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Fischer, Eva

Hauber, Mark

Bell, Alison

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Author for correspondence:
Eva K. Fischer
e-mail: efischer@illinois.edu

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Back to the basics? Transcriptomics offers integrative insights into the role of space, time and the environment for gene expression and behaviour

Eva K. Fischer[1,2], Mark E. Hauber[1,2] and Alison M. Bell[1,2]

[1]Department of Evolution, Ecology, and Behavior, School of Integrative Biology, and [2]Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign, IL 61801, USA

EKF, 0000-0002-2916-0900; MEH, 0000-0003-2014-4928

Fuelled by the ongoing genomic revolution, broadscale RNA expression surveys are fast replacing studies targeting one or a few genes to understand the molecular basis of behaviour. Yet, the timescale of RNA-sequencing experiments and the dynamics of neural gene activation are insufficient to drive real-time switches between behavioural states. Moreover, the spatial, functional and transcriptional complexity of the brain (the most commonly targeted tissue in studies of behaviour) further complicates inference. We argue that a Central Dogma-like 'back-to-basics' assumption that gene expression changes cause behaviour leaves some of the most important aspects of gene–behaviour relationships unexplored, including the roles of environmental influences, timing and feedback from behaviour—and the environmental shifts it causes—to neural gene expression. No perfect experimental solutions exist but we advocate that explicit consideration, exploration and discussion of these factors will pave the way toward a richer understanding of the complicated relationships between genes, environments, brain gene expression and behaviour over developmental and evolutionary timescales.

1. Introduction

Understanding how genes influence behaviour remains an outstanding challenge for biology. This is in part because it is not genes *per se*, but rather when, where and to what degree genes are expressed, that shapes behaviour across immediate, developmental and evolutionary timescales [1]. As a result, behavioural ecologists are increasingly using RNA-sequencing (hereafter: RNA-seq) to explore gene expression changes associated with behaviour. Indeed, the twenty-first century has ushered in an era when genomic approaches are ever more feasible—and even expected—in studies exploring behavioural diversity in and beyond traditional model species. Moreover, sequencing technologies provide unprecedented opportunities to explore behaviour across Tinbergen's levels of analysis [2] by integrating temporal (ontogenetic) and physiological (molecular) shifts with questions about adaptive context (function) and phylogenetic diversity (evolution). Thus, whether alone or alongside related technologies, the RNA-seq explosion has seemingly allowed behavioural ecologists to identify gene expression differences causing behaviour and to increasingly unify Tinbergen's four questions.

Yet, as RNA-seq studies become increasingly ubiquitous, we caution that the path from gene expression to behaviour is neither unidirectional, nor necessarily causal. We argue that behavioural traits offer a unique set of challenges for interpretation, given the importance of both space (i.e. the complex structural

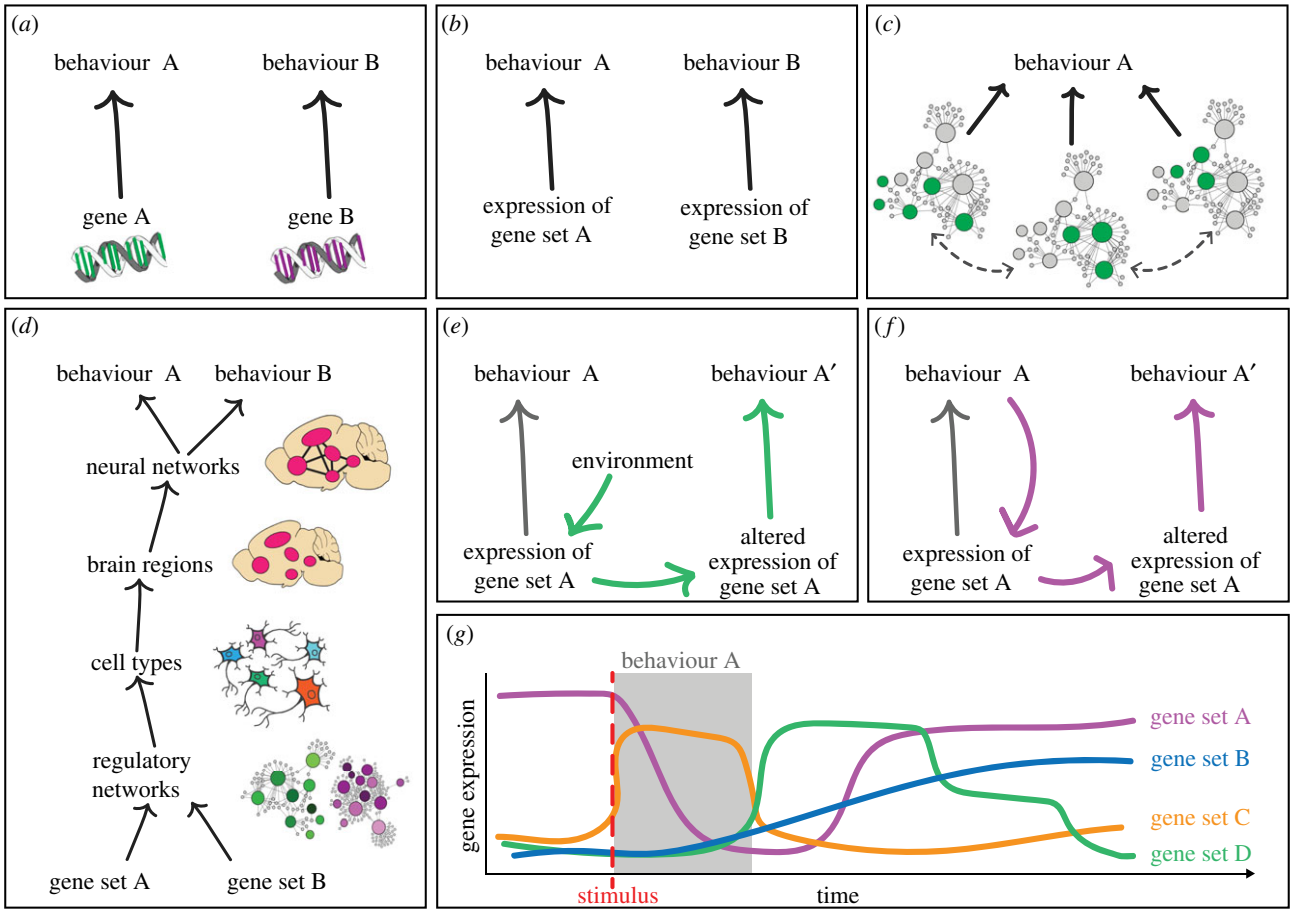


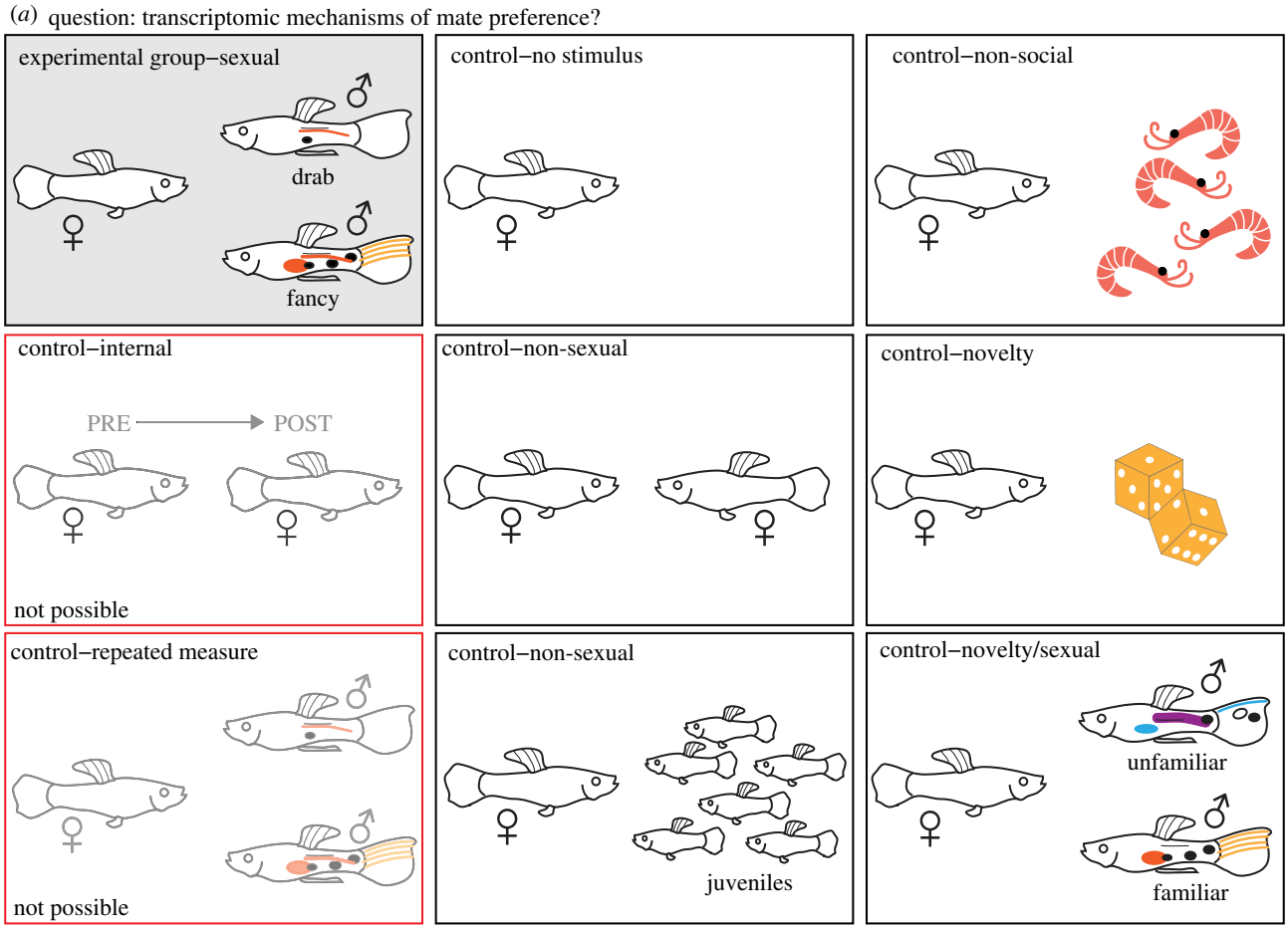
Figure 1. Complexities in interpreting brain gene expression–behaviour relationships. RNA-seq is increasingly applied in behavioural ecology to identify genomic mechanisms of behaviour. However, (*a*) the basic view that gene expression causes behaviour is overly simplified. A (*b*) transcriptomic view of behaviour inherently surveys the expression of many genes, and expression patterns are in turn influenced by (*c*) robustness and redundancy in the transcriptional states underlying behaviour, (*d*) interactions across hierarchical levels of organization, (*e*) environmental influences on gene expression and behaviour, as well as (*f*) the feedback loop from behaviour to gene expression and back. Moreover, (*g*) both behaviour and gene expression change over time, and distinct expression patterns may be associated with different timepoints during and following a behaviour and the transition between distinct behavioural states.

and strongly hierarchical organization of the brain) and time (i.e. acute, developmental and evolutionary influences) for gene expression. We advocate that careful study design and a nuanced, critical view of the factors influencing brain gene expression are essential components of studies linking transcriptional variation to behaviour. Here, we draw inspiration from behavioural endocrinology [3] and its experimental sophistication to establish the reciprocal relationships among physiology, behaviour and the environment. We focus our discussion on the interpretation of bulk RNA-seq studies that target coding sequences (mRNA), as this approach is most common in behavioural studies; however, we note that other classes of transcripts can also play important, albeit less understood, roles in shaping behaviour (e.g. micro-RNAs; [3–5]), and that transcriptome or genome assembly and annotation are prerequisite for this work and carry their own complexities.

2. Experimental design matters

Over the past two decades, studies have identified genes associated with behaviour by comparing the brain gene expression profiles of individuals of different behavioural types [6–8], animals in different behavioural states (e.g. parenting versus non-parenting [9], nurses versus foragers [10]) or in response to a stimulus that provokes a behavioural response (e.g. a predator [11,12], a potential mate [11] and an intruder ([13], or food [14]). An insight from this expanding literature is that behaviour is associated with large-scale changes in brain gene expression, often with hundreds to thousands of genes in the genome differing in expression between treatment groups. The challenge remains discerning how these differences are related to the phenotype of interest as there exist a large number of intervening players between mRNA and the behavioural phenotype (e.g. gene regulatory network, protein, cell type, neural circuit, etc.), with opportunities for feedback and environmental influences at each level (figure 1).

An additional challenge is that because RNA-seq captures most of the genes being expressed in a tissue at a given point in time, genes that are expressed following a behavioural state or action likely reflect multiple influences, only some of which are directly related to the target behaviour. Therefore, to be maximally informative, gene expression should ideally be compared relative to one or more control groups (figure 2). For example, to identify genes associated with female mate preference, Cummings *et al*. [15] compared brain gene expression between female swordtail fish presented with (i) an attractive versus an unattractive male (expected to elicit preference behaviour); (ii) two equally unattractive males (to control for reaction to males); (iii) two females (to control for reaction to conspecifics); and (iv) no stimuli (control). This experimental



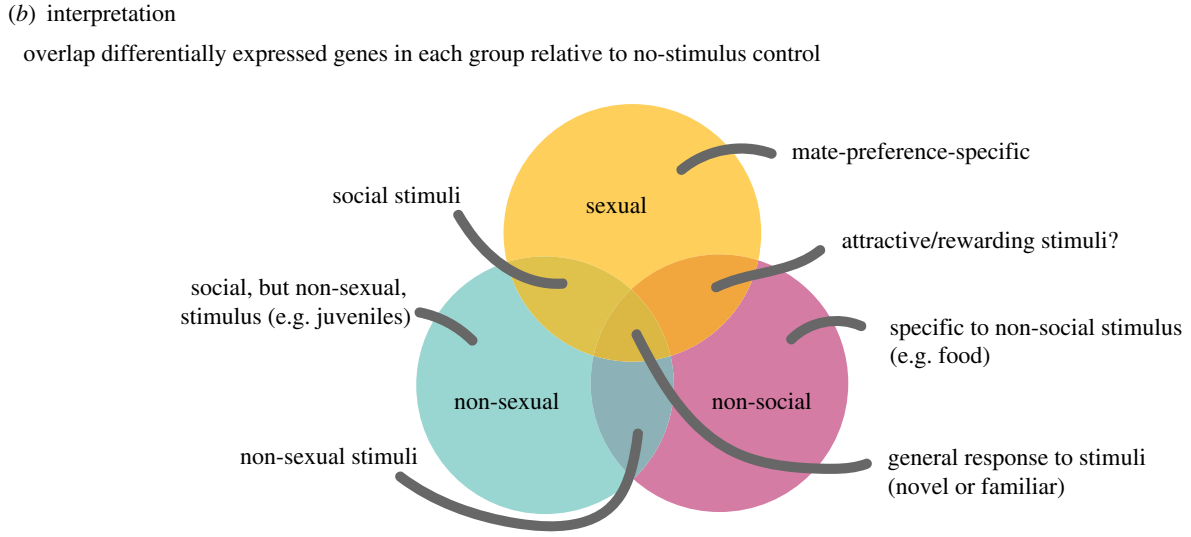


Figure 2. Complexities in behavioural RNA-seq experimental design. The opportunity to choose a mate presumably elicits many different biological responses, only some of which reflect mate preference *per se*. For example, a female guppy (*Poecilia reticulata*) presented with an opportunity to choose between two potential mates experiences not only her preference, but also potentially the process of responding to a conspecific, the reaction to a novel stimulus, stress and arousal. (*a*) As a result, identifying appropriate control group(s) is multivariate and dependent on the particular research question of interest. This process is further complicated by the impossibility of repeated measurements from the same individual when sampling is destructive (red boxes). (*b*) In order to isolate the transcriptomic signal of mate preference independent of these confounds, a researcher could measure the transcriptomic response to stimuli that are intended to evoke these different reactions and then subtract out those genes from the genes that are differentially expressed during mate preference. For simplicity, (*b*) shows an example containing only a subset of the conditions in (*a*).

design allowed the authors to control for multiple confounding influences on the outcome of interest. However, given the price and considerations about animal welfare (behavioural RNA-seq studies most often entail terminal sampling of brain tissue), such studies often require tough *a priori* decisions about the number of groups and subjects, and results must be thoughtfully interpreted in light of these constraints.

Although time-course RNA-seq experiments remain rare, when brain gene expression patterns have been compared at different timepoints after a behaviour of interest, distinct

patterns have been detected across time (figure 1*g*). For example, only a small number of genes were consistently differentially expressed in male sticklebacks 15, 30 and 120 min following a territorial challenge [16]. The majority of genes were expressed in waves, with gene ontology analyses suggesting that some biological functions (e.g. endocrine activity) peak early and other biological functions (e.g. immune response) peak hours after the challenge [16]. Such results underscore the dynamic nature of gene expression in the brain and illustrate that a single sampling timepoint may capture only a brief, non-representative glimpse into molecular outcomes. Moreover, it seems reasonable to assume that genes causally related to behaviour are expressed prior to its production, but current brain gene expression profiling technologies are terminal, such that gene expression is by necessity measured after, rather than before, the behaviour of interest. Finally, even when a time-course design is employed, destructive sampling means that repeated measures on the same individual are not possible and individual variation across time cannot be assessed (figure 2).

For all the above reasons—including the causal gap between mRNA and behaviour, the unbiased, genome-wide nature of RNA-seq, the environmentally responsive and dynamic nature of gene expression, and terminal sampling—it is likely that the genes identified by this burgeoning literature reflect highly heterogeneous processes, only some of which are responsible for generating the target behaviour. What this means is that it may be premature to assume a classical, 'back-to-the-basics' causal relationship between genes identified in RNA-seq experiments and behaviour.

Instead, it might be more helpful to conceptualize gene expression patterns detected with RNA-seq as having more in common with hormones than with DNA sequence variation. Like neural gene expression patterns, endocrine levels change over time and in response to diverse stimuli (e.g. a territorial challenge) [17]. Decades of work in the endocrinology literature have taught us how behaviour, the environment, physical substrates, social cues and internal state can interact with one another. Lehrman's studies on reproduction in ring doves, *Streptopelia risoria*, [18,19] provide a classic example: interactions during courtship trigger the release of oestrogen, which stimulates the secretion of progesterone, which facilitates egg laying and incubation. The presence of eggs induces the secretion of prolactin, which induces physiological changes enabling the transition from incubation to feeding offspring. This and other (e.g. [20]) work has taught us that not only do hormones cause behaviour, but stimuli arising from the behaviour feed back to influence these same hormones [21], phenomena that likely also apply to gene expression (figure 1).

3. Profiling brain tissue matters

The brain is functionally, structurally and transcriptionally complex. In humans and mice, approximately 80% of all genes in the genome are expressed in the brain [22], and this transcriptional complexity is embedded in spatial and structural heterogeneity that is central to brain function; distinct brain regions have distinct (behavioural) functions, regions can be further divided into subregions, and cells within regions can be categorized into distinct cell types of classes that may subserve specific behaviour. For example, distinct subsets of preoptic area galanin neurons mediate pup-seeking versus pup-licking and -grooming in parental mice [23]. As a result of this structural and transcriptional complexity, behaviourally relevant gene expression specific to one brain region or cell type may be masked when the expression is surveyed at a coarse level of analysis (e.g. whole brain). Moreover, current analytical techniques are biased toward identifying the largest gene expression differences, even though many molecules with critical functions (e.g. transcription factors) and specific influences on behaviour (e.g. oxytocin) are known to be transcribed at low levels [24]. Technological advances over the past decade have facilitated increasingly fine-grained transcriptional surveys (e.g. single-cell RNA-seq), yet these advances have not freed us from limitations. Rather, they have revealed additional levels of complexity, demonstrating that cells of the same transcriptional type may be functionally distinct [25], that cells of the same functional type may be transcriptionally distinct [25,26], and that transcripts may be localized even at the subcellular level [27].

Rapid behavioural responses are mediated by signals transduced electrically along neurons and electrically or chemically across synapses. Gene expression changes—in which molecules must be transcribed—are simply too slow for this job. Thus, as discussed above, gene expression changes associated with behaviour are largely occurring in *response* to activity in behaviourally relevant neurons and circuits. While they do not drive the immediately preceding behaviour, the transcriptional responses triggered by rapid electrical and chemical responses can alter neural circuit tuning and structure to facilitate ongoing and shape future behaviour. This has two, non-mutually exclusive consequences for the interpretation of differentially expressed genes. First, as transcriptomic activity is linked to neural activity, we can still reasonably interpret differences as behaviourally relevant. Second, because transcriptional responses alter future circuit function, we may view gene expression changes as predictive of future behaviour. Taken together, the inherent feedback between nervous system activity and gene expression provides a means to integrate experience-dependent behavioural outcomes across contexts and timescales, which in turn allows for individual variation and environmentally induced plasticity in behaviour. Indeed, brain gene expression profiling has the potential to open the black box of so-called 'integrator mechanisms' [28] by which individuals combine information from different sources to make adaptive decisions [29,30].

The idea that gene expression responses provide a means of integrating acute experiences into longer lasting responses provides a synthetic framework for understanding the role of gene expression changes in behaviour across timescales [31]. Importantly for our discussion here, this means that some of the gene expression variation associated with behaviour reflects outcomes of previous experiences that have altered individuals' behavioural propensity and/or ability for the behaviour, while other gene expression responses reflect the performance of the behaviour in question and may thereby drive behavioural performance in the future [32]. These alternatives are critical to consider—even when they are difficult to distinguish—in particular because the same genes may be central to organizational, responsive and evolutionary processes, a phenomenon that has important implications for the evolution of phenotypic plasticity [1,32,33].

As with 'organizational' versus 'activational' effects of hormones [34], transcriptional changes associated with a

particular behaviour can reflect changes resulting from its acute performance, changes resulting from previous experiences, or a combination of both. Moreover, it is likely that, as with hormones, transcriptional mechanisms themselves have evolved in species-specific ways. For example, nonapeptides facilitate social behaviour in multiple species of estrildid finches but the direction of this association depends on sex and social system (gregarious versus territorial) [35,36]. In brief, additional work is needed to distinguish the precise influence of individual gene expression changes on behaviour.

Finally, brain gene expression differences associated with behaviour may reflect robustness, rather than change (figure 1). This could be the case when transcriptional differences are compensatory or homeostatic, allowing individuals to produce consistent behaviour despite—rather than in response to—changes in the internal and external environment. In other words, transcriptional responses may in some cases stabilize higher-level outputs, a phenomenon elegantly demonstrated by work showing consistent circuit-level activity arising from variable underlying gene expression configurations in the crab stomatogastric ganglion [26,37,38]. Though behavioural plasticity and robustness appear initially contrasting, by mediating experience-dependent influences on the structure and tuning of neural circuits, gene expression changes may in fact be critical precisely for mediating this balance [39]. Indeed, the phenomena described above are likely occurring simultaneously rather than sequentially or exclusively. The central challenge remains distinguishing between alternatives to understand how and when gene expression changes do (or do not) propagate to behavioural responses and what consequences these phenomena have for fitness outcomes and evolutionary trajectories.

4. Where do we go from here?

Here we have highlighted problems with the implicit assumption that gene expression differences associated with behaviour identify genes that cause behaviour, issues with linking brain gene expression to behaviour, and the notion that the gambit RNA-seq can elucidate this relationship directly. So how can we reap rewards from this gambit?

First, and most importantly, only careful study design and thoughtful interpretation will allow us to leverage the dynamic nature of the transcriptome to discern (rather than confound) the influences of time, experience and the environment on gene expression and behaviour.

Second, while they are not a panacea, increasingly sophisticated technologies continue to provide new insights [39,40]. For example, recent advances allow transcriptomic profiling specifically of neurons active during behaviour [41,42], spatial-transcriptomics facilitates gene expression surveys with stunning spatial resolution [25], and ATAC-seq can identify regions of open chromatin in bulk tissue or within single cells. Importantly, the concurrent expansion of tools for gene expression surveys and gene expression manipulation (e.g. CRISPR/Cas9 gene editing) is facilitating causal testing of gene–behaviour relationships identified via transcriptomic surveys, even outside of traditional model systems [43,44].

Finally, additional answers will come from comparative studies that provide the opportunity to discern core principles from species-specific patterns [45–47] and from studies of behavioural variation in genetically identical individuals that provide the opportunity to disentangle behavioural from genetic variation [48,49]. As the twentieth century brought hormone measurements to a wide array of species in and outside the laboratory, we are poised to apply RNA-seq and related approaches to diverse species and, thereby, advance evolutionary perspectives on the biological basis of behaviour. These approaches will be most powerful when they are combined into integrative studies that link transcriptional variation with neural activity, physiology and genetic variation. As ever more sophisticated genomic technologies continue to revolutionize behavioural studies, it is critical that we go back to the basics of careful study design, sufficient sample sizes and thoughtful interpretation in order to build a beyond-the-basics view of the complex, dynamic and bidirectional relationship between genes and behaviour.

Data accessibility. This article has no additional data.

Authors' contributions. All authors contributed to the conception and writing of the manuscript.

Competing interests. We declare we have no competing interests.

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References

1. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press.
2. MacDougall-Shackleton SA. 2011 The levels of analysis revisited. *Phil. Trans. R. Soc. B* **366**, 2076–2085. (doi:10.1098/rstb.2010.0363)
3. Marler P. 2005 Ethology and the origins of behavioral endocrinology. *Horm. Behav.* **47**, 493–502. (doi:10.1016/j.yhbeh.2005.01.002)
4. Zhang X, Xu YN, Chen B, Kang L. 2020 Long noncoding RNA PAHAL modulates locust behavioural plasticity through the feedback regulation of dopamine biosynthesis. *PLoS Genet.* **16**, e1008771. (doi:10.1371/journal.pgen.1008771)
5. Narayanan R, Schratt G. 2020 miRNA regulation of social and anxiety-related behaviour. *Cell. Mol. Life Sci.* **77**, 4347–4364. (doi:10.1007/s00018-020-03542-7)
6. Todd EV, Liu H, Lamm MS, Thomas JT, Rutherford K, Thompson KC, Godwin JR, Gemmell NJ. 2018 Female mimicry by sneaker males has a transcriptomic signature in both the brain and the gonad in a sex-changing fish. *Mol. Biol. Evol.* **35**, 225–241. (doi:10.1093/molbev/msx293)
7. Hale MC, McKinney GJ, Thrower FP, Nichols KM. 2016 RNA-seq reveals differential gene expression in the brains of juvenile resident and migratory smolt rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. D* **20**, 136–150. (doi:10.1016/j.cbd.2016.07.006)
8. Fraser BA, Janowitz I, Thairu M, Travis J, Hughes KA. 2014 Phenotypic and genomic plasticity of alternative male reproductive tactics in sailfin mollies. *Proc. R. Soc. B* **281**, 20132310. (doi:10.1098/rspb.2013.2310)
9. Parker DJ, Cunningham CB, Walling CA, Stamper CE, Head ML, Roy-Zokan EM, McKinney EC, Ritchie MG, Moore AJ. 2015 Transcriptomes of parents identify parenting strategies and sexual conflict in a subsocial beetle. *Nat. Commun.* **6**, 8449. (doi:10.1038/ncomms9449)
10. Whitfield CW, Cziko AM, Robinson GE. 2003 Gene expression profiles in the brain predict behavior in individual honey bees. *Science* **302**, 296–299. (doi:10.1126/science.1086807)

11. Carney GE. 2007 A rapid genome-wide response to *Drosophila melanogaster* social interactions. *BMC Genomics* **8**, 288. (doi:10.1186/1471-2164-8-288)
12. Sanogo YO, Hankison S, Band M, Obregon A, Bell AM. 2011 Brain transcriptomic response of threespine sticklebacks to cues of a predator. *Brain Behav. Evol.* **77**, 270–285. (doi:10.1159/000328221)
13. Shpigler HY *et al.* 2017 Behavioral, transcriptomic and epigenetic responses to social challenge in honey bees. *Genes Brain Behav.* **16**, 579–591. (doi:10.1111/gbb.12379)
14. McNeill MS, Kapheim KM, Brockmann A, McGill TAW, Robinson GE. 2016 Brain regions and molecular pathways responding to food reward type and value in honey bees. *Genes Brain Behav.* **15**, 305–317. (doi:10.1111/gbb.12275)
15. Cummings ME, Larkins-Ford J, Reilly CRL, Wong RY, Ramsey M, Hofmann HA. 2008 Sexual and social stimuli elicit rapid and contrasting genomic responses. *Proc. R. Soc. B* **275**, 393–402. (doi:10.1098/rspb.2007.1454)
16. Bukhari SA *et al.* 2017 Temporal dynamics of neurogenomic plasticity in response to social interactions in male threespined sticklebacks. *PLoS Genet.* **13**, e1006840. (doi:10.1371/journal.pgen.1006840)
17. Wingfield JC, Hegner RE, Dufty AM, Ball GF. 1990 The 'Challenge Hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* **136**, 829–846. (doi:10.1086/285134)
18. Lehrman DS. 1959 Hormonal responses to external stimuli in birds. *Ibis* **101**, 478–496. (doi:10.1111/j.1474-919X.1959.tb02405.x)
19. Lehrman DS. 1964 The reproductive behavior of ring doves. *Scient. Am.* **211**, 48–54. (doi:10.1038/scientificamerican1164-48)
20. Safran RJ, Adelman JS, McGraw KJ, Hau M. 2008 Sexual signal exaggeration affects physiological state in male barn swallows. *Curr. Biol.* **18**, R461–R462. (doi:10.1016/j.cub.2008.03.031)
21. Rubenstein DR, Hauber ME. 2008 Dynamic feedback between phenotype and physiology in sexually selected traits. *Trends Ecol. Evol.* **23**, 655–658. (doi:10.1016/j.tree.2008.07.010)
22. Lein ES *et al.* 2007 Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**, 168–176. (doi:10.1038/nature05453)
23. Kohl J *et al.* 2018 Functional circuit architecture underlying parental behaviour. *Nature* **556**, 326–331. (doi:10.1038/s41586-018-0027-0)
24. Hudson NJ, Dalrymple BP, Reverter A. 2012 Beyond differential expression: the quest for causal mutations and effector molecules. *BMC Genomics* **13**, 356. (doi:10.1186/1471-2164-13-356)
25. Moffitt JR *et al.* 2018 Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. *Science* **362**, eaau5324. (doi:10.1126/science.aau5324)
26. Northcutt AJ, Kick DR, Otopalik AG, Goetz BM, Harris RM, Santin JM, Hofmann HA, Marder E, Schulz DJ. 2019 Molecular profiling of single neurons of known identity in two ganglia from the crab. *Proc. Natl Acad. Sci. USA* **116**, 26 980–26 990. (doi:10.1073/pnas.1911413116)
27. Middleton SA, Eberwine J, Kim J. 2019 Comprehensive catalog of dendritically localized mRNA isoforms from sub-cellular sequencing of single mouse neurons. *BMC Biol.* **17**, 5. (doi:10.1186/s12915-019-0630-z)
28. Martin LB, Liebl AL, Trotter JH, Richards CL, McCoy K, McCoy MW. 2011 Integrator networks: illuminating the black box linking genotype and phenotype. *Integr. Comp. Biol.* **51**, 514–527. (doi:10.1093/icb/icr049)
29. Dall SRX, McNamara JM, Leimar O. 2015 Genes as cues: phenotypic integration of genetic and epigenetic information from a Darwinian perspective. *Trends Ecol. Evol.* **30**, 327–333. (doi:10.1016/j.tree.2015.04.002)
30. Stein LR, Bukhari SA, Bell AM. 2018 Personal and transgenerational cues are nonadditive at the phenotypic and molecular level. *Nat. Ecol. Evol.* **2**, 1306–1311. (doi:10.1038/s41559-018-0605-4)
31. Clayton DF, Anreiter I, Aristizabal M, Frankland PW, Binder EB, Citri A. 2020 The role of the genome in experience-dependent plasticity: extending the analogy of the genomic action potential. *Proc. Natl Acad. Sci. USA* **117**, 23 252–23 260. (doi:10.1073/pnas.1820837116)
32. Traniello IM, Robinson GE. 2021 Neural and molecular mechanisms of biological embedding of social interactions. *Annu. Rev. Neurosci.* **44**, 109–128. (doi:10.1146/annurev-neuro-092820-012959)
33. Rittschof CC, Hughes KA. 2018 Advancing behavioural genomics by considering timescale. *Nat. Commun.* **9**, 489. (doi:10.1038/s41467-018-02971-0)
34. Arnold AP, Breedlove SM. 1985 Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. *Horm. Behav.* **19**, 469–498. (doi:10.1016/0018-506X(85)90042-X)
35. Goodson JL, Kelly AM, Kingsbury MA. 2012 Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. *Horm. Behav.* **61**, 239–250. (doi:10.1016/j.yhbeh.2012.01.005)
36. Goodson JL, Schrock SE, Klatt JD, Kabelik D, Kingsbury MA. 2009 Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science* **325**, 862–866. (doi:10.1126/science.1174929)
37. Schulz DJ, Goaillard JM, Marder E. 2006 Variable channel expression in identified single and electrically coupled neurons in different animals. *Nat. Neurosci.* **9**, 356–362. (doi:10.1038/nn1639)
38. Goaillard JM, Taylor AL, Schulz DJ, Marder E. 2009 Functional consequences of animal-to-animal variation in circuit parameters. *Nat. Neurosci.* **12**, 1424–1430. (doi:10.1038/nn.2404)
39. Hoke KL, Adkins-Regan E, Bass AH, McCune AR, Wolfner MF. 2019 Co-opting evo-devo concepts for new insights into mechanisms of behavioural diversity. *J. Exp. Biol.* **222**, jeb190058. (doi:10.1242/jeb.190058)
40. Ruiz-Ortiz J, Tollkuhn J. 2021 Specificity in sociogenomics: identifying causal relationships between genes and behavior. *Horm. Behav.* **127**, 104882. (doi:10.1016/j.yhbeh.2020.104882)
41. Fischer EK, Roland AB, Moskowitz NA, Tapia EE, Summers K, Coloma LA, O'Connell LA. 2019 The neural basis of tadpole transport in poison frogs. *Proc. R. Soc. B* **286**, 20191084. (doi:10.1098/rspb.2019.1084)
42. Baran NM, Streelman JT. 2020 Ecotype differences in aggression, neural activity and behaviorally relevant gene expression in cichlid fish. *Genes Brain Behav.* **19**, e12657. (doi:10.1111/gbb.12657)
43. Louder MIM, Balakrishnan CN, Louder AAN, Driver RJ, London SE, Hauber ME. 2019 An acoustic password enhances auditory learning in juvenile brood parasitic cowbirds. *Curr. Biol.* **29**, 4045–4051.e3. (doi:10.1016/j.cub.2019.09.046)
44. Gallant JR, O'Connell LA. 2020 Studying convergent evolution to relate genotype to behavioral phenotype. *J. Exp. Biol.* **223**, jeb213447. (doi:10.1242/jeb.213447)
45. Fischer EK, Nowicki JP, O'Connell LA. 2019 Evolution of affiliation: patterns of convergence from genomes to behaviour. *Phil. Trans. R. Soc. B* **374**, 20180242. (doi:10.1098/rstb.2018.0242)
46. Jourjine N, Hoekstra HE. 2021 Expanding evolutionary neuroscience: insights from comparing variation in behavior. *Neuron* **109**, 1084–1099. (doi:10.1016/j.neuron.2021.02.002)
47. Saul MC *et al.* 2019 Cross-species systems analysis of evolutionary toolkits of neurogenomic response to social challenge. *Genes Brain Behav.* **18**, e12502. (doi:10.1111/gbb.12502)
48. Laskowski KL, Doran C, Bierbach D, Krause J, Wolf M. 2019 Naturally clonal vertebrates are an untapped resource in ecology and evolution research. *Nat. Ecol. Evol.* **3**, 161–169. (doi:10.1038/s41559-018-0775-0)
49. Loughry WJ, McDonough CM. 1994 Scent discrimination by infant nine-banded armadillos. *J. Mammal.* **75**, 1033–1039. (doi:10.2307/1382486)