Is plasticity caused by single genes?

ARISING FROM C. K. Ghalambor *et al. Nature* **525,** 372–375 (2015); doi:10.1038/nature15256

Whether phenotypic plasticity generally facilitates or hampers adaptive evolution is a matter of much debate. By comparing gene expression changes in guppy populations, Ghalambor *et al.*[1] suggest that 'adaptive' plasticity hampers adaptive evolution, whereas 'non-adaptive' plasticity facilitates adaptive evolution[2]. Here, we argue that the classification of individual gene expression changes as representing either adaptive or non-adaptive plasticity is problematic. Instead of indicating adaptive evolution, the expression changes of suites of genes may be caused by potentially random changes in underlying regulatory processes. There is a Reply to this Comment by Ghalambor, C. K. *et al. Nature* **555,** http://dx.doi.org/10.1038/nature25497 (2018).

Ghalambor *et al.*[1] did not directly measure organismal plasticity or evolutionary adaptation at the organismal level[2], but inferred evolutionary changes in plasticity from transcriptomic data (gene expression profiles). They first selected genes in the derived fish populations that are concordantly differentially expressed with respect to the ancestral population, thereby accounting for possible non-independencies in their expression data. They then continued their analysis by evaluating the selected genes one by one, thereby assuming that each gene is an independent realization of plasticity and that all convergent gene expression changes are adaptive. Yet, genes do not act in isolation to shape the organismal phenotype, but are part of regulatory networks[3,4]. A change in the expression of one gene can propagate through the entire network, thereby affecting the expression of many downstream regulatory genes, many of which might have no consequences for the organismal phenotype. Moreover, changes in the expression of one gene strongly depend on the action of other genes[5–7]. As a result, divergent changes at the gene expression level can be associated with convergent changes (or lack of change) at the level of organismal plasticity; whereas convergent gene expression changes do not exclude diversity in organismal response patterns. To illustrate this, we ran replicate evolutionary simulations, in which a simple gene regulatory network was selected to launch an adequate organismal response to the presence and absence of predators. In each simulation, the same optimal response readily evolved, but the network structures mediating this response differed substantially across replicate simulation runs (Fig. 1). Thus, without accounting for regulatory interactions, it is often impossible to infer the organismal consequences of gene expression changes and, hence, their adaptive value.

By stating that selection acts "more strongly to decrease plasticity in those transcripts showing the greatest non-adaptive plasticity," Ghalambor and colleagues[1] suggest that individual genes can be classified as expressing adaptive or non-adaptive plasticity, and that the expression changes of each gene between the ancestral and derived population resulted from adaptive evolution. This gives the false impression that the observed expression changes of many genes can be regarded as a large number of independent data points, whereas a single regulatory change could generate the same expression profiles. We illustrate this by a simple model that demonstrates how a single regulatory change can yield the same results as those shown by Ghalambor *et al.*[1] without indicating adaptive evolution. To motivate this model, we need to have a closer look at their classification of adaptive and non-adaptive plasticity. In their experiment, Ghalambor *et al.*[1] transplanted Trinidadian guppies (*Poecilia reticulata*) from a habitat with predators to two predatory-free habitats. After a few generations, fish from all three locations were reared in the laboratory to examine the degree of evolutionary divergence and the plastic responses of fish towards the presence and absence of predators. Genes in the ancestral population were classified as expressing adaptive plasticity when, in a predator-free environment, they exhibit an up- or downregulation in the direction of gene expression in the derived populations in this environment, but only if the degree of regulation is weaker in the ancestral population. Thus, plastic responses that occur in the same direction in both ancestral and derived populations but have a smaller effect size in the derived fish are considered to be non-adaptive. According to this definition, any factor that weakens the change in gene expression in the derived populations would be a source of non-adaptive plasticity. Let us assume, for example, that a large set of genes is under (partial) control of a regulatory mechanism, which affects the expression of these genes in response to different environments. An example is the hormone cortisol, which in guppies typically has higher levels in the presence than in the absence of predators[8]. Cortisol can have a major influence on gene expression[9] and it affects organismal phenotypes in a variety of ways[10]. The simplest model for the action of a hormone on many genes posits that the expression level of gene i is given by $G_i = B_i + w_i \times H(E) + \varepsilon$, in which B_i is the baseline expression level of this gene, $H(E)$ is the environment-specific level of the hormone, and ε subsumes all stochastic effects. The effect w_i of the hormone on gene

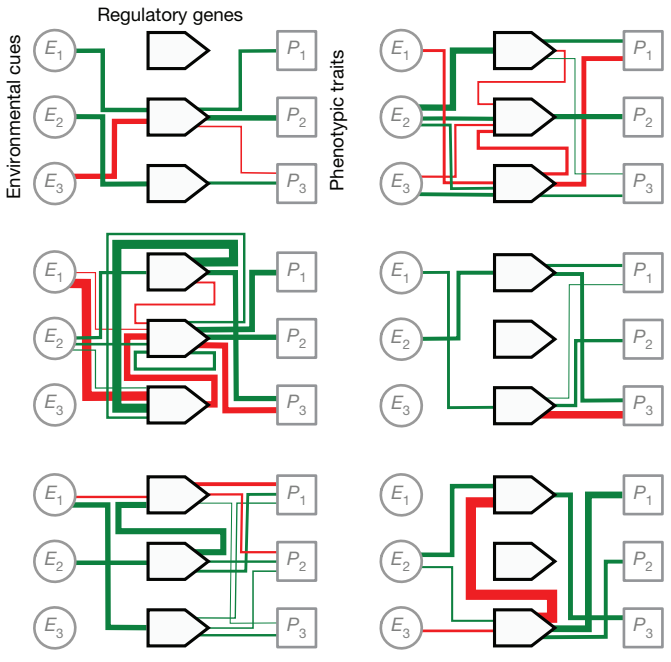

Figure 1 | Alternative gene regulatory network with identical plastic responses. Networks were selected to show an adequate phenotypic response to the presence (cues: $E_1 = 1.5$, $E_2 = 2.2$, $E_3 = 0.3$) or absence of predators (cues: $E_1 = 1$, $E_2 = 2$, $E_3 = 0.5$). The optimal response is to express three different phenotypic traits as $P_1 = P_2 = 0$, $P_3 = 1$ in the presence of predators, and as $P_1 = P_2 = 1$, $P_3 = 0$ in the absence of predators. The networks shown are the result of six replicate evolutionary simulations; all networks produce the same optimal phenotypic response, but the stimulating (green) and inhibitory (red) interactions within the networks and their strength (thickness) differ considerably. In accordance with previous models[12], we used a gene regulatory network implementation with Boolean gene expression and continuous connection weights.

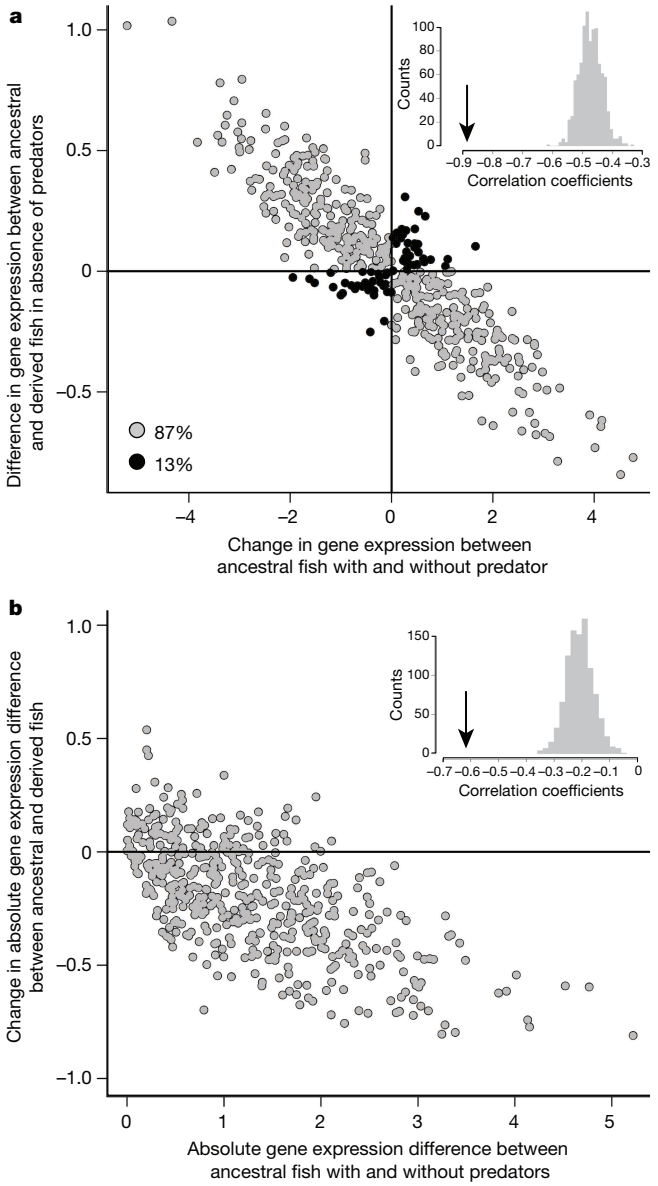

Figure 2 | Effect of small hormonal differences on gene expression patterns. a, **b**, In an ancestral and a derived population, the hormone-modulated expression pattern of 500 genes was assumed to follow the model $G_i = B_i + w_i \times H(E) + \varepsilon$ (see text). All parameters were the same in both populations, with the exception of hormone levels in a predator-free environment (ancestral population: $H = 1.6$; derived population: $H = 1.3$). Conducting the same analyses as in Fig. 2 and 3 of ref. 1 (panels **a** and **b**, respectively) produces very similar patterns (of high statistical significance) as those reported by Ghalambor *et al.*[1] Parameters: baseline levels (which do not affect the results) $B_i = 0.0$; $H = 0.0$ in the presence of predators; ε normally distributed with mean of 0 and s.d. of 0.1.

i can be positive or negative and large or small (in our simulation, the w_i effects are drawn from a standard normal distribution). If we now assume that fish from a derived population have a slightly lower hormone level when reared in the absence of a predator than fish from the ancestral population, this simple model reproduces the main results of Ghalambor *et al.*[1] (compare figures 2 and 3 from ref. 1 to Fig. 2a, b). Hence, a single regulatory change provides a much more parsimonious explanation for the observed transcription patterns than the large-scale evolutionary changes postulated by Ghalambor *et al.*[1] Our alternative explanation does not refer to adaptive or non-adaptive plasticity, and it does not require evolution of gene regulation patterns (the w_i effects remain constant). It does not even require adaptive evolution of the hormone level, because the lowered hormone level of the derived populations in a predator-free environment might just reflect a random change, for example, one caused by a founder effect[11].

We conclude that the evolutionary analysis of transcriptomics data remains a major challenge. Ghalambor *et al.*[1] present an intriguing experiment to investigate the role of phenotypic plasticity on evolutionary adaptation, in which they show that gene expression patterns can rapidly diverge between independently evolving populations. We do not question this rapid divergence or that non-adaptive plasticity could potentially underlie this divergence, instead we want to point out that one should be cautious when inferring evolutionary changes in plasticity from transcriptomic data, especially when the underlying regulatory mechanisms are unknown. Because many loci are involved, the rapid divergence in gene expression found by Ghalambor *et al.*[1] probably reflects only a few regulatory changes, making it impossible to classify individual genes as expressing either adaptive or non-adaptive plasticity. Thus, owing to the complex relation between an organism's genotype and phenotype, it is hard to understand adaptive evolution by focusing on single genes alone.

Data Availability All data are available from the corresponding author upon reasonable request.

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Contesting the evidence for non-adaptive plasticity

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The effect of phenotypic plasticity on the evolution of traits is a highly controversial subject. Ghalambor *et al.*[1] added a new spin to this debate as they contrasted gene expression patterns of guppy populations evolved at two different predation levels. After four generations in a new environment, gene expression plasticity evolved with two interesting key features: (1) in environments without predation, gene expression in the evolved populations changed in a direction that decreases ancestral plasticity; and (2) the ancestral level of plasticity and the magnitude of change in plasticity are negatively correlated. However, this pattern could be an artefact of the analysis procedure rather than reflecting selection, and simple computer simulations that assume no divergence in gene expression between populations and only stochastic variation are able to replicate the pattern. There is a Reply to this Comment by Ghalambor, C. K. *et al. Nature* **555,** http://dx.doi.org/10.1038/nature25497 (2018).

The analysis of genome scale data in evolutionary experiments is very challenging because it is important to distinguish genetic drift from directional forces (selection). Ghalambor *et al.*[1] approach this problem in two steps. First, they identify genes that are differentially expressed between ancestral and evolved populations in the new predator-free environment. Second, they condition on the average effect of the two evolved populations to go in the same direction as for one additional population that naturally colonized a predator-free environment. This step is included to distinguish between genetic drift and selection, because only selection is directional. Although we agree with the rationale of the testing procedure, we caution that the implementation could generate a signal of 'concordantly differentially expressed (CDE)' genes, even in the absence of selection.

Assuming no genetically based differences in gene expression and random differences in gene expression following a normal distribution, we simulated 37,493 transcripts and applied the testing procedure of Ghalambor *et al.*[1] We show that CDE transcripts can be detected even in the absence of selection. As in ref. 1, for CDE transcripts we also detect a negative correlation between ancestral plasticity and divergence in gene expression (Fig. 1a).

This negative correlation is the result of a statistical phenomenon known as the regression towards the mean (RTM), which was first described by Sir Francis Galton[2]: he observed that tall parents have, on average, children that are smaller than them (and vice versa). In this example, the parents at the extreme end of a distribution have children whose heights are closer to the mean of the distribution: that is, they regress towards the mean.

In our simulations, all transcripts have the same distribution in both environments. CDE transcripts have by chance diverged from this mean and thus were kept for further analysis. Yet, when contrasted to their expression in the second environment, they will probably regress towards the mean. Here, RTM creates the negative correlation as illustrated in Fig. 2: because three independent means are required for one group, but only one for the other, we expect that the first group will have values closer to the mean than the latter one, creating an asymmetry. Because random draws of the ancestral population under predation conditions are expected to regress towards the mean, the initial conditioning of the CDE transcripts causes the observed negative correlation (Fig. 2). Using the CDE transcripts, we also reproduce the negative correlation between the magnitude of plasticity in the source population and the evolved mean change in plasticity (Fig. 1b). As in Fig. 1a, we think that this pattern can be explained by the conditioning in the analysis. The more extreme the expression of the ancestral population at low predator conditions is, the less deviation from the mean is needed for the evolved populations to be significant in the first step of the analysis. Hence, drawing a random sample (for the predator environment) will result in small differences between the expression values for the evolved populations.

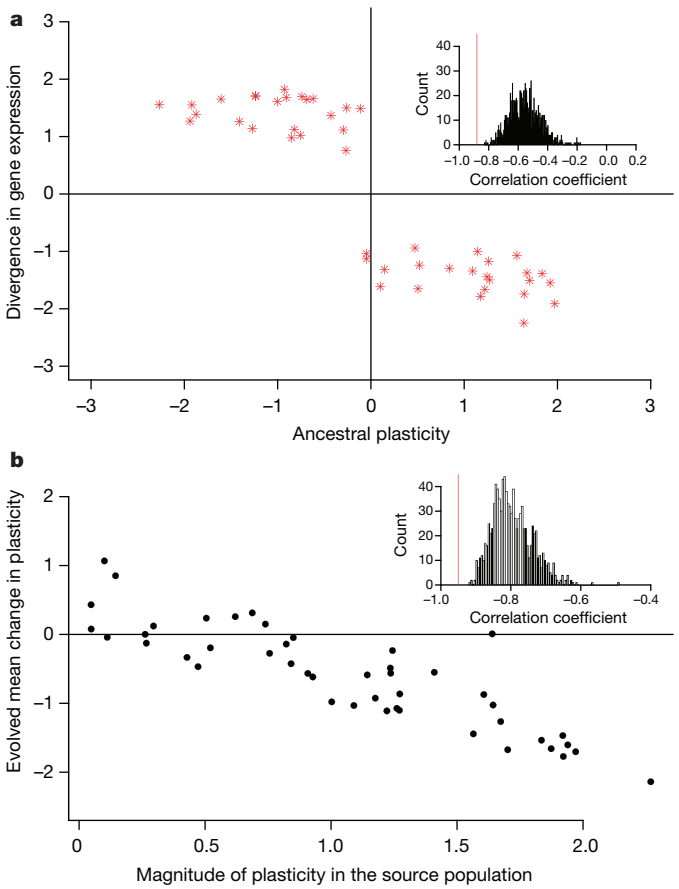

Figure 1 | Negative correlations between ancestral plasticity and divergence in gene expression, and between the magnitude of plasticity in the source population and the evolved mean change in plasticity. **a**, **b**, Negative correlation of the ancestral plasticity with the divergence in gene expression (**a**) and negative correlation of the mean change in plasticity in the introduced population with magnitude of plasticity in the source population (**b**) for CDE transcripts assuming no genetic divergence among all populations. Both insets show the distribution of Spearman's rank correlation for the CDE transcripts from 1,000 random permutations of the CDE transcripts. The correlation in the originally simulated (that is, non-permuted) dataset (red vertical line) is always more negative than in the permuted dataset.

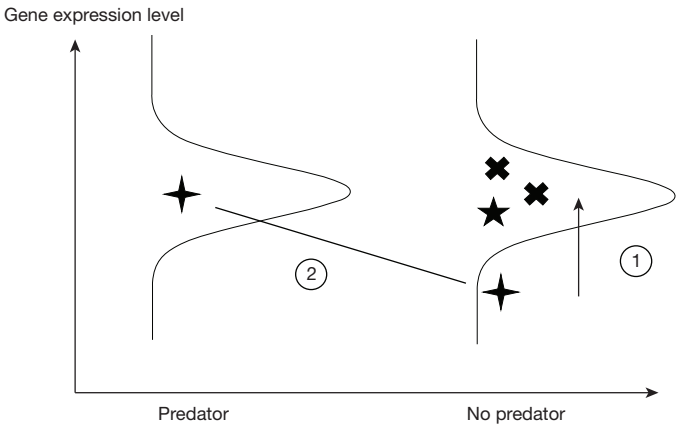

Figure 2 | Schematic representation of the negative correlation between the changes in gene expression observed in the predator-free environment and the ancestral plasticity when all values are randomly sampled from a normal distribution. The analysis of Ghalambor *et al.*[1] identifies genes expressed at one end of the distribution in the ancestral population, and closer to the mean for the three evolved ones (1). As the values in the predator environment are independent, they are also grouped around the mean and correlate negatively (2) with the evolved response: they regress towards the mean.

We showed that the key results of Ghalambor *et al.*[1] could be obtained without any genetically based difference in mean expression between the populations. Nevertheless, the results of our simulations differ in one aspect from the original study—the number of CDE transcripts in the simulated data are identical to the ones in the permuted data (all are randomly sampled). Reasoning that small differences due to sampling or even directional selection could explain this discrepancy, we performed additional simulations assuming small random changes in mean expression between populations. We confirmed that the number of CDE transcripts is higher than in the permuted dataset, exhibiting the same negative correlations without selection on plasticity (see Supplementary Information). The effect of RTM decreases with increasing between-population divergence relative to within-population variation[3]. For the data of Ghalambor *et al.*[1], the effect of RTM is difficult to quantify because each transcript has specific mean expression values and within and between population variances. Hence, we contrast the results of computer simulations using different parameter combinations to the experimental data (Supplementary Fig. 4).

The experimental data from Ghalambor *et al.*[1] were most similar to computer simulations in which the between-population divergence estimator was lower than the within-population variation; that is, the simulation data that show the RTM effect.

In any case, our simulations were not designed to match the experimental details of Ghalambor *et al.*[1] but instead to highlight that even without evolution of gene expression plasticity, we are able to recapitulate the key results of their study. Thus, we do not claim that the guppies have not evolved their plasticity of gene expression, but we feel that the authors would need to provide additional analyses to support their conclusions.

Methods

We randomly sampled gene expression values from a normal distribution for 37,943 transcripts in 32 samples. For each population comparison, we computed Student's *t*-test on 250 randomly permuted datasets and identified transcripts for which the simulated data were located in the 5% tails of the *t*-statistic distribution. We then calculated the mean differences in simulated expression between treatments and populations to determine the correlations between ancestral plasticity and divergence in gene expression (Fig. 1a) and magnitude of plasticity in the source population and evolved mean change in plasticity within introduced populations (Fig. 1a).

Code availability. The R code for the simulations are provided in the Supplementary Information.

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Supplementary Information accompanies this Comment.

Author Contributions F.M. performed simulations and analysed the data. F.M., A.M.J. and C.S. conceived the study and wrote the manuscript.

Competing Interests Declared none.

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Ghalambor *et al.* reply

REPLYING TO J. van Gestel & F. J. Weissing *Nature* **555,** http://dx.doi.org/10.1038/nature25495 (2018); F. Mallard, A. M. Jakšić & C. Schlötterer *Nature* **555,** http://dx.doi.org/10.1038/nature25496 (2018)

The concerns raised in the accompanying Comments by Mallard *et al.*[1] and van Gestel and Weissing[2] that natural selection could not generate the transcriptomic results reported in our Letter[3] overlook the larger context of previous work documenting rapid parallel evolutionary changes in guppies[4–7]. Here we also show compelling evidence that their alternative interpretations simply do not match our published datasets.

Mallard *et al.*[1] argue that the negative correlation between plasticity and evolution reflects neutral processes rather than selection, but they only report simulations using a subset of criteria our conclusions were based on; when all criteria are applied, their simulations support our conclusions. We based our conclusions on four criteria with analyses that appropriately accounted for stochastic variation and non-independence of our data: (1) we found more concordantly differentially expressed (CDE) transcripts than in permuted datasets; (2) we did not find more genes that diverged in opposite directions than in permuted datasets; (3) the association between plasticity and divergence was more negative than in permuted datasets; and (4) the direction of association between plasticity and divergence was more extreme than in permuted datasets. Had our data not met both of the first two criteria, we would not have concluded that our CDE genes were enriched for genes evolving under selection and would not have proceeded to assess the relationship between ancestral plasticity and divergence. The results presented in figures 1 and 2 of Mallard *et al.*[1] do not meet criterion (1) and thus provide no evidence that weakens our conclusions. The authors acknowledge this potential flaw and present simulations in their supplementary methods that meet criterion (1), yet they omit criterion (2) in those analyses. In fact, only 26 of their 400 parameter sets replicate all four criteria, and only a fraction of these yield datasets with distributions that might reasonably match our results (Fig. 1 and ref. 8). Therefore, the simulations of Mallard *et al.*[1] are not only consistent with our interpretation, but also other studies concluding that rapid evolution of genes exhibiting non-adaptive plasticity is more likely due to selection and very unlikely to arise by chance[3,9].

We agree with van Gestel and Weissing[2] that individual transcripts are not independent, and recognize the robustness of gene expression networks to produce similar phenotypes via different mechanisms[10]. We explicitly incorporated non-independence among transcripts in designing our permutations to preserve entire transcriptional profiles. The model proposed by van Gestel and Weissing[2] positing a single regulatory change underlying patterns in our set of CDE transcripts simply does not match our data, as such a model would generate a strongly correlated set of CDE transcripts. Correlational analyses show that few of our CDE genes are strongly correlated, and that numerous clusters of transcripts independently evolved CDE expression patterns and negative associations between plasticity and divergence (see ref. 8) to compare correlations in experimental data and simulated datasets with a single regulatory change). We thus refute the claim that variation in a single modulator can account for the rapid evolution of our CDE transcripts, just as it is unlikely to explain the rapid parallel evolution of other complex phenotypes in guppies.

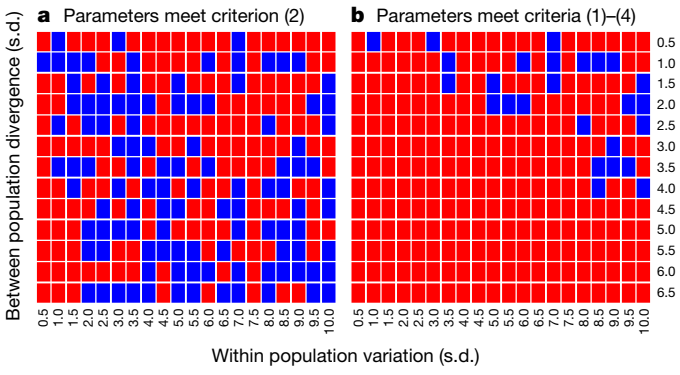

Figure 1 | A small fraction of simulations performed by Mallard *et al.*[1] meet all four criteria that support our conclusion that CDE genes diverged under selection, and that they diverged in the opposite direction as ancestral plasticity. Blue boxes indicate parameter sets that met the criteria, and red boxes indicate parameter sets that did not meet the criteria. **a**, Only a subset of parameters meet criterion (2), positing that differentially expressed genes that diverge in opposite directions should not be overrepresented compared to the permuted datasets. **b**, All four criteria merged together shows that only 26 parameter sets recreate the results found in Ghalambor *et al.*[3] This contrasts with the much higher fraction claimed by Mallard *et al.*[1], as they did not consider criterion (2) in their analyses.

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