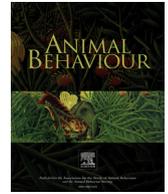




Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Special Issue: Kin Selection

Genomics: moving behavioural ecology beyond the phenotypic gambit



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ARTICLE INFO

Article history:

Received 1 November 2013

Initial acceptance 14 January 2014

Final acceptance 18 February 2014

Available online 1 April 2014

MS. number: ASI-13-00918

Keywords:

adaptive trade-off

behavioural genetics

behavioural modelling

behavioural optimization

genetic toolkit

phenotypic plasticity

Researchers studying the adaptive significance of behaviour typically assume that genetic mechanisms will not inhibit evolutionary trajectories, an assumption commonly known as the 'phenotypic gambit'. Although the phenotypic gambit continues to be a useful heuristic for behavioural ecology, here we discuss how genomic methods provide new tools and conceptual approaches that are relevant to behavioural ecology. We first describe how the concept of a genetic toolkit for behaviour can allow behavioural ecologists to synthesize both genomic and ecological information when assessing behavioural adaptation. Then we show how gene expression profiles can be viewed as complex phenotypic measurements, used to (1) predict behaviour, (2) evaluate phenotypic plasticity and (3) devise methods to manipulate behaviour in order to test adaptive hypotheses. We propose that advances in genomics and bioinformatics may allow researchers to overcome some of the logistical obstacles that motivated the inception of the phenotypic gambit. Behavioural ecology and genomics are mutually informative, providing potential synergy that could lead to powerful advances in the field of animal behaviour.

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Behavioural ecologists have always recognized that knowing the relationship between genes and behaviour is important for understanding how selection is operating on a trait (Brockmann, 2001; Grafen, 1984; Roff, 2007). However, the gene–behaviour connection has not been a focal point for behavioural ecology, particularly for studies of the adaptive significance of behaviour. Behavioural ecologists study natural behaviours that are often regulated by many genes and influenced by individual genotype, environmental factors, and their interaction (Caro & Bateson, 1986). These types of behaviours, particularly for nonmodel species, are largely outside of the scope of traditional genetic analyses. Behavioural ecologists instead prioritize the study of the ecological selection pressures that shape behaviour, assuming that genetic mechanism will not inhibit evolutionary trajectories, an assumption commonly known as the 'phenotypic gambit' (Grafen, 1984; Roff, 2007).

The phenotypic gambit has proven to be a useful heuristic for behavioural ecology. It has allowed researchers to establish general rules for the evolutionary processes that shape behavioural phenotypes while having little or no knowledge of the genetic basis of these traits (e.g. Brockmann, 2001; Parker, Ball, Stockley, & Gage,

1997; Roff & Fairbairn, 2007; Vollrath & Parker, 1992). Indeed, in some contexts, knowledge of genetic mechanism is not necessary to address questions about the adaptive significance of behaviour. The success of the phenotypic gambit, in combination with the often-narrow applicability of genetic analyses to questions of interest to behavioural ecologists, continues to limit the role of modern genetics in behavioural ecology studies today.

In recent years, however, genetics has been transformed by genomics, a subdiscipline that has greatly expanded both the philosophy and experimental approaches of the field. This expansion has allowed researchers to begin to reshape the relationship between genetics and behavioural ecology (e.g. Bell & Robinson, 2011; Fitzpatrick et al., 2005; LaFreniere & MacDonald, 2013; Linksvayer, Busch & Smith, 2013; van Oers & Mueller, 2010; Roff, 2007; Zayed & Robinson, 2012). Two major features of genomics seem to strongly resonate with behavioural ecologists. First, genomic approaches emphasize the dynamic nature of the genome, correlating gene expression patterns, not just allelic variation, with behavioural phenotype. This perspective emphasizes the fact that the genome, like behaviour, is both heritable and environmentally responsive (Flint, 2003; Mackay et al., 2005; Robinson, 2004). Second, genomics has the tools to sequence or measure the expression of thousands of genes simultaneously, which has given researchers the ability to experimentally account for the long-known fact that behaviours often involve many genes of small effect that interact in complex ways (Flint, 2003; Kultz et al., 2013; Robinson, Fernald, &

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Clayton, 2008; Sokolowski, 2001). These advances, along with the expansion of genomic resources for nonmodel organisms, create an opportunity for behavioural ecologists to apply a genomic perspective to their research questions.

In this essay we describe some analytical tools and conceptual approaches from genomics, discuss their current usage in behavioural studies, and put forward new ideas as to how these approaches might apply to questions of interest to behavioural ecologists. Rather than provide a comprehensive review of the behavioural applications of genomics, our goal is to prompt behavioural ecologists to think creatively about possible insights to be gained by moving beyond the phenotypic gambit. For instance we discuss the possibility that there are genetic toolkits for behaviour, an area of research founded on the observation that convergent phenotypes sometimes evolve using similar genetic mechanisms. Then, in the section Gene Expression Profiles: Analytical Tools, we focus on the utility of genomic approaches beyond the traditional goal of identifying the causal genetic basis of behaviour. We propose ways in which gene expression profiles can be used to (1) predict behaviour, (2) evaluate plasticity and (3) devise methods to manipulate behaviour in order to test adaptive hypotheses.

BACKGROUND AND TERMINOLOGY

Genomic approaches have been applied to a broad scope of natural behaviours in nonmodel organisms (Bell & Aubin-Horth, 2010; van Oers & Mueller, 2010; St-Cyr & Aubin-Horth, 2009; Wong & Hofmann, 2010; Zayed & Robinson, 2012). These studies typically use whole genome transcriptomic analyses of gene expression or mRNA abundance in the brain, comparing expression profiles across individuals with different behavioural phenotypes. Because gene expression analyses have been used most extensively to couple genome dynamics to behaviour, we will primarily focus on this type of measurement throughout the essay. Other 'omic' levels of organization (e.g. proteomics and metabolomics) are also useful in interpreting behaviour (Brockmann et al., 2009; Wong & Hofmann, 2010), but are beyond the scope of this essay.

Transcriptomic analyses can be used to identify the genes that show dynamic expression in correlation with variation in behaviour. Behaviour-specific gene expression could occur as a result of transient environmental change, epigenetic changes, and/or changes at the DNA sequence level. These data can be used to study proximate genetic and genomic mechanisms at different levels of biological organization. For researchers interested in understanding DNA sequence variants that affect behaviour, gene expression data provide a set of targets for further analysis (Joshi, 2005; Kelley et al., 2012; Mackay et al., 2005). Researchers can also group genes with correlated expression levels into networks and evaluate how these networks are modulated in real time or over evolutionary time (Hyduke & Palsson, 2010; Linksvayer, Fewell, Gadau, & Laubichler, 2012). Assessing multiple genes simultaneously also allows one to draw inferences about the molecular pathways or physiological processes that are implicated in the expression of a behaviour. We describe these higher inference levels collectively as 'molecular functions' and 'biological processes', after the common terminology in the field of genomics (the Gene Ontology Consortium, 2000, <http://www.geneontology.org>). Molecular functions indicate precisely what a gene protein product does at the molecular level (e.g. it catalyses a particular biochemical reaction), and biological processes describe the consequences of these functions, at higher levels of biological organization (e.g. 'learning and memory' or 'signal transduction'). Because of the modular and combinatorial nature of biology, a gene can be involved in many molecular functions, and the same molecular functions can be

involved in a variety of different biological processes. Throughout this essay, we discuss ways in which inferences across these organizational levels (genes, gene networks and physiological processes) can be used to evaluate behaviour and its evolution.

IDENTIFYING GENETIC TOOLKITS FOR BEHAVIOUR

Behavioural ecologists have long been fascinated by the observation that remarkably similar behaviours and strategies can evolve convergently across distantly related species (Bell & Aubin-Horth, 2010; Brockmann, 2001; Fawcett, Hamblin, & Giraldeau, 2012). Furthermore, behavioural convergence appears to occur despite differences at the level of mechanism (e.g. differences in the hormone and neurotransmitter systems or neural structures that regulate behaviour). These observations support the phenotypic gambit perspective, which assumes that ecological selection pressures ultimately drive phenotypic convergence, and underlying mechanisms neither constrain nor facilitate behavioural evolution in the long term. Increasingly, however, studies reveal that similar sets of genes are often associated with the expression of convergent phenotypes (Arendt & Reznick, 2008). Homology at the level of genes, gene networks and molecular functions occurs despite differences at other mechanistic levels. One of the most well-known examples of this kind of phenotype is the eye. The eye is an organ with tremendous structural diversity, including image-forming eyes in vertebrates and invertebrates that are products of convergent evolution. However, eye development is consistently regulated, at least in part, by the transcription factor *Pax-6* (Halder, Callaerts, & Gehring, 1995). This type of consistent relationship between phenotype and genetic mechanism across phylogenetically diverse species could have interesting implications for behavioural ecologists studying behavioural adaptations.

Repeated evolution of both phenotype and mechanism highlights the complementary role of ecological conditions and genetic architecture in shaping adaptations. From one perspective, this phenomenon may indicate that behavioural outcomes are constrained by a mechanistic framework with finite capacity for variation. Thus, in contrast to the concept of the phenotypic gambit, there may be limitations to behavioural optimization, much like the constraints imposed by competing ecological selection pressures, or context-dependent fitness costs. Alternatively, the occurrence of shared mechanism may simply indicate that, over evolutionary time, certain genes or gene networks are particularly responsive to changes in ecological conditions (Martin & Orgogozo, 2013). Although these genes are repeatedly used to reach certain adaptive behavioural end points, they do not necessarily facilitate or constrain behavioural evolution. Knowledge of these highly responsive genes and gene networks, however, would provide a more complete understanding of the ways ecological conditions act on the genome to shape behavioural phenotypes.

Some studies have begun to assess whether there are shared genes that underlie the repeated evolution of behavioural phenotypes. Candidate gene approaches have shown that certain genes regulate similar types of behaviours across a number of different species and contexts (Fitzpatrick et al., 2005). Allelic variants of the *foraging* gene regulate foraging behaviour in *Drosophila melanogaster*, while the orthologue of this gene in the honeybee, *Apis mellifera*, is differentially expressed in the brain of foraging versus preforaging bees. Similarly, *FoxP2*, a transcription factor, is involved in language and song learning across a variety of vertebrates (Campbell, Reep, Stoll, Ophir, & Phelps, 2009; Haesler et al., 2004). The observation that certain genes are used and reused over evolutionary time to regulate complex but similar behavioural phenotypes suggests there may be 'toolkit' genes that underlie behaviours across species (Toth & Robinson, 2007), analogous to

the genetic toolkit model from developmental biology (Gellon & McGinnis, 1998).

The efficacy of a genetic toolkit for behaviour can be evaluated more generally by using data from genome-wide transcriptomic studies to model the subtler relationships between genes and behavioural phenotypes. It is possible to identify networks or modules of genes that are reliably coexpressed or coregulated in the brain in association with behaviour generally (Chandrasekaran et al., 2011), or with specific types of behaviours (Barron & Robinson, 2008; O'Connell & Hofmann, 2012; Oldham, Horvath, & Geschwind, 2006). For instance, using whole-brain gene expression data in the honeybee, Chandrasekaran et al. (2011) built a brain transcription regulatory network to model and hypothesize hierarchical relationships between genes that encode transcription factors and their putative regulatory targets. This network incorporated information from behavioural phenotypes that were grouped into three major behavioural contexts: foraging, aggression and behavioural maturation. The model results led to a prediction that some transcription factors regulate brain gene expression across the three major behavioural contexts while others regulate gene expression in just one or two behavioural contexts. These global and context-specific transcription factors, or possibly their targets, could represent toolkit genes. For instance, if a gene or module of coexpressed genes is repeatedly associated with aggression across multiple contexts in honeybees, perhaps it also regulates aggression generally across species (Toth et al., 2014). Genomic resources for an ever-increasing number of species now allow such hypotheses to be tested.

The above examples examine shared mechanism at the level of a specific gene or gene network. However, comparative gene expression analyses could identify shared molecular functions or biological processes that are repeatedly associated with a behavioural phenotype, and inferences at this level may also provide evidence for a behavioural toolkit. For example, in the honeybee, transcriptomic analyses with microarrays found that decreased brain oxidative phosphorylation activity was associated with higher aggression levels across multiple aggressive contexts: comparing bees exposed to an aggression-inducing cue to unexposed bees, comparing young, less aggressive adult bees to older, more aggressive adults, and comparing relatively docile European bees to genetically distinct highly aggressive Africanized bees (Alaux, Sinha, et al., 2009). Although these differences in aggression occur across different timescales, they appear to be modulated by a similar biological process. It is important to note that if the unit of comparison is a biological process, there can be cross-species variation in the specific genes driving the correlation between this process and a behaviour.

These results suggest some possible lines of study for the future. If similar proximate mechanisms regulate behaviour across species, perhaps particular mechanisms are also required for certain behaviours to evolve in the first place. A comparative framework (across populations or species) could be used to infer the critical mechanisms that facilitate or constrain the evolution of a behavioural phenotype. This information, with knowledge about similarities and differences in ecological conditions, could inform hypotheses about the selection pressures that shape the phenotype. For instance, highly conserved mechanisms may facilitate the evolution of one phenotype at the expense of other better solutions that are genetically constrained (e.g. parallel evolution; Losos, 2011; Stern, 2013). Parallel evolution most often manifests across closely related species where mechanistic conservation is more likely (Cresko, McGuigan, Phillips, & Postlethwait, 2007; Losos, 1998; Martin & Orgogozo, 2013); however, currently available genomic resources can evaluate the extent to which this phenomenon occurs across phylogenetically distant species.

The phenotypes of significance to behavioural ecologists may make particularly interesting tests of the utility of the genetic toolkit concept. Indeed recent studies suggest that the concept can be applied to complex behavioural phenotypes, for example sociality, which is multifaceted and has several phylogenetic origins (Fischman, Woodard, & Robinson, 2011; Toth & Robinson, 2009; Toth et al., 2007). Behavioural ecologists have uncovered a surprising diversity of complex phenotypes that have arisen repeatedly across taxa, for example, reproductive strategies like male chorusing and female-mimicking male sneaking behaviours, and foraging behaviours like echolocation (Hanlon, Naud, Shaw, & Havenhand, 2005; Hartbauer, Siegert, Fertschai, & Romer, 2012; Jordão, Fonseca, Amorim, & Janik, 2012; Parker et al., 2013; Taborsky, 2008). A genomic approach could begin to address whether shared mechanisms, in addition to ecological factors, are in part responsible for this type of convergence (Arendt & Reznick, 2008).

GENE EXPRESSION PROFILES: ANALYTICAL TOOLS

Genomic analyses have utility beyond identifying the genes that modulate a behavioural trait. A whole-genome gene expression profile of a particular tissue is a powerful general physiological measure, and similar to behaviour itself, it represents the integration of a complex array of factors (both internal and environmental). Gene expression profiles can retain stable signatures of past events in an organism's life (Cole, 2009; Miller et al., 2009; Weaver, Meaney, & Szyf, 2006), and they can also be used to predict behaviours (Chandrasekaran et al., 2011; Whitfield, Cziko, & Robinson, 2003). Thus, although gene expression profiles do not directly discriminate among adaptive hypotheses, we propose they can be used as analytical tools to aid in the study of behaviour and behavioural adaptations. In this section we discuss how gene expression profiles are typically linked to behavioural phenotypes. We then offer three possible contexts in which gene expression profiles may be implemented to address questions in behavioural ecology.

Linking Gene Expression Profiles to Behavioural States

Gene expression profiles are correlated with an organism's 'behavioural state' (i.e. its behavioural phenotype at the time gene expression values are measured; Chandrasekaran et al., 2011). Any shift in behaviour can be described as a change in 'behavioural state'. A life-history theorist may be interested in one behavioural transition, comparing the juvenile growth state (phase) to the reproductive state (Roff, 2007; Roff & Fairbairn, 2007). In contrast, a researcher studying reproductive tactics may care more about behavioural state shifts that occur within the reproductive phase (e.g. shifting from being a nomadic male to a territory holder). Differences in behavioural state can be defined across any timescale: a behavioural response to a brief social cue can be characterized as a shift in state, as can variation in a trait that is fixed within a population but variable across populations. In the latter case the shift in state occurs over evolutionary time. Implicitly, a behavioural state (and the corresponding gene expression profile) arises from the integration of individual status (e.g. condition, genotype, body size, mating status) and environmental factors (e.g. conspecific density, prey availability, predator presence).

Across a number of contexts, studies have successfully connected shifts in behavioural states to gene expression changes in the brain and other tissues. Significant gene expression differences are associated with relatively stable variation in behaviour, for instance comparing across species (Cresko et al., 2007), life-history transitions (Aubin-Horth, Landry, Letcher, & Hofmann, 2005;

Whitfield et al., 2003) and behavioural states associated with morphological changes (Badisco et al., 2011). However, gene expression changes also occur in response to brief stimuli that cause temporary shifts in behaviour (Alaux, Sinha, et al., 2009; Cummings et al., 2008; Sanogo, Band, Blatti, Sinha, & Bell, 2012). Notably, gene expression is sensitive to abiotic and biotic factors that are relevant to behavioural ecologists, such as infection with a parasite or fungus (Rosenblum, Poorten, Settles, & Murdoch, 2012; Zhu, Yang, Zhang, Wu, & Yang, 2013), changes in microenvironment (Unal, Bucklin, Lenz, & Towle, 2013) and temperature stress (Smith, Bernatchez, & Beheregaray, 2013). Thus, there seems to be broad utility in employing gene expression measurements to help describe behavioural phenotypes.

There are a number of possible limitations to coupling shifts in behaviour to gene expression changes. The degree of genome responsiveness to behaviourally relevant stimuli can be variable (Drnevich et al., 2012). Moreover, behaviourally relevant stimuli can induce a molecular response at multiple interacting organizational levels, and the time courses for both behavioural and molecular changes are complex and not always aligned (Gerber et al., 2005; Sandoval et al., 2004; Saunders, Core, & Lis, 2006). The majority of studies that have assessed the relationship between behaviour and the dynamic genome measure gene expression in the brain (e.g. O'Connell & Hofmann, 2011; Zayed & Robinson, 2012), since the brain is the ultimate regulator of behavioural and physiological change. Brain gene expression studies require destructive sampling, which can limit the applicability of genomics to certain types of behavioural studies. However, behaviourally relevant transcriptional changes need not be limited to the brain, and behavioural studies are beginning to incorporate information from other cell types (e.g. blood cells) to provide gene expression measurements without killing the organism (Cole, 2010; Miller et al., 2009). This latter approach has even made genomic approaches relevant to the study of human behavioural states (Cole, 2009). Despite these limitations, there appears to be a lot potential for linking gene expression profiles to behaviour. In the following sections we discuss some ways in which gene expression patterns can be used as analytical tools to aid in the study of the adaptive significance of behaviour.

Using Gene Expression Profiles to Predict Behaviour and Describe Strategies

A number of studies have used brain gene expression profiles to identify subsets of genes whose expression levels can be used to predict behaviour. In some cases, this approach has identified single predictive genes (Cummings et al., 2008; Rittschof & Robinson, 2013; Sanogo et al., 2012), making it possible to use whole-genome expression data to discover 'biomarkers' for a particular behavioural state. While it is possible to use single genes to predict behaviour, more generally a set of genes is required and is more accurate. Class-prediction analysis and related techniques (for review, see Leung & Cavalieri, 2003) measure correlations between a phenotype and expression values for multiple genes simultaneously, and then derive a subset of genes that are the best predictors. This type of approach is used extensively in the medical field (e.g. to describe tumour subtypes and predict individual response to drug interventions; Ooi & Tan, 2003). Moreover it has been successfully used to develop predictive genes for certain types of behaviours (e.g. Whitfield et al., 2003).

Predictive gene sets could be useful for behavioural ecologists trying to measure behavioural tendencies in natural contexts. In many cases, it is possible to measure behaviour in an experimental context in the laboratory, but then difficult to apply this assay to natural field conditions. Measuring behavioural tendencies under

natural conditions requires either monitoring individuals until they encounter a context that elicits the behaviour, or manipulating and thus disturbing the environment in order to assay the behaviour. A gene expression approach, which allows for instantaneous sampling of individuals, preserves the behavioural and thus the transcriptomic state at the time of collection. Gene expression and behavioural associations derived from laboratory analyses can then be used to infer behavioural tendencies without further behavioural assessment. For example, in rodents, it can be difficult to use behavioural assays to determine how environmental factors (e.g. habitat fragmentation or predation risk) influence stress response because the act of collecting individuals is itself stress inducing. However, brain mRNA levels of corticotropin-releasing factor are predictive of an individual's stress response (Meaney, 2001). Thus mRNA levels of this gene, sampled at the time of collection and used as a biomarker, could help to evaluate how ecological and environmental variables influence this phenotype.

Genomic approaches that assess predictive brain gene expression patterns could also help dissect complex behavioural strategies. Organisms are tuned to a wide array of cues and environmental conditions to optimize their behaviour, and they must do so across multiple behavioural dimensions. For example, reproductive tactics account for predation risk, energetic demands, as well as the quality and number of available mates. Moreover, adult behaviours are shaped by experiences that occur during development (Kasumovic, 2013). It can be difficult to characterize behavioural patterns and identify the major sources of individual variation in behaviour. However, gene expression profiles could be used to cluster individuals into putative 'strategies' on the basis of molecular profile. Because variation in brain gene expression and behaviour are often linked, such an approach could reduce a continuum of behavioural complexity into a small number of major categories. Coupled with knowledge of behavioural histories, individual fitness values and ecological conditions, molecular information could help identify key behavioural characters that define strategies, compare the relative success of these strategies and determine the ecological conditions that lead to these behavioural end points. This ability to discretize strategies that may otherwise manifest as continuous variation in behaviour could advance the study of alternative strategies and decision rules.

Using Gene Expression Profiles to Study Plasticity

In behavioural ecology, there is broad interest in understanding how phenotypic plasticity affects the efficiency of selection on a phenotype, and how phenotypic plasticity itself evolves (Aubin-Horth & Renn, 2009; Dingemanse & Wolf, 2013; Fuller, 2003; West-Eberhard, 2003). In this section we discuss possible genomic approaches that could aid in these types of studies.

Throughout this essay so far, we have discussed single measurements of mRNA transcript abundance following a shift in behaviour. However, changes in expression following a behaviourally relevant stimulus could show a dynamic pattern if measured across multiple time points. For labile phenotypes, studying the temporal dynamics of mRNA abundance following phenotypic change (e.g. if and when transcript levels return to baseline) may be a way to make inferences about the plasticity of that phenotype. Furthermore, coupling time course expression analyses with variation in individual genotype or environmental conditions could reveal insights about the causes and consequences of plasticity. For example, in the killifish, *Fundulus heteroclitus*, mRNA expression levels in the gill shift when individuals are exposed to low-salinity environments. The temporal dynamics of the gene expression changes depend on the severity of the salinity change. A modest decrease in salinity causes only transient changes

in mRNA levels, while a more extreme decrease results in sustained changes in mRNA, presumably because more new proteins are required to adjust to the physiological conditions. Furthermore, within a given degree of salinity change, individuals from a population with low tolerance to salinity change are more likely to show a sustained change in mRNA expression levels, which suggests that the population-dependent rate of adjustment to salinity change manifests and can be measured at the molecular level (Whitehead, Roach, Zhang, & Galvez, 2012).

Identifying the genes and molecular functions that respond to the salinity shift in killifish, particularly those that show variation in temporal dynamics, could serve as a first step towards understanding the genetic architecture of plasticity. For example, genes that respond differently to salinity changes across populations (i.e. genes that show sustained changes in the low-tolerance population and transient changes in the high-tolerance population) could represent genes whose expression levels (and transcriptional regulation) facilitate plasticity in the high tolerance population. Identifying the transcriptional control elements that regulate these genes (e.g. transcription factors, cofactors or enhancers) could provide putative targets of selection for plasticity. Moreover, examining the biological processes and molecular functions that show temporal variation in response to perturbation could be a way to assess the trade-offs to plasticity at the cellular or molecular level, which may be important for understanding phenotypic optimization.

Using genomic approaches to identify the types of biological processes that accompany a change in behavioural state could be another way to make inferences about plasticity. Certain types of physiological or morphological changes may limit an organism's ability to move reversibly between states. To give one well-studied case, the cichlid fish *Astatotilapia burtoni* is highly responsive to social rank; males that shift from subordinate to dominant status, and vice versa, undergo conspicuous phenotypic changes, including changes in body coloration, behaviour, neuron volume and testis size. These changes have a range of temporal profiles. Changes in testis and brain morphology are some of the slowest to occur (Burmeister, Jarvis, & Fernald, 2005; Kustan, Maruska, & Fernald, 2012). A brain microarray study found that genes associated with changes in cellular function (presumably rapid and reversible) as well as changes in cell structure (potentially slower and more persistent) accompany the shift in dominance status (Burmeister et al., 2005; Kustan et al., 2012; Renn, Aubin-Horth, & Hofmann, 2008), consistent with behavioural and morphological observations. This relationship suggests that in other species where less is known about the neurobiological basis of a behavioural phenotype, it may be possible to use a genomic signature of certain tissues to generate hypotheses about the relative stability of a shift in behaviour.

A wide array of changes in the brain can accompany a shift in behavioural state. It is intuitive to think that certain types of changes (e.g. changes in cellular metabolism) are more plastic compared to others (e.g. changes in synaptic morphology). However, our inferences are currently limited to our knowledge of the mechanisms that encode experience in the brain, and the extent to which these mechanisms are labile. Moreover, in most cases, the link between mRNA levels and higher levels of organization like protein concentration and cell structural changes is not known. Nevertheless, in some cases suggestive inferences are possible using transcriptomic data.

Using Gene Expression Profiles to Identify Tools to Test Adaptive Hypotheses

Simultaneously measuring all genes that are modulated in correlation with a shift in behavioural state has clear benefits for

researchers interested in discovering candidate genes that are important predictors of behavioural phenotypes. Precise techniques can then be used to knockout or knockdown these candidates and thus manipulate behaviour (Wong & Hofmann, 2010). For a behavioural ecologist, the utility of a candidate gene approach may be limited for reasons discussed above: behaviour is often regulated by multiple genes of small effect, and genetic knockdowns and knock-outs can be difficult to apply to nonmodel systems, although this is changing with improvements in methodology, especially RNA interference (Ament et al., 2011; Nelson, Ihle, Fondrk, Page, & Amdam, 2007; Sifuentes-Romero, Milton, & Garcia-Gasca, 2011).

Perhaps more important from the standpoint of behavioural ecology, lists of genes that are differentially expressed across behavioural states can be grouped into molecular functions and biological processes that provide higher-level information on putative regulators of behaviour (Alaux, Le Conte, et al., 2009; Barron, Brockmann, Sarma, & Robinson, 2012; Gaudet, Livstone, Lewis, & Thomas, 2011; Naeger et al., 2011). Like candidate genes, 'candidate processes' (e.g. hormone or neurotransmitter production and signalling) can be manipulated. In addition, techniques that target candidate processes are often broadly applicable and may not require as much method development as techniques targeting single genes.

A study of novelty-seeking behaviour in honeybees illustrates the utility of using whole-genome expression profiles to identify candidate biological processes amenable to manipulation to test causality. In honeybees, foragers that consistently seek out novel food sources regardless of resource availability are called 'scouts'. Nonscouts, in contrast, forage only on food patches previously identified and communicated by scouts (Liang et al., 2012). A whole-genome microarray analysis reported over 1200 genes differentially expressed in the brain comparing scouts and nonscouts. Bioinformatic functional analysis of these differentially expressed genes implicated several neurotransmitter systems in the propensity to scout, including catecholamine, glutamate and GABA signalling. Authors used this information to conduct pharmacological experiments that established causal connections between these neurotransmitter systems and scouting behaviour, providing a means to manipulate the probability that an individual will behave as a scout (Liang et al., 2012). This study did not explicitly address the adaptive significance of scouting behaviour, but it is possible to imagine that such studies would benefit from the ability to manipulate the level of scouting within a colony. For instance, it would be possible to manipulate the level of scouting in order to evaluate the colony-level fitness consequences of variation in scouting activity across ecological contexts.

The ability to identify candidate biological processes associated with a behavioural trait may also allow researchers to assess adaptive trade-offs associated with different phenotypes. For instance, males of the Atlantic salmon, *Salmo salar*, exist in two morphs that differ in life-history strategy. One morph matures at an early age and small size and adopts a sneaking strategy. The other morph matures later and at a larger size. These two alternative strategies represent a classic life-history trade-off of reproduction for growth. Aubin-Horth et al. (2005) showed that these adaptive trade-offs are reflected at the level of brain gene expression. These authors compared brain gene expression signatures of immature males (which would have presumably continued to grow and reproduce later in life had they not been sacrificed for the analysis) to sneaker males, which matured and began to reproduce at a smaller size. Immature males showed upregulation of genes involved in growth and energy storage relative to sneakers, while the sneakers showed upregulation of genes related to reproduction and mate acquisition, including hormone production and neural plasticity. These data

demonstrate a neurogenomic signature of the life-history trade-off, indicating that certain types of adaptive trade-offs can be measured by assessing differences in brain gene expression.

In the salmon, the researchers used gene expression patterns to provide insight into the evolution of alternative mating strategies. Comparisons of gene expression patterns among immature females, immature males and sneakers in the salmon system suggested, perhaps counterintuitively, that maturing early at a small size is the default developmental pathway in this species (Aubin-Horth et al., 2005). This type of insight, which is not possible if molecular mechanisms are ignored under the phenotypic gambit, is useful to researchers interested in identifying the ecological variables and selection pressures that favour the evolution and maintenance of alternative tactics and of behavioural phenotypes generally (e.g. Linksvayer et al., 2013).

The approach used to study the salmon, that is, using neurogenomic signatures to generate or test adaptive hypotheses, could be applied to systems where less is known about an organism's life history, or where trade-offs are subtler. The study of adaptive trade-offs using genomic approaches may be particularly useful for cryptic adaptations that are not readily observable (Bussiere, Hunt, Jennions, & Brooks, 2006; Huber, 2005; Reinhold, Kurtz, & Engqvist, 2002; Snow & Andrade, 2005). One growing area of research that uses genome-enabled methods to evaluate cryptic adaptive trade-offs is the study of accessory proteins in male seminal fluid. Seminal fluid proteins, which are transferred to females along with sperm during mating, play critical roles in both male–male competition and male–female sexual conflict. In *Drosophila melanogaster*, proteins affect female remating and feeding behaviour, as well as ovulation, oviposition rate and sperm storage (Chapman, 2001; Ravi Ram & Wolfner, 2009; Wigby et al., 2009; Wolfner, 2002). Furthermore, because the composition of seminal fluid can change depending on male health, mating history and the social environment (Wigby et al., 2009), altering ejaculate components is a cryptic reproductive tactic with consequences that may not be apparent without knowledge of the specific seminal proteins involved or their functions. The components of seminal fluid and their functions have been assessed in detail in *D. melanogaster* using traditional genetic and molecular methods (Chapman, 2001; Sirot, Buehner, Fiumera, & Wolfner, 2009; Wolfner, 2002). Using this foundational work as a springboard, other studies have begun to use proteomics to identify the seminal fluid proteins, elucidate their functions, assess male reproductive trade-offs and study seminal protein evolution across species with a range of ecologies (Andres, Maroja, Bogdanowicz, Swanson, & Harrison, 2006; Andres, Maroja, & Harrison, 2008; Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011; Bailey et al., 2013). This type of approach could be applicable to other types of cryptic variation with adaptive consequences (e.g. variation in immunocompetence or physiological plasticity).

CONCLUDING REMARKS

With increased availability of genomic technology, behavioural ecologists can begin to assess mechanisms for natural and complex behaviours that were previously studied solely under the framework of the phenotypic gambit. Implementing genomic methods in the framework of behavioural ecology requires computational and bioinformatics expertise, and preferably a sequenced genome. Genome sequencing is becoming cheaper, and it has been predicted that the genomes of all species of interest in biological research will be sequenced within 10 years (Robinson et al., 2010). Behavioural ecologists can take advantage of the resources that have already developed around genomic approaches. Many resources are now available online, including statistical tools for data analysis and

tools to identify genetic homology, associate genes with functional categories and perform basic gene expression association analyses (Blankenberg et al., 2010; Giardine et al., 2005; Goecks, Nekrutenko, Taylor, & Galaxy, 2010). Other recent reviews discuss some additional tools necessary to achieve the general goals of integrative biology, including technological and community development (Kültz et al., 2013; Losos et al., 2013; Robinson et al., 2010). Behavioural ecologists can benefit from the infrastructure surrounding this rapidly advancing field.

When considering the possible contributions of genomics to behavioural ecology now and in the future, it is useful to highlight the conceptual synergy between the two fields in at least three domains. First, genomics and behavioural ecology both describe and dissect the critical elements of complex phenotypes: genomics reduces the complexity of the genome to predictive gene sets, key modules and functional processes, while behavioural ecology similarly reduces behavioural complexity to the key ecological and phenotypic factors that influence fitness. Second, both disciplines are interested in understanding how the environment modulates a dynamic phenotype, and both use tools to infer outcomes in cases where phenotypes cannot be easily assayed. Genomics assesses behavioural states by describing gene expression profiles, which are products of individual state and experience and can be used to predict behaviours. Similarly, behavioural ecology uses modelling approaches (e.g. dynamic state optimization modelling; Clark & Mangel, 2000) that integrate individual state and ecological variables to hypothesize behavioural strategies. Third, both disciplines try to understand the occurrence of common patterns of behaviour across diverse species, whether these patterns are due to genetic conservation, ecological convergence, or both, and similarly, both advocate an important role for gene-by-environment interactions in behavioural evolution. In light of this conceptual synergy, we believe the prospects of a productive synthesis between behavioural ecology and genomics are very bright.

Acknowledgments

We thank A.M. Bell, two anonymous referees and members of the Robinson laboratory for comments that improved the manuscript. Supported in part by a National Institutes of Health Pioneer Award (1DP10D006416) to G.E.R.

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