Neurogenomics of Behavioural Plasticity in Socioecological Contexts

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Social and ecological challenges often elicit behavioural and physiological responses that are adaptive and subject to selection. The varying behavioural states and traits of animals are a direct output of the nervous system and underlying molecular substrates. Changes in gene expression in response to a variety of contexts such as mate choice, aggression and developmental experience can alter a number of cellular and neural pathways that lead to changes in behaviour. A common framework has emerged to understand the role of the transcriptome in animal behaviour. Behavioural plasticity describes both an individual's ability to modify behavioural states and correlated suites of behaviour in populations, which may constrain variance across contexts. By integrating the study of behavioural plasticity with genome scale, bioinformatics and candidate gene analyses, we are rapidly expanding our understanding of this kind of organismal flexibility, its relationship with the genome and its evolutionary implications.

Introduction

Within- and between-individual behavioural variation in animals is commonly observed in response to ecological and social challenges and opportunities. Behavioural plasticity can be thought of as both an individual's capacity to display varying behavioural responses to both internal and external stimuli and distinct suites of behaviour seen in populations, which may constrain variance across contexts on a broader scale (Bell *et al*., 2009; Dingemanse and Wolf, 2013). Is a behaviour innate, flexible or modifiable by environment? Such questions on the causes, consequences and limitations of behavioural plasticity have fascinated researchers for some time. To better understand variation in behaviour, Tinbergen (1963) argued that both the proximate (e.g. causation and ontogeny) and the ultimate (e.g. function and evolution) mechanisms must be considered, ideally in an integrative manner (Hofmann *et al*., 2014). Due to the diversity of questions addressed by Tinbergen's framework, many fields from molecular genetics and neuroscience, to population and evolutionary ecology, can all contribute to how animals behave in a certain way. **See also**: **Tinbergen, Nikolaas**; **Behavioural Genomics: An Organismic Perspective**

Animal behaviour is immediately controlled through specific neural activity patterns in the brain. These circuits are both inherited and modified throughout life by experience, making the brain an ideal region to investigate the underlying mechanisms of behavioural plasticity. Further, we are discovering that the genome, which has long been considered static, is dynamic and flexible in response to different social and environmental cues (**Figure 1**). Differential gene expression can facilitate a wide range of behavioural variations within and between individuals, which in variable environments may offer functional utility in survival and reproduction. By understanding how genes mediate neural activity patterns in specific brain regions and pathways, we can gain insight into the functional value of a behaviour and how it may have evolved in species or populations. Additionally, similar behaviours have frequently evolved multiple times across taxonomic groups (convergent evolution), which may be parsimoniously explained by the repeated recruitment of common genetic and molecular mechanisms. Thus, comparative studies will be essential in identifying shared mechanisms and give insight into the relationship between genome complexity and behaviour. Modern genomic techniques have allowed researchers to integrate across proximate and ultimate mechanisms through

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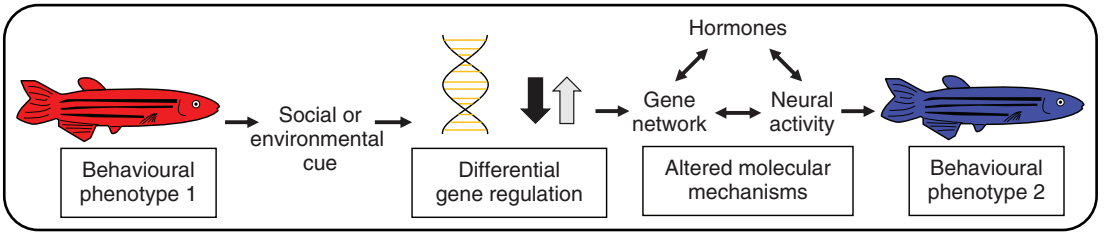

Figure 1 Induction of behavioural plasticity. On the individual level, behavioural plasticity begins with an animal receiving a social or environmental cue. This cue induces a change in the transcriptome, resulting in suites of genes that are up- or downregulated. This change in gene expression can affect hormone levels, neural activity patterns and other transcriptional pathways, ultimately altering long- or short-term behavioural phenotype depending on the mechanism.

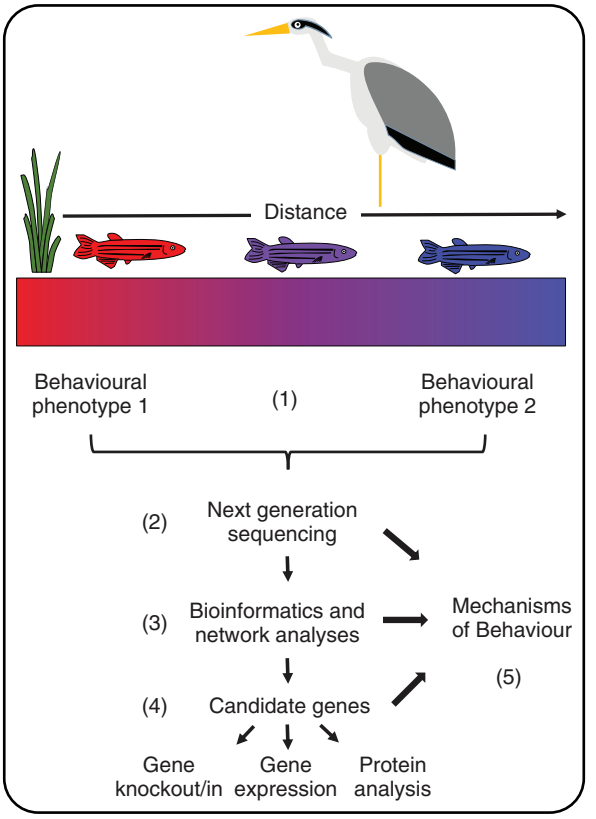

Figure 2 Framework for studying behavioural plasticity and mechanisms of behaviour. (1) Identify divergent phenotypic states (e.g. tendency to take risks in presence of predators). These can be distinct behaviours or represent two extremes on the continuum of a single behaviour. (2) Next-generation sequencing of tissue or cell samples. (3) Bioinformatics and network analyses identify key functional groups of genes, proteins or other cellular pathways. (4) Candidate gene approaches isolate and characterise specific genes' roles in behaviour. (5) Integrate proximate and ultimate mechanisms of behaviour.

large-scale genomic studies in a variety of ecological and evolutionary model systems. In this article, we highlight a framework for studying the role of gene expression in animal behaviour (**Figure 2**). We then review current studies that examine the neurogenomics of behavioural plasticity as it relates to a variety of social and reproductive behaviours that have had success employing this framework. Lastly, we highlight several comparative studies across diverse species and consider the relationship between genome structure and transcription regulatory complexity and the evolution of social behaviours. **See also**: **The Evolution of Animal Personality Variation**; **Animal Personality**

Neurogenomic Framework for Investigating Behavioural Variation

The study of complex behaviour at the genetic and molecular levels has drastically improved in the past decade with the advent of large-scale sequencing technologies. During this time, a common approach has emerged for identifying some causal mechanisms of behaviour with regard to distinct behavioural states or extremes on the continuum of a single behaviour (**Figure 2**). Advances in forward genetics (i.e. phenotype to genotype) have allowed thousands of genes to be simultaneously examined in response to changes in the environment. Microarrays have been the standard method in behavioural genomics and are used as a powerful tool in relating gene expression to certain behaviours such as mating, foraging, aggression and others. However, over the last decade, next-generation sequencing has largely replaced microarrays for comprehensive views of the transcriptome (e.g. RNA (ribonucleic acid) sequencing). This is driven in large part by increased sensitivity, higher throughput capabilities and decreasing costs that are now comparable with microarrays. RNA sequencing provides a powerful discovery aspect, allowing analysis of novel transcripts, splice junctions and noncoding RNAs, for which no prior knowledge is required. This method is also readily used in species that lack genomic resources, vastly expanding the range of phenotypes (e.g. behaviour) that can be analysed at the genomic level. Next-generation sequencing is also used to characterize how genes can be differentially expressed without changes in DNA (deoxyribonucleic acid) sequences (e.g. epigenomics) and which genes are translated (e.g. ribosome profiling). These other genome-scale regulatory processes have only begun to be explored in the field of behavioural genomics (Wang *et al*., 2013; Lenkov *et al*., 2015). **See also**: **Next Generation Sequencing Technologies and Their Applications**; **Ribosome Profiling: Principles and Variations**; **Epigenetic Factors and Chromosome Organization**

One challenge with global gene analyses is the massive amounts of data that they generate. Bioinformatics is a rapidly changing field that develops computational tools to interrogate large data sets from different perspectives. When relating RNA-sequencing data from neural tissue to a behaviour, a general workflow involves aligning the reads to a reference genome (if available), followed by quantifying of the number of reads for each gene or transcript through either commercial or open-source software. After controlling for total number of reads across samples, both univariate and multivariate statistical analyses can be applied to detect differences in the number of reads between groups (e.g. behavioural phenotypes). In the event, no genome is available as a reference, a *de novo* transcriptome can be assembled, annotated and then utilised for differential gene expression analyses (Ockendon *et al*., 2016). Often such differential gene expression analyses result in hundreds, if not thousands, of candidate genes linked to a behaviour of interest. To gain a broad system-level perspectives of what the data may indicate, subsequent analyses can be performed to identify overrepresented categories of gene functions or pathways. **See also**: **Bioinformatics**

Although candidate gene and pathway studies have been successful in characterising important genes related to behaviour, we are beginning to appreciate that complex animal behaviour is affected by many genes simultaneously. With the increased capabilities and frequency of obtaining large data sets, many new computational tools and pipelines are currently being developed to facilitate the analysis of 'big data' from multidimensional and multivariate perspectives. One particularly exciting avenue in the field of neurogenomics has been the application and extension of graph theory to be able to identify emergent and interacting properties of suites of genes. The general premise is that a relationship between genes (or proteins) can be mathematically represented as a graph. The relationship between genes in the resulting gene network is determined through the development of statistical and mathematical methodologies of correlation of expression, or through known interactions from empirical research. Further characterisation of the network is done through analysis of a variety of network properties (e.g. node degree, centrality, path length and modularity). In the context of neurogenomics, gene network analyses can provide some insights that include which suites of genes share similar expression profiles, identity of modules or clusters of genes associated with a phenotypic trait (e.g. behaviour) and identity of influential genes of the network (Chandrasekaran *et al*., 2011; Oliveira *et al*., 2016; Whitney *et al*., 2014; Wong *et al*., 2015). Although a standardised methodology to compare network dynamics rigorously across multiple networks is still being actively developed, such capabilities promise to provide more accurate insights into underlying neuromolecular mechanisms of animal behaviours. An ongoing challenge for bioinformaticians and biologists is to identify which genes, gene networks or molecular pathways can account for a significant proportion of variation in behaviour. **See also**: **Gene Expression Networks**

Detailed characterisation of candidate genes, networks or pathways is a necessary and crucial step to assess direction of causality between neurogenomic and behavioural responses. Although quantifying and localising messenger ribonucleic acid (mRNA) or protein expression through quantitative reverse transcriptase polymerase chain reaction (qPCR) or neurohistochemistry (e.g. *in situ* hybridisation or immunohistochemistry) has increased spatial resolution of gene expression, these results remain largely correlational. In order to move towards causality, a combination of forward and reverse genomic approaches should be considered and used in conjunction (Harris and Hofmann, 2014). Reverse genetic approaches offer powerful analyses of a single gene's role in a behaviour. Traditional methods have relied on gain- or loss-of-function experiments through pharmacological agonists or antagonists. Similarly, 'knock in' and 'knock out' models (e.g. RNA interference and transgenics) allow for direct observation of resulting phenotypes. A recent discovery has allowed researchers extensive efficiency, specificity and control of *in vivo* gene expression through the utilisation of clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system (Doudna and Charpentier, 2014). In principle, researchers can utilise this system to edit the genomes of any organism effectively. This emerging genome engineering technology has the potential to provide insights into how genes can influence a variety of phenotypes (e.g. behaviour, morphology and secondary sexual characteristics) in any animal (Doudna and Charpentier, 2014). Combining these candidate gene approaches with large-scale sequencing techniques allows us to identify and characterise genes of interest involved in complex animal behaviour better. Below, we review some of the progress that has already been made with these techniques in the fields of mate choice, aggression and behavioural plasticity across time and contexts. Each of these fields represents different challenges and opportunities to a diverse range of species, and researchers have already had success utilising the proposed framework discussed above (**Figure 2**). **See also**: **Polymerase Chain Reaction (PCR): Specialised Applications**; ***In Situ* Hybridization**; **Gene Inactivation Strategies: An Update**

Mate Choice

Behavioural and phenotypic variation of secondary sexual characteristics between individuals is a key for the evolution of sexually selected traits. Behavioural variation in courtship can indicate a variety of attributes such as relative fitness or ability to provide and defend resources to mate and offspring. Poeciliid fishes represent a well-studied vertebrate system to understand the proximate and ultimate mechanisms of female mate choice. Specifically in *Xiphophorus nigrensis*, a swordtail fish, and its related species, studies have examined the evolution, adaptive value and causal (e.g. neurogenomic, neural, neuroendocrine and molecular) mechanisms underlying female mate preferences. *X. nigrensis* mate preferences vary between females; however, they generally prefer males that have a large body size, perform courting displays and possess ultraviolet ornamentation (Cummings *et al*., 2003; Wong *et al*., 2011). Utilising variation in female mate preferences, Cummings *et al*. (2008) analysed whole-brain transcriptomic responses in females exposed to different social conditions. They found that not only do many genes rapidly alter expression within 30 min of stimulus exposure but also identified genes that showed differential regulation unique to the

mate choice condition (e.g. candidate genes). These candidate genes have been linked to social and reproductive behaviours and synaptic plasticity. Several studies have subsequently detailed specific neuromolecular mechanisms linked to female mate preference in the poeciliid system. Focusing on two genes linked to synaptic plasticity, neuroserpin (*Ns*) and neuroligin-3 (*Nlg-3*), Wong *et al*. (2012a,b) and Wong and Cummings (2014), identified significant covariation of gene expression and female preference strength in several brain regions. In addition to identifying candidate brain regions linked to variation in mate preference, they also demonstrated, through network analyses, that functional network connectivity of gene expression across the brain differed by social context. Although these studies showed coordinated changes in gene expression for two genes across multiple brain regions depending on social context, Ramsey *et al*. (2012) identified that specific social contexts can be linked to different covariation patterns of suites of genes (11 genes) and behaviour. Supporting the role of plasticity in courting behaviours, Ramsey *et al*. (2014) also found that blocking NMDA (*N*-methyl-D-aspartate) receptors, a key component in learning-induced synaptic plasticity, dramatically reduced female preference behaviours.

To obtain neuromolecular insights into the evolution of female mate preference in poeciliid fishes, studies have examined whether similar molecular mechanisms observed in *X. nigrensis* are also utilised in a closely related species, *Gambusia affinis*. This species displays a coercive mating system where females exhibit relatively weaker preference for larger males compared with *X. nigrensis* (Lynch *et al*., 2012). Interestingly, although there was a positive correlation between *Ns* and *Nlg-3* expression and strength of mate preference in *X. nigrensis*, there was a negative correlation in *G. affinis* females (Lynch *et al*., 2012). In fact, when *G. affinis* females were exposed to a courting or coercive heterospecific *Poecilia latipinna* male, there was a positive and negative correlation between *Ns* and *Nlg-3* expression and female preference, respectively (Wang *et al*., 2014). It is an intriguing possibility that in this system, variation in female mate preferences across species may be utilising a common set of molecular components. Collectively, the initial investigation of neurogenomic mechanisms underlying female mate preference in poeciliid fishes highlights the potential of genomic analyses and subsequent steps for understanding both proximate and ultimate mechanisms of a behaviour.

Within invertebrates, fruit fly (*Drosophila melanogaster*) mating behaviours have similarly been investigated at the genomic levels. Courtship in fruit flies involves a complex exchange of visual, acoustic, olfactory and tactile signals between the sexes (Yamamoto and Koganezawa, 2013). To date, several studies have examined genomic responses of the courtship ritual, including copulation (Lawniczak and Begun, 2004; McGraw *et al*., 2004). Recently, a study has investigated female genomic responses to courtship within just the acoustic sensory modality. Using microarrays, Immonen and Ritchie (2012) identified 41 genes associated with only hearing a courtship song. Since courtship in *Drosophila* is a multimodal process, it is likely that this modest genomic response is a result of being exposed to just the acoustic cues of males. The majority of these genes were involved in antenna olfactory signalling, hormone and neuropeptide signalling and immunity. As a mounting immune response and hormone signalling are characteristic of postmating changes in females (Immonen and Ritchie, 2012), it suggests that during courtship, a proportion of genes is regulated to prepare females for copulation. The authors also reported a genomic response to recognising conspecific male songs. Although specific neurons and genes have been thoroughly studied in the context of male courtship (e.g. *Fru* splice variants, circuitry, Manoli *et al*., 2005), it will be interesting to see whether similar fine-detailed characterisation of candidate genes or reverse genetics approaches can support a female-specific genomic basis for sexual selection.

In some species, the preference and choice of a particular mate can result in the formation of long-term pair bonds between the sexes. Although not much is known about mate choice and precopulatory mate preferences in voles, the neuromolecular mechanisms underlying the formation and maintenance of pair bond have been well studied in the prairie vole, *Microtus ochrogaster*. Formation and maintenance of pair bonds involve oxytocin and arginine vasopressin and their receptors as well as the dopaminergic system (Young and Wang, 2004). To date, genomic resources have been limited to BAC (bacterial artificial chromosome) libraries; however, a genome project is underway (Larry Young, personal communication) and next-generation sequencing will offer new insights into other genes related to formation of pair bonds in voles (McGraw *et al*., 2010). These prospects seem especially promising as a study by Wang *et al*. (2013) showed that increasing histone acetylation at the promoter site for oxytocin and vasopressin receptor genes could induce pair bonding even in the absence of mating. A more recent study utilised RNA sequencing in and near the oxytocin receptor (*Otr*) gene and identified noncoding single nucleotide polymorphisms that contribute to differences in *Otr* expression and social attachment between individuals (King *et al*., 2015). The authors identified a single polymorphism near a cis-regulatory element explaining a remarkable 74% of *Otr* expression variance in the striatum, a prominent forebrain region in the reward system, where *otr* signalling had previously been shown to influence social attachment. Similarly, polymorphisms near the arginine vasopressin 1a receptor (*Avpr1a*) locus predict the epigenetic status and neural expression of *Avpr1a* (Okhovat *et al*., 2015). These studies suggest that variation in regulatory processes may be selected for and promote molecular and behavioural diversity. Although candidate pathway approaches have obviously been successful in characterising single genes in great detail, they often can overlook multitudes of genes with smaller effects on behaviour or effects that manifest elsewhere in the brain and/or under slightly different social conditions. This highlights the need for utilising both large-scale and candidate approaches along with diverse behavioural paradigms to fully discover underlying mechanisms.

Aggression

From establishing dominance in a social hierarchy to defending resources or offspring, antagonistic interactions are another ecological challenges encountered by animals. For example, throughout its life, the cichlid fish, *Astatotilapia burtoni*, can fluctuate between behavioural spectrums of aggression within

an individual. Males can undergo several switches between a dominant and subordinate social status. Dominant males are ornamented, defend territory and reproduce with females. When a dominant male is defeated by another male, behavioural, physiological and molecular changes occur in social descent to submissive status (Hofmann, 2003). In 2008, Renn *et al*. identified a suite of genes that were upregulated specific to the dominant and subordinate phenotypes. Intriguingly, utilising system analysis of the gene expression profiles uncovered modules of genes that may underlie variation in aggressive behaviour in cichlids. Recently, Renn *et al*. (2016) performed a metaanalysis of gene expression data sets and found that females that were experimentally permitted to form similar hierarchies showed similar gene modules and regulation patterns affecting dominance. One proposed mechanism for gene expression changes is DNA methylation, where animals with induced, increased methylation rates were significantly more likely to ascend in rank (Lenkov *et al*., 2015).

One gene differentially regulated during change in social status is arginine vasotocin (*Avt*), which is important in mediating social behaviour in teleost fish (Huffman *et al*., 2015). Huffman *et al*. (2015) measured gene expression levels of the AVT-related *V1a2* receptor in cichlids exposed to AVT or an antagonist. They found that males ascending from subordinate to dominant status showed reduced aggressive and increased courting behaviours in conjunction with increased gene expression of *Avt* and *V1a2* in the brain. In addition to neuropeptides, sex hormones have also been implicated in regulating hierarchical patterns of dominance. Particularly, changes in sex hormone receptors have been found in several socially important brain regions following social opportunity or between statuses (Maruska *et al*., 2013; O'Connell *et al*., 2013). Further, aromatase, an enzyme that converts testosterone into oestradiol, is the product of the *Cyp19* gene, which has been shown to promote social hierarchy-related aggressive behaviours (Huffman *et al*., 2013). In a different cichlid species (*Oreochromis mossambicus*), sex steroids in the urine of males are used to convey chemical information reflecting dominance status and induce transcriptomic changes in receiving individuals (Simoes *et al*., 2015). Depending on receptor density of these sex steroids (i.e. sensitivity), transmission could reinforce differences in dominance status. These studies demonstrate the complex interaction between genes and hormones in the generation of behaviour.

Territorial animals will also exhibit aggression when an intruder enters its territory. The resolution of this aggression can have significant fitness consequences. In response to conspecific territorial intrusion, male sticklebacks (*Gasterosteus aculeatus*) display aggression and have unique transcriptomic responses in several brain regions (Sanogo *et al*., 2012). Interestingly, the authors identified a set of genes that were regulated in opposing directions across different brain regions. These genes were found to have a role in a variety of biological processes including peptide hormone processing, adult feeding and mating and social behaviours. In 2016, Bell *et al*. compared gene profiles between consistently aggressive and nonaggressive male sticklebacks. This study also found uniquely regulated gene expression profiles in several brain regions. Implicated genes (e.g. those involved in glutamatergic transmission, dopaminergic neurons and several G-protein receptors among others) have been linked to aggression in other species. Of particular note, both Bell *et al*. (2016) and Sanogo *et al*. (2012) identified five genes showing the same expression pattern with intruder-elicited aggression, which suggests that they may be key genes involved in this response. Utilising and comparing these genome level analyses not only reveal specific molecular and behavioural processes involved but they also allow for formation of more specific hypotheses for future studies.

Fruit flies also display aggression when competing for resources, territory, access to mates and establishing social hierarchies. Several studies have investigated the genomics behind natural variation in aggression in *Drosophila* (Edwards *et al*., 2009; Shorter *et al*., 2015). Both Edwards *et al*. and Shorter *et al*. used microarray and genome-wide association, respectively, on selectively bred strains of *Drosophila* with high and low aggression levels. These studies identified suites of known and novel candidate genes related to aggressive behaviour. Implicated genes were also involved in metabolism, nervous system development and function, and other behavioural traits. Similar to reproductive behaviours in male *Drosophila*, *Fru*-expressing neurons are involved in aggression. Several studies have shown that expressing *Fru* in female, but not in male, *Drosophila* resulted in distinct aggression behaviours appropriate for the opposite sex (Vrontou *et al*., 2006). More recently, a specific group of *Fru*-expressing neurons containing the neuropeptide tachykinin have been implicated in male-specific aggression (Asahina *et al*., 2014). Overall, these studies demonstrate that in both vertebrates and invertebrates, neuropeptides and other hormones may play a large role in communicating dominance relationships and regulating aggressive behaviours.

Life History Plasticity

Eusocial insects display behavioural plasticity throughout defined periods of their lives in distinct reproductive and provisioning roles. This is clearly seen in several caste systems that determine functional, social and behavioural roles in certain Hymenoptera species. Honeybees (*Apis mellifera*) undergo the first part of life as 'nurses' that care for offspring and hive maintenance. As they age, social and hormonal cues can push development into 'foragers' that collect food for the nest (Robinson *et al*., 2008). Several studies have examined the gene expression of these distinct phenotypic states. Whitfield *et al*. (2003) used microarrays and found 2670 genes that were significantly regulated between 'nurse' and 'forager' bees, 50 of which were predictive of caste role. Remarkably, transition from nurses to foragers has been shown to be a reversible process after altering DNA methylation (Herb *et al*., 2012). In this same model system, researchers have investigated how genetically identical eggs can diverge into worker or queen phenotypes. Mao *et al*. (2015) found that queen-destined larvae had an exclusive diet of a glandular secretion from nurse bees, whereas worker-destined larvae were given three days of the same 'royal jelly' followed by honey and beebread. RNA-seq was performed showing that a phytochemical present in honey and beebread, p-coumaric acid, differentially regulates genes involved in caste determination. Indeed, Vaiserman (2014) showed that larval dietary differences lead to

differential DNA methylation, which may result in caste-biased gene expression and resulting phenotypes. The importance of nutrition in development of eusociality is also seen in another Hymenoptera species, the paper wasp (*Polistes metricus*), which displays a more primitive caste system with distinct provisioning and reproductive roles that can vary throughout their lifetime. Berens *et al*. (2015) used RNA-seq on *P. metricus* castes finding 285 nourishment-responsive transcripts, many of which are involved in lipid metabolism and oxidation-reduction activity. These studies suggest that through mechanisms such as DNA methylation, food availability and nutrition, in general, may play a large role in determining the functional role of an individual in a social hierarchy. **See also**: **Ecology and Social Organisation of Bees**

Although these Hymenoptera species undergo behavioural changes across their lifetime, behavioural plasticity can occur cyclically on a shorter time scale (e.g. annual breeding seasons). Songbirds undergo dramatic seasonal changes in vocal behaviour and the underlying neuroendocrine mechanisms. Male song sparrows (*Melospiza melodia*) maintain territories year-round, even though breeding season lasts only a few months each year. Males can signal either aggressive or reproductive intentions through vocalisations. Mukai *et al*. (2009) explored hypothalamic gene expression associated with territorial aggression behaviours during simulated intrusions in breeding and nonbreeding seasons. Using microarrays, they found a number of genes that were differentially expressed between spring and autumn in the control birds. Interestingly, they found nearly three times as many genes that were differentially regulated during autumn than in the spring mating season. Two of the major brain regions that control vocalisation behaviours in songbirds are the HVC (used as a proper name) and the robust nucleus of the arcopallium (RA). These regions are known to change in volume across seasons through different cellular mechanisms. Thompson *et al*. (2012) used microarrays to examine gene expression differences between these regions across breeding and nonbreeding seasons in Gambel's white-crowned sparrows (*Zonotrichia leucophrys*), a related songbird. The authors found hundreds of genes varied by 1.5-fold expression across seasons, either coexpressed by or specific to the HVC and RA. Gene ontology analyses implicated many functional processes that may be relevant in seasonal song change in the HVC and RA, including neurogenesis, apoptosis, cell growth, dendrite arbourisation and axonal growth, angiogenesis, endocrinology, growth factors and electrophysiology. These studies support the idea that gene expression can be specific to time across different breeding and nonbreeding seasons and that distinct molecular programs contribute to different behaviours. As a genome-wide DNA methylation map has recently been established in songbirds (Steyaert *et al*., 2016), it will be interesting to see the effect of photoperiod on gene methylation, expression and behavioural shifts in songbirds. **See also**: **Neural Control of Birdsong**

Consistent Individual Differences

Behavioural plasticity is commonly studied as flexibility in a particular context, across individuals or over time. Animals, however, can be exposed to multiple contexts within a short period (e.g. foraging, predation, territory defence and mate attraction). Many studies have documented that across different ecological contexts, an individual will display correlated suites of behaviours that are consistent across time and contexts (e.g. behavioural syndromes and animal personality) (Sih *et al*., 2004). Behaviours displayed in one context can predict how the individual will behave in a disparate context. The bold-shy behavioural continuum is an example of different suites of highly correlated behaviours that are observed in many vertebrates (Sih *et al*., 2004). Bold (or proactive) individuals are characterised by displaying more risk-prone behaviours (e.g. high exploration in novel environments, increased aggression and decreased fear responses to predators) and having a lower glucocorticoid stress response. In contrast, individuals on the shy (or reactive) end of the spectrum generally display correlated suites of traits that are opposite to bold individuals (e.g. risk-adverse and high-stress response). Although individuals can be qualitatively categorised into different behavioural types (e.g. bold or shy), consistent individual differences can limit phenotypic (e.g. behaviour and morphology) plasticity, thereby constraining evolution of those traits (Dochtermann and Dingemanse, 2013). Only recently researchers have begun to examine the neurogenomic mechanisms that may explain consistent individual differences.

Zebrafish (*Danio rerio*) are a promising system to investigate the underlying mechanisms of consistent individual differences (e.g. bold-shy). Both wild and laboratory strains of zebrafish display the bold and shy behavioural phenotypes and have distinct genetic architectures that can be heritable (Ariyomo *et al*., 2013; Martins and Bhat, 2014; Oswald *et al*., 2013; Rey *et al*., 2013; Wong *et al*., 2012a,b). Bold zebrafish are typically dominant and have higher reproductive success (Ariyomo and Watt, 2012; Dahlbom *et al*., 2011). Within two recently wild-derived zebrafish lines selectively bred for bold and shy behaviours (Wong *et al*., 2012a,b), the more 'risky' behaviours of the bold line across multiple contexts may be facilitated in part by having a larger caudal region and a quicker fast-start escape response than shy individuals, which suggests that selection for a behavioural trait can constrain morphological evolution (Kern *et al*., 2016). A recent study used RNA sequencing to investigate the whole-brain neurotranscriptomic profiles in wild-derived, selectively bred bold and shy zebrafish lines (Wong *et al*., 2015). The authors identified that 13% of the transcriptome varied between bold and shy lines at baseline. Gene ontology analysis showed that these genes are implicated in neurometabolism and synaptic plasticity, amongst other functions. Gene coexpression network analysis revealed that network connectivity dramatically differed for neurometabolic gene ontology categories between the bold and shy zebrafish. Collectively, these results represent the groundwork to identifying key neuromolecular mechanisms differentiating bold and shy zebrafish and examining potential gene coexpression regulatory mechanisms. In another study, Rey *et al*. (2013) behaviourally screened a laboratory strain of zebrafish to identify bold and shy individuals before examining their baseline whole-brain gene expression profiles via microarrays. Intriguingly, although 9% of genes differed between bold and shy zebrafish, the probability for the number of genes shared between Rey *et al*. (2013) and Wong

et al. (2015) is not significantly different from chance. It is possible that different genetic architectures stemming from different genetic backgrounds (e.g. recently wild-derived vs commercial wild-type) can lead to the same behavioural phenotypes. It will be interesting to see how these genes directly impact behavioural variation via functional manipulation, influence neural activity patterns in zebrafish or influence behaviours in other contexts (e.g. candidate gene analyses, **Figure 2**).

Other studies have examined whether correlated suites of behaviours can be influenced by external cues, such as predation levels. Bell and Sih (2007) and Sin-Yeon (2016) found that correlated profiles only emerge in populations of three-spined stickleback fish under high predation conditions. Interestingly, females that were exposed to predation risk were found to produce offspring with altered genetic profiles, behaviour, metabolism and stress physiology (Mommer and Bell, 2014). Gene ontology analysis implicated pathways involved in metabolism, epigenetic inheritance and neural proliferation and differentiation in promoting these differences. Remarkably, this suggests that divergent phenotypes could derive from a single maternal genetic background, depending on the environmental context. One important factor in establishing divergent stress physiological profiles has been shown to be sensitive to glucocorticoids through differential expression of receptors in the brain (*Gr1* and *Gr2*) (Aubin-Horth *et al*., 2012). Altogether, utilising a systems-to-candidate mechanism approach (**Figure 2**) will likely lead to key insights into the proximate and ultimate mechanisms of consistent individual differences. **See also**: **Predator-induced Plasticity**

Comparative Transcriptomics

Similar behaviour patterns and mating systems have evolved independently multiple times. Certain neurochemical pathways regulating these behaviours have ancient origins, suggesting that as similar novel behavioural traits arose independently (convergently) in diverse lineages, similar (and potentially conserved) neuromolecular mechanisms were recruited repeatedly (O'Connell and Hofmann, 2011a,b; 2012). Recent research has begun to test the hypothesis that some of the gene modules that underlie certain aspects of social behaviour constitute an ancient 'neurogenomic code' that regulated complex behaviour already in the last common ancestor of all major vertebrate lineages and possibly even beyond. In 2014, Pfenning *et al*. investigated the transcriptomes in the brains of humans and song-learning birds. They identified convergent gene expression patterns in specific speech and song production brain regions in humans and songbirds, respectively. This is especially remarkable as the closest common ancestor dates back over 300 million years ago. Similar to learned vocalisations, electric organs have evolved independently several different times to produce electric fields for communication, navigation, predation or defence. Comparing the transcriptomes from electric eels and three other lineages that independently evolved electric organs, Gallant *et al*. (2014) identified overlapping transcription factors and cellular pathways in the evolution of electric organs. Other studies have investigated eusociality across lineages in hymenopteran insects. By comparing paper wasp and honeybee transcriptomes, Toth *et al*. (2010) found significant overlap of genes related to foraging and provisioning behaviours. However, comparing honeybee, paper wasp and fire ant transcriptomes revealed only a few shared genes (Berens *et al*., 2015).They did note significant overlap at the biological and pathway functions, suggesting that convergent molecular functions could be present without an exactly conserved gene set. Across a broader range of taxonomic groups, Rittschof *et al*. (2014) compared stickleback transcriptomic responses to territorial intrusion to the house mouse (*Mus musculus*) and honeybee (*A. mellifera*). The authors identified several homologous transcription factors and similar brain functional processes across these species. Although they compared whole-brain transcriptomes from honeybees to nonhomologous brain regions in the house mouse and stickleback fish, this study provides evidence that diverse species may recruit similar 'genetic toolkits' and functional processes in behaviours that have evolved independently across species. Overall, there is still much to be learned from comparative studies. Not only can these studies aid in elucidating the underlying mechanisms of conserved animal behaviour but they can give a unique insight into its evolution across lineages.

Social Evolution and Genome Complexity

Although determining the genomic mechanisms of social behaviour is important (Hofmann *et al*., 2014), we must also consider how social behaviour influences and is influenced by the structure of the genome and the rate at which it evolves (Rubenstein and Hofmann, 2015). Examining the relationship between genome structure and social behaviour has thus far been investigated in invertebrates. Despite genome size being hypothesised to be constrained by sociality (Koshikawa *et al*., 2008), comparative studies in eusocial insects have not found a clear link between sociality and genome size (Tsutsui *et al*., 2008; Ardila-Garcia *et al*., 2010). However, changes in the regulatory structures of the genome (e.g. amount of transcription factor binding sites, methylation states and transposable elements) are linked to the evolution of eusociality in bees. This suggests that social and genomic regulatory complexity is linked and may explain why more social species in some taxonomic groups have relatively smaller genome sizes (Kapheim *et al*., 2015). Although recombination rates have also been suggested to link genome size and sociality in some way (Kent *et al*., 2012), the relationship between genome structure and sociality remains largely unknown, particularly in vertebrates (Rubenstein and Hofmann, 2015). At this point, we are just starting to uncover how the structure of the genome and its rate of evolution relate to the evolution of social behaviours.

Conclusion

Overall, these studies demonstrate how behavioural plasticity contributes to variation in animal behaviour. On the individual level, behavioural plasticity involves rapid changes in the

transcriptome in response to a number of socioecological challenges. Affiliative behaviours have implicated neural plasticity as essential for mate preference behaviours and have identified both functional and temporal mechanisms that may contribute to evolution through sexual selection. Neural plasticity in relevant brain networks should be an important focus on other behaviours and species in the future. Variation in aggression or dominance highlights the complex role of genes and hormones either through production or sensitivity to neuropeptides and sex steroids. Although phenotypic plasticity can be constrained by consistent individual differences, they may ensure diversity among populations and be adaptive for different combinations of selective pressures. We are at the beginning stages of identifying whether consistent individual differences are mediated by a common set of neuromolecular mechanisms or whether there are independent mechanisms that result in the same suite of traits. Advancements in sequencing techniques are allowing researchers to monitor the flexible nature of the genome and identify networks of candidate genes with increasing resolution and specificity. Combining bioinformatics, systems network analyses and candidate gene approaches across species, we are starting to have the capabilities to extend correlational findings into characterising causal mechanisms underlying variation in behaviour. Through these mechanisms, we may elucidate the functional utility and ultimately the evolution of complex animal behaviours.

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Glossary

Aggression An antagonistic encounter with another individual with regard to defence or acquisition of resources, mates or territory, protection of offspring, defence of predation or establishing dominance.

Behavioural plasticity The ability of an individual to display varying responses to external stimuli, as well as distinct correlated suites of behaviour that occur in populations.

Consistent individual differences Correlated suites of behaviour across time and ecological contexts. These consistent traits can constrain behavioural or phenotypic variation.

Gene regulation A mechanism that allows for cells to increase or decrease the amount of gene product (protein or RNA). It is essential in all fundamental biological processes, such as cellular differentiation, growth, behaviour and behavioural plasticity.

Mate choice An intersexual selection process involving the detection, preference, courtship and copulation with another individual. Choice is influenced by secondary sexual characteristics that indicate relative amounts of direct or indirect benefits and arise from competitive access to mates.

Next-generation sequencing Modern, high-throughput technologies used to characterize the entire genome at the cellular, tissue or organismal levels.

Plasticity The ability to induce change in an organism, tissue or cell in response to external cues. These changes can occur at different levels of biological organisation and time scales (e.g. gene expression, neural activity and behaviour).

Temporal plasticity Changes in behaviour across life history stages (e.g. caste systems) or more cyclical seasonal changes (e.g. annual breeding season).

Transcriptome The complete set of mRNA (messenger ribonucleic acid), rRNA (ribosomal ribonucleic acid), tRNA (transfer ribonucleic acid) and noncoding RNA. In response to different environmental factors, the transcriptome can vary as a result of gene regulation.

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