Behavioural Genomics: An Organismic Perspective

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Article Contents

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The behavioural patterns observed in many organisms generally result from the integration of both external and internal cues. Why do animals behave the way they do? The study of the proximate and ultimate mechanisms underlying animal behaviour tries to answer this question. Although various approaches have been developed for examining – often quantitatively and with increasing specificity and resolution – the roles genes play in the regulation of behaviour, until recently they were limited to individual candidate genes and often neglected ultimate mechanisms. Advances in genomic approaches in recent years have made it possible to examine gene expression patterns (in the brain and elsewhere) on a genomic scale even in nontraditional, yet ecologically and evolutionarily important model systems. As behavioural genomics begins to integrate proximate and ultimate mechanisms of animal behaviour, we may finally understand why animals behave the way they do.

Introduction

Ethology

All animals interact with their environment, including individuals or groups of either the same or different species. These behavioural interactions, whether with the environment or other animals, have fascinated researchers for a long time, and scientific efforts to understand behaviour have developed into a discipline of modern biology: the study of animal behaviour. This field was internationally recognised in 1973 when the Nobel Prize in Physiology or Medicine was awarded to Karl von Frisch, Konrad Lorenz and Nikolaas Tinbergen 'for their discoveries concerning organisation and elicitation of individual and social behaviour patterns' (The Nobel Prize in Physiology or Medicine 1973 Press Release). Many would argue that these three are the most prominent historical figures in the field of behavioural biology. Karl von Frisch (1967) pioneered the research on the communication mechanisms amongst bees about a food source, discovering the honeybee 'dance language'. Konrad Lorenz (1952) conducted many groundbreaking studies examining instinctual and fixed action patterns of behaviours in animals as well as imprinting. Nikolaas Tinbergen (1951) examined the degree of behavioural responses to various stimuli in many animals; some behavioural responses could be elicited more strongly using an exaggerated stimulus (supernormal stimulus) compared to the natural stimulus. However, perhaps the most lasting contribution, which to this day inspires students of animal behaviour, is the framework proposed by Tinbergen (1963) to answer the question: Why do animals behave the way they do? **See also**: Lorenz, Konrad Zacharias; Tinbergen, Nikolaas; Von Frisch, Karl

The mechanisms underlying animal behaviour can be broadly divided into four categories, known as Tinbergen's (1963) four questions: causation, ontogeny, survival value and evolution. The processes underlying causation and ontogeny (development) of behaviour are considered to be proximate mechanisms, whereas the processes underlying survival value (function) and evolution of behaviour are known as ultimate mechanisms. Studying causal mechanisms aims to understand the underlying internal factors (e.g. neural, genetic and hormonal) for a behaviour. Ontogenetic mechanisms of behaviour examine how behaviour develops in relation to genetic and experiential factors. A behaviour is said to have survival value if it has important survival and fitness consequences. Finally, research into the evolutionary mechanisms of behaviour requires an understanding of the origins of the behaviour in question within a comparative and phylogenetic context. Although Tinbergen proposed these four categories as useful guidelines for research, he emphasised that these areas are not mutually exclusive in explaining animal behaviour. To get a complete understanding of an animal's behaviour, we need to understand both proximate and ultimate mechanisms (**Figure 1**). It is this integration across

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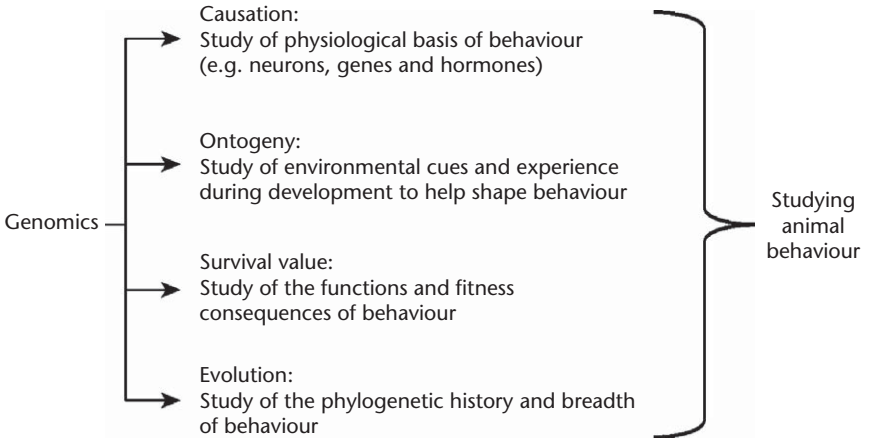

Figure 1 Relationship between genomics and studying animal behaviour. Genomic tools have the potential to address questions in Tinbergen's four levels of analyses (causation, ontogeny, survival value and evolution) of animal behaviour.

levels of biological organisation where modern genome-scale approaches have opened exciting new avenues of research, as they allow us in freely behaving animals to explain the function and evolution of behaviour in molecular terms.

Tools of transcriptomics

Behaviour emerges from specific electrophysiological activity patterns of neurons and neural circuits within the animal. The action of hormones and genes can both influence and be influenced by this electrical activity which in turn may effect behaviour (Robinson *et al*., 2008). In this article, we will focus on the relationship between genes and behaviour. Genes can influence behaviour through presence or absence of allelic gene variants in the genome or through differential gene expression. The majority of studies that examine these dynamic expression patterns as they relate to behaviour quantify messenger ribonucleic acid (mRNA). Northern blot analysis (Alwine *et al*., 1977), RNase protection assay (Gilman, 2001; Melton *et al*., 1984) and reverse transcription polymerase chain reaction (RT-PCR), all measure in a more or less quantitative manner the relative abundance of mRNAs of interest in the brain, specific brain regions or other organs of interest to behaviour. For targeted analyses of candidate genes, quantitative real-time RT-PCR (qPCR) has become the method of choice (Vanguilder *et al*., 2008) because it is relatively sensitive, robust, economical and allows the researcher to simultaneously assess multiple genes in very small samples (for reviews see Bustin, 2000, 2002; Valasek and Repa, 2005; Vanguilder *et al*., 2008). Relative measures of mRNA abundance are commonly obtained by normalising to the expression of so-called housekeeping genes (which often can be misleading; Bustin, 2002) or quantifying the total amount of starting RNA of each sample (Hashimoto *et al*., 2004; Suzuki *et al*., 2000). A variety of studies have used these tools to relate changes in gene expression with behaviours such as foraging, aggression and mate choice (Aubin-Horth *et al*., 2005; Cummings *et al*., 2008; Mukai *et al*., 2009; Toth *et al*., 2007; White *et al*., 2002). However, mRNA levels do not always correspond to the amount of protein present in a cell (Gygi *et al*., 1999), and as a consequence, methods that can estimate protein levels (e.g. immunohistochemistry and mass spectrometry) have become increasingly important (Pandey and Mann, 2000). Finally, recent technological advances in transgenic technology, including virus-based gene delivery, have allowed researchers to 'knockout' or 'knockin' gene and regulatory sequences in animals to assess their roles on various behaviours such as maternal care, social memory and male reproductive behaviours (Brown *et al*., 1996; Demir and Dickson, 2005; Ferguson *et al*., 2000; Pitkow *et al*., 2001). Although these techniques allow for testing the causal connection between genes and behaviour, they currently are most effective in traditional model systems, and in combination with genome-scale analyses can become even more powerful. **See also**: Knockout and Knock-in Animals; Polymerase Chain Reaction (PCR); Quantitative Trait Loci (QTL) Mapping

Technological advances have allowed researchers to simultaneously examine the expression of thousands of genes either through microarrays or, recently, through next-generation mRNA sequencing (RNA-Seq). This genomic-level analysis allows for an unbiased view of genes potentially underlying a behaviour; thousands of genes are simultaneously assessed as opposed to a select few. For microarrays, nucleic acid probes representing genes of interests (often called features or spots) are printed or synthesised on an appropriate substrate (e.g. a glass slide). In dual channel microarray technology, RNA from different samples are then labelled with different fluorescent dyes and allowed to competitively hybridise to the features on the microarray (Duggan *et al*., 1999). Depending on the expression level of a transcript between samples, one can assess the relative expression by examining the intensities of each feature for the two fluorescence channels. Single-channel platforms allow for the hybridisation of only one

sample per array (Lipshutz *et al*., 1999). In either case, after applying some stringent thresholding criteria to account for background noise, one can assess differential regulation of the genes represented on the microarray. A microarray designed for a single species also has the potential to be used for relatively closely related species (Renn *et al*., 2004; Sen Sarma *et al*., 2007). This is necessarily a very short overview of microarray technology, and we cannot discuss the multitudes of experimental designs or analytical methods. The most recent technological development in gene expression profiling utilises next-generation sequencing which promises unprecedented speed, efficiency and cost effectiveness (for review see Metzker, 2010). In the case of RNA-Seq, next-generation sequencing is used to identify and quantify transcripts on a genomic scale (Wang *et al*., 2009). Few studies have utilised RNA-Seq in the context of behavioural genomics, though this is changing rapidly. However, despite the excitement for this new technology, microarrays will likely continue to be a valuable tool for the foreseeable future. Because array technology and RNA-Seq offer a more global and comprehensive view (e.g. thousands of genes) of genome dynamics in relation to behaviour, we focus here on examples utilising these approaches (**Figure 2**). **See also**: Microarray Bioinformatics

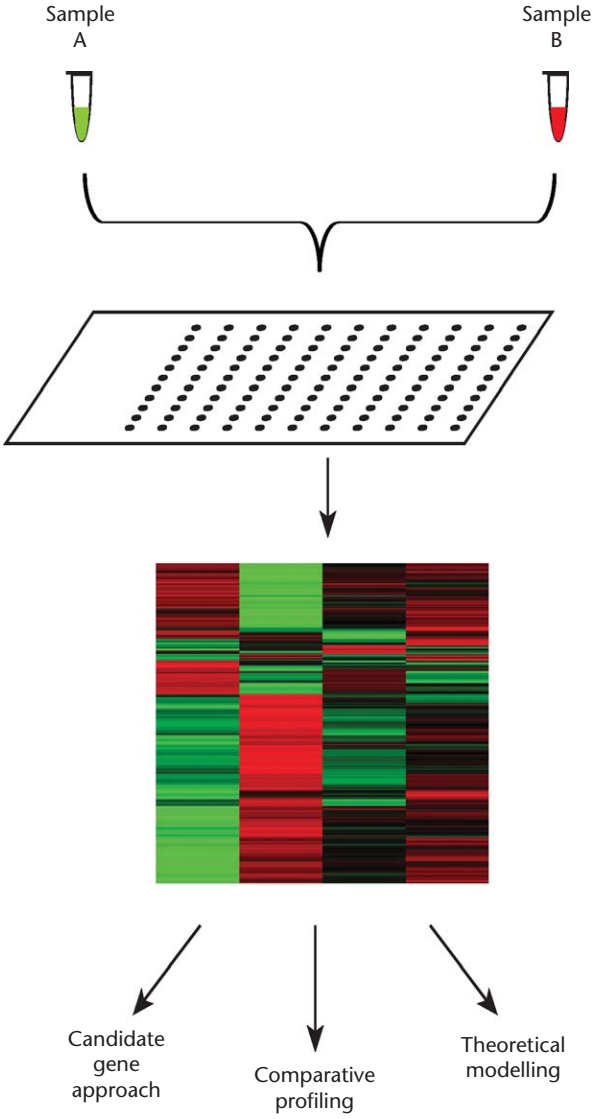

Figure 2 Use of microarrays and the potential of behavioural genomics. Two independent samples that are uniquely fluorescently labelled are competitively hybridised on a microarray. After assessing the relative expression via signal intensities of each spot on the microarray, we can determine which genes are differentially regulated and organise them on a heat map for visual representation. After identifying differentially regulated genes, future studies can further characterise the behaviour within the animal or across species using a candidate gene approach, comparative profiling or theoretical modelling. Heat map reproduced with permission by The Royal Society Publishing from Cummings *et al.* (2008).

Genomic Analysis of Animal Behaviour

A typical animal endures a variety of challenges throughout its life. From birth, the ecological and social environment it was raised in may influence how the animal behaves as it ages (ontogeny). To survive and pass along its genes (survival value), the animal will have to obtain and defend resources and mates. Specific affiliative or aggressive behaviour patterns to ensure survival and reproduction may have been selected for over time and may have diverged in form or function in closely related species (evolution). All of these behaviour patterns can be influenced by various physiological mechanisms (causation, e.g. gene expression, neural activity and hormones). In the following examples, we explore studies that ask a common question: Does this particular behaviour exhibit a unique gene expression profile? In the following text, we discuss these studies in three broadly defined behavioural categories: affiliation, aggression and life history transitions. This classification not only provides a functional context, but also facilitates comparisons across the diverse model systems and experimental paradigms used in behavioural genomics.

Affiliation

The tendency to engage in social behaviour or belong to a social group can be seen in a variety of animals and the motivations for such behaviour range from sexual to survival (e.g. group benefits). To understand the function and evolution of such behaviour patterns pose a fundamental problem in biology. It is thus remarkable that in vertebrates, there are very few published studies that examine the genomic responses in the context of affiliation. Cummings *et al*. (2008) examined female mate choice in a swordtail fish, *Xiphophorus nigrensis*. Swordtails provide a powerful model system for understanding sexual selection by female choice (Basolo, 1990; Ryan and Rosenthal, 2001). Females prefer to mate with males that are large, court and possess ultraviolet ornamentation

(Cummings *et al*., 2003; Ryan and Wagner, 1987). To explore the whole brain genomic response, Cummings *et al*. (2008) exposed females to mate choice conditions or other social controls. Remarkably after only 30 min of social stimulation, the female brain exhibited significant differential expression for approximately 9% of the genes on the microarray (306 of 3422 genes randomly selected from a brain-specific complimentary deoxyribonucleic acid, cDNA, library) with some surprising patterns linked to social context. The expression pattern of 128 of those genes could be used to distinguish between the different treatments (mate choice and controls). This analysis identified 77 genes associated with mate choice conditions. Surprisingly, the majority of these mate choice-associated genes were downregulated compared to other social conditions. Also unexpected was the opposing gene expression patterns in females exposed to mate choice conditions compared with those exposed to other females. For example, genes that were downregulated in females in the mate choice treatment were upregulated in the female social control and vice versa. Given the surprising number of genes downregulated during mate choice conditions but were upregulated during female exposure, perhaps there is a molecular manifestation of the release of a default inhibitory control of gene expression on female mate choice in this species.

Genomic responses have also been examined during sexual behaviour in the fruitfly, *Drosophila melanogaster* (Lawniczak and Begun, 2004). *Drosophila* courtship involves multimodal sensory integration for both sexes for a successful mating (Greenspan and Ferveur, 2000). Using mRNA from whole animals, Lawniczak and Begun (2004) explored which genes were differentially regulated in females that had either successfully or unsuccessfully mated. Out of the 14 000 genes examined, 23 genes showed significant differential expression when courted but not mated and 38 different genes were regulated in mated females. Considering that males in addition to sperm, transfer seminal fluid proteins which alter the female's behaviour and physiology (Ravi Ram and Wolfner, 2007), it is not surprising that many of the genes differentially regulated in mated females were related to immunity, serine proteases and sodium–phosphate symporters. To which extent these molecular changes occur in the brain remains to be seen.

Vocalisations can signal affiliative or aggressive intentions, often depending on social context. Songbirds have become a powerful model system for understanding how complex social vocalisations are learned and memorised (Bolhuis and Gahr, 2006), and several studies have examined the transcriptomic correlations underlying song learning and production in zebra finches, *Taenopygia guttata* (Dong *et al*., 2009; London *et al*., 2009; Lovell *et al*., 2008; Wada *et al*., 2006). Although the transcriptomic responses to a singing territorial intruder have been examined in the brains of male song sparrows, *Melospiza melodia* (Mukai *et al*., 2009, described in the following text), it is surprising that no study has used a genomic approach in the context of females when exposed to a conspecific male (and his song) in an affiliative context, although several studies have described immediate early gene responses in various brain areas (Goodson and Wang, 2006; Woolley and Doupe, 2008). Given the functional and evolutionary insights that could be gleaned from a genomic analysis of responses to mating opportunities – for example, in sexual selection research – we are confident that future work will soon fill this void. **See Also**: Neural Control of Birdsong

Besides affiliation in a sexual situation, we can also view affiliation in the context of maternal care. In *Polistes*, a primitively eusocial wasp species, individuals show provisional behaviour before specialising into a caste. Specifically in *Polistes metricus* when a female 'foundress' starts a new colony, she cares for both the eggs and the resulting offspring (Toth *et al*., 2007). After one generation, female offspring become 'workers' and take over caring for their siblings, while remaining nonreproductive; the foundress becomes a 'queen' and serves just a reproductive role. 'Gynes' are females that do not reproduce or care for siblings in the colony. After mating, 'gynes' will turn into 'foundresses' and leave to establish a colony elsewhere (Hunt, 2007). Taking advantage of the fact that individuals can vary along two major axes (provisioning and reproduction) in this species, Toth *et al*. (2007) examined whether the four castes (foundresses, workers, queens and gynes) exhibited distinct gene expression profiles using RNA-Seq. The experiment focused on 32 genes selected from the RNA-Seq data because of their previous implication in division of labour in the exceptionally eusocial honeybee, *Apis mellifera*, which has a highly specialised caste system. 'Workers' had similar gene expression profiles to the behaviourally similar (e.g. provisioning) 'foundresses' as opposed to the other two castes. These results suggest that specialised nonreproductive castes in advanced eusocial insects (such as honeybees) may have evolved from individuals that once provided maternal care.

Aggression

Antagonistic encounters can occur in a variety of contexts including, but not limited to, establishment of a dominance hierarchy, acquisition and defense of resources and protection of offspring. In the cichlid fish, *Astatotilapia burtoni*, a male may undergo a dynamic change of social status multiple times throughout its life (Hofmann, 2003). Dominant males are ornamented, establish and defend territories and are reproductively active (Hofmann, 2003). If a dominant male is defeated by a competing male, he will quickly descend into the nonreproductive subordinate phenotype and adjust his behaviour, physiology and molecular processes in the brain to indicate submissiveness (Burmeister *et al*., 2005; Hofmann and Fernald, 2000; White *et al*., 2002). A recent study examined the brain genomic profiles of the different social phenotypes, utilising a custom-built microarray platform to examine the activity of 3647 unique cichlid genes in established dominant and

subordinate males as well as females (Renn *et al*., 2008). The authors showed that expression patterns of a priori candidate genes were consistent with expectation from results of previous studies, and uncovered a suite of novel genes associated with dominance and reproduction. Although the brain gene expression profiles were largely different between the sexes, 87 and 84 genes were significantly upregulated in the dominant and subordinate individuals independent of sex, respectively. However, when accounting for both sex and social status, several sets of tightly co-regulated genes (modules) appear: modules that may be involved in reproduction, submissive behaviour, 'super-male' dominance and opposing 'super-male' dominance. These modules may represent a core set of genes important for expressing such behaviour.

Another example of aggressive behaviour related to territory defense can be found in songbirds. Male song sparrows (*M. melodia*) maintain territories year round even though breeding is restricted to a few months in the year. Males signal either aggressive or reproductive intentions through vocalisations. As in some other songbirds, the choice of a song by each individual in antagonistic encounters predicts whether the level of aggression will escalate to an attack or de-escalate until one flees (Searcy and Beecher, 2009). Mukai *et al*. (2009) used simulated intrusions into a male song sparrow's territory to explore the hypothalamic gene expression profiles associated with aggressive behaviour. During and outside the breeding season, territorial males were presented with either a simulated territorial intrusion or control. Of the 17 214 unique sequences on the microarray, 727 showed significant differential regulation across treatments, controls and seasons. There appears to be an effect of season on the genomic response to a territory intrusion as 283 genes showed differential regulation between the seasons. Examining within a season, 67 and 173 genes were differentially regulated between the experimental and control conditions in the spring and autumn, respectively. Interestingly, there are more regulated genes during the nonbreeding season (autumn) compared to the breeding season (spring) which indicates different suites of genes respond according to time of year. Behavioural responses to (territorial) threats are of fundamental importance for understanding the function and evolution of male–male competition. We therefore predict that future work will substantially expand on this study.

Similarly, honeybees can also show heightened aggression in response to disturbances to the hive. Depending on the species, there are varying levels of aggressive behaviour towards potential threats to the hive (Breed *et al*., 2004). A recent study investigated the genomic expression profiles between the highly aggressive Africanized honeybee (*Apis mellifera scutellata*) and the less aggressive European honeybee (*Apis mellifera ligustica*) for 26 800 features on microarray (Alaux *et al*., 2009). They examined relative genomic expression of the two species in several castes of bees (forager, guard and soldier) that were co- and cross-fostered and that were either exposed or not exposed to an alarm pheromone. For bees not exposed to an alarm pheromone, there was an increasing number of differentially regulated genes related to aggression tendency (i.e. soldier bees had the greatest number of regulated genes with 538). Approximately 5–10% of these genes were also differentially regulated in bees that were exposed to an alarm pheromone. Moreover, there were significant positive correlations for each caste's expression profile between bees exposed to an alarm pheromone and under nonexposed conditions. Looking at regulatory elements of upregulated genes for the more aggressive individuals or treatments, Alaux *et al*. (2009) found a common set of motifs. All this suggests that for aggression in honeybees, hundreds of genes may be involved in regulating the behaviour and a subset of those genes are critical regardless of environment or lineage (i.e. a conserved set of aggression-related genes).

Life history transition

The honeybee, *Apis mellifera*, exhibits remarkable socially regulated phenotypic plasticity between behavioural phenotypes, as individual workers express the characteristics of different functional castes as they age (Robinson, 1992). For the first part of their lives, *A. mellifera* are 'nurses' which provide brood care and hive maintenance. Then, depending on social and pheromonal cues, 'nurse' bees transition to 'foragers' which leave the nest to collect food (Leoncini *et al*., 2004; Robinson, 1992). Several studies have examined the neural gene expression profiles that characterise the distinct phenotypes and the transition period within *A. mellifera* and closely related species (Sen Sarma *et al*., 2007; Whitfield *et al*., 2003, 2006). Whitfield *et al*. (2003) used microarrays to examine the expression of $\sim$5500 genes in the brains of bees that differed in both age and behaviour (e.g. 'nurses' versus 'foragers') as well as experimentally produced colonies of bees of the same age that only differed in behaviour. For those that differed in both age and behaviour, 2670 genes were significantly regulated. The majority of differentially regulated genes could be explained by differences in behaviour and not age. When examining individual gene expression profiles, Whitfield *et al*. (2003) identified 50 genes whose expression were predictive of either a 'nurse' or 'forager' bee. Some of these genes have been implicated in behavioural and neural plasticity, and metabolism. These major differences between nurses and foragers are also apparent across species (Sen Sarma *et al*., 2007). Comparing day old bees to foragers in four different species, the expression of 218 genes could differentiate the four species. Sen Sarma *et al*. (2007) found that genes differentially regulated in day old bees of one species were likely to be similarly regulated in other species. This suggests that the genes involved in maturation of bees into foragers are highly conserved across species. Although many genes differed in expression between nurses and foragers (Whitfield *et al*., 2003), these differences in genomic profiles are not due to a sudden shift in expression but rather a gradual process during the maturation stage (Whitfield *et al*., 2006).

Many animal species exhibit another form of phenotypic plasticity in the context of life history transitions, such that males will pursue alternative reproductive tactics (e.g. holding a territory versus sneak mating) in the quest for reproduction. Although in some species alternative reproductive tactics are genetically determined, in many others it is the environmental and social cues that determine which reproductive and/or social phenotype a maturing individual will express. A famous example is the Atlantic salmon (*Salmo salar*). After hatching, salmon spend the first years of their lives in freshwater. All the females and most males then migrate to the sea directly (early migrants) or wait a year prior (late migrants) to entering the sea, where they grow considerably in size before returning to their native stream for reproduction. A subset of males, however, will sexually mature into a small sneaker phenotype in their second year of life and remain in freshwater for their entire lives (Verspoor *et al*., 2007). These male phenotypes (sneaker, early, and late migrants) differ in the genes expressed in the brain (Aubin-Horth *et al*., 2005, 2009). The brains of sneaker males, and 1-year-old (Aubin-Horth *et al*., 2005) and 2-year-old immature males (early and late migrants; Aubin-Horth *et al*., 2009) were collected from the wild to analyse differential gene expression. Of the 2917 genes examined, 432 were differentially expressed between sneaker and immature males. Furthermore, comparing sneaker males and 1-year-old males, there appears to be a molecular correspondence to the trade-offs between reproduction and growth. Genes responsible for feeding, reproduction and neural plasticity were significantly upregulated in sneaker males, whereas those involved in protein synthesis and neurodegeneration were upregulated in 1-year-old males. There also are unique gene expression profiles between early and late migrants and some of those genes were also differentially regulated between individuals representing the two reproductive tactics (Aubin-Horth *et al*., 2009). Interestingly, there is a set of 20 genes that were differentially regulated in a similar fashion in both life history transitions, between sneakers and those that remained immature, on the one hand, and between early and late migrants, on the other (Aubin-Horth *et al*., 2009). Interestingly, the majority of genes in this 'life history transition module' show inverse expression patterns between the transition to early reproduction, on the one hand, and the preparation to migrate, on the other. These results suggest that there might be genes that play a general role in shaping brain function every time an animal undergoes plastic change, whether it is in the context of reproduction or migration. It will be interesting to examine whether such a module (possibly even involving similar genes) also exists in, for example, songbirds with seasonal reproduction and migration.

Common Themes

After reviewing the current state of behavioural genomics across a range of model systems, we can identify several characteristics that appear to be common to genomic responses to social stimuli. It is remarkable, for example, how rapidly the genome – once thought of as rather static – can respond to a social stimulus that signals threat or opportunity. Ranging from as little as 30 min to 2 h after exhibiting a behaviour or stimulus presentation, a considerable proportion of genes are differentially regulated (Cummings *et al*., 2008; Dong *et al*., 2009; Lawniczak and Begun, 2004; Mukai *et al*., 2009). We suggest that this dynamic flexibility of the genome in the face of ongoing changes in the social environment is likely a reflection of continuous and fast paced adjustments in the activity of gene networks in response to – and in preparation for – changes in the activity of both neural circuits and neuroendocrine systems (Hofmann, 2010).

Another unexpected result emerging from several neurogenomic studies is the observation that co-regulated gene sets can exhibit diametrically opposed expression patterns between different – and possibly opposing – behavioural phenotypes (**Figure 3**; Alaux *et al*., 2009; Aubin-Horth *et al*., 2009; Cummings *et al*., 2008). In swordtails, Cummings *et al*. (2008) found that most of the genes downregulated in females exposed to a mate choice condition were upregulated in the brains of females exposed to other females and vice versa. Similarly, in honeybees, genes associated with brain metabolism were downregulated in aggressive individuals compared with more passive ones (Alaux *et al*., 2009). Finally, co-regulated gene set associated with transitions from one life history stage to another in Atlantic salmon was upregulated in sneaker males and downregulated in the premigratory phenotype (Aubin-Horth *et al*., 2009). We suggest that such opposing gene regulatory patterns could be widespread, as suites of genes associated with the behaviour exhibited in one context may inhibit the production of behaviour in another. That is, genes involved in one behaviour may prevent the production of another behaviour not only in different phenotypes but also within the same phenotype, possibly depending on social and environmental context.

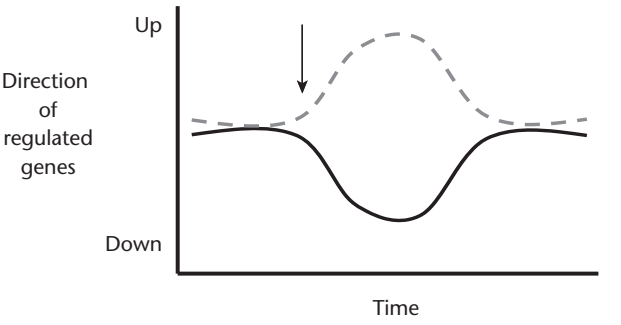

Figure 3 Opposing transcriptomic responses. For a set of genes regulated in one direction for a particular behaviour (or context), the same (or similar) set of genes can sometimes be regulated in the opposite direction for a different behaviour (or behavioural context). Solid line represents the pattern of expression in one condition and dashed line represents the pattern of expression in a different condition (e.g. different behaviour or life history stage). Arrow indicates onset of behaviour or stimulus presentation.

Clearly, the genome is highly plastic, providing the basis for organismic change throughout life in an ever-changing environment. However, given the ever-increasing number of studies in behavioural genomics, we can now begin to ask whether the involvement of some of the genes, or gene sets, in regulating social interactions is in fact conserved across species. All animals show behavioural responses in functional contexts such as territorial threat or mating opportunity which are fundamental to life on earth. It is thus conceivable that conserved suites of genes could underlie behaviour patterns such as aggression or affiliation even across distantly related species, even though the motor patterns and display are species-specific (Toth and Robinson, 2009). It is too early to answer this exciting question, though it is already clear that behavioural genomics provides us with the tools to advance this idea.

Potential of Genomics in Behaviour

Neurogenomic analyses enable us to identify suites of genes associated with a behaviour pattern in an unbiased manner. Further analyses of these genes as well as the products they encode and their distribution using multiple gene expression analysis techniques (e.g. qPCR, *in situ* hybridisation and/or immunohistochemistry) can solidify their association with the behaviour of interest. Once we know which genes may be involved in a behaviour, we can assess their importance for the behaviour through perturbing their function, for instance via transgenic or pharmacological techniques. Transcriptomics also provides insight into the molecular basis of behavioural variation across individuals and species. Specifically, we can ask whether variation in certain genes explains variation in behaviour, whether these same genes vary across species that differ in this behaviour or whether similar gene sets are recruited in diverse taxa in behavioural responses to similar social stimuli (e.g. intruder threat and mating opportunity). Decreasing costs of newer technologies will allow researchers to utilise transcriptomic tools on an ever-increasing number of organisms. We predict that comparative transcriptome profiling using microarrays and next-generation sequencing in nontraditional model organisms (Renn *et al*., 2004; Toth *et al*., 2007) will allow us to integrate for the first time causal and ontogenetic mechanisms with survival value and evolution of a behaviour pattern. The limited studies to date that have examined neurogenomic responses across different species have shown conserved differential expression patterns in behavioural maturation in honeybees (Sen Sarma *et al*., 2007) and identified genes that may underlie different mating systems in cichlids (Machado *et al*., 2009). From a systems level perspective, as genes often influence each other, we can view them as dynamic networks regulating behaviour (London *et al*., 2009; Mukai *et al*., 2009). Once identified, such gene expression networks can then be manipulated and further examined using theoretical models and simulations to determine how changes in gene networks may change behavioural outcomes. These are exciting times, now that behavioural genomics is beginning to take on Tinbergen's charge to integrate proximate and ultimate mechanisms of animal behaviour. We may finally truly understand why animals behave the way they do.

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