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The evolution of gene expression in *cis* and *trans*

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Abstract

There is abundant variation in gene expression between individuals, populations, and species. The evolution of gene regulation and expression within and between species is thought to frequently contribute to adaptation. Yet considerable evidence suggests that the primary evolutionary force acting on variation in gene expression is stabilizing selection. We review here the results of recent studies characterizing the evolution of gene expression occurring in *cis* (via linked polymorphisms) or in *trans* (through diffusible products of other genes) and their contribution to adaptation and response to the environment. We review the evidence for buffering of variation in gene expression both at the level of transcription and translation, and the possible mechanisms for this buffering. Lastly, we summarize unresolved questions about the evolution of gene regulation.

Keywords

cis, trans; regulatory evolution; stabilizing selection; buffering

Evolution of gene expression between and within species

Regulation of gene expression is complex, and involves interactions between DNA, RNA, proteins, and the environment. Variation in gene expression is ubiquitous within populations and between species, however discerning the functional implications of that variation remains a challenge. Interpreting the functional implications of variation requires understanding how variation in gene expression propagates through gene regulatory networks, and ultimately results in complex phenotypes and disease. A common approach to analyzing the evolution of gene expression is to break it into its *cis* and *trans* components. That is, gene expression differences in *cis* that are due to linked polymorphisms (allele-specific and local to the affected gene) and differences in gene expression in *trans*, or due to diffusible products that needn't be linked with the affected gene (in diploids, in the absence of *cis*-regulatory differences, *trans*-regulatory changes are expected to affect both alleles equally) (Fig. 1). Studies on the evolution of gene expression have found abundant variation within and between natural populations for both *cis* and *trans*-regulation of gene expression

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[1-12]. Both of these modes of gene expression regulation likely contribute to adaptation and divergence, however evidence to date suggests they may have different genetic and evolutionary properties.

When intra- and interspecific variation in gene expression is decomposed to *cis* and *trans* components, *trans* factors have often been found to make larger intraspecific contributions [12-16]. The larger contribution of *trans* factors within species is sometimes attributed to *trans* factors having a larger mutational target, thus being more likely to arise in comparison with *cis* regulatory mutations. As genes affect each other through many genetic and metabolic networks, *trans* factors could potentially arise anywhere in the genome (though the actual number of positions that could affect a particular gene in *trans* is smaller than the entire genome) [12,16,17]. Between species *cis*-regulatory differences are thought to have a greater contribution to divergence, suggesting that under selection they preferentially accumulate overtime [14,18-20]. A greater contribution of *cis* factors between species could be due to a number of reasons, such as *trans* factors having more deleterious **pleiotropic**-side effects, or because *trans* effects are more frequently recessive [21,22].

In several systems, and with different experimental approaches, work on *cis-trans* decomposition has found that *cis* and *trans* regulatory differences often influence the same gene, and when they do more often than not they act in opposite directions **[4,5,7,11,12,23,24]**. While both *cis* and *trans* factors might destabilize the transcriptome, *cis-trans* compensation – if common – will serve to re-stabilize the overall expression level of genes despite the presence of segregating – and putatively adaptive – regulatory variation [4,7]. If *cis-trans* compensation stabilizes overall gene expression this is consistent with other work which suggests that **stabilizing selection** is the predominant mode of evolution for gene expression (see **Glossary**) [14,25]. Overall, recent work on *cis* and *trans* differences in gene expression within populations, between species, and in different environments is creating a general picture of the importance of each type of regulatory difference in adaptation and speciation; providing a framework for advancing the understanding of evolution of gene regulation and expression.

Experimental approaches

There are several approaches to characterize the evolution of gene expression at single loci due to factors in *cis* and in *trans*. In diploids, *cis* differences will be found local to the affected gene and be allele specific, while *trans* changes can be linked or unlinked, but affect both alleles. There are three main approaches to characterizing *cis* and *trans* variation, two of which are closely related: comparing allele-specific expression between homozygote parents and their F1 hybrids and a common reference design (Fig. 1). In F1 hybrids *cis* and *trans* effects are partitioned by comparing the expression level of individual alleles in the hybrid (*cis*) and overall expression of both alleles compared between the parental strains (*cis* + *trans*) [16]. The common reference design is similar, but involves crossing a panel of genotypes to a common strain (i.e. creating a panel of F1 individuals where ½ of their genome is identical across all crosses). Within this cross design *cis* components of expression variation are quantified by comparing between the common allele and the population allele within a genotype, while comparing expression of the common allele

across the population's genomic backgrounds estimates variation due to *trans* factors [26,27]. These approaches are different in that the F1 hybrid approach relies upon a comparison with parental expression levels while the common reference design uses population samples. Both approaches are agnostic with regard to the source of *trans* differences. While these approaches have been used extensively for individual genes, we will focus primarily on genome-wide studies. Genome-wide, these designs have been successfully used to characterize *cis* and *trans* differences in yeast, plants, *Drosophila*, birds, and mice [1,2,10,28-31].

The third approach to understanding *cis* and *trans* variation is eQTL analysis, which correlates a molecular phenotype such as gene expression with genetic variation [11,32-35]. However, the definition of *cis* and *trans* in eQTL versus allele-specific expression studies is not entirely the same as **eQTLs** primarily characterize physical proximity (Fig. 1) [11,36-38]. The strength of the eQTL approach is in the ability to approximate the number of regulatory differences involved in changes in mRNA expression (though the authors note that a single eQTL does not imply a single genetic change), and to estimate the effect sizes of these regulatory differences. However, the eQTL approach has less power to detect *cis*-eQTLs that have *cis* and *trans* effects in the opposite direction. Further, because mapping *trans* factors requires correcting for a larger number of statistical tests, it has less power in comparison with mapping *cis* factors [39]. One exception is finding '*trans* hotspots', i.e. genomic regions that appear to affect a disproportionate number of genes [39,40].

One approach to the study of *cis*-*trans* differences in gene expression that the authors believe warrants further investigation is to understand their distribution within gene regulatory networks. Very little is known about how *cis* and *trans* differences are distributed throughout gene regulatory networks, though several approaches are available to analyze expression variation in the context of gene regulatory networks, for example Structural Equation Models (see [26] for review). However, understanding these patterns requires detailed knowledge of the gene regulatory networks that does not exist for more than a handful of cases, and there has not yet been any systematic investigation of the distribution of *cis* and *trans* changes within networks across multiple systems. One noteworthy attempt found that the number of *trans* regulators negatively correlates with the evolution of *cis* regulation, though the phenotype was limited to the number of upstream and downstream targets [39]. We do not generally know how regulatory perturbations are distributed throughout gene regulatory networks, if these effects are local or do they propagate through gene networks, or if they are frequently dampened or amplified. Indeed, there are many unanswered questions about the evolution of gene expression, and it remains an area with abundant opportunities for future research.

Adaptation in *cis* and *trans*

Adaptive evolution of individual or limited groups of genes affecting expression in *cis* and *trans* has been investigated extensively, more so than can be summarized here, and a number of excellent reviews are available [22,40-42]. There is considerable support for expression of individual genes often evolving in *cis*, though the exact adaptive significance is not always established [32,43-47]. In general, it is thought that *cis*-regulatory changes will be less

pleiotropic and more commonly found at ‘structural’ genes that do not directly impact the expression of other genes (meaning they are involved in catalyzing enzymatic reactions or other structural roles rather than regulating other genes) [41,43,46,48]. However, such genes still belong to gene regulatory networks, and thus directly or indirectly are expected to affect the expression of other genes. In plants, for example, the structural genes required for producing the compounds that create flower color also produce other compounds necessary for proper organismal function, while the transcription factors that activate them are tissue specific, thus even though they are structural genes evolution of flower color is biased towards *trans*-regulatory changes [49]. Therefore, there is still a lot to understand about the importance of *cis*-regulatory changes within gene networks, and how adaptive differences are spread throughout gene networks.

In genome-wide surveys of gene expression, adaptation is often inferred as an excess of either eQTLs that change gene expression in the same direction or *cis*-regulatory differences biased towards the alleles of one parent [50-53]. In the case of *cis*-regulatory expression this is limited to particular pathways or functionally enriched categories, and can also include an excess of *cis/trans* pairs that are in the same direction, if *trans* differences are investigated [51]. If the eQTLs or *cis*-regulatory differences are neutral then changes in the direction of expression or differences between parental alleles would be expected to be roughly equal. For example, a recent application of this method to yeast eQTLs found an excess of up-regulated biofilm suppressor genes associated with adaptation to human hosts [54]. In addition, by expanding these types of analyses to outgroup species lineage specific selection can be inferred [52,55]. It is important to note that while some genome-wide studies have found support for the contribution of *cis*-factors to divergence and adaptation, in many cases *trans*-factors are not investigated [56-59]. There is some evidence that they contribute to adaptation, for instance, in a study on the evolution of cichlid opsins *trans* differences were found to contribute more to adaptation between species than *cis* differences [60]. Note that these inferences of adaptation generally apply to particular subsets of genes and eQTLs, and do not necessarily imply that the general mode of evolution of gene expression is adaptive. It is also possible that many of the observed differences in *cis* and *trans* are due to context specific effects, as the environment will invariably effect gene expression in some way (Box 1).

Stabilizing selection on gene expression

Due to the frequency with which compensatory *cis-trans* pairs are observed in genome-wide studies, it has been theorized that in general selection on gene expression may be stabilizing. Stabilizing selection requires the maintenance of a mean, non-extreme phenotype in a population or species (Fig. 2a). There has been abundant evidence for stabilizing selection on gene expression, in a number of model and non-model systems, however the relative contribution of *cis* and *trans* differences, or compensatory *cis-trans* effects, have not been explicitly examined. We will summarize recent evidence for stabilizing selection on gene expression, and note that the contribution of *cis-trans* compensation to stabilizing selection is an area of potential interest. In *Drosophila*, comparisons between three species with different divergence times found that while the number of genes with evidence for *cis* regulatory divergence increased over divergence time, the number of differences in total

expression did not [14]. This would imply that *cis* regulatory differences are being compensated in some fashion. In addition, in this study less than 2.2% of genes showed differences in expression consistent with genetic drift [14]. Both of these observations are consistent with widespread stabilizing selection on gene expression levels. Without decomposing the contribution of *cis* or *trans* this type of approach was extended to larger phylogenetic distances and similar results were found, though with the additional caveat that at shorter time scales both types of evolution of gene expression will appear neutral, and that our ability to detect both stabilizing and directional selection is dependent upon the scale of divergence at which it is measured [19]. Between flycatcher species variance in gene expression was correlated with between species divergence, which also suggests stabilizing and/or neutral evolution in gene expression [61]. Between humans and primates the amount of inter-species variation in gene expression can be explained by variation in gene expression within species – which is consistent with stabilizing selection on gene expression [62-64].

Another approach to evaluating the prevalence of stabilizing selection is to investigate the distribution of mutational effects in standing variation relative to new mutations [65-67]. For example, under the house-of-cards theory of stabilizing selection mutations are expected to be infrequent, and the effects of mutations are expected to far exceed standing variation [25,68]. The house-of-cards theory of stabilizing selection is referred to as such because new mutations are thought to disrupt multiple processes and are subject to strong selection, compared to related models in which new mutations have weak effects. In contrast, neutral evolution would predict that selection is negligible and equilibrium genetic variance is a balance between mutation and drift. The predictions for house-of-cards stabilizing selection were found to best fit the distribution of mutational effects in *D. melanogaster*, *S. cerevisiae*, and *C. elegans* [25]. Many lines of evidence thus suggest that stabilizing selection on gene expression may be an important factor influencing inter and intra-species gene expression evolution. Stabilizing selection on gene expression can occur through different molecular mechanisms, including purifying selection – wherein deleterious mutations are selected against (Fig. 2b), and/or compensatory evolution – where small mutations affecting gene expression in *cis* (*trans*) are compensated for through *trans* (*cis*) factors that stabilize overall gene expression level (Fig. 2c). Below we will focus on the latter of these two potential hypotheses.

Buffering gene regulatory differences with *cis-trans* compensation

Between species

Between species *cis-trans* effects that compensate one another for individual genes are more commonly observed than those with the same direction of effect. However, despite common observation, the potential significance of this patterns in terms of selection and/or adaptation has not been investigated. Fixation of *cis-trans* factors between species could occur as a result of stabilizing selection on overall gene expression or to ameliorate the negative pleiotropic side effects of a selected mutation. Stabilizing selection on overall gene expression could result in the fixation of compensatory *cis* (*trans*) factors in response to mildly deleterious *trans* (*cis*) factors to maintain gene expression levels. It is also possible

that compensatory mutations are selected for to mitigate the side effects of an otherwise beneficial mutation, as has been observed in the evolution of antibiotic resistance [69-74]. It is clear from a number of different studies and systems that compensatory *cis-trans* pairs are more common than those that are not compensatory. For example, when two divergent yeast species were crossed, 67% of genes with both *cis-trans* effects were compensatory, consistent with buffering of regulatory divergence [75]. In a different study that focused on genome-wide *cis* and *trans* effects for a single gene in yeast, compensatory effects were two to three times more likely than to have *cis* and *trans* effects with the same direction [18]. In interspecific crosses between *D. simulans* / *D. sechellia* and *D. melanogaster* / *D. simulans*, compensatory *cis/trans* pairs accounted for 73% and 87% of cases respectively [14]. In house mice hybrids the majority of *cis-trans* pairs were compensatory [9], thus from yeast to vertebrates *cis-trans* pairs are more likely to be compensatory. It is possible that this type of *cis-trans* compensation results from co-adaptation within species, and contributes to reproductive barriers. For a thorough review of the potential for co-adapted gene complexes to contribute to speciation see [76].

Within species

While between species *cis-trans* differences that are compensatory will (or can) be fixed, within species *cis-trans* differences that compensate one another will be polymorphic and not necessarily co-inherited. However, it has been recognized that within species there are abundant coupled *cis-trans* factors contributing to buffering of gene expression variation, though again the importance of this pattern for selection or adaptation is not known [2,11,27]. For example, in conifers, abundant *cis-trans* compensation was observed within a population using an eQTL study design that characterized local *trans* effects and *cis*-regulatory differences [11]. Although the study was not designed to detect unlinked *trans* effects, the presence of linked compensatory *trans* effects lead the authors to suggest that this compensation was due to self-regulation or closely linked *trans* modifiers. **Linkage disequilibrium** is high in organisms such as conifers, and it is unclear how likely it is that linkage between *trans* modifiers and *cis* variants would be maintained in more readily recombining species such as *Drosophila*. However, *cis-trans* compensation has been characterized in studies of within species variation in *Drosophila*. One study used a common reference design to characterize *cis* and *trans* variation within *D. melanogaster*, and found that 85% of *cis-trans* combinations are compensatory [27]. The combination of many studies finding *cis-trans* compensation within species suggests that *cis-trans* compensation can accumulate intra-specifically, though it does little to suggest a potential mechanism for this compensation.

Buffering gene regulatory differences at the level of translation

While differences in the *cis* and *trans* regulation of gene expression are abundant, it is also possible that these differences have little functional implication beyond the level of translation. Buffering at the level of translation could be another source of what is essentially *cis-trans* compensation if changes in transcription in *cis* are countered by changes in translation in *trans*. Translational buffering could be regulated in *cis, trans*, or both, though most commonly the source is not mapped. This would be an interesting area of future

research. At the level of translation, it was found that in hybrids between two species of yeast, differences in translation efficiency and transcription occurred seven times more frequently in opposite directions – essentially *cis-trans* compensation at the level of translation [75]. Due to this, 20-80% of non-conserved transcription was buffered at the level of translation [75]. The observation of buffering at the level of translation is similar to earlier reports in two yeast species that found that aberrant expression of mRNA in hybrids was not reflected in ribosome occupancy [77]. However, another study found no evidence of buffering at the level of translational efficiency including when they reanalyzed the earlier referenced data [78]. In another study on yeast the results were more mixed, as there was some concordance between eQTLs and protein QTLs but the relationship was variable. Two large eQTL hotspots that overlapped with protein QTL found effects in opposite directions which would serve to buffer the overall level of gene expression/protein, but this effect was not general [79,80]. In a study on specific genes in snakes no evidence was found of post-transcriptional buffering of mRNA expression differences, rather a high concordance was found between mRNA abundance and the proteome [81]. Overall, the evidence for posttranscriptional compensation for mutations affecting transcription remains inconclusive. It maybe be that buffering at the level of translation is important for particular subsets of genes or developmental pathways, and that a more detailed understanding of translational dynamics will illuminate these differences.

Mechanisms underlying the appearance of compensation

Inherent mutational and inference biases

It is possible that the appearance of *cis-trans* compensation is due to biases in mutation or ascertainment. A recent study found that there was no bias in the frequency of *cis* or *trans* effects in either direction (increase or decrease), however overall *cis* regulatory differences have a larger effect size [12]. In addition, for *cis* regulatory mutations, variants that decreased expression had a larger magnitude of effect than those that increased expression. The opposite was true for *trans* regulatory mutations, with larger effect sizes when they increased expression [12]. These results are from a screening for *cis* and *trans* effects on a single gene (genome-wide) so it is not known whether these results are generalizable. If these patterns are generalizable, it will have two effects, first that we will be more able to detect *cis* effects than *trans* effects, because larger effect sizes are easier to detect. This is also true because it is more difficult to detect effects that are distal, or not linked to the affected gene, in eQTL studies due to the larger burden of multiple testing. This is because the potential location for a proximal eQTLs is limited, while the potential location of a distal eQTL is the entire genome. We will also more frequently detect *trans* effects that increase expression, and *cis* effects that decrease expression (Fig. 3a). Which is to say that the observed patterns of *cis-trans* compensation may be due to the analysis method used and its sensitivity to detect changes and/or mutational bias rather than selection, which is especially true for eQTL studies (Fig. 3a). In one instance in which this could be investigated however, the opposite was true, and 65% of *cis/trans* pairs had a positive *cis* effect and a negative *trans* effect [27]. Additional problems might result from confounding sampling error or from estimation biases with either *cis* or *trans* effects. However, every method of examining *cis-*

trans differences finds an excess of compensatory effects, in every system, lending some confidence to the pattern.

Compensatory mutations

It has been hypothesized that a *trans* mutation spreads jointly with a *cis* mutation to compensate for their slightly deleterious effects (Fig. 3b, c) [7]. While the joint spread of *cis-trans* mutations is possible in some species with extensive linkage disequilibrium, or if mutations within the target genes act in *trans*, as a general mechanism these factors will not be co-transmitted within species [11]. For example in *Drosophila* even when co-localized on the same arm long-range linkage disequilibrium in flies is not a general feature [82]. Such a strong excess of ~80% compensatory *cis-trans* interactions within species cannot be explained by co-evolution without co-inheritance.

If a *trans* mutation downregulates an allele only in the presence of a *cis* mutation, with the *cis* effects compensated, it is possible that these *cis-trans* mutations may exist jointly and underlie widespread compensation. Indeed, multiple studies have detected abundant *cis-trans* epistasis [83-86], however the statistical methodology has not been developed to test whether those interaction terms are of a compensatory nature [87,88]. Conceptually, this scenario is akin to Wright's arguments on the evolution of dominance, where a slightly deleterious mutation (in *cis*) is allowed to segregate due to a secondary mutation (in *trans*) that renders the first mutation recessive. Population genetic analyses have established that the strength of selection for such secondary modifiers is most frequently vanishingly small [89]. Given that the actual genes or mechanism of *trans* effects is generally not known, these patterns warrant further investigation.

Gene network feedback

It is possible that many *cis-trans* compensatory interactions are due to feedback within gene regulatory networks (Fig. 3a, d). There is some support for this in single gene and genome-wide studies, though there is room for this to be more extensively investigated. For example, two single gene studies in yeast found support for gene-network feedback: at ROX1 where negative feedback confers robustness to the expression despite naturally occurring allelic variants [90], and at AMN1 where a local *trans*-eQTL was found to operate through a regulatory feedback loop involving several additional genes [91]. Genome-wide, one study on buffering by feedback found that approximately 15% of allelic differences are compensated through this mechanism [78], and work in conifers also found that 10% of local eQTLs acted in *trans*, suggesting they may work through self-regulation [11]. The potential for gene network feedback to stabilize overall gene expression levels is likely to be important for at least some genes, and investigating its importance more broadly is in interesting avenue for future research.

Transvection

In both mammals and dipterans, a type of inter-chromosomal communication has been observed, termed transvection, where the regulatory information on one chromosome can be used to regulate the expression of the allele on the other chromosome (Fig. 3a, e) [92-96]. Initially considered an oddity, transvection is now understood to be widespread [92,93,95]. It

involves both up and down regulation of alleles through coordination of expression between alleles [97], and it affects a large majority of tested regulatory regions [95]. The effect of transvection on natural transcriptome variation has never been tested, but given the evidence for its ability to work in *trans* and compensate for deficiency mutations it is a candidate mechanism for compensation. Some instances of transvection require pairing between homologous chromosomes, a peculiar feature of insects, but other instances appear to be independent of pairing [92,97]. While the principles of chromosome architecture are an area of intense research at the moment, it is unclear how important transvection is for gene regulation in different systems. However, there is evidence that insulator proteins involved in establishing inter-chromosomal contacts in mammals and insects may facilitate transvection [98-100]. This is an interesting area for future research, as there is currently very little understanding of the role of transvection in natural populations, and no understanding of its potential role in *cis/trans* effects.

Concluding Remarks and Future Perspectives

While many individual cases of the evolution of gene expression have been investigated, a broad view of the evolution of gene regulation remains to be formulated. Genome-wide approaches have failed to create a consensus about how gene expression evolves in general, in part because there are likely different answers for different subsets of genes or systems (see Outstanding Questions). The path by which any particular gene is going to evolve is going to be affected by its role within its gene network and its developmental context. However, both gene networks and developmental context are known for only a very few systems, making it difficult to place any patterns that are found in the evolution of gene expression into a larger picture. For example, genes with structural roles within networks may have inherently different evolutionary dynamics than those involved in transcription, or those with physiological effects. As more gene regulatory networks are characterized in non-model systems and we gain a better understanding of their developmental role we foresee many historically observed patterns fitting into new paradigms. Emerging technologies make this an exciting time to study the evolution of gene expression, including new techniques to plumb the effect of chromatin organization, gene neighborhood, and cell or tissue specific differences in gene regulation. In the coming years, these emerging technologies and the increasing tractability of non-model systems promises many new and exciting insights about the evolution of gene expression.

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Glossary

Adaptive evolution

Evolutionary changes that increase survivorship or reproduction.

eQTLs

Expression quantitative trait loci, regions of the genome that contribute to variation in expression levels of RNA.

Linkage disequilibrium

The non-random association of alleles within a population.

Pleiotropic

A gene is referred to as pleiotropic if it effects more than one phenotype.

Stabilizing selection

The favoring of individuals in the population with mean, rather than extreme, phenotypes. Stabilizing selection generally reduces existing phenotypic variation, and is measured at the level of phenotypes.

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Box 1 Environmental effects on *cis-trans* variation

Gene expression robustness in the face of environmental variation can be an important component of system homeostasis, while gene regulatory differences in response to the environment can be an important component of adaptation, and potential for adaptation. It is currently unclear how important *cis* and *trans* effects are for the response to environmental differences, and whether they are maintained within populations due to gene by environment interactions. In yeast these questions have been approached in a variety of ways, for example in interspecific yeast crosses local *cis* effects tended to be less condition dependent than *trans* effects [99]. In another case, allele specific expression was examined in normal and heat-stressed environments for two yeast species, one of which was adapted to higher temperatures, and abundant *cis*-regulatory divergence was uncovered between species [3]. However, these *cis* effects were not environment dependent and were not adaptive for thermal tolerance [3]. A recent study in yeast that did not examine *trans* factors found that induced mutations in *cis* had different effects depending upon the environment, but their effect on fitness and relevance to adaptation are not known [100]. These conclusions are corroborated in other systems, for example in *C. elegans* while abundant *cis* effects were detected between strains, they were not environment dependent and the response to heat stress was mediated largely by *trans*-effects [38]. Several studies have found that *cis* effects are concordant between environments in intra-specific crosses of *Drosophila* and inter-specific differences in *Arabidopsis* [27,101]. In contrast to these studies, in grass while *cis*-effects changed in magnitude but not direction with treatment (drought stress) very few *trans* effects were detected, though this may be largely related to issues of power [102]. Overall, the current evidence suggests, but is not conclusive, that *trans* regulation might be more important for environment dependent differences in gene expression, and it is unclear what the relationship is between these *trans* differences and long term adaptation to the environment [15,27].

Outstanding Questions

How are *cis* and *trans* effects distributed within gene regulatory networks, and what implications does their position have for their effect and potential to contribute to adaptation?

How do *cis* and *trans* effects, including those that respond to the environment, contribute to the long-term divergence and adaptation of species?

How important are different sources of buffering for variation in gene expression?

What mechanism is responsible for the wide-spread *cis-trans* compensation observed across techniques and organisms – is it an artifact of our methods, due to mutational compensation, gene network feedback, or transvection?

Trends Box

cis regulatory differences appear to be more commonly responsible for adaptive evolution, though there are exceptions that illustrate the importance of gene network context in the path by which evolution proceeds.

Current evidence supports the supposition that genome-wide gene expression evolves under stabilizing selection. There is limited evidence that some of this stabilizing selection is due to compensatory *cis-trans* evolution, but more research is needed.

Overall when *cis-trans* contributions to gene expression differences are investigated there is an excess of compensatory *cis-trans* pairs.

The observation of an excess of *cis-trans* pairs that are compensatory could be due to mutational and ascertainment bias, selection for compensatory mutations, buffering from gene network feedback, or potentially communication between alleles (transvection).

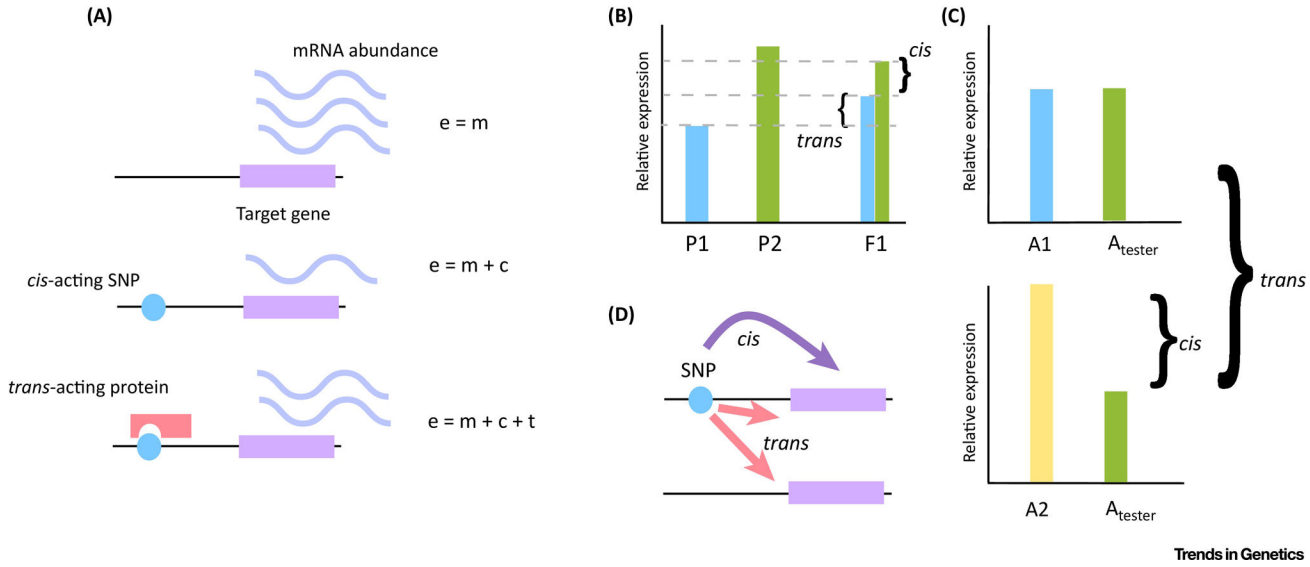

Figure 1:

cis and *trans* differences in gene regulation and the ways that *cis* and *trans* differences can be experimentally detected **a)** An allele is shown with average expression due to the presence of wild-type *cis*-regulatory modules and *trans*-regulatory factors. When these *cis*-regulatory modules are mutated, this will cause deviation from normal expression by a value c – *cis* factor – with the expression level m + c. This is shown in the second portion of the panel, where a *cis*-acting SNP is shown as a blue circle that alters the regulatory output of the associated gene, indicated as periwinkle squiggles. This same mutant allele might be represented in a genetic background where it is additionally affected in *trans*, with the resulting expression level m + c + t. In the example on the figure, the c is negative, but the t is positive. This is represented by a *cis*-mutation in blue, with a *trans*-acting protein interacting with the SNP in coral. Because c is negative and t is positive overall expression is closer to the average expression represented in the first panel. **b)** The earliest approach to *cis-trans* decomposition was to characterize expression of parental alleles in an F1 hybrid, often using techniques such as pyrosequencing. In the F1 hybrid differences in the expression of only one allele relative to the other allele is a *cis* effect. *trans* effects can be detected by comparing the expression ratio of each allele in hybrids to the ratio of expression between parents - if the ratio of expression is different in the F1 hybrid this is a *trans* effect. Here the bracket labeled *cis* indicates the differences between the two alleles in the F1. The *trans* bracket measures the amount of expression which is altered relative to the expression of the parental alleles. **c**) In the common reference design, a panel of individuals from a population sample are crossed to a single ‘tester’ strain (shown in green as A_{tester}). Differences between the expression of the ‘tester’ allele and the population alleles (A1 and A2, blue and yellow) within an individual are *cis* effects, while differences in the expression of the tester allele between individuals are *trans* effects. In the top panel, there is no *cis* effect as the A_{tester} and A1 allele are expressed at the same level. In the bottom panel, there is a *cis* effect between A2 and A_{tester}, and there is a *trans* effect because the A_{tester} allele is expressed at a different level in different individuals (between the A1 and A2 background). Note that this does not measure all *trans* effects, only those originating from the A1 and A2

backgrounds but not the A_{tester} background. This section of the figure is based off of a figure from Fear et al. (2016). **d)** The eQTL approach to *cis-trans* decomposition. In eQTL studies the characterization of *cis* and *trans* is somewhat different, often meaning that the SNP effecting expression is either linked to the locus it effects or unlinked, though in some cases the contribution of specific alleles to eQTLs has been decomposed. In general, a SNP that has an effect of a gene expression phenotype will be mapped, and if it is within the gene that it effects it is called a *cis*-eQTL. If it is unlinked, these are termed *trans*-eQTLs. When the effect of individual alleles on expression is characterized the definition is the same as for F1 crosses.

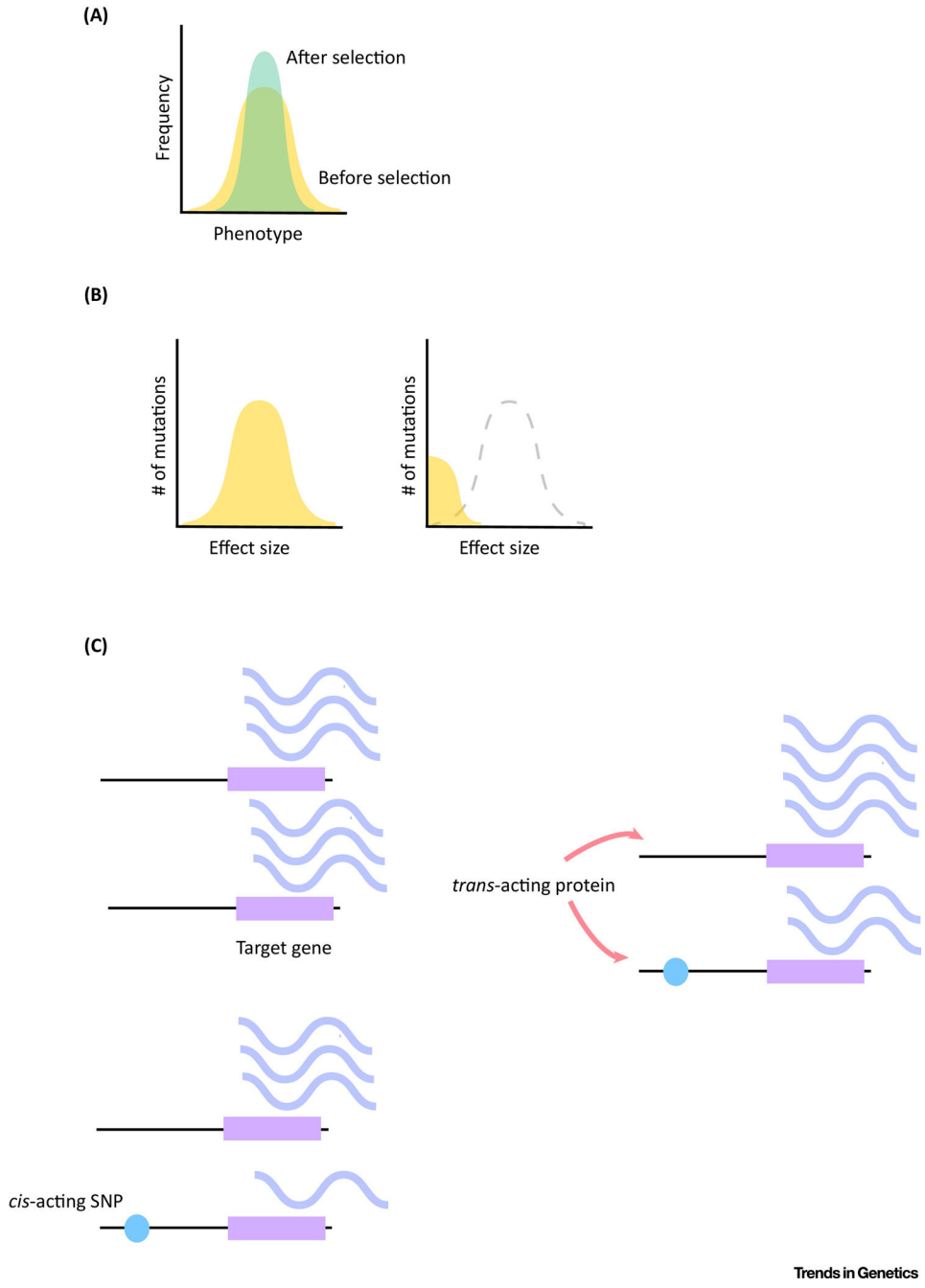

Figure 2).

Selection and *cis-trans* compensation **a)** Stabilizing selection on phenotypes within a population. Prior to selection the distribution of phenotypes is broader (shown in yellow), and after selection extreme phenotypes have been selected out (green). **b**) A hypothetical illustration of purifying selection, with the effect size of mutations on the *x* axis and the number of mutations with that effect size on the *y*. The distribution of mutations with different effect sizes is approximated in yellow. The panel on the left illustrates essentially neutral evolution, as mutations with larger effect sizes (and, by inference, more deleterious effects) are not being selected out of the population. On the right is an example of purifying

selection, in which mutations of larger effect size are selected out of the population. This is one potential way to for stabilizing selection to act on gene expression. **c**) *cis-trans* compensation. The upper left panel represents a wild type individual with equal expression between the two alleles at a single gene, with the regulatory region shown as a black bar and the coding region a purple square. Relative transcript abundance is illustrated by the periwinkle squiggles. The lower left panel shows how a *cis*-acting SNP could potentially appear on one of the alleles, reducing gene expression allele-specifically. This SNP is illustrated with a blue circle. In the panel on the right, a *trans*-acting mechanism upregulates both alleles to preserve the total mRNA output from the gene (compensation). In this panel, the arrows indicate that a *trans* acting protein is interacting with the regulatory regions of both copies of the gene.

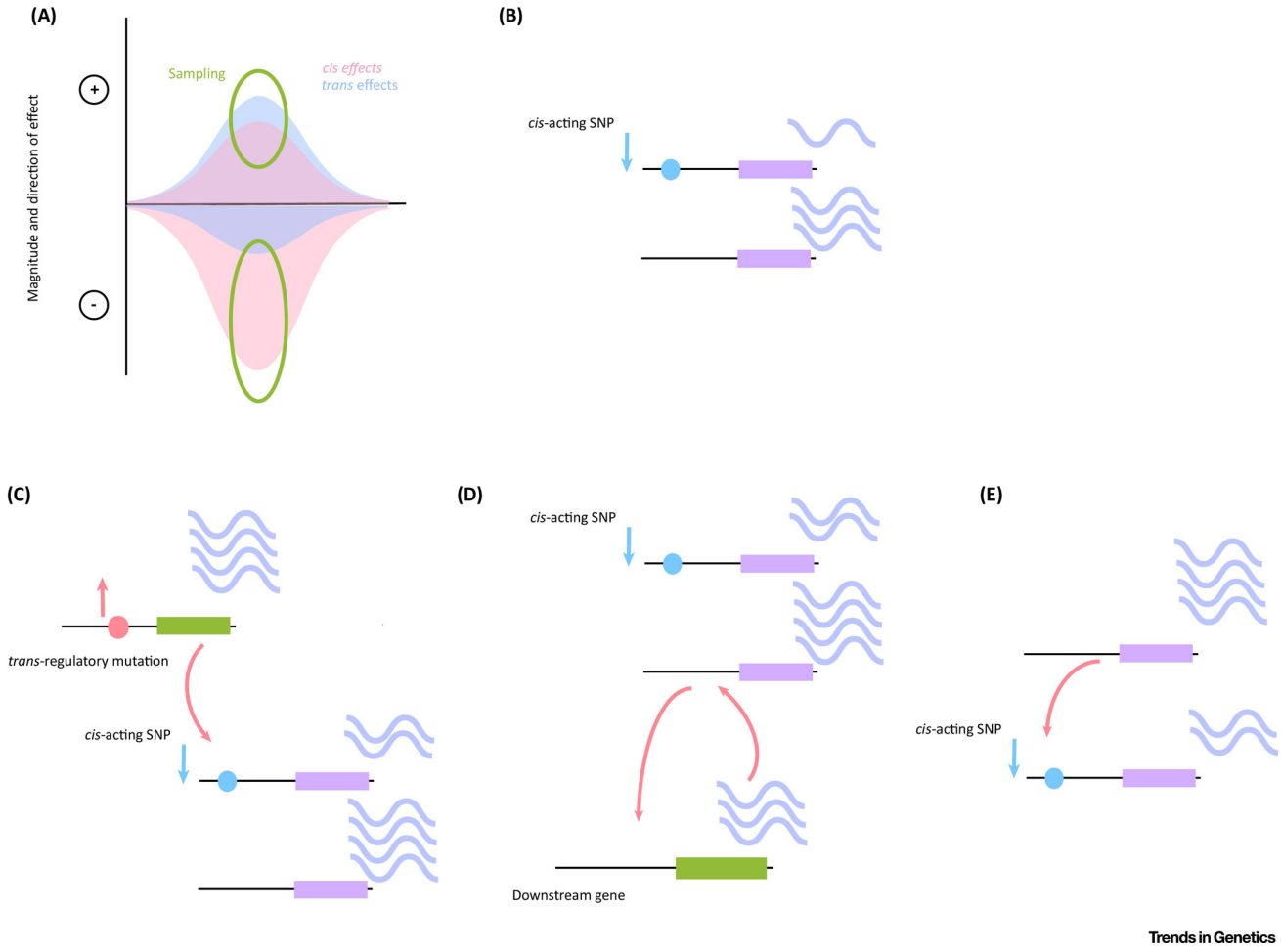

Figure 3).

Potential causes and mechanisms for *cis-trans* compensation. **a**) It is possible that the apparent widespread prevalence of *cis* and *trans* effects that compensate one another is due to ascertainment bias. Large *cis* effects are more commonly negative, and large *trans* effects are more commonly positive. Thus, given that we are biased towards detecting differences of large effect, it is possible that it is a methodological artifact. In this figure, the frequency of *cis* and *trans* differences are shown, where the frequency distribution of *cis* effects is shown in pink and *trans* effects in blue. The green circles indicate what is being sampled by any given study from the distribution. **b**) A wild type allele (bottom) where the number of transcripts is illustrated as an arbitrary number of periwinkle squiggles. An allele with a *cis*-regulatory mutation (top) is shown, where the mutation is illustrated as a blue dot. The total number of transcripts produced is reduced by 2/3. This illustration will serve as a baseline for c) through e). **c)** It is possible that the observation of *cis-trans* compensation is due to the accumulation of effects in *cis* (*trans*) that are then compensated for by a mutation in *trans* (*cis*). Here a *trans* regulatory difference is shown at the gene in green, where a SNP illustrated by the pink dot results in upregulation and an increase in the number of transcripts. This acts on its downstream target, the gene shown in purple, which has a SNP in *cis* illustrated by the blue circle that downregulates the gene. The two mutations together

stabilize the overall level of gene expression. Note that the authors are agnostic as to whether *cis* or *trans* mutations appear first, this is one example. **d**) It is also possible that *cis-trans* compensation is caused by gene regulatory network feedback (which could be positive or negative, shown here is positive). In this case activation of a downstream gene may feedback on the target gene and normalize gene expression levels in spite of a *cis*-regulatory mutation. Gene network feedback can happen locally, through self-regulation, or essentially as a *trans* effect that does not require a mutation. Here the downstream gene is shown in green, and there is no mutation at the locus. Feedback on the upstream gene with a *cis*-acting SNP shown in blue serves to stabilize gene expression. **e**) Transvection is the most hypothetical explanation for *cis-trans* compensation, and it is also the least well understood. It has been observed across species and genes that the regulatory information from one copy of an allele has the potential to regulate the other copy. Enhancers show *cis*-preference, but if for any reason the enhancer on the other allele was not regulating its target as expected they can act in *trans*. Here the genes in purple are two copies of the same locus, one of which has a mutation in *cis* that is shown as a blue circle. Communication between each copy of the allele stabilizes gene expression output (periwinkle squiggles).