equal expression in hybrid species is inversely correlated with the level of parental differential expression, which is an expected consequence of different levels of parental differential expression, in turn likely reflecting different timeframes of genetic divergence between the various parental species. Importantly for this study, this factor results in different

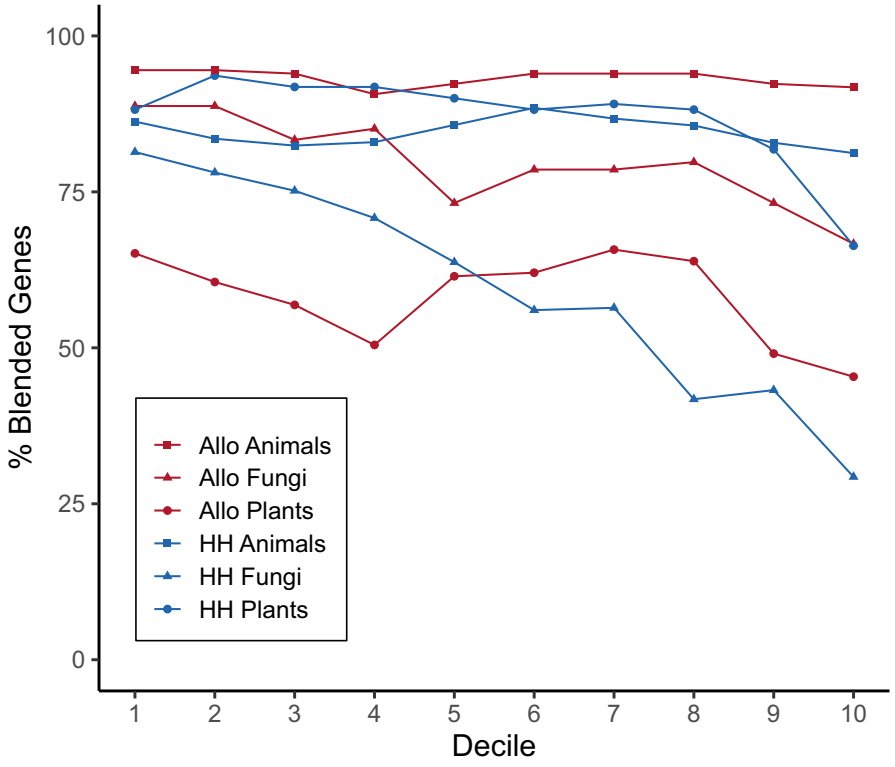

FIGURE 4 The proportion of blended genes is not dependent on the level of parental differential expression, except for the most highly differentially expressed genes. Genes were divided into deciles by parental expression difference, and the proportion of blended genes for each system are plotted for each decile. Allo, Allopolyploid; HH, Homoploid hybrid

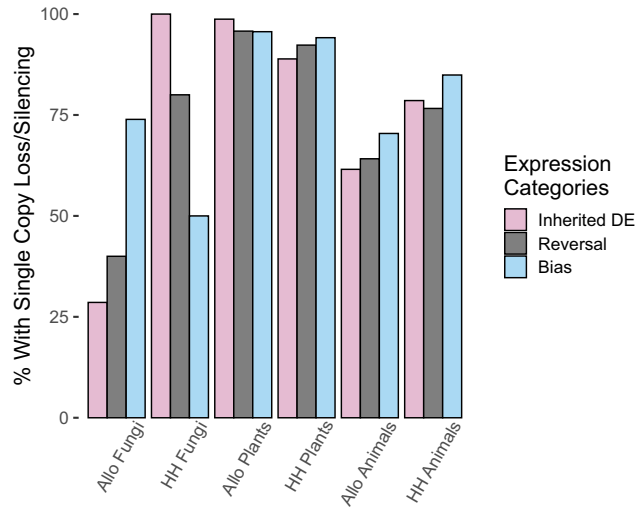

FIGURE 5 Most extremely differentially expressed homeologs are due to gene loss or silencing of one homeolog in the hybrid. Extreme differential expression was defined as >50-fold difference in expression. Plots are shown separately for extremely differentially expressed homeologs in the inherited differential expression, bias and reversal categories. Allo, Allopolyploid; HH, Homoploid hybrid; DE, Differential expression

distributions of genes in the expression categories, thus making direct comparisons of category counts between systems an inaccurate means of comparing transcriptomic differences in hybrids. To account for this, we separately investigated the 'fates of genes' with equal or with differential parental gene expression in allopolyploid and homoploid hybrid species across our three kingdom study systems.

4.1 | Consistent patterns of hybrid gene expression are likely the cumulative outcome of interactions between homeologs and their regulatory factors

A gene with approximately equal expression between parental species has two possible fates when the orthologs are combined in a hybrid nucleus: either this equal expression is inherited, or an expression bias may arise in the hybrid. Our results show that inheritance of approximately equal expression is most common. In contrast, differentially expressed parental genes have three possible fates following hybridization: either the parental differential expression is inherited; differential expression is inherited but its direction is reversed; or the differential expression is lost in the hybrid (blending). Unlike equal parental expression, we found that the predominant outcome for differentially expressed parental genes was not inheritance, but blending of their expression in the hybrid. This major trend is species independent, in addition to being independent of the extent of differential expression between the parental species and any change in ploidy.

It is of interest to understand why similar patterns of hybrid gene expression are observed in systems separated by vast phylogenetic distances. One possible explanation is that these represent adaptive changes in expression; for example, by certain types of genes being deleterious when expressed at different levels in a hybrid. However, we found no evidence for functional gene types displaying a consistent pattern of hybrid expression, as might be expected if the adaptive hypothesis were true. The absence of such a pattern is consistent with previous observations among allopolyploid and homoploid hybrid species (Adams, 2007;

Bassene et al., 2010). Instead, as we have previously proposed (Cox et al., 2014), we suggest that consistent patterns of hybrid gene expression are primarily driven by interactions between homeologs and their regulatory factors in the hybrid nucleus. For example, in the case of blending, if parental differential expression is determined by the presence of a *trans*-acting gene regulatory factor (such as a transcription factor) in one parental species that is absent in the other, the interaction of this regulatory factor with both homeologs within the hybrid nucleus may result in equal expression of both homeologs (Figure 7). Our observation of the predominance of blending of hybrid gene expression is consistent with this mechanism. Also consistent is our result suggesting that hybridization, rather than change in ploidy level, is the major driver of hybrid expression patterns, as this mechanism is dependent on genome merger, but independent of an increase in ploidy. Data from studies on other allopolyploid and homoploid hybrid species including *Senecio* (Hegarty et al., 2005, 2006), *Brassica* (Albertin et al., 2006), *Arabidopsis* (Wang et al., 2006), *Spartina* (Chelaifa et al., 2010), wheat (Jung et al., 2015) and Cyprinidae fish (Li et al., 2018) have also suggested that genome merger has a greater effect on gene expression patterns than an increase in ploidy.

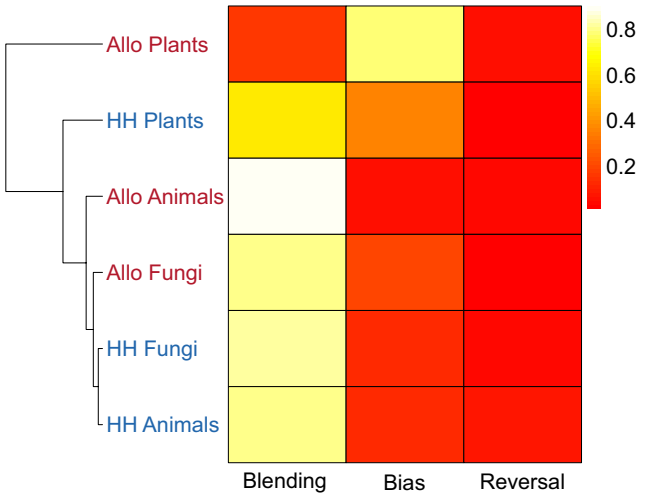

FIGURE 6 Change in genome ploidy does not strongly influence the distribution of hybrid gene expression patterns. Hierarchical clustering of noninherited (blending, bias and reversal) expression category counts does not group systems based on whether they have undergone a change in genome ploidy or not. Category count data were normalized by the total number of genes in the three respective expression categories. The heatmap colour scale corresponds to the normalized count data. Allo, Allopolyploid; HH, Homoploid hybrid

The occurrence of expression blending as a consequence of sharing of gene regulatory systems in the hybrid might be expected to occur only when there is the 'right' level of evolutionary divergence between parental species. With too little divergence, both parents would likely carry the same gene regulatory factors and have approximately equal levels of expression, leading to inherited equal expression in the hybrid. With too much parental divergence, a gene regulatory factor from one parent may not recognize the binding sequence of the homeolog inherited from the other parent, leading to inherited differential expression between homeologs. Only in a 'Goldilocks' zone might there be a parent-specific gene regulatory factor that can act on both homeologs in the hybrid. This idea is similar to that of Gruber et al. (2012), who suggested that regulatory differences between more closely related organisms should mostly be *trans*-acting, while transcriptional variation

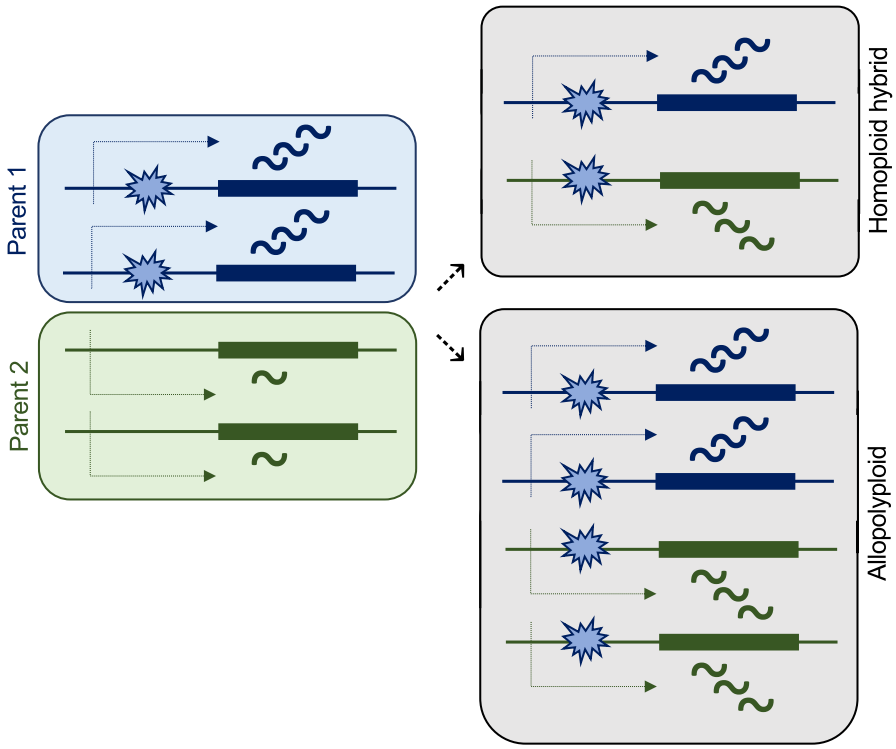

FIGURE 7 Blended hybrid gene expression is likely driven by interactions between homeologs and their regulatory factors. A representative gene in the nucleus of parent 1 (left) shows *trans*-activation via transcription factor binding (blue star bursts) and subsequent high levels of expression of the gene (wiggly lines). In contrast, the lack of this transcription factor in parent 2 results in low levels of expression of the orthologous gene in parent 2. When both parental gene copies and the transcription factor are combined in a single homoploid hybrid nucleus (top right) or allopolyploid nucleus (bottom right), the ability of the transcription factor to bind to both parentally derived homeologs results in a more similar, or 'blended', level of expression

between distantly related organisms would more likely be explained by *cis*-acting factors. Although insufficient data sets are available currently to look for these patterns across multiple hybrid systems with different levels of parental divergence, genes with extremely differential expression between parent species can act as a proxy for this high parental divergence effect. Genes with low parental expression differences, which would be predicted to be mostly *trans*-acting under the Gruber model, predominantly tend to show blending effects (Figure 4). Conversely, there is a trend for rates of blending to decrease for extremely differentially expressed genes, which instead appear to show more inherited differential expression (Figure S8), as would be predicted to result from *cis*-acting processes under the Gruber model. Thus, the hybrid patterns that we observe resulting from high- and low-expression differences between parent species broadly match the expectations of the Gruber model, and it will be interesting to see whether this is also observed for temporal divergence between parent species when sufficient data become available.

Another common phenotypic pattern among hybrids is intermediacy: a condition where hybrids develop a phenotype that is intermediate to their parental species (Hermansen et al., 2011; Rubini Pisano et al., 2019; Salamone et al., 2013; Schneider et al., 2011). Phenotypic intermediacy can be conceptualized as a blending of parental phenotypes, and impacts particular morphological traits. Our observation of preferential blending of parental differential expression in hybrid species is interesting in this regard, as this blending at the transcriptional level may, in part, underlie blending–intermediacy– at the phenotypic level. However, the predominant trend towards blending of hybrid gene expression is not likely to solely explain phenotypic intermediacy, as a number of hybrid species display transgressive phenotypes (Dittrich-Reed & Fitzpatrick, 2013; Koide et al., 2019; Rieseberg et al., 1999; Stelkens et al., 2009). In addition, Bartoš et al. (2019) recently proposed that phenotypic intermediacy in hybrids is caused by the combination of individual genes that are expressed at levels more similar to one or other parent. It would be interesting to see whether the degree of intermediate versus transgressive phenotypes correlates with the level of blending when looking across many different hybrid systems, but this will require more systems and better phenotyping than are currently available.

4.2 | The impact of genome shock limits blending of differentially expressed genes

We found many more extremely differentially expressed genes in the hybrids compared to the parents, and most of these genes in the hybrid are extremely differentially expressed because reads map to only one homeolog. By definition, no genes with reads mapping to only one homeolog can fall into the blended category. Thus, if these genes were removed, the proportion of differentially expressed parental genes that are blended would be even greater, further emphasizing the preponderance of the blending trend across these hybrid systems. Nonexpression of one homeolog in hybrids could be the result of gene loss or complete gene silencing. Both phenomena have been observed in hybrid species as a response to genome merger (Buggs et al., 2009, 2010; Cox et al., 2014; Feldman et al., 2012; Lashermes et al., 2016; Nasrallah et al., 2007). It is, however, difficult to infer the relative proportions of each, something that would be interesting to determine across different hybrid systems in a similar manner as done here with expression, for example, by using targeted PCR or by obtaining whole genome sequences of the hybrids.

4.3 | Building a more robust picture of generalized hybrid transcriptomic responses

In this study, we have identified similar transcriptomic trends in allopolyploid and homoploid hybrid systems across eukaryotes. However, our ability to generalize these findings in eukaryote hybrid systems is limited by our analyses being restricted to a single allopolyploid and homoploid system from each of the plant, animal and fungi kingdoms. Despite the widespread uptake of RNA-seq analysis, the striking lack of suitable data sets to perform robust comparisons of expression patterns in hybrids and their parent species remains the main limitation to extending this kind of analysis to a broader set of hybrid systems. Future work would benefit from a more standardized approach to collecting transcriptomic data across animal, plant and fungal hybrids to facilitate systematic cross-study comparisons, and thus further examine the generality of the expression patterns across kingdoms identified in this work. Moreover, the general expression trends found here in hybrid species across a wide phylogenetic range are an important factor for studies to take into consideration when interpreting the expression of genes in individual hybrid species.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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DATA AVAILABILITY STATEMENT

All data used in this study are publicly available, with accession numbers listed in File S2.

ORCID

Anna H. Behling https://orcid.org/0000-0002-1170-5087
Murray P. Cox https://orcid.org/0000-0003-1936-0236

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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