Trends in Genetics

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Letter

Inferring Compensatory Evolution of *cis*- and *trans*-Regulatory Variation

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Genetic variation in gene regulation is an important source of phenotypic variation, contributing to human phenotypes and diseases [1,2] as well as evolution within and between species [3,4]. Expression variation between two individuals can be partitioned into diffusible/*trans* elements (e.g., transcription factors) or non-diffusible/*cis* elements (e.g., linked regulatory sequences such as promoters or enhancers) [4]. By taking advantage of genetic crosses, we can gain insight into the mechanistic basis of expression variation that differentiates individuals [5,6]. Because parental genotypes share a single cellular compartment in F1 hybrids, they also share all diffusible regulatory factors. Thus, expression variation between alleles in an F1 hybrid reflects the portion of variation between the parents due to *cis* factors alone. The remaining portion of variation between parents not explained by variation in the F1 hybrids is due to variation in *trans* factors. Conceptually, this leads to the mechanistic perspective that allele-specific expression (ASE) variation in F1 hybrids is equivalent to variation in *cis* elements, whereas ASE variation in parents is a combination of variation in *cis+trans* factors [5]. By measuring the expression variation in both parents and their F1 hybrids, we can estimate the contribution of *cis* elements and *trans* factors to expression variation.

This ASE perspective facilitates estimation of important expression parameters on a genome scale [7,8], providing abundant fodder for making mechanistic inferences on the genetic basis of expression variation within and between species. However, an article in this issue of *Trends in Genetics* points out that, when *cis* and *trans* estimates share common F1 hybrid samples, they will be negatively correlated via error shared from the hybrid data [9]. One important consequence of this observation is that spurious inferences of compensatory evolution between *cis* and *trans* factors will occur when correlated error is not accounted for. This is because this type of compensatory evolution is defined as a negative relationship between *cis* and *trans* variation. As [9] points out, many studies continue to make precisely this error regarding compensatory evolution; consequently, a solution is urgently needed. Fraser [9] argues that the simplest solution to this problem is to estimate *cis* and *trans* parameters from independent replicates of hybrid data so that error is no longer correlated. Indeed, an ASE inference framework formulated by Emerson *et al.* [8] recommends correcting for error in just this way (cf. Figures 2 and S2 from [8]). To demonstrate the utility of this approach, we investigate two ASE data sets. The first is an artificial data set designed to be devoid of genetic variation in gene expression and is constructed purely from biological replicates of the same strain from [10] (for methods, see the supplemental information online). The second involves genetically distinct strains from the study by Emerson *et al.* [8] and therefore potentially exhibits compensatory variation in gene regulation.

Figure 1A,B illustrates the estimation of *cis* and *trans* expression parameters both with and without correcting for correlated error in a representative random partition of the ASE data set constructed to have no genetic variation between the parents [cf. (A) to (B)]. The negative correlation in Figure 1A is large in magnitude and highly significant ($r = -0.48$, $P < 0.0001$), while that of Figure 1B is small and marginally significant ($r = -0.02$, $P = 0.02$). Overall, when full biological replication is used, correlations cluster around zero (Figure 1C). In addition, given that the approach in the study by Emerson *et al.* [8] places *cis* and *trans* expression parameters in a likelihood testing framework, it can address questions of compensatory evolution on a gene-by-gene basis in a way purely correlative approaches cannot. For example, in the study by Emerson *et al.* [8], the overall correlation between *cis* and *trans* was near zero in the independent estimates, offering no evidence for compensatory evolution ($r = -0.028$, $P = 0.076$), compensating for a spurious conclusion of rampant compensatory evolution suggested by the correlated estimates ($r = –0.46$, $P < 10^{-15}$). However, by using independent estimates of *cis* and *trans*, individual genes with evidence for differential expression can be identified. Of the 850 genes significant for *cis* and/or *trans* in the independent data set of the study by Emerson *et al.* [8], 55% (466/850 with a 95% binomial confidence interval on the proportion 51–58%) fall into the compensatory category at a significance threshold of 1%. Under a model of random expression variation, only 50% (425) are expected to fall in compensatory categories, quadrants II and IV, by chance (16 genes were excluded that have a *cis* estimate of zero and cannot be classified as compensatory or reinforcing). Thus, while no evidence for a negative correlation between *cis* and *trans* is apparent at the genome level, the statistical evidence might support the action of compensatory evolution above the background expectation for at most a small number of genes (~41). Alternatively, because of the nature of replication in the study by Emerson *et al.* [8] (replicate cultures were pooled before library preparation and

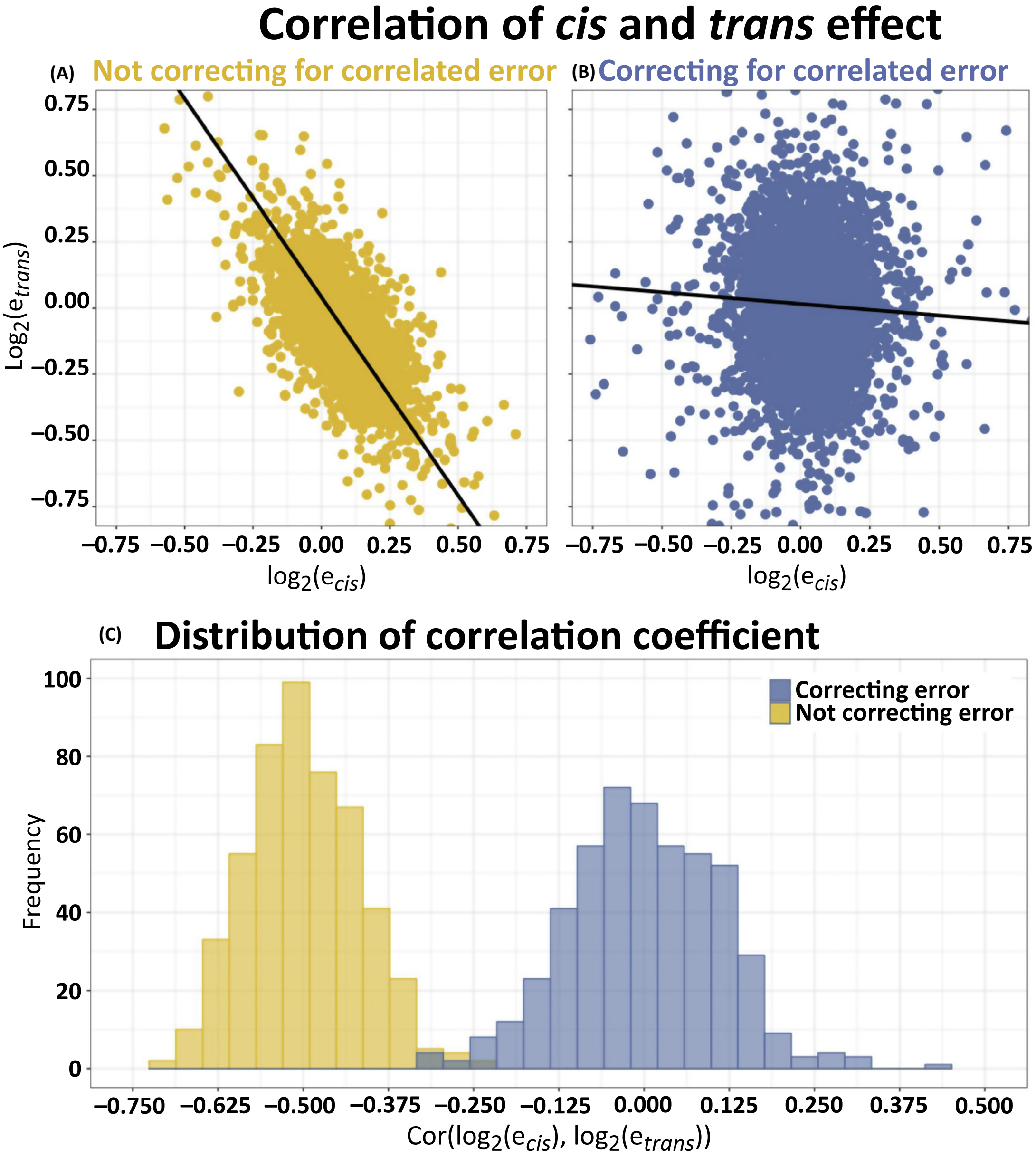

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Figure 1. Effect of Correlated Error on Estimation of *cis* and *trans* Expression Variation Ratios. The data considered in the figure were compiled from partitions of a highly replicated expression data set in yeast [10]. (A) Both *cis* and *trans* parameter estimates share a common sample of 11 hybrid individuals. (B) The *cis* parameters are estimated from one set of seven hybrid individuals, and the *trans* parameters are estimated from a different set of seven individuals. (C) Summary of Kendall rank correlation coefficient (τ) for 500 randomly chosen partitions of both the correlated and independent estimation schemes. (A) and (B) are representative instances of these random partitions.

subsequent replicates came from the same library), the variation associated with library preparation was not controlled, perhaps explaining the remaining small magnitude of excess compensatory evolution observed in the study. Clearly, however, a substantial proportion of the signal of compensatory variation was caused by correlated error arising from sequencing, as the method of the study by Emerson *et al.* [8] reduced the correlation from −0.46 to −0.028.

This approach illustrates the utility of accounting for correlated error in a statistical inference framework. The ability to make inferences on individual genes is an important advantage in carefully measuring the extent of compensatory evolution. Indeed, any time estimates of *cis* and *trans* are considered jointly to make biological conclusions, correlated error should be considered, not just in cases of compensatory evolution. Modern data sets should be even better suited to addressing such questions, as lower sequencing costs allow us to achieve higher and higher replication, not only eliminating the correlated error problem but also improving statistical power. Indeed, it would be irresponsible not to replicate parental and hybrid treatments in future ASE studies.

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Supplemental Information

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