

Reply to: Existing methods are effective at measuring natural selection on gene expression

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Peter D. Price¹✉, Daniela H. Palmer Drogue^{1,2}, Jessica A. Taylor¹, Dong W. Kim³, Elsie S. Place⁴, Thea F. Rogers¹, Judith E. Mank^{5,6,7}, Christopher R. Cooney¹ & Alison E. Wright¹✉

REPLYING TO: Hunter B. Fraser *Nature Ecology & Evolution*
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We read with great interest the commentary by Fraser¹ and wholeheartedly agree that understanding how selection acts on patterns of gene expression is key to identifying mechanisms of adaptive change. In Price et al.², we identified significant challenges to testing how the transcriptome evolves, specifically that shifts in tissue composition can bias inferences of selection over long evolutionary time frames. However, Fraser¹ suggests the problems we outline have been ‘largely solved by the research community’ in two ways—through studies of cell lines and interspecific hybrids. We are in complete agreement that both approaches circumvent issues arising from varying cell type abundance that we highlight and so are useful tools to accurately measure expression change. However, neither represents a panacea for detecting natural selection on gene expression that Fraser¹ suggests.

Contrasts of cell lines can be used to accurately identify regulatory variation and in principle can be applied over greater evolutionary distances to quantify the mode of gene expression evolution among distantly related species. However, creating cell lines is non-trivial and likely not feasible for many species. Importantly for multicellular model systems, the diversity of cell types that can be cultured is severely limited and the costs in doing so prohibitive if all cell types in a tissue are to be included. This means that this approach is unlikely to extend across the tree of life in the near future.

Most importantly, organisms are far more than the sum of their parts. Changes in tissue composition are key to the evolution of many adaptive phenotypes (for example, refs. 3–6) and likely the product of differences in expression across development⁷. Therefore, by their very nature, cell types analysed individually have limited potential for studying the developmental regulatory changes that produce variation in

cell type abundance and complex adaptive traits. Consistent with these limitations, the vast majority of the cell line research cited by Fraser¹ does not test for selection on gene expression levels, with the exception of three studies using primate cell lines^{8–10}. Examining cell types one at a time is therefore unlikely to yield a comprehensive picture of differences between species.

The second approach highlighted by Fraser¹ is the sign test of selection¹¹, which was extended by Fraser et al.¹² to test for selection on gene expression. This method has provided important insight into how gene expression evolves¹³, including the first known example of polygenic gene expression adaptation¹², and we have no wish to diminish this important contribution to the field. However, since this approach relies on prior knowledge of the directionality of genetic changes affecting a quantitative trait, to our knowledge, it has exclusively been applied to species that can produce viable hybrids, namely very closely related species^{14–16} or subspecies^{12,17,18}. Therefore, while informative for understanding expression evolution over very short evolutionary time frames, its potential to study many instances of adaptive change over the full breadth of evolutionary time is limited.

Together, neither approach suggested by Fraser¹ is widely applicable outside of model systems, limited cell types that are readily cultured, or relatively narrow evolutionary windows, leaving large gaps in both the scope of questions that can be addressed and the range of organisms that can be studied. Instead, we see a number of important points emerging from Fraser¹. First, developmental context matters for the evolution of many adaptive phenotypes, particularly in multicellular organisms. For these traits we should focus not on eliminating differences in cellular composition but instead properly

¹Ecology and Evolutionary Biology, School of Biosciences, University of Sheffield, Sheffield, UK. ²Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, USA. ³Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ⁴Development, Regeneration and Neurophysiology, School of Biosciences, University of Sheffield, Sheffield, UK. ⁵Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada. ⁶Beaty Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, Canada. ⁷Centre for Ecology and Conservation, University of Exeter, Penryn, UK. ✉e-mail: pprice3@sheffield.ac.uk; a.e.wright@sheffield.ac.uk

accounting for such differences when testing for selection on gene expression, potentially through the use of single-cell RNA sequencing. Second, it is likely that selection pressures vary over short versus long evolutionary time frames and limiting our analyses to closely related species will bias our understanding of how the transcriptome evolves. Comparative approaches that sample a range of evolutionary scales are clearly essential to understand the full spectrum of evolutionary responses to selection. Therefore, addressing the confounding issues of cellular composition, as discussed in Price et al.², is a major priority for the field.

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Author contributions

All authors contributed equally.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Peter D. Price or Alison E. Wright.

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