Hybridization and gene expression:

beyond differentially expressed genes

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Abstract

Gene expression has a key role in reproductive isolation, and studies of hybrid gene expression have identified mechanisms causing hybrid sterility. Here, we review the evidence for altered gene expression following hybridization, and outline the mechanisms shown to contribute to altered gene expression in hybrids. Transgressive gene expression, transcending that of both parental species, is pervasive in early generation sterile hybrids, but also frequently observed in viable, fertile hybrids. We highlight studies showing that hybridization can result in transgressive gene expression, also in established hybrid lineages or species. Such extreme patterns of gene expression in stabilized hybrid taxa suggests that altered hybrid gene expression may result in hybridization derived evolutionary novelty. We also conclude that while patterns of misexpression in hybrids are well documented, the understanding of the mechanisms causing misexpression is lagging. We argue that jointly assessing differences in cell composition and cell specific changes in gene expression in hybrids, in addition to assessing changes in chromatin and methylation, will significantly advance our understanding of the basis of altered gene expression. Moreover, uncovering to what extent evolution of gene expression results in altered expression for individual genes, or entire networks of genes, will advance our understanding of how selection molds gene expression. Finally, we argue that jointly studying the dual roles of altered hybrid gene expression, serving both as a mechanism for reproductive isolation and as a substrate for hybrid ecological adaptation, will lead to significant advances in our understanding of the evolution of gene expression.

Introduction

Gene expression is regulated by a complex interaction of regulatory elements (Dover and Flavell 1984; see Hill et al. 2021 for a review). The molecular co-adaptation of gene regulatory elements is thought to result from stabilizing selection (Hodgins-Davis et al. 2015). Mutation accumulation assays in flies, yeast, and worms suggest that mutations often have effects that exceed the standing genetic variation, selecting for a cascade of compensatory changes between *cis*- and *trans*-regulatory factors to maintain the level of mRNA product produced by the gene regulatory network (Denver et al. 2005; Rifkin et al. 2005,Landry et al. 2007b). Based on these findings, mutations are suggested to typically have large effects on gene expression and be balanced by stabilizing selection ("house of cards" model; Hodgins-Davis et al. 2015). Balancing selection on gene expression, in combination with drift, may give rise to the same pattern of gene expression from divergent regulatory underpinnings, referred to as developmental systems drift (True and Haag 2001), and these different regulatory bases may lead to new extreme expression phenotypes when combined in hybrids. In contrast, divergent patterns of gene expression in parental species frequently give rise to either intermediate expression profiles in hybrids, potentially due to the more similar trans-acting environment (Behling et al. 2022), but may also lead to malfunctioning gene expression (Mack and Nachman 2017; Wu et al. 2022). Both systems drift and expression divergence (Behling et al. 2022), but may also lead to malfunctioning gene expression (Mack and Nachman 2017; Wu et al. 2022). Both systems drift and expression divergence contribute to the unique role divergence in gene regulatory networks may play in speciation (Mack and Nachman 2017). When divergent gene expression networks are recombined in hybrid genomes, negative pairwise epistatic interactions between loci can lead to reduced hybrid fitness (Orr and Presgraves 2000; Rifkin et al. 2003; Lemos et al. 2005), a form of Bateson Dobzhansky Muller Incompatibilities (BDMIs, Bateson 1909; Dobzhansky 1937; Muller

1942). Disrupted expression in hybrid genomes can provide insight into the nature of reproductive barriers, through the association of misregulated genes or gene expression networks with sterile or inviable hybrids. While studies of gene expression in hybrids have hitherto focused on incompatibilities, studies of coding genetic variation have focused on adaptive sorting of variation, and hybridization is now recognized as an important source of novel variation for selection (Marques et al. 2019; Runemark et al. 2019; Taylor and Larson 2019).

Extreme patterns of gene expression have been recorded in many allopolyploid (Freeling et al. 2015; Edger et al. 2017, 2019) and a few homoploid (Lai et al. 2006; Hegarty et al. 2009; Brouillette and Donovan 2011; Czypionka et al. 2012; Yazdi et al. 2022; White et al. 2023) hybrid species without reduced viability, fertility, or fitness. This suggests that gene expression could have a dual role in hybrids, leading to both incompatibilities and novel patterns of expression, potentially giving rise to new adaptive variation. Thus, while the traditional focus of studies of hybrid gene expression has been to determine the basis of hybrid sterility (Landry et al. 2007a; Ortíz-Barrientos et al. 2007; Civetta 2016; Mack and Nachman 2017; Patlar and Civetta 2021), the same mechanisms may also contribute to evolutionary novelty through altered gene expression. Transgressive hybrid phenotypes or gene expression may result in mismatches with the environment, resulting in ecological incompatibilities (Thompson et al. 2023) and it is possible that novel transgressive patterns of gene expression and phenotypes may be better adapted to novel environments.

Hybrid gene expression has been studied for more than half a century (Denis and Brachet 1969), but there are still many outstanding questions (**Box 1**). The majority of eukaryotic genomes are non-coding and these vast stretches of DNA contain sequences, such as promoters, enhancers, non-coding RNAs (ncRNAs), and transposable elements (TEs), that can influence gene expression (Patlar and Civetta 2021, **Figure 1**). Gene expression studies

have traditionally sequenced only a small subset of the genome and we still know little about how divergent non-coding regions of the genome influence expression. We also know little about how divergent gene regulatory networks interact in hybrid genomes across different organisms - patterns which could provide insight into the nature of hybrid incompatibilities and hybrid novelty. For example, is there a tendency for gene expression in hybrids to be transgressive (outside of the range of both parent species) or additive (intermediate to the expression divergence of the two parents)? When expression in hybrids is transgressive are genes typically over or underexpressed (often referred to as overdominant and underdominant expression), or a combination of both? Finally, the outcomes of divergent gene expression have been best studied in the context of reproductive barriers (Ortíz-Barrientos et al. 2007; Civetta 2016; Mack and Nachman 2017), while the role of altered regulation of gene expression in hybrid novelty is an outstanding question (Hegarty et al. 2013; Yazdi et al. 2022).

Here, we review the current state of knowledge of the role of gene expression in hybrid incompatibilities, and its potential to contribute to phenotypic novelty over both shorter and longer evolutionary time. We conduct the first systematic survey of hybrid gene expression studies and summarize major patterns. We further evaluate evidence for different mechanisms underlying these patterns. We use our survey to identify areas where significant progress can be made, including applying network approaches to increase our understanding of whether novel patterns of expression commonly arise on the network or gene level, and using methods that disentangle the effects of differences in cell composition from those derived from altered regulation of expression. We propose that studies uncovering how hybridization derived patterns of gene expression may serve as a substrate for evolutionary adaptation would significantly advance our understanding of hybridization derived novelty.

Patterns of gene expression in early generation hybrids

We performed a comprehensive search of the hybrid gene expression literature related to speciation. We used Web of Science (accessed July 26, 2023) with the following search terms (hybrid* OR F1) AND (speciation OR 'reproductive isolation') AND ('gene expression' OR 'transcriptom* OR 'allele specific expression OR RNAseq). We recovered 571 records, then narrowed this list to 81 original research publications that examined patterns of gene expression in 58 unique homoploid hybrid species pairs (*e.g.*, F1, F2, hybrid populations, or introgression lines, **Supplementary Data 1**). We excluded studies that examined allopolyploid hybrid genomes because these datasets have been reviewed extensively elsewhere (Freeling et al. 2015; Bird et al. 2018; Cheng et al. 2018; Edger et al. 2018; Alger and Edger 2020). In our survey we summarized overall trends in gene expression in hybrids as transgressive if the hybrid expression patterns differed from both parents (either over or under expressed in hybrids), additive if the hybrid expression patterns were higher than one parent but lower than the other, and dominant if gene expression was similar to one parent (following McManus et al. 2010). If hybrids were compared only to each other, rather than to parents, we summarized whether there was over or under expression in at least one hybrid type relative to the other. Hybrids have more complex regulatory dynamics that are a mix of all of these patterns, but given the focus on transgressive expression in the hybrid literature, our goal was to provide a general sense of key findings as reported in each manuscript. To summarize divergence time between parental lineages, we used reported values from the manuscript if available, otherwise the species pairs were input into the TimeTree5 database to estimate the relative relatedness of hybridizing taxa (Kumar et al. 2022).

Our survey identified 58 species pairs where hybrid gene expression has been studied across 81 studies (**Table S1, Figure 2**). These spanned 9 classes, with most studies in insects. Overall, most of these studies focused on F1 hybrids (57/81), but there were 30 studies that

contrasted other hybrid types, such as F2s and backcrosses (13), introgression lines (6) and naturally occurring hybrids or hybrid populations (8). The majority of studies also focused on taxa where there are reproductive barriers between species and assayed tissues where barriers are likely to manifest (52/81). However, there were 29 studies on species pairs where there were either no barriers, barriers were prezygotic, or the studies focused on gene expression in the fertile/viable hybrid. In studies that focused on reproductive barriers, the most common type of barrier was hybrid sterility (38/52), particularly hybrid male sterility, with only two studies addressing hybrid female sterility. Fewer studies have examined hybrid inviability (15/52), likely because it is challenging to assay the developmental stage immediately prior to developmental dysregulation.

It was common for patterns of gene expression in hybrid genomes to differ from parental gene expression (69/81). In hybrids that are inviable or sterile, gene expression tended to be transgressive to some extent (40/52), while fewer studies reported additive (8/52) or dominant (5/52) expression in sterile or inviable hybrids. Hybrids that were viable and fertile tended to also have transgressive expression (13/24), although they were more likely to have additive (6/24) or dominant (5/24) expression than sterile or inviable hybrids. Across different types of hybrids (F1, F2 and backcross, etc), this same pattern held, with transgressive expression more common than additive or dominant expression. This is consistent with studies that have also found altered patterns of expression in intraspecific hybrids where there are no reproductive barriers (Gibson et al. 2004; Coolon et al. 2014). It is an important reminder that altered patterns of gene expression may be an outcome of hybridization and not intrinsic to hybrid incompatibilities (Mack and Nachman 2017).

Typically sterile hybrids were compared to one or both parent species, but in 24 studies sterile and fertile hybrids were also compared directly. The advantage of this study design is that hybrids share the same genetic background, making it possible to differentiate

altered patterns of expression due to hybridization or hybrid incompatibilities (Wei et al. 2014; Mack et al. 2016; Kerwin and Sweigart 2020). These methods included comparing fertile/viable hybrids and sterile/inviable hybrids using reciprocal F1 hybrids from crosses with asymmetric hybrid incompatibilities (Gomes and Civetta 2015; Larson et al. 2017; Brekke et al. 2021), hybrid introgression lines (Guerrero et al. 2016), or fertile and sterile tissues within a single hybrid (Rottscheidt and Harr 2007; Kerwin and Sweigart 2020). There were also more direct associations between hybrid incompatibility phenotypes and gene expression that used introgression of known hybrid incompatibility loci (Lu et al. 2010; Wei et al. 2014; Kerwin and Sweigart 2020) or mapping hybrid incompatibilities using expression QTL or GWAS (Dion-Côté et al. 2014; Turner and Harr 2014; Turner et al. 2014; Zuellig and Sweigart 2018; Tsuruta et al. 2022). In studies that controlled for hybridization, some found widespread misregulation in sterile or inviable hybrids (13/24), while others found limited or no misexpression associated with hybrid incompatibilities (Wei et al. 2014; Guerrero et al. 2016). This suggests that disrupted gene expression is not an inherent outcome of hybrid incompatibilities, but does not rule out that gene regulation of specific gene(s) is associated with hybrid incompatibilities (Guerrero et al. 2016). Only a few studies reported enrichment of particular regions of the genome associated with hybrid incompatibilities, and many of these found different patterns of expression on the sex chromosomes relative to the autosomes. In some cases there were more differentially expressed genes on sex chromosomes (Good et al. 2010; Davis et al. 2015; Brekke et al. 2016) and in other cases there were fewer (Moehring et al. 2007; Llopart 2012). There were also studies that found no differences across the sex chromosomes and the autosomes (Rafati et al. 2018). A promising direction is for studies to test the genomic distribution of differential expressed genes and look for hotspots of regulatory divergence (e.g., Larson et al. 2017, 2022). Further

investigating the importance of sex linked genes in altered patterns of expression is an exciting direction for future research.

Mechanisms causing misexpression in early generation hybrids

While patterns of misexpression in hybrids are well documented, the mechanisms causing misexpression are less well understood. Gene expression is determined by an intricate regulatory machinery (**Figure 1**), which jointly determines the amount of transcripts produced. In first generation F1 hybrids, transgressive gene expression can arise from dominance (Thompson et al. 2021) or heterosis (Barreto et al. 2015). While altered expression in F1 is not necessarily expected from interactions between autosomal *cis*- and *trans* regulatory elements, the frequent occurrence of cross-direction and sex dependent asymmetry in hybrid gene expression points to a potentially important role for such interactions involving parts of the genome with parent-of-origin dependent inheritance, such as imprinting, sex chromosomes, and interactions with organellar genomes. In addition to intricate interactions among regulatory elements, alterations in methylation and chromatin, presence of transposable elements, different levels of regulatory microRNAs in hybrids, and changes to cell compositions could contribute to hybrid misexpression. Below, we briefly review the evidence found for altered hybrid gene expression for each of these mechanisms based on the studies recovered in the search above (see **Tables S1** and **S2**).

Break-up of interacting cis *and* trans *regulatory elements*

Gene expression is determined by an interacting network of regulatory factors and sequences. Whereas some regulatory elements are *cis*-acting, localized on the same chromosome and often close to the target gene, other elements act in *trans*, including RNA-molecules and proteins that are encoded elsewhere in the genome (Hill et al. 2021). In F2- and later

generation hybrids, co-adapted parental regulatory networks are broken up and combined in novel ways, with the potential to give rise to more extreme patterns of gene expression (**Figure 3**). The proportion of divergent *cis*- and *trans*-acting regulators can be determined by examining the relative expression of parent specific alleles in F1-hybrids, taking advantage of the fact that the *trans*-regulatory environment is intermediate in these hybrids (Cowles et al. 2002; Wittkopp et al. 2004). Within species, there is a pattern of a stronger contribution of *trans*-regulatory mutations on variation in gene expression. In contrast, differences in gene expression among species are often equally or even more affected by *cis*-regulatory variants (reviewed in Hill et al. 2021). There is evidence in *Saccharomyces cerevisiae* that *trans*-regulatory mutations are more pleiotropic, causing larger fitness effects and potentially reducing the likelihood they become fixed (Vande Zande et al. 2022). This could perhaps contribute to the greater divergence of *cis*-regulatory elements between species. The state of the art method for evaluating *cis*- and *trans* regulation is to evaluate allele specific expression, where an expressed gene can be traced back to the subgenome from which it was transcribed. Of the literature in our survey, only half of the studies that collected RNAseq data used allele-specific expression analysis methods (28/56), indicating that these methods can be more widely employed in studies of hybrid gene expression.

Gene expression is often under stabilizing selection (Bedford and Hartl 2009) and has been found to best fit a "house of cards" model (Hodgins-Davis et al. 2015). In this model, mutations are infrequent, with effects exceeding the standing genetic variation, and stabilizing selection maintains a mutation-selection balance (Hodgins-Davis et al. 2015). The phenotypes may remain stable, while small changes may accumulate in the underlying gene regulatory networks (systems drift, see Schiffman and Ralph 2022). Transgressive expression may occur in hybrid genomes depending on the regulatory network structure in parental genomes and the selection acting on regulatory elements. Likewise, stabilizing selection on

polygenic traits in parental genomes may lead to the accumulation of compensatory mutations that maintain similar levels of gene expression, which in turn, may cause transgressive gene expression when combined in F2 hybrid genomes (Fraser et al. 2021, **Figure 3**). Phenotypically partial parental dominance is a pervasive pattern in F1 hybrids and this may result in unfit hybrid phenotypes due to mismatch (Thompson et al. 2021). Uncovering to which extent novel combinations of autosomal trans-regulatory elements contribute to misexpression in hybrids remains an empirical challenge.

Methylation and histone modifications

Methylation of DNA, including of promoter regions, introns and genes, is known to regulate gene expression (Moore et al. 2013; Bewick et al. 2016; Anastasiadi et al. 2018). Whereas a regulatory effect of methylation is well documented in vertebrates (Lowdon et al. 2016) and plants (Zhang et al. 2018; plants, Muyle et al. 2022) levels of methylation are lower and more variable in invertebrates (Bewick et al. 2017). The role of methylation in invertebrates is less well understood and varies across different organisms. . For example, intragenic methylation regulates gene expression in *Bombyx mori (Xu et al. 2021)*, while *Drosophila* has nearly undetectable levels of methylated cytosines in DNA (Dunwell and Pfeifer 2014) and gene regulation through histone methylation remains intact (Bonnet et al. 2019). The various ways in which methylation regulates gene expression have been extensively reviewed elsewhere (Moore et al. 2013; Bewick et al. 2016; Lowdon et al. 2016; Anastasiadi et al. 2018; Zhang et al. 2018; Muyle et al. 2022). Despite the critical role of methylation as a mechanism shaping the gene regulatory landscape, examination of epigenetic marks in hybrids has largely been limited to well-studied taxa. A recent review raised the interesting possibility of epigenetic changes contributing to incompatibilities, making its role an exciting area for future research (Reifová et al. 2023). Among the studies recovered in our literature search, “methylation” is

included in the title, keywords, or abstract in ten. Six of these present data on methylation and/or histone modifications in F1-hybrids compared to parental species. Two studies on mammalian hybrids reveal parent-of-origin dependent placental growth, associated with imprinted genes showing transgressive methylation and gene expression (Wiley et al. 2008; Arévalo et al. 2021). In fish hybrids between the self-fertilizing *Kryptolebias hermaphroditus* and outcrossing *K. ocellatus*, 2.2% of the loci that had different patterns of methylation in the parent species were transgressively methylated (77 loci overdominant, and 54 loci underdominant) (Berbel-Filho et al. 2022). In plants, transgressive methylation has been shown to give rise to transgressive gene expression in interpoloidy *Mimulus (Kinser et al. 2021)*. There is also indirect evidence, suggesting that methylation could be altered in F1 hybrids between *Brassica napa* and *B. carinatus*, as the function macromolecule methylation is enriched among genes that are differentially expressed compared to both parent species (Chu et al. 2014). Jointly, these studies suggest that changes in methylation contribute to the novel patterns of gene expression observed in early generation hybrids.

We identified two studies that directly addressed histone modifications and their effects on gene expression (Zhu et al. 2017; Bodelón et al. 2022). In hybrids between *Drosophila buzzatii* and *D. koepferae*, the ovarian transcriptome was found to be underexpressed, and downregulated TEs and differentially expressed ovarian genes had differential chromatin mark combinations, consistent with an effect of histone marks on gene expression (Bodelón et al. 2022). In *Arabidopsis thaliana* and *A. lyrata* hybrids, CHH DNA methylation was elevated in transposon rich regions , and *A. thaliana* genes enriched for H3K27me3 histone modifications tended to be differentially expressed in hybrids (Zhu et al. 2017). CHH refers to methylated cytosines outside of CpG context in the genome, and are used to regulate both gene and TE expression in plants (Martin et al. 2021) and in a tissue-dependent manner in vertebrates (Chow et al. 2023). H3K27me3 is a dynamically regulated,

repressive histone modification (Cai et al. 2021). Uncovering general patterns among the diverse ways in which methylation and chromatin modifications may be altered in hybrids, and the effect of these modifications on gene expression, remains a challenge for researchers.

Imprinted genes

One class of genes with parent-of-origin methylation (genomic imprinting) has evolved independently in at least two taxa, and likely plays a role in hybrid incompatibilities in both flowering plants and mammals. In plants, the triploid endosperm is an extraembryonic tissue formed when a haploid sperm fertilizes a diploid central cell to form the tissue that provisions the developing embryo (Haig and Westoby 1989). In mammals, the extraembryonic placenta is an organ derived from both maternal and embryonic tissue with functional layers that regulate critical pregnancy functions including immune recognition, endocrine signaling, and nutrient transport (Roberts et al. 2016). Notably, both the endosperm and the placenta have asymmetries in maternal and paternal genomes, as one of the three placental layers is derived entirely from maternal tissue and the triploid endosperm has a 2:1 maternal to paternal genomic ratio. As such, these tissues are the site of unique co-evolutionary dynamics that favor the evolution of genes expressed in an imprinted, parent-of-origin manner (Haig and Westoby 1989; Moore and Haig 1991; Haig 1997; Zeh and Zeh 2000) and are potential hotspots for hybrid inviability (Wiley et al. 2008; Brekke et al. 2016; Garner et al. 2016; Coughlan et al. 2020; Coughlan 2023).

The mechanisms by which hybridization disrupts imprinted gene expression is an open question, and likely varies within and across taxa. Some imprinted genes show binary parent-of-origin specific expression of alleles (Wilkins and Haig 2003) while others have appreciable yet differential levels of expression of parental alleles, a pattern referred to as the differential dosage hypothesis (Dilkes and Comai 2004). Removal of imprints in hybrids

should then alter gene expression in particular ways, where loss of imprinting should increase expression of the parental allele previously silenced. This appears to the the case in the flowering plant *Mimulus*, where dosage and rescue of allelic imbalance can rescue hybrid inviability (Coughlan et al. 2020); however, evidence from mammals suggests that imprinting disruption may be less predictable, with imprinting loss accompanying suppression of both parental alleles in *Phodopus* dwarf hamsters (Brekke et al. 2016, 2021). To what extent differential dosage of expressed alleles contributes to the pattern of partial phenotypic dominance of one parent in F1 hybrids (Thompson et al. 2021) is an outstanding question for future research. Disrupted methylation has also been linked to imprinting disruption and hybrid inviability in Arabidopsis crosses (Josefsson et al. 2006; Kirkbride et al. 2015), but can be rescued by methyltransferase inhibitors (Huc et al. 2022). An interesting question is to what extent global removal of methylation marks in primordial germline cells (Messerschmidt et al. 2014) could be disrupted in homoploid hybrids, contributing to hybrid sterility.

Interactions with organellar genomes and sex chromosomes

Regulatory interactions among different parts of the genome are known to be important for altered expression in F1 hybrids. Epistatic effects of mitochondrial genotype on nuclear expression have been documented in e.g. *Drosophila (Mossman et al. 2016)* and humans (Barshad et al. 2018) , and recent research suggests that these epistatic interactions between mitochondrial genomes may have an important role in both disease and speciation (Hill 2017; Rand and Mossman 2020; Papier et al. 2022). Moreover, recent discoveries suggest that mitochondrial sncRNA influences nuclear gene expression (Passamonti et al. 2020). Interactions between the chloroplast and nuclear genomes are also known to affect gene expression in plants (Joseph et al. 2013; Petrillo et al. 2014; de Souza et al. 2017). Consistent

with a role for epistatic interactions with organellar genomes in gene expression, cross-direction dependent asymmetry in the reproductive barriers was reported in at least 21 of the gene expression studies we surveyed, while symmetric barriers were reported only in seven (**Table S1**). The number of studies where asymmetric barriers were reported could suggest that mitochondria or sex chromosomes, parts of the genome with parent-of-origin dependent inheritance (see above), may be important in hybrid misexpression, but it remains a challenge to disentangle the organellar contributions to this pattern. Promising approaches for exploring epistatic interactions between the nucleus and organellar genomes and their influence on gene expression include the use of cytoplasmic hybrids (Nuzhyna et al. 2016) and mitochondrial substitution lines that directly assess the effects of mito-nuclear interactions(Brekke et al. 2021).

In addition, a role for interactions between sex chromosomes and the nuclear genome in regulating gene expression has been increasingly recognized.Epistatic interactions between sex chromosomes and mitochondrial genomes can also regulate gene expression (Ågren et al. 2020) and disrupted imprinted gene expression can be dependent on sex chromosome genotype (Brekke et al. 2021). Separate analysis on male and female hybrids, enabling inference about putative roles of sex chromosomes in regulation of gene expression, were performed in six studies in our survey. In three of these, hybrid males (or male tissues) had more strongly altered patterns of gene expression relative to parents (two with XY (Llopart et al. 2018; Kerwin and Sweigart 2020,Banho et al. 2021a) and in three of these hybrid females had more strongly altered patterns of gene expression (two with XY and one ZW sex (Ponnanna et al. 2021; Sánchez-Ramírez et al. 2021; Yazdi et al. 2022). Overall, there are still too few studies that contrast patterns in hybrids across different sexes and tissues and future work should focus on the role of epistatic interactions among autosomes, sex chromosomes, organelles, and imprinted genes. An exciting, alternate new venue to explore

this is the use of attached-X hybrid females with the same degree of autosome-sex chromosome mismatch as that of hybrid males, enabling documenting the effects of X-linked recessive factors (Llopart et al. 2018).

Transposable Elements (TEs)

Transposable elements contribute to regulation of transcription, as they may contain sequences that can recruit the host transcription machinery to promote their own transposition and expression (Fueyo et al. 2022). Importantly, some TEs retain their regulatory ability after losing the ability to transpose, contributing promoter and enhancer sequences that may regulate expression in host genomes (Fueyo et al. 2022), affecting DNA folding and chromatin organization (reviewed in Lawson et al. 2023). TEs may become de-repressed when introduced into a hybrid if the parental repression mechanism is not inherited, which can lead to novel insertions and/or expression in the host genome (transcriptomic shock, McClintock 1984), causing hybrid reproductive isolation (Maheshwari and Barbash 2011; Serrato-Capuchina and Matute 2018; Gebrie 2023). As TE insertions and expression in hybrids has been reviewed elsewhere (Serrato-Capuchina and Matute 2018; Hénault 2021; Gebrie 2023), we will only briefly refer to some studies documenting the mechanism altering TE presence and/or expression and the effects of altered TE presence or expression on mRNA expression in hybrids retrieved from the search (**Table S2**).

In *Drosophila*, germline transposition and expression is controlled both by transcriptional silencing, (e.g., due to heterochromatin), and through post transcriptional silencing mediated by Piwi-interacting RNAs (piRNA). For instance, sequence divergence in the piRNA pathway in parental taxa at least partially explain deregulation of TEs in F1 hybrids and backcrosses between *D. buzzatii* and *D. koepferae (Romero-Soriano et al. 2017)*., The mechanism of TE deregulation differed between sexes in F1 hybrids between *D. arizona*

and *D. mojavensis wrigleyi (Banho et al. 2021b)*. In ovaries, TE upregulation was inferred to result from the combination of a lack of piRNAs mapping to the region where the TE resides and divergence of copies inherited from the two parental genomes of that region. In contrast, divergent expression of genes in testes was associated with the piRNA pathway and chromatin state (Banho et al. 2021b). In hybrids between *Arabidopsis thaliana* and *A. lyrata*, hybrid specific modifications of TE expression have instead been shown to arise when TEs are inserted close to genes (Göbel et al. 2018) providing another mechanism for increased TE expression in hybrids. Finally, there is evidence for TEs affecting mRNA expression in *Arabidopsis* hybrids. In crosses between the *Arabidopsis thaliana* accessions Columbia and Landsberg erecta, non-additive mRNA inheritance, specifically lower expression compared to the mid-parent expectation, was related to the presence of TEs and with the presence of small RNA from both strands (Li et al. 2015). This finding is intriguing as it potentially suggests that TEs could lead to novel patterns of expression of other genes in hybrids, which is an exciting venue for future research.

Small RNAs

Small RNAs (sRNAs) are known to regulate gene expression (Castel and Martienssen 2013; Plawgo and Raczynska 2022) and have long been known to have a non-additive expression in hybrids and allopolyploids (Lu and Chen 2011). Misexpression of microRNAs (miRNAs) during spermatogenesis has been shown to be predictive of sterility, with several miRNAs predictive of sterility for African clawed *Xenophus* frogs also being expressed during spermatogenesis in mice (Michalak and Malone 2008). Small RNAs have also been found to affect gene expression in allopolyploid hybrids, both in plants and animals. For instance, novel patterns of expression of miRNAs associated with developmental processes and downregulation of repeat-associated small interfering RNAs (siRNAs), have been

documented in allopolyploid *Spartina* hybrids (Cavé-Radet et al. 2020). Allopolyploid hybrids resulting from a cross between a female red crucian carp *Carassius auratus* and male common carp *Cyprinus carpio* had upregulated miRNAs associated with phenotypic changes in traits regulated by the pathways the miRNAs were involved in, affecting female fertility (Zhou et al. 2015). In plants, sRNA has been implicated in hybrid seed failure. For instance, homoploid F1 tomato hybrids show reduced abundance of some sRNA transcripts in the endosperm, resulting in overexpression of specific genes, likely contributing to hybrid seed failure (Florez-Rueda et al. 2021). Small RNA divergence between parental maize lineages is even predictive of grain production in hybrid progeny (Seifert et al. 2018). As described above, small RNAs may also mediate alternative expression patterns in conjunction with other mechanisms; TEs were associated with altered allele-specific expression in F1 (*Capsella*) flower buds only when found in conjunction with unique siRNA sequences (Steige et al. 2015).

In addition to the mechanisms outlined above, an exciting venue for future research is to address the effects of novel structural variants arising from hybridization on gene expression. For instance, are Topologically Associating Domains altered in hybrids, and does this lead to novel patterns of expression? Does hybridization induced novel genome rearrangements including inversions, deletions and insertions lead to novel patterns of gene expression? While some pioneering studies have shown a conservation of interacting regions, even in the face of genomic rearrangements (Galupa et al. 2022), more research is clearly needed to establish general patterns. A study on *Drosophila* revealed that two inversions each alter transcript abundance for hundreds genes across the genome, including genes that are not in LD with the inversion (Lavington and Kern 2017). In cottonwood hybrids (*Populus*) at least two genes have been identified with new splice variants in hybrid genomes that may

contribute to novel patterns of hybrid expression (Scascitelli et al. 2010). Few other studies of hybrid gene expression have examined alternative splicing, and assessing it in more species to assess its role in novel hybrid gene expression is another interesting topic for future studies.

New directions

Disentangling the roles of cell composition and alterations to gene expression

In most hybrid gene expression studies, we estimate an average gene expression in a complex tissue, such as testes or brains. Tissues are composed of many different cell types, each with a specific pattern of gene expression. Cellular composition can vary greatly between species, particularly for tissues that are highly divergent between species and may be of greatest interest for reproductive isolation. Cell composition can also differ between hybrids and parent species, particularly when there is hybrid dysfunction or heterosis or when parents and hybrids develop at different rates. As a result, novel patterns of expression in hybrids may reflect both changes in gene expression and changes in the relative abundance of individual cell types (Hunnicutt et al. 2022; Price et al. 2022). There are several good examples where cellular composition confounds estimates of hybrid gene expression. Hybrids of the yeast *Saccharomyces cerevisiae* and *S. paradoxus* have earlier meiosis relative to the parental species, which leads to a temporal signal of hybrid misexpression driven by differences in cell composition (Lenz et al. 2014). In sterile hybrid house mice, there are fewer late stage spermatogenesis cell types, and this difference in cell composition between sterile and fertile males leads to completely different patterns of differential expression compared to whole testes (Hunnicutt et al. 2022). Ideally, RNAseq studies would isolate RNA from individual cell populations at the same stages of development and environmental conditions, but this isn't possible for all organisms. Single-cell sequencing approaches can be

a useful alternative, if paired with good functional annotation and knowledge of cell-specific expression in the focal tissue (i.e. marker genes). For example, single cell sequencing made it possible to discover how both meiotic arrest and the differentiation stage of spermatogonia contributed to sterility in cattle and yak hybrids (Mipam et al. 2023). In another study, single-cell RNAseq of testes in the pied and collared flycatchers identified meiotic failures associated with hybrid sterility (Segami et al. 2022), that were not detected using whole tissue RNAseq (Mugal et al. 2020).

Beyond single genes - a network perspective

Studies of hybrid gene expression have focused primarily on identifying differentially expressed genes, often with the goal of identifying a few key genes that may contribute to reproductive isolation or hybrid phenotypes. At best, pairwise gene expression studies average across these axes of variation and most comparisons result in a long list of differentially expressed genes and it can be difficult to move beyond these gene lists. In addition, gene expression is the result of complex interactions among many different parts of the genome (see Mechanisms above). RNAseq studies sequence a small subset of the protein-coding genome , while the vast remainder of the genome contains DNA sequences that can play some role in modulating gene expression. We expect that many of the traits of interest in speciation are shaped by complex multi-loci interactions (Satokangas et al. 2020). These interactions can be depicted using a gene expression network approach, where sets of genes with similar expression patterns across individuals are identified and summarized (Ovens et al. 2021). Gene coexpression networks may be used to approximate the developmental genetic cascades that shape individual cell types, as genes interacting in a biological network will likely show higher levels of coordinated expression which can then be detected by the statistical network. Genes with the highest connectivity (correlation with the other

coexpressed genes in a gene set) are often called “hub genes;” while the “hub gene” terminology evokes transcription factors serving as key regulators of a developmental process, it it important to remember that these are simply the genes that most resemble the statistical pattern of expression.

Network approaches can allow for analyses that take into account the transcriptional changes that occur across the development of organisms and/or tissue specific development (*e.g.,* gametogenesis). With careful experimental design, they may also be used to detect the regulatory variation or daily and seasonal rhythms, across environmental contexts, and plasticity during development (Pfennig and Ehrenreich 2014). Gene coexpression networks also have the power to detect sets of genes that behave differently in parental species and their hybrids (Morgan et al. 2020; Brekke et al. 2021). However, network approaches have been little used to study hybrid gene expression in our literature survey (3/81 studies). By planning experiments with more replicates and deeper sequencing, we can detect co-regulated genes using gene network approaches, or examine more nuanced regulatory divergence such as alternative splicing (Todd et al. 2016). Moreover, long-read RNA sequencing will enable capturing the identities and abundances of different isoforms, which can have important biological implications even when the change in proportional expression is small. The ability to increase the resolution of RNAseq studies to better understand the complexity of divergent and hybrid genomes should be a major goal of future work, and will help to address outstanding questions about the nature of gene regulatory evolution and hybridization (**Box 2**). The more data we have about how genes are expressed, spliced, and co-regulated, the better we will be able to integrate phenotypes at different biological levels to get at adaptive function.

Hybrid gene expression evolution as a potential source of evolutionary novelty

Gene expression is not only altered in early generation hybrids, but also in stabilized hybrid lineages or species, following long periods of natural selection. In allopolyploid hybrids, studies of gene expression have documented sub-genome dominance where the expression in the hybrid is more similar to that of one of the parental species (Woodhouse et al. 2010; Parkin et al. 2014). This phenomenon has to date been documented in at least 73 studies (recovered in a Web of Science search, accessed August 16, 2023, with "sub-genome dominance" as search term). Typically, the parental subgenome that has the lowest density of TEs has been suggested to be the dominantly expressed subgenome (Freeling et al. 2015; Edger et al. 2017, 2019). The change in gene expression that polyploidization can bring about has also long been recognized as a potential source of novel variation (Hegarty et al. 2013) and some studies have highlighted interesting variation in patterns of gene expression in allopolyploids. For instance, the herbaceous weed *Capsella bursa pastoris* has a floral tissue gene expression similar to the parent species *C. orientalis* which also is a selfing plant with small flowers, whereas the gene expression is more similar to the outcrossing parent species *C. grandiflora,* which has a lower genetic load, in leaves and roots (Kryvokhyzha et al. 2019). While this pattern could arise as a consequence of many different processes including chance, an exciting possibility would be if gene expression in allopolyploid hybrids can evolve in an adaptive manner not only through minimizing the genetic load, e.g. in the form of TEs, expressed but also through using parental variation for ecological adaptation. While gene expression and its evolution in allopolyploid species and its potential role in evolutionary novelty in hybrids (Paun et al. 2011), allopolyploid novel gene expression is well documented (Freeling et al. 2015; Edger et al. 2017, 2019), the evolution of gene expression in homoploid species remains a field where significant progress could be made.

We performed a Web of Science literature search (accessed August 22nd, 2023), tailored to identify any study of gene expression in a stabilized hybrid species. The search

terms were ("hybrid species" OR "hybrid speciation" OR "hybrid lineage" OR "homoploid") AND ("gene expression" OR transcriptom* OR “allele specific expression” OR RNAseq). This search recovered 30 studies; we included only original data papers containing RNA data (20) and further excluded studies of polyploids (5), one study on chimeric genes, studies examining only TE expression (2), studies using RNA-seq for population genomics (4) and two papers on F1-hybrids. This leaves only three plant studies on Asteraceae species, from which one is a 300 year old hybrid lineage introduced to Britain (Lai et al. 2006; Hegarty et al. 2009; Brouillette and Donovan 2011; White et al. 2023), one study on a bird (Yazdi et al. 2022), one study on experimental hybrids from the approximately 840th generation following a cross between two species of *Drosophila (Ponnanna et al. 2021)*, and one study on 200th generation hybrid sculpins (*Cottus*) (Czypionka et al. 2012). There is also a study of nitrogen adaptation in Asteraceae based on a similar data set. In spite of the intermediate genome composition of these hybrid species or lineages, all show evidence of some degree of transgressive gene expression, where genes are either more or less expressed than both parental species. Two studies of Asteraceae estimated 1.5% and 2% of the genes to be transgressively expressed in *Helianthus deserticola* seedlings (Lai et al. 2006) and in *Senecio squalidus* mature flower buds (Hegarty et al. 2009), respectively. Interestingly, expression was more transgressive in the naturally occurring homoploid species than in lab generated early generation hybrids in *Senecio squalidus (Hegarty et al. 2009)*. In an RNAseq study of *Argyranthemum lemsii* and *A. sundingii*, 2% of the loci that were differentially expressed among parent species were transgressively expressed in both or either of the hybrid species (White et al. 2023). In contrast, the two studies on animals show much higher levels of transgressive gene expression in at least some tissues. In the Italian sparrow *Passer italiae,* 22% of the genes in testes, but only 0.3% of the genes in the ovaries, were found to be transgressively expressed in a RNAseq study (Yazdi et al. 2022). In the 840th generation

experimental hybrids arising from a *Drosophila nasuta nasuta* male and *D. n. albomicans* female cross, 11% of the ovarian transcriptome and 6% of the testes transcriptome were transgressively expressed in a microarray study (Ponnanna et al. 2021). Potentially, the differences between plants and animals could partly be explained by the fact that gene expression generally evolves faster in the gonads (Brawand et al. 2011), which are the focal parts in the animal studies. However, the mature flower buds of *S. squalidus* also contain reproductive tissues, which may mean that other differences between plants and animals may lead to increased transgressive expression in animal hybrids.

One line of evidence that suggests that the transgressive expression observed in homoploid hybrid species and lineages could be adaptive is the enrichment of functions that reflect adaptation to novel environments. The functions and networks that show transgressive expression include fatty acid biosynthesis, enriched in both *A. sundingii* and *A. lemsii*, biotin metabolism, flavonoid biosynthesis and N-Glycan biosynthesis enriched in *A. sundingii*, and carbon fixation which was enriched in *A. lemsii (White et al. 2023)*. In *S. squalidus*, two genes known to be genes known to be up-regulated in response to sulphur deficiency, glutathione S-transferase and ATP- sulfurylase were transgressively expressed (Hegarty et al. 2009), putatively suggesting that the novel expression patterns could be adaptive. Similarly, *H. deserticola* seedlings show an enrichment of ion transportation related genes among those that are transgressively expressed, speculated to be adaptive in the extremely arid environment in the desert floor where they grow (Lai et al. 2006). In a study of nitrogen adaptation in the homoploid hybrid *H. anomalus* transgressively expressed genes showed an overrepresentation of stress-response genes and genes involved in responses to biotic or abiotic stimuli (Brouillette and Donovan 2011). In the Italian sparrow, genes that were over-dominantly expressed in testis were enriched for functional categories involved in mitochondrial protein complex, binding of sperm to zona pellucida and genes involved in

forming the T-complex protein Ring Complex (TRiC). Subunits of this complex are required for spermatogenesis (Counts et al. 2017). There is ample evidence for selection against mitonuclear incompatibilities in the Italian sparrow (Hermansen et al. 2014; Trier et al. 2014; Elgvin et al. 2017; Runemark et al. 2018) and the TRiC complex was found to be under positive selection in the parental species (Rowe et al. 2020).

The evidence for transgressive gene expression in stabilized homoploid hybrids, especially the surprisingly high frequencies of novel expression patterns of genes in animal hybrids following many hundreds or thousands of generations, is striking. Experimental studies of purging of incompatibilities in the copepod *Tigropus californicus* hybrids also suggest that misexpression is highest at the F2 stage and reduced already in F4-F5 hybrids (Barreto et al. 2015), although only 1.2% of the transcriptome was transgressive in this study based on whole individuals from a variety of developmental stages. Moreover, population fitness of experimental *T. californicus* hybrids is regained as quickly as in the F9 generation judging from population growth rates (Pereira et al. 2021). In another study, invasive sculpins collected from the wild share patterns of transgressive gene expression with lab-reared F2 hybrids, indicating that novel gene expression patterns were maintained for at least 200 generations of natural selection, and may also contribute to the success of these fish exploiting novel environments (Czypionka et al. 2012). A very interesting question is to what extent transgressive gene expression in stabilized hybrid genomes could be selectively neutral or even adaptive. The evidence for altered expression of ion transport genes enabling the colonization of extremely arid environments in *H. deserticola* suggests that gene expression can result in evolutionary novelty in hybrid species. Another example is the expression of visual pigments, opsin genes, in cichlid fishes where hybridization has remixed *cis* and *trans*-acting factors that remodel the suites of visual pigments expressed in the eye (Nandamuri et al. 2017). Understanding of the mechanisms and selective pressures

determining gene expression evolution, and under what circumstances these contribute to evolutionary novelty (as opposed to incompatibilities) in hybrids, is an outstanding question. Research on the evolution of gene expression taking place during genome stabilization in homoploid hybrid taxa also holds great potential to generate general insights into regulation and evolution of gene expression.

In addition to individual hybrid taxa, adaptive radiations can provide insight into the mechanisms that generate novel gene expression, as recurrent hybridization and introgression in nascent lineages can expand the range of expression variation, allowing for novel traits to emerge (Seehausen 2004). In the *Cyprinodon* pupfish radiation, craniofacial gene expression F1 hybrids between recently diverged (6-19 kya) lineages have a high proportion of transgressive expression during development (McGirr and Martin 2019). This radiation is noted for the rapid evolution of craniofacial structure related to dietary adaptation, which has occurred through selection on ancient alleles that were remixed to expand trophic phenotypes (Richards et al. 2021). Transgressive phenotypes also dominate the cichlid fish adaptive radiation (Stelkens et al. 2009), which has been well reviewed elsewhere (Marques et al. 2019). In order to connect the mechanisms of gene expression evolution with selection for adaptive transgressive phenotypes in these radiations, we encourage more studies that examine expression remodeling in models of adaptive radiation.

Conclusions

We now know that altered gene expression in sterile or inviable hybrids is pervasive. To understand the mechanisms resulting in altered expression or transgressive expression, it is necessary to disentangle the relative contributions from changes in the cell type composition and altered expression within cells. Assessing to what extent entire networks or only specific genes are differentially expressed is also crucial for understanding how gene expression

evolves. Transgressive gene expression was not only found in sterile hybrids, but documented in more than half of the studies of viable and fertile hybrids. Furthermore, the few studies of stabilized homoploid hybrid lineages to date show that transgressive gene expression is common, and some find enrichment of transgressive expression in genes relevant to ecological adaptations. Jointly, this suggests that novel patterns of gene expression in hybrids have dual roles, both contributing to reproductive isolation and serving as substrate for novel adaptation.

Box 1: Outstanding questions in the field of gene expression and hybridization

Gene regulation in hybrid genomes
What regulatory mechanisms contribute to altered hybrid gene expression?
Is disruption of- and selection on gene expression acting mainly at a gene to gene level or a network level?
Are novel forms post-transcriptional modifications (e.g. alternative splicing) in hybrids common, and what are their effects on gene expression?
To which extent are patterns of expression in parental species predictive of hybrid gene expression?
Hybrid incompatibilities
Does hybrid misexpression more frequently arise from differences in tissue composition or changes in specific genes or gene regulatory networks?

How does hybrid misexpression manifest across a developmental timeline and does it lead to a cascade of disrupted gene expression?
Does the role of gene expression in reproductive isolation depend on the sex determination system?
What are the roles of sex chromosomes and mitochondria in altered expression leading to hybrid incompatibilities?
Hybrid novelty
Is altered gene expression an important component for hybrid novelty?
Are novel patterns of gene expression in hybrids molded by ecological selection or remnants of selection for regaining fertility in early generation hybrids?
Are genes that are expressed in a sexually dimorphic, plastic or polyphenic manner in the parental species more likely to have novel expression patterns in hybrids? Under what conditions do novel patterns of gene expression lead to incompatibility and under what circumstances do they lead to novel adaptive variation?

Box 2: Beyond DE genes in non-model organisms

Connecting Hybrid Gene Expression to Genetic Variation	
eQTL in a hybrid context	Quantitative Trait Locus (QTL) mapping uses crosses to identify regions of the genome statistically associated with a phenotype. In expression QTL (eQTL) studies, the phenotype being mapped is gene expression. This strategy is often used in model organisms due to the large numbers of samples needed, though recent experiments have used eQTL strategies to get at the regulatory basis of adaptive traits in non-models(see Carruthers et al. 2022).
eQTL using eigengene vectors	The main reason eQTL studies require so many individuals is that mapping the more than 10,000 genes expressed in a tissue or 20,000 genes expressed in a whole organism requires a great deal of statistical power. If network approaches are used to reduce the number of statistical tests by using module eigengene values rather than gene expression values, regions of the genome directly related to hybrid transgressive gene expression can be detected with many fewer individuals(see Brekke et al. 2021).
TWAS	For naturally occurring hybrid zones, historical recombination has remixed the genome creating an *in situ* mapping panel. Genome wide association studies (GWAS) use these populations to connect genotype to phenotype, and as with QTL studies, may use gene expression as the phenotype in a transcriptome wide association study (TWAS). Strategies for TWAS are still more widely used in biomedical contexts(Li and Ritchie 2021), but have great promise for hybrid systems as sequencing costs continue to go down(see Turner and Harr 2014)).
Identifying Regulatory Elements in Hybrid genomes	
ASE	The next step beyond looking at DE is to evaluate how individual parental alleles are expressed in hybrid tissues (allele specific expression, ASE). Evaluating ASE requires RNAseq reads at sufficient sequencing depth and genetic differentiation between the parental genomes to call variants within the sequenced read(Castel et al. 2015). For many hybrid studies, there should be enough fixed SNPs to call alleles for most genes in the genome, so the data being generated for expression studies can also be analyzed for ASE.
Splicing	Another mechanism for transcriptional control, alternative splicing can be quantified from high coverage RNAseq data provided alternative splice junctions have been sequenced at sufficient depth(see Mehmood et al. 2020). Newer long read sequencing of transcripts is also promising for better characterization of splice isoforms, and higher probability of including the splice junctions in reads for quantification(see Wright et al. 2022).

Allele specific epigenetics	One of the ways we can interrogate gene regulation in hybrids is to identify how parts of the genome inherited from different parents are methylated (allele specific methylation, ASM). The most accessible way to evaluate DNA methylation is through bisulfite conversion, which requires pipelines that can distinguish species variation from that generated in the conversion process(see Rodriguez-Caro et al. 2023). Allele specific chromatin configuration can be evaluated with assay for transposase accessible chromatin sequencing (ATAC-seq), which captures the total signal of open chromatin coming from both DNA methylation and histone modifications(see Devens et al. 2023).
Integrating methylation and expression networks	We can generate large data sets at different biological levels (organismal and tissue-level phenotypes, gene expression, epigenetics, genetics), but the challenge remains about how to integrate these data from different experiments into single analyses. New methods are being developed that integrate gene expression and epigenetic experiments(Itai et al. 2023), which will be useful as we advance analysis of hybrid gene regulation with complementary methodologies.
Understanding Variation Across Tissues	
Single cell sequencing, single cell eQTL	One of the challenges to understanding how hybridization shapes gene expression is that within a single organism expression is highly variable across tissues. Single cell transcriptomics allows for the evaluation of expression cell-by-cell, which can be used in hybrid systems to disentangle the effects of hybrid genomes on expression to that of cell composition in tissues(Hunnicutt et al. 2022). If this is done within the context of a mapping panel, single cell expression profiles can be connected to genotype(Ben-David et al. 2021). As of yet, this technology is expensive, so feasibility for these types of experiments will be expanded once cost comes down.
Spatial transcriptomics	As we begin to identify the critical tissues that are remodeled by transgressive expression in hybrids, the location of gene expression in the tissue or organ can become informative for understanding mechanisms underlying phenotype. Spatial transcriptomics is another way to account for heterogeneity in tissue gene expression, either by using spatially-limited marker genes to infer the location form single cell sequencing(Satija et al. 2015) or by using labeled probes with known spatial orientation during tissue preparation for sequencing(Williams et al. 2022).
Integrating single cell and regulatory networks	Connecting single cell sequencing datasets to putative regulatory networks facilitates the identification of the regions of the genome responsible for cell-type specific changes. If we want to be able to understand the mechanisms that alter and expand gene expression in hybrids, methods that overlay single cell sequencing with surveys of regulatory architecture in the genome are needed(Bravo González-Blas et al. 2023).

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Author Contributions

AR conceived of the study and all authors performed the literature surveys, data analysis, and wrote the manuscript. EM drew all artwork not attributed to Phylopic.

Data Accessibility and Benefit-Sharing

This study builds on published data only.

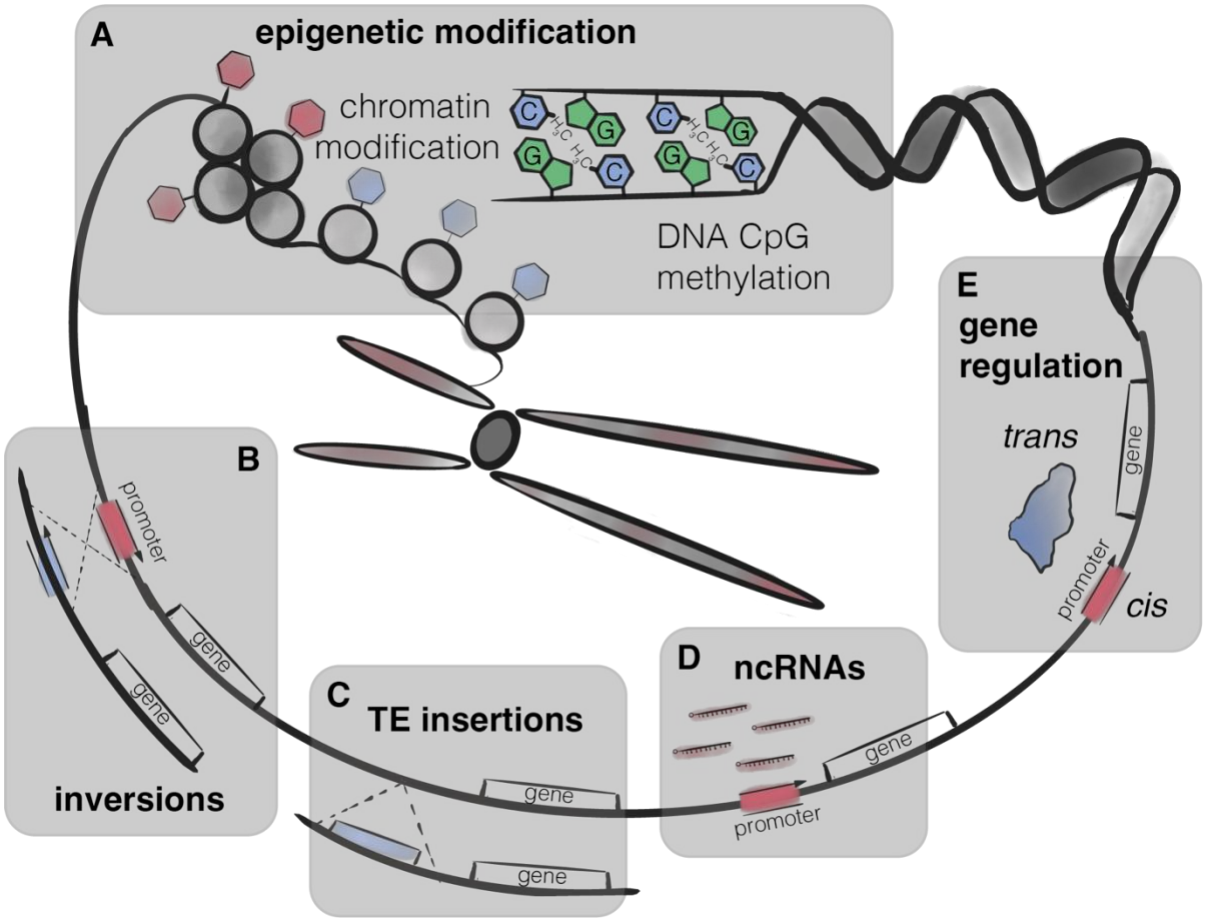

Figure 1. Factors affecting gene expression. A) Epigenetic modifications can occur at the histone level, with specific modifications generating open (blue) and closed (red) chromatin states. Alternatively, cytosine DNA methylation at regulatory CpG islands, short stretches of palindromic cytosine followed by guanine nucleotides (CpG) that are often near promoters, can alter gene expression. **B)** Inversions and other structural variants can remodel the regulatory landscape surrounding genes, altering the orientation of promoters relative to genes (ancestral promoter orientation in red, inverted orientation in blue). **C)** Allele-specific transposable element (TE) insertions can alter expression by inserting into the regulatory element of the gene adding or disrupting transcription factor binding sites, or by triggering TE silencing mechanisms that silence the gene as well. Regulatory elements from other locations in the genome, **D)** noncoding RNAs (ncRNAs), such as Piwi-interacting RNAs (piRNA) or short interfering RNA (siRNA), and **E)** *trans*-acting transcription factors interact with *cis*-acting allelic variation to fine-tune gene expression.

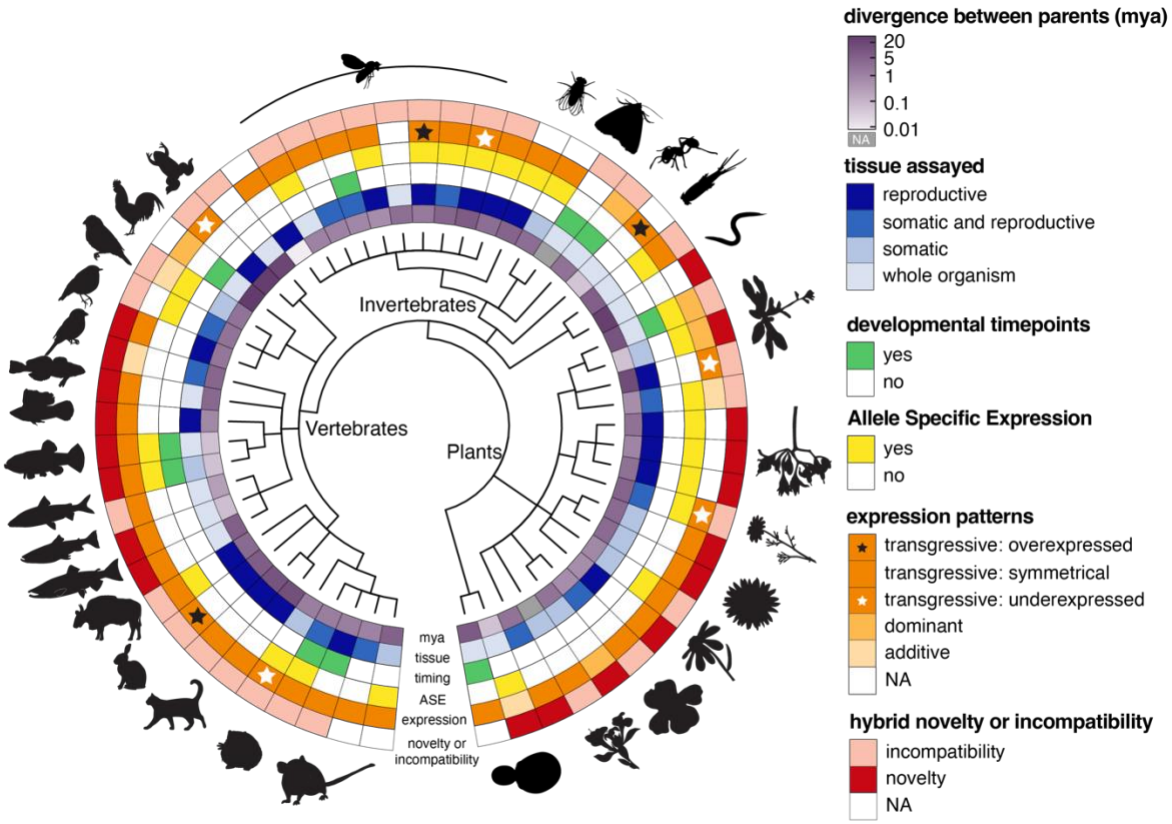

Figure 2. Survey of hybrid gene expression studies. Each tip of the tree represents two hybridizing taxa, with divergence time between parental taxa indicated in millions of years (mya). The tissue that hybrid gene expression has been evaluated was summarized as a reproductive tissue (e.g. testes or ovaries) or a somatic tissue (e.g. brain, leaf). We indicated whether studies used novel methods such as allele specific expression (ASE) or assaying expression over a developmental timeline. We further categorized studies by expression patterns found in hybrids and whether hybridization led to novelty or incompatibility. Where no information on tissue was given, this is denoted by NA.

https://www.phylopic.org/permalinks/1286be6838cef3d4d7afbcff2d2baa8d184a026eb85dcde64db00ff61457b82d

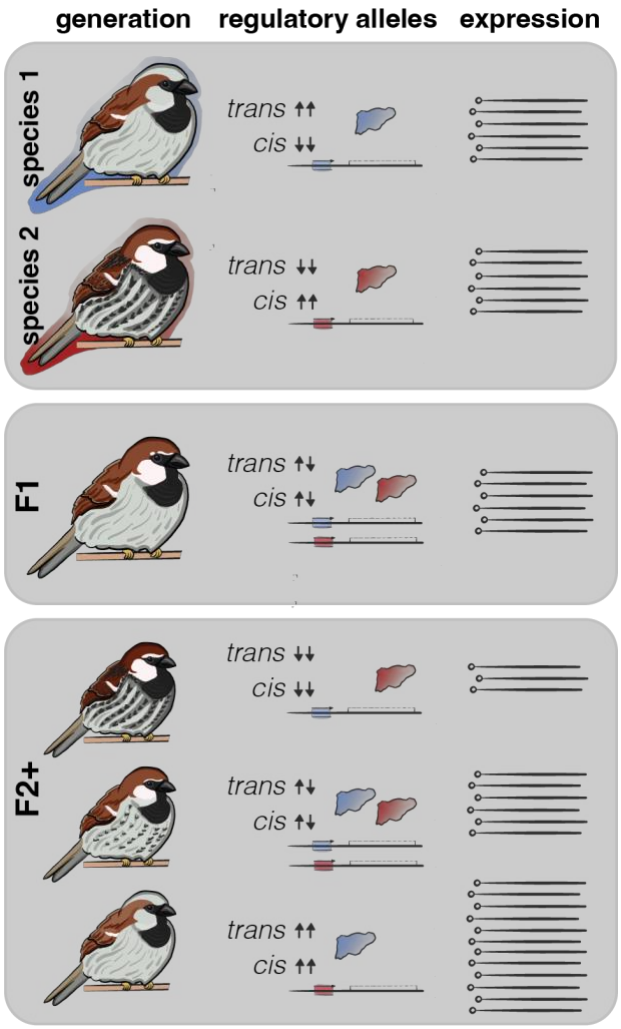

Figure 3. Co-evolved *cis*- and *trans*-regulatory elements can be broken up in hybrids, expanding the range of variation in gene expression. First generation hybrids (F1) may have expression within the range of parental variation if alleles are additive (*shown here*), but may also show transgressive expression if alleles have epistatic interactions. Later generation hybrids (F2+) thus have expanded expression variation that can be acted upon by selection.

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