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Insight

Intraspecific gene regulation in *cis*- and *trans*-

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Abstract

Changes in gene expression underlie much of evolution and occur via either *cis*-acting mutations, which lie near the affected gene and act in a context-specific manner, or *trans*-acting mutations, which may be far from the affected gene and act through diffusible molecules such as transcription factors. A commonly held view is that most expression variation within species is controlled in *trans*- while expression differences between species are largely controlled in *cis*-. Here, we summarize recent intraspecific gene regulation studies and find, contrary to this widely held view, that many studies in diverse taxa have revealed a large role for *cis*-acting mutations underlying expression variation within species. A review of the existing literature also shows that preparations using whole organisms rather than individual tissues may be biased toward identifying *trans*-regulation. Moreover, we note several examples of predominantly *cis*-acting regulation in recently diverged populations adapted to different environments. We highlight the challenges of drawing general conclusions from comparisons among studies that use different methodologies and we offer suggestions for studies that will address outstanding questions concerning the evolution of gene regulation.

Keywords: gene regulation, *cis*, *trans*, intraspecific variation

Current understanding of the contribution of *cis*- and *trans*-regulation to expression variation

Variation in gene expression is known to play a major role in evolution. Mutations causing gene regulatory changes can be either *cis*-acting or *trans*-acting; *cis*-regulatory mutations are typically located near the gene they influence and are thought to act in a modular, context-specific manner, whereas *trans*-regulatory mutations are typically located far from the gene they influence, and act in a diffusible manner via molecules such as transcription factors. Changes in gene expression have been associated with differentiation across a diverse set of organisms and situations including coat color differences in rodents (Linnen et al., 2013), hybrid infertility among species pairs (Mack & Nachman, 2017), colonization of freshwater by sticklebacks (Jones et al., 2012), and environmental adaptation in various species (Phifer-Rixey et al., 2018; Zhao et al., 2015). Furthermore, in some cases, functional studies have shown that changes in gene expression directly underlie differences in specific traits, such as pelvic spine reduction in three-spine sticklebacks (Chan et al., 2010; Shapiro et al., 2004) and species-specific wing patterning in insects (Gompel et al., 2005). Describing patterns of gene regulatory divergence is essential for understanding the genetic basis of evolutionary change.

Both *cis*- and *trans*-acting mutations contribute to changes in gene expression, and understanding the relative contribution of these mutations in different contexts has been a major focus of evolutionary genetics research (Hill et al., 2021; Signor & Nuzhdin, 2018). Early studies comparing interspecific and intraspecific variation suggested a general pattern of *trans*-acting mutations making a larger contribution to intraspecific variation, with *cis*-acting mutations making a larger contribution to interspecific differences (Coolon et al., 2014; Emerson et al., 2010; Metzger et al., 2017; Schaefke et al., 2013; Wittkopp et al., 2008). Wittkopp et al. (2008) first demonstrated this pattern using crosses between *Drosophila melanogaster* and *D. simulans*, finding that *cis*-acting regulation accounted for a larger proportion of expression differences in interspecific crosses as opposed to intraspecific crosses. This pattern was corroborated primarily in other *Drosophila* and *Saccharomyces* studies, which generally demonstrated greater *cis*-acting regulation for interspecific comparisons (Coolon et al., 2014), and sometimes increasing proportions of *cis*-acting regulation with increasing divergence times (Metzger et al., 2017).

However, the commonly held view that expression variation within species is primarily controlled in *trans*- (e.g., (Hill et al., 2021; Signor & Nuzhdin, 2018) is belied by a number of recent studies that have found a predominance of *cis*-acting mutations underlying expression variation within species, even at the earliest stages of population differentiation (Ballinger et al., 2023; Verta & Jones, 2019) or among individuals within the same population (Osada et al., 2017; Puixeu et al., 2023). Many studies have now been conducted on a variety of organisms assessing the relative contributions of *cis*- and *trans*-acting changes within species. Here, we review these studies focusing exclusively on patterns of expression variation within species; we do not explore the patterns of *cis*- and *trans*-regulation between species, which are discussed elsewhere (e.g., Guerrero et al., 2016; Hill et al., 2021; Signor & Nuzhdin, 2018).

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Factors governing the relative contribution of *cis*- and *trans*-acting mutations to expression variation

When considering the expected proportion of *cis*- and *trans*-changes, it is useful to think about the dynamics of mutations in populations. Mutations arise in individuals. A fraction of new mutations may increase in frequency and give rise to variation among individuals within a species. A smaller fraction may eventually increase in frequency, become fixed, and thereby give rise to differences between species. Strongly deleterious mutations are expected to be eliminated quickly by selection. However, weakly deleterious mutations may become polymorphic within populations, contributing to variation among individuals, but rarely becoming fixed, and thus contributing little to differences between species (Kimura, 1983; Ohta, 1973, 1992). In contrast, unconditionally beneficial mutations are expected to sweep quickly to fixation, thereby contributing more to differences between species than to variation within species (e.g., Sawyer & Hartl, 1992).

Because *trans*-acting mutations can affect a higher number of downstream genes compared to *cis*-acting mutations, they are expected to be more pleiotropic and therefore more deleterious, on average (Emerson et al., 2010; Signor & Nuzhdin, 2018). Experimental studies in yeast support this logic, showing that the negative fitness effects of *trans*-acting mutations affecting the expression of TDH3, a gene encoding a key metabolic enzyme, are greater than the fitness effects of *cis*-acting mutations affecting this same gene (Vande Zande et al., 2022). If *trans*-acting mutations are, in general, more deleterious than *cis*-acting mutations, then the proportion of *trans*- to *cis*- mutations is expected to be greater within species than between species.

On the other hand, some mutations affecting gene expression are undoubtedly beneficial. Many authors have suggested that *cis*-acting mutations may contribute more to adaptation than *trans*-acting mutations, specifically because they are less likely to be pleiotropic (Coolon et al., 2014; Lemos et al., 2008; Vande Zande et al., 2022; Wittkopp et al., 2008). Such mutations are expected to contribute disproportionately to differences between species. Indeed, many studies have recognized the importance of positive selection in shaping the relative amount of *cis*- versus *trans*-regulation in comparisons between species (Coolon et al., 2014, 2015; Emerson et al., 2010; Schaefke et al., 2013). For example, Coolon et al. (2014) found that despite being more recently diverged, *D. simulans* and *D. sechellia*, which are separated by about 250,000 years, showed a greater proportion of *cis*-acting changes than seen between *D. melanogaster* and *D. simulans*, which are separated by about 2.5 million years. Coolon et al. (2014) noted that *D. sechellia* is adapted to a very distinct environment and suggested that the large number of *cis*-acting changes seen in this recently diverged pair might reflect positive selection.

In summary, deleterious mutations (whether *cis*-acting or *trans*-acting) are expected to contribute more to variation within species than to differences between species, while beneficial mutations (whether *cis*-acting or *trans*-acting) are expected to become fixed and thereby contribute to differences between populations (in the case of local adaptation) or to differences between species (in the case of unconditionally beneficial mutations). Thus, *cis*-acting mutations that are involved in local adaptation might underlie intraspecific expression variation more than previously appreciated.

Another factor that may affect the relative proportion of *cis*-acting versus *trans*-acting mutations is methodological. Specifically, samples of whole organism RNA may be biased toward the detection of *trans*-acting mutations due to the inability to detect tissue-specific *cis*-changes in these samples and the possibility that differences in cellular composition could present as *trans*-regulated expression divergence, as described in more detail below. Here, we evaluate recent studies in light of these expectations.

Methodologies to study *cis*- and *trans*-acting gene regulation

The first efforts to identify the genetic basis of variation in transcript abundance used one of two main approaches (Figure 1). First, recognizing that gene expression level could be treated as a quantitative trait, a number of studies sought to map loci controlling expression variation using the existing

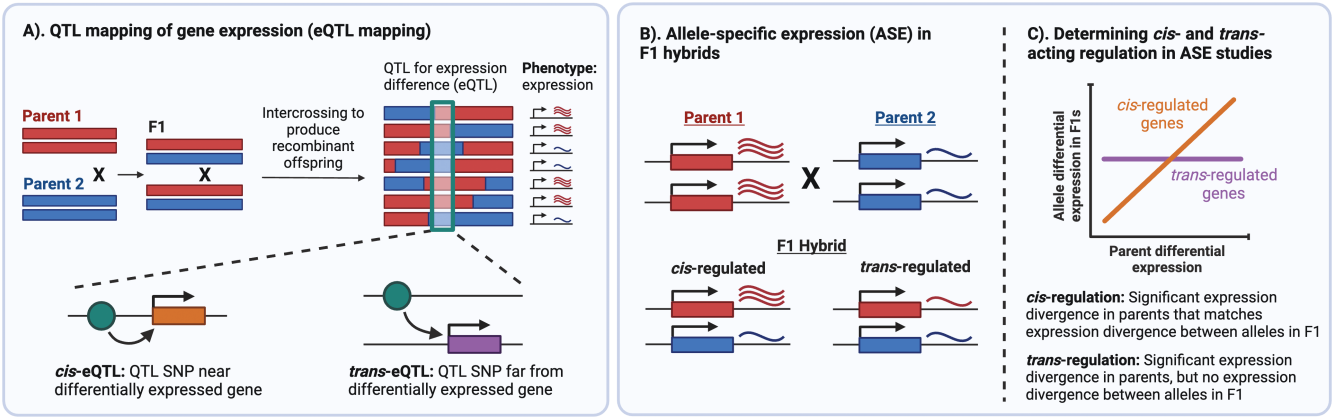

Figure 1. Methodologies to measure *cis*- and *trans*-acting gene regulation. (A) eQTL mapping can be used to identify genomic regions associated with differential expression by creating a recombinant mapping population from two starting parental groups. eQTL that are near the differentially expressed gene are categorized as *cis*-regulated, while eQTL far from the differentially expressed gene are categorized as *trans*-regulated. (B–C) *cis*- and *trans*-regulation can be inferred using allele-specific expression (ASE) in F1 hybrids. *Cis*-regulation is categorized as a differential expression between two parents that is recapitulated as a differential expression between the two alleles in an F1 hybrid. *Trans*-regulated genes will not show this allele-specific expression pattern. Figure was partially created with BioRender.com.

framework of quantitative trait locus (QTL) mapping. Applied to gene expression data, these studies identified expression QTL (eQTL) affecting the expression of single genes in *cis*- as well as eQTL affecting the expression of multiple genes in *trans*- (e.g., Brem et al., 2002). A second approach uses the expression levels of individual alleles in F1 hybrid crosses. In an F1 hybrid, chromosomes from both parents are exposed to the same *trans*-acting environment. Because *cis*-regulatory elements are linked to the genes they influence, differences in gene expression between the two alleles in an F1 hybrid can be attributed to *cis*-acting mutations (Cowles et al., 2002; Wittkopp et al., 2004).

The mapping resolution of the first approach is often limited owing to the limited number of informative meioses in typically sized mapping populations (e.g., Turner et al., 2014), but see Albert et al. (2018). However, the second approach can directly identify genes whose expression is controlled in *cis*-. Furthermore, by comparing allele-specific expression (ASE) in F1s to expression differences between parents, *trans*-effects can be inferred. Genes whose expression differs between the parents but not between alleles in an F1 are likely controlled by variation that acts in *trans*- (Figure 1 B–C). The simplicity and power of this approach have led to its widespread adoption in a wide variety of organisms involving crosses between parents separated by varying amounts of time. For these reasons, we focus on studies using ASE in F1 hybrids to understand patterns of intraspecific gene regulatory divergence. Additionally, the primary focus of eQTL studies is often to identify the general location of regulatory mutations, as opposed to quantifying the relative contribution of *cis*- and *trans*-acting regulation to expression divergence. Lastly, a benefit of ASE is that *cis*-regulation is detected when the causal regulatory mutation and the affected gene segregate together on the same haplotype, whereas in eQTL studies, genes are defined as *cis*-regulated if the regulatory SNP is within a predefined, subjective distance from the affected gene.

Empirical studies of *cis*- and *trans*- gene regulation within species

Table 1 summarizes results from intraspecific studies that have inferred *cis*- and *trans*-acting gene regulation by looking at ASE patterns in F1 hybrids. These studies are diverse, including both unicellular and multicellular organisms and spanning plants, animals, and fungi of varying divergence times. Comparisons among these studies offer a detailed view of the contribution of *cis*- and *trans*-regulation to intraspecific gene expression variation.

However, there are several important caveats to keep in mind when comparing results across different expression studies. First, the power to detect ASE depends on read depth (Albert et al., 2018), and therefore studies of differing sequencing depth may report varying degrees of *cis*- and *trans*-regulation that are not directly comparable. Second, there are multiple ways of assessing the relative importance of *cis*-acting and *trans*-acting mutations to gene expression differences, and different studies have emphasized different approaches. For example, many studies have identified the proportion of genes at which expression differences are governed exclusively in *cis*- or exclusively in *trans*-, as described in McManus et al. (2010). Others have compared the proportion of genes showing any significant *cis*- or *trans*-regulation. Finally, some studies have emphasized the amount of expression variation that is explained by *cis*- and *trans*-acting mutations, which accounts for differences in effect size between mutations. We have noted the method used to determine the predominant regulatory type in Table 1.

Contrary to the view that most intraspecific gene expression variation is controlled in *trans*-, of the 37 studies included in Table 1, 19 had comparisons showing predominantly *cis*-acting regulation, and 14 showed predominantly *trans*-acting regulation, with the others having approximately equal amounts or inconclusive results. Importantly, this pattern is seen across a range of diverse taxa and in studies that used various methodologies to determine the predominant regulatory type. Below we discuss factors influencing the relative contribution of *cis*- and *trans*-acting mutations in the studies shown in Table 1, exceptions to these patterns, and future directions for the field.

Whole organism preparations create a bias toward the detection of *trans*-regulation

Various experimental designs have been used to study intraspecific expression differences, and the use of single-tissue vs. whole-organism samples has the potential to distort the measured contribution of *cis*- and *trans*-regulation to expression differences. Whole organism preparations may lead to a biased over-detection of *trans*-acting regulation. This bias could arise in two ways. First, changes in cellular composition (i.e., changes in the proportions of different cell types) can lead to differences in overall expression between groups that do not reflect expression differences in individual cells (Brawand et al., 2011). In whole-organism samples, these expression differences will appear as *trans*-regulated though they are not mediated by a *trans*-regulatory element (Ranz et al., 2023). For example, an organism with a larger brain may have increased expression of brain-associated transcripts because of having more brain cells, not due to mutations upregulating these brain-associated transcripts. Second, *cis*-acting changes are often associated with tissue-specific gene expression (Carroll, 2005; Durkin et al., 2024; Mack et al., 2023; Wray, 2007). If RNA is measured at the level of the entire organism, this could dampen the signal of tissue-specific *cis*-acting changes, therefore minimizing the level of *cis*-regulation detected. Importantly, this effect may be present even within single tissues that are heterogeneous and contain many cell types, such as testes, highlighting the utility of single-cell RNA sequencing approaches (Hunnicutt et al., 2022). Therefore, the degree of cellular heterogeneity of the focal tissue is an important consideration when evaluating the biases that may arise from sampling design.

Support for this effect comes from *Drosophila* studies that used single tissues (Osada et al., 2017; Puixeu et al., 2023; Ramirez-Corona et al., 2021) vs. whole flies (Chen et al., 2015; Coolon et al., 2014; Huang et al., 2021; Suvorov et al., 2013) where the single-tissue analyses revealed more *cis*-acting regulation whereas whole-fly studies did not. Also consistent with a tissue effect, in their investigation of locally adapted *Drosophila mojavensis*, Benowitz et al. (2020) found that the degree of *trans*-regulation is elevated in whole bodies in comparison to brain samples, and they conclude that the use of whole bodies blunted their ability to detect *cis*-acting changes. Similar trends are seen in fish and plant studies using single-tissue (Cubillos et al., 2014; Verta & Jones, 2019) and

Table 1. Intraspecific and sub-species comparisons using ASE in F1 hybrids to investigate the relative contribution of *cis*- and *trans*-regulation to gene expression differences.

Study System[1]	Comparison details and divergence time, if known[2]	Tissue	Predominant regulatory type reported[3]	Percent *cis*-only[4]	Percent *trans*-only	*cis:trans* ratio	Citation
Fungi							
Saccharomyces cerevisiae	Laboratory vs. wild strain	NA, unicellular	*trans*[a,b]	6.7%	25.1%	0.27	(Sung et al., 2009)
Saccharomyces cerevisiae	Laboratory vs wild strain	NA, unicellular	*trans*[a,b]	0.7%	2.8%	0.25	(Emerson et al., 2010)
Saccharomyces cerevisiae	Laboratory vs wild strain	NA, unicellular	*trans*[a]	13.8%	21.1%	0.65	(Schaefke et al., 2013)
Insects							
Drosophila melanogaster	Inbred laboratory strains	Whole fly	*cis*[c]				(Wittkopp et al., 2008)
Drosophila melanogaster	Locally adapted strains, 10,000 years	Whole fly	*trans*[b]				(Coolon et al., 2014)
Drosophila melanogaster	Inbred laboratory strains	Whole fly, room temp. condition	*trans*[a]	5.2%	6.7%	0.78	(Chen et al., 2015)
Drosophila melanogaster	Geographically distinct laboratory strains	Head and body separated	*cis*[b,c]				(Osada et al., 2017)
Drosophila melanogaster	Isofemale lines from the same locality	Malpighian tubule, comparison 1	*trans*[a]	1.6%	9.6%	0.17	(Glaser-Schmitt et al., 2018)
Drosophila melanogaster	Ancestral vs derived strains	Malpighian tubule, comparison 2	*trans*[a]	0.9%	9.8%	0.09	(Glaser-Schmitt et al., 2018)
Drosophila melanogaster	Ancestral vs derived strains	Malpighian tubule, comparison 3	*trans*[a]	1.1%	10.6%	0.10	(Glaser-Schmitt et al., 2018)
Drosophila melanogaster	Locally adapted populations, 1,000 – 2,000 years	Whole fly	*trans*[a]				(Huang et al., 2021)
Drosophila melanogaster	Geographically distinct lines	Fat body, pre-infection condition	*cis*[a]	1.75%	0.32%	5.47	(Ramirez-Corona et al., 2021)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Head, comparison 1, sex averaged	*cis*[a]	~3.45%[5]	~2.45%[5]	1.41	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Head, comparison 2, sex averaged	*cis*[a]	~4.6%[5]	~2.95%[5]	1.56	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Head, comparison 3, sex averaged	*cis*[a]	~4.7%[5]	~2.25%[5]	2.09	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Ovaries, comparison 1	*cis*[a]	~5.1%[5]	~3.8%[5]	1.34	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Ovaries, comparison 2	*cis*[a]	~4.7%[5]	~1.6%[5]	2.94	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Ovaries, comparison 3	*cis*[a]	~5%[5]	~2.2%[5]	2.27	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Testes, comparison 1	*cis*[a]	~6.3%[5]	~3.9%[5]	1.61	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Testes, comparison 2	*cis*[a]	~7.2%[5]	~2.6%[5]	2.77	(Puixeu et al., 2023)
Drosophila melanogaster	Isofemale laboratory strains (DGRP)	Testes, comparison 3	*cis*[a]	~8.25%[5]	~2.85%[5]	2.89	(Puixeu et al., 2023)
Drosophila mojavensis	Locally adapted strains	Whole fly, brain	inconclusive[a,c]				(Benowitz et al., 2020)
Drosophila pseudoobscura	Inbred laboratory strains	Whole fly	*trans*[a]	7.9%	16.9%	0.47	(Suvorov et al., 2013)

Table 1. Continued

Study System[1]	Comparison details and divergence time, if known[2]	Tissue	Predominant regulatory type reported[3]	Percent *cis*-only[4]	Percent *trans*-only	*cis:trans* ratio	Citation
Drosophila simulans	Inbred laboratory strains	Whole fly	*cis*[c]				(Wittkopp et al., 2008)
Tetranychus urticae	Strains of differing toxin resistance	Whole mite, comparison 1	*trans*[a]	13.07%	22.04%	0.59	(Kurlovs et al., 2022)
Tetranychus urticae	Strains of differing toxin resistance	Whole mite, comparison 2	*trans*[a]	10.69%	34.57%	0.31	(Kurlovs et al., 2022)
Tetranychus urticae	Strains of differing toxin resistance	Whole mite, comparison 3	*trans*[a]	11.03%	30.85%	0.36	(Kurlovs et al., 2022)
Tetranychus urticae	Strains of differing toxin resistance	Whole mite, comparison 4	*trans*[a]	10.75%	31.03%	0.35	(Kurlovs et al., 2022)
Mammals							
Mus musculus domesticus × *M. m. castaneus*	Sub-species, 250,000 – 500,000 years	Liver	*cis*[a]	14%	0.6%	23.34	(Goncalves et al., 2012)
Mus musculus domesticus × *M. m. castaneus*	Sub-species, 250,000 – 500,000 years	Retina	*cis*[a]	30.8%	7.4%	4.43	(Shen et al., 2014)
Mus musculus domesticus × *M. m. musculus*	Sub-species, 250,000 – 500,000 years	Testes	*cis*[a]	24%	9%	2.67	(Mack et al., 2016)
Mus musculus domesticus	Locally adapted strains, 500 years	Liver, room temp. condition	*cis*[a]	7%	4%	1.75	(Ballinger et al., 2023)
Mus musculus domesticus	Locally adapted strains, 500 years	Brown adipose tissue, room temp. condition	*cis*[a]	8%	4%	2	(Ballinger et al., 2023)
Mus musculus domesticus[6]	Locally adapted strains, 500 years	Liver	*cis*[a]	8.0%	4.6%	1.74	(Durkin et al., 2024)
Mus musculus domesticus[6]	Locally adapted strains, 500 years	Brown adipose tissue	*cis*[a]	6.3%	4.0%	1.58	(Durkin et al., 2024)
Fish							
Astyanax mexicanus	Locally adapted populations, 161,000 – 191,000 years	Embryos (10 hours post fertilization)	*cis*[a]	3.5%	0%	NA	(Leclercq et al., 2024)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Pharyngeal tooth plates, comparison 1	*trans*[c]	13.9%	15.8%	0.88	(Hart et al., 2018)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Pharyngeal tooth plates, comparison 2	*trans*[c]	12.4%	11.7%	1.06	(Hart et al., 2018)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Gills, comparison 1	*cis*[a]	~9%[5]	~4%[5]	2.25	(Verta & Jones, 2019)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Gills, comparison 2	*cis*[a]	~13%[5]	~8%[5]	1.63	(Verta & Jones, 2019)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Gills, comparison 3	*cis*[a]	~11%[5]	~8%[5]	1.24	(Verta & Jones, 2019)
Gasterosteus aculeatus	Locally adapted populations, 10,000–20,000 years	Gills, comparison 4	*cis*[a]	~20%[5]	~13%[5]	1.54	(Verta & Jones, 2019)
Menidia menidia	Locally adapted populations	Whole larvae, condition 1	*trans*[a]	3.4%	8.4%	0.40	(Jacobs et al., 2024)
Menidia menidia	Locally adapted populations	Whole larvae, condition 2	*trans*[a]	2.5%	15.8%	0.16	(Jacobs et al., 2024)

Table 1. Continued

Study System[1]	Comparison details and divergence time, if known[2]	Tissue	Predominant regulatory type reported[3]	Percent *cis*-only[4]	Percent *trans*-only	*cis:trans* ratio	Citation
Birds							
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Brain, comparison 1, sex averaged	*trans*[a]	3.60%	4.28%	0.84	(Wang et al., 2019)
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Brain, comparison 2, sex averaged	*trans*[a]	2.47%	4.22%	0.59	(Wang et al., 2019)
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Liver, comparison 1, sex averaged	*trans*[a]	7.86%	13.43%	0.59	(Wang et al., 2019)
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Liver, comparison 2, sex averaged	*trans*[a]	6.36%	13.51%	0.47	(Wang et al., 2019)
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Muscle, comparison 1, sex averaged	*trans*[a]	5.16%	16.27%	0.32	(Wang et al., 2019)
*Gallus gallus domesticus**	Artificially selected breeds, ~100 years	Muscle, comparison 2, sex averaged	*trans*[a]	3.22%	18.23%	0.18	(Wang et al., 2019)
Plants							
Arabidopsis thaliana	Diverged accessions	Seedling	approximately equal amounts[b]				(Zhang & Borevitz, 2009)
Arabidopsis thaliana	Diverged accessions	Rosette	*cis*[c]				(Cubillos et al., 2014)
*Capsicum annuum**	Cultivated vs. wild strain	Placenta and pericarp pooled	*trans*[a]	0.9%	17.8%	0.05	(Diaz-Valenzuela et al., 2020)
Cirsium arvense	Diverged accessions	Leaf	approximately equal amounts[a,b]				(Bell et al., 2013)
*Gossypium hirsutum**	Cultivated vs. wild strain	Cotton fibers, midpoint reported	inconclusive[a,c]	1.65%	1.00%	1.65	(Bao et al., 2019)
Oryza sativa indica × *O. s. japonica*	Sub-species, 440,000 years	Panicle (rice, stem, leaf pooled)	*trans*[a]	13.1%	14.6%	0.90	(Xu et al., 2014)
Oryza sativa indica	Locally adapted ecotypes	Leaf, non-stress	*cis*[a]	11.2%	8.9%	1.26	(Ereful et al., 2021)
Panicum hallii	Locally adapted ecotypes	Leaf	*cis*[a]				(Lovell et al., 2016)
Salix purpera	Clones from the same locality	Internode, F1 Family	*cis*[a]	2.80%	1.60%	1.75	(Carlson et al., 2017)
Salix purpera	Clones from the same locality	Shoot tip, F1 Family	*cis*[a]	5.90%	4.80%	1.23	(Carlson et al., 2017)
*Zea mays**	Domesticated inbred lines	Immature ear, seedling	*cis*[a]				(Stupar & Springer, 2006)
*Zea mays**	Domesticated inbred lines	Immature ear, B73 x Mo17 comparison	*cis*[a]	30.3%	3.4%	8.91	(Springer & Stupar, 2007)
*Zea mays**	Domesticated inbred lines	Embryo, B73 x Mo17 comparison	*cis*[a]	37.2%	1.8%	20.67	(Springer & Stupar, 2007)
*Zea mays**	Domesticated inbred lines	Seedling, B73 x Mo17 comparison	*cis*[a]	34.2%	1.5%	12.32	(Springer & Stupar, 2007)
*Zea mays**	Domesticated inbred lines	Meristem	*cis*[b]				(Guo et al., 2008)

Table 1. Continued

Study System[1]	Comparison details and divergence time, if known[2]	Tissue	Predominant regulatory type reported[3]	Percent *cis*-only[4]	Percent *trans*-only	*cis:trans* ratio	Citation
*Zea mays** (Maize × Teosinte)	Cultivated vs. wild strain, 9,000 years	Ear	approximately equal amounts[a,c]	19.5%	19%	1.03	(Lemmon et al., 2014)
*Zea mays** (Maize × Teosinte)	Cultivated vs. wild strain, 9,000 years	Leaf	*trans*[a,c]	15.2%	21.3%	0.71	(Lemmon et al., 2014)
*Zea mays** (Maize × Teosinte)	Cultivated vs. wild strain, 9,000 years	Stem	*cis*[a,c]	20.1%	13.8%	1.46	(Lemmon et al., 2014)

Note.
*Artificial selection examples.
[1]Restricted to only intraspecific studies that explicitly quantified both *cis*- and *trans*-acting regulation using allele-specific expression in F1 hybrids. Information not reported in the original paper is left blank, and we did not include separate studies that utilized identical datasets.
[2]When available, the divergence time noted in the cited paper is given, otherwise it is left blank.
[3]"Predominant regulatory type reported" refers to the mode of regulation determined to be dominant. We have noted the method used to determine predominant regulatory type: [a]the relative number of *cis*-only and *trans*-only genes, [b]the relative number of genes with significant *cis*-regulation and *trans*-regulation, or [c]the relative contribution of *cis*- and *trans*-acting regulation to parental differential expression.
[4]In studies that quantified the percent of *cis*-only and *trans*-only genes (as in McManus et al., 2010), those values are reported along with the ratio of *cis*-only to *trans*-only genes. Studies with multiple comparisons or multiple tissues that had information on *cis*-only and *trans*-only genes are listed on separate rows.
[5]Number had to be estimated from Figure. Exact numbers not reported.
[6]For Durkin et al. (2024), only the EDMA comparison is listed as the SARA comparison is reported in Ballinger et al. (2023).

whole-organism preparations (Diaz-Valenzuela et al., 2020; Jacobs et al., 2024; Xu et al., 2014).

Many studies assessed the relative contribution of *cis*- and *trans*-acting mutations to expression variation by comparing expression differences between parents, comparing expression differences between alleles in the F1, and comparing the ratio of parental and F1 allelic expression differences (as in McManus et al., 2010). From these comparisons, most studies reported the number of *cis*-only and *trans*-only genes. *Cis*-only to *trans*-only ratios greater than one indicate a preponderance of *cis*-only genes, while values less than one indicate a preponderance of *trans*-only genes. To assess the potential bias of sample preparation, we compared this ratio across studies that used single tissues and studies that used whole organisms. All studies using whole organisms reported a predominance of *trans*-acting regulation. In contrast, all studies except two (Glaser-Schmitt et al., 2018; Hart et al., 2018) using single tissues reported a larger proportion of *cis*- compared to *trans*-acting regulation (Figure 2).

Role of positive selection in driving *cis*-acting regulation

Another key determinant of the proportion of *cis*- or *trans*-acting mutations may be the average fitness effects of variants, as noted by previous authors (e.g., Coolon et al., 2014; Wittkopp et al., 2008). If many *cis*-acting mutations are fixed by positive selection, we might expect that a large proportion of *cis*-acting changes would also be seen at short time scales (within a single species) in situations where populations have experienced strong selection. There are several examples in which recently diverged populations, ranging from a few hundred to 10,000 years, show a greater proportion of *cis*-acting changes. For example, this pattern was seen in locally adapted populations of *Mus musculus domesticus* from tropical and temperate habitats (Ballinger et al., 2023; Durkin et al., 2024), in Mexican tetra (*Astyanax mexicanus*) from surface and cave environments (Leclercq et al., 2024), in sticklebacks (*Gasterosteus aculeatus*) from marine and freshwater environments (Verta & Jones, 2019), and in diverged accessions of *Arabidopsis thaliana* (Cubillos et al., 2014; Zhang & Borevitz, 2009) among others.

While we do not have information on the evolutionary history of the focal populations from enough studies to make inferences on the relationship between selection and *cis*-regulation more broadly, we note that the importance of positive selection in fixing *cis*-regulatory changes does not appear to be limited to comparisons between species but can also be seen between populations within species over very short timescales. Understanding how the strength of selection influences the relative proportion of *cis*- and *trans*-acting regulation and the relationship between divergence time and the proportion of *cis*-regulation remain important topics for future studies.

Exceptions and other considerations

While we found that *cis*-regulation dominates expression variation in studies that used single tissues, we did find exceptions. First, Wang et al. (2019) found that single-tissue expression differences between domesticated chicken breeds that diverged within the past 100–150 years are primarily governed by *trans*-acting changes, perhaps indicating that strong artificial selection can fix pleiotropic mutations that would be deleterious and unlikely to fix under more natural conditions. However, we note that not all examples of artificial selection find predominantly *trans*-acting regulation (Table 1).

Second, Hart et al. (2018) found *trans*-acting changes to underlie a majority of expression differences between marine and freshwater stickleback pharyngeal tooth plates, in contrast to the finding by Verta and Jones (2019) of predominantly *cis*-acting changes between marine and freshwater sticklebacks

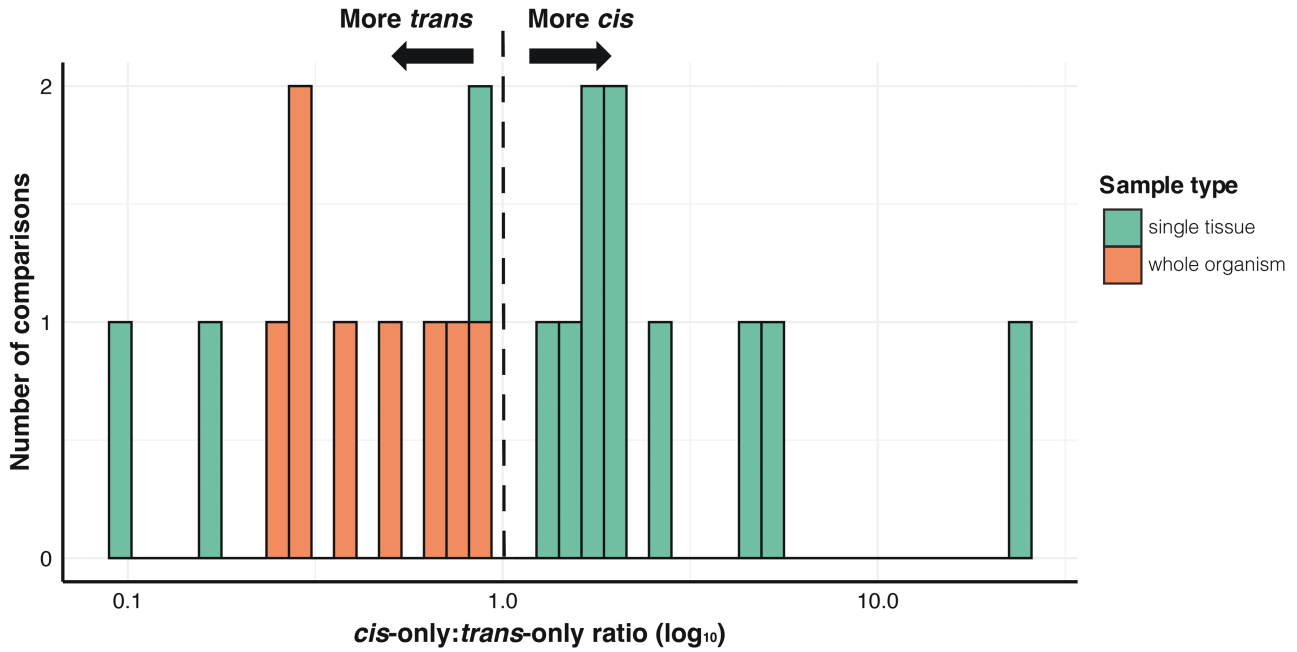

Figure 2. Influence of sample preparation on the relative contribution of *cis*- and *trans*-acting regulation to intraspecific expression variation. The X-axis represents the ratio of *cis*-only to *trans*-only genes. Comparisons with a ratio less than one had predominantly *trans*-regulation, while those with a ratio more than one had predominantly *cis*-. This figure only includes studies that measured the number of *cis*-only and *trans*-only genes (as in McManus et al., 2010). Studies that had multiple tissues or replicates are represented as an average across the multiple examples (see Table 1). Glaser-Schmitt et al. (2018) included comparisons between divergent and non-divergent populations, and these are both represented. Studies of artificial selection are not included.

for genes expressed in gill tissue. Verta and Jones suggested that this difference is due to tooth plates being less multifunctional than gills, and thus less susceptible to the deleterious side effects of otherwise pleiotropic *trans*-acting changes. A similar situation may apply to gene regulation in *Drosophila* malpighian tubules (Glaser-Schmitt et al., 2018). Future studies focused on comparing tissues of differing function within the same organism could help resolve these issues. Interestingly, while Hart et al. found that *trans*-acting mutations contributed to expression differences to a greater degree than *cis*-acting mutations, when categorizing genes into various regulatory categories (as in McManus et al., 2010) they found roughly equal numbers of *cis*-only and *trans*-only genes (Table 1). A similar pattern was also found in Bao et al. (2019). Thus, an important distinction exists between the number of *cis*- and *trans*-regulated genes and the effect sizes of these mutations, and both are important when considering the contribution of *cis*- and *trans*-effects to overall expression differences.

Future directions

Here, we provide an overview of intraspecific studies investigating the relative contribution of *cis*- and *trans*-acting regulation to gene expression divergence. A prevailing view is that intraspecific variation is largely regulated by mutations in *trans*-, while interspecific divergence is largely due to *cis*-regulatory mutations. However, there is now a large body of evidence supporting the importance of *cis*-acting mutations in governing intraspecific variation and some evidence that whole organism preparations lead to a biased inference of *trans*-regulation. The conditions under which positive selection favors *cis*-acting or *trans*-acting mutations is an important open question.

Future studies could help clarify the roles of pleiotropy, tissue specificity, and positive selection in shaping gene regulatory evolution. In particular, studies could use single-cell RNA sequencing in heterogeneous tissues to avoid biased detection of *trans*-regulation. Further, studies investigating multiple tissues of differing function and integration may help address whether *cis*-changes are more likely to evolve in some tissues than in others. Lastly, to further understand the relationship between positive selection and the proportion of *cis*-acting changes, studies comparing individuals from the same population to individuals sampled from divergent populations experiencing directional selection may be particularly informative. For example, inbred mouse strains (Phifer-Rixey et al., 2018) or isofemale fly lines (Svetec et al., 2016; Zhao et al., 2015) from the ends of geographic transects could be used to generate crosses between individuals from the same locality and crosses between individuals from divergent localities. Alternatively, experimental evolution studies could be used to generate cohorts of organisms that have experienced controlled selective regimes, including no selection, that can then be crossed back to the starting population to determine the regulatory architecture of the resulting expression divergence. Study designs such as these could provide a thorough and careful analysis of the contribution of *cis*- and *trans*-acting regulation to expression differentiation in various evolutionary contexts.

Author contributions

S.M. Durkin and M.W. Nachman conceived the work. S.M. Durkin made figures and tables. S.M. Durkin and M.W. Nachman wrote the manuscript.

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