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Review

Conservation of gene function in behaviour

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Behaviour genetic research has shown that a given gene or gene pathway can influence categorically similar behaviours in different species. Questions about the conservation of gene function in behaviour are increasingly tractable. This is owing to the surge of DNA and 'omics data, bioinformatic tools, as well as advances in technologies for behavioural phenotyping. Here, we discuss how gene function, as a hierarchical biological phenomenon, can be used to examine behavioural homology across species. The question can be addressed independently using different levels of investigation including the DNA sequence, the gene's position in a genetic pathway, spatial–temporal tissue expression and neural circuitry. Selected examples from the literature are used to illustrate this point. We will also discuss how qualitative and quantitative comparisons of the behavioural phenotype, its function and the importance of environmental and social context should be used in cross-species comparisons. We conclude that (i) there are homologous behaviours, (ii) they are hard to define and (iii) neurogenetics and genomics investigations should help in this endeavour.

Keywords: gene function; behaviour; conservation; homology; genetics; genomics

1. INTRODUCTION

It is well known that gene sequences are conserved even between distantly related species. For example, genes in the nematode *Caenorhabditis elegans* or the fly *Drosophila melanogaster* exhibit sequence similarity to versions of human genes. But does such DNA sequence similarity reflect a functionally conserved role for the genes in question? The answer is yes for developmental genes such as *hox* genes that specify anterior–posterior morphology in organisms from flies to mammals suggesting that *hox* genes had this function in a common ancestor of arthropods and chordates [1]. Here, we ask if evolutionary developmental (evo-devo) approaches can be extended to behavioural phenotypes that exhibit extensive plasticity and are subject to real-time interaction with the environment.

2. HOMOLOGY AND THE CONSERVATION OF BEHAVIOURAL TRAITS

A phenotype is homologous when two (or more) species share a common ancestor that exhibits the phenotype. Distinguishing between evolutionary conservation and convergence is challenging [2,3]. Descriptions of homology have traditionally been the purview of embryologists, anatomists and systematists. More recently, evo-devo biologists have addressed morphological homology and the role that gene function plays in specifying conserved phenotypes across species. Molecular phylogenies have been used as important baseline data for tests of homology and morphology [4]. Identifying homologous behavioural phenotypes is challenging because (i) behaviour exhibits plasticity in response to the environment and (ii) behaviour can show homology at one level of biological organization (e.g. gene pathway), but not at another (e.g. neural circuitry; see below) [5].

Homologous behaviours are hard to define. In developmental biology, researchers consider the modular nature of an organisms' body plan (e.g. a limb or an organ) in order to relate morphological features to phenomena at the cellular level to patterns of gene expression [6]. Similar to this is the concept of endophenotypes, where complex behaviours are constructed from simpler components or modules [7–9]. Endophenotypes and their behavioural components are useful for approaching investigations of homology in behaviour. For example, 'courtship behaviour' in *D. melanogaster* comprises component behaviours, including orientation, tapping, wing extension and song [10]. Examining behavioural components is not only important for tractability and interspecific comparisons of behaviours but can also help identify species-specific modifications in complex behavioural phenotypes that might not have been apparent.

How can behavioural phenotypes be compared in species with drastically different morphology and natural history? We can start with similar categories of behaviour that are shared across species such as feeding, mating, parental care, aggression, learning, memory, circadian rhythms and sleep. Each category can be quantified using descriptions of the behaviours performed (e.g. for aggression: kick, lunge, punch, bite) as well as information about the frequency, duration and sequencing of behaviours. A significant challenge is to develop and standardize informative and unbiased assays of behaviour across species using a comparative approach [11]. Part of this challenge is

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One contribution of 10 to a Theme Issue 'Evolutionary developmental biology (evo-devo) and behaviour'.

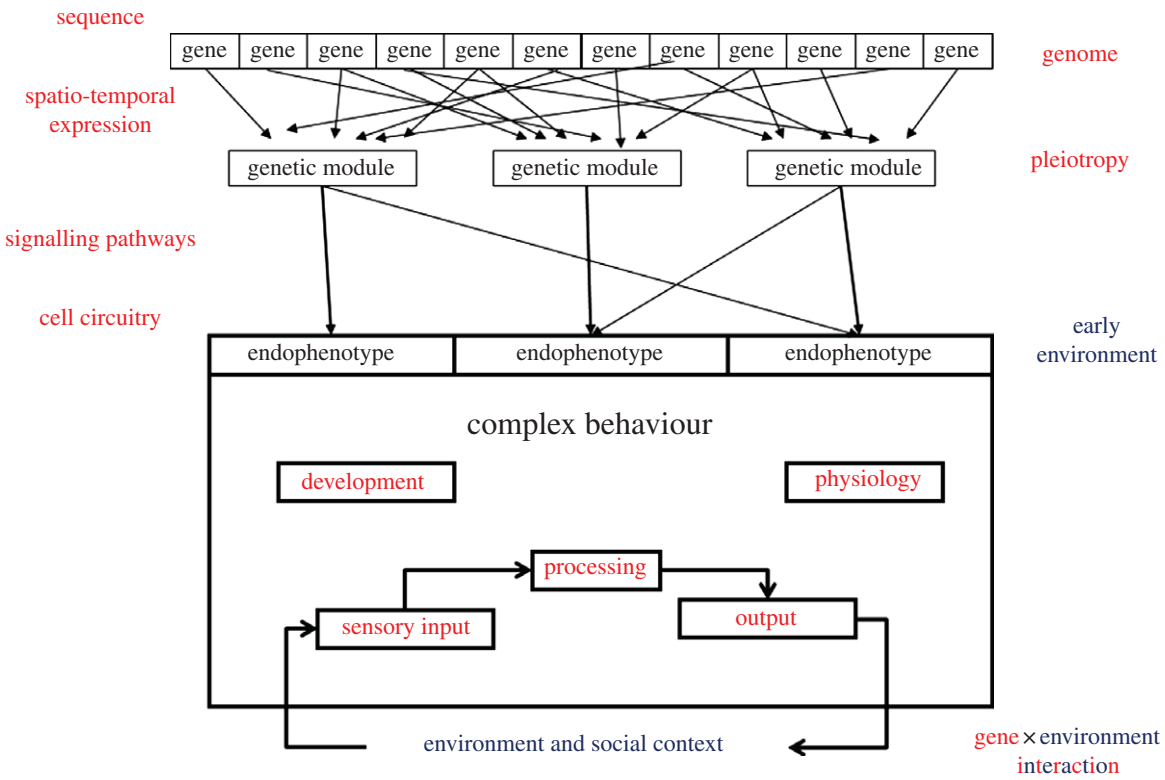

Figure 1. Schematic diagram of the hierarchical nature of gene function in behaviour. Included are a number of factors that can be examined for homology in influencing behaviour through gene function. Terms coloured in red indicate factors that are intrinsic to the organism that are involved in gene function in behaviour while those that are coloured blue are extrinsic to the organism. Epistasis and epigenetic processes are not shown in the figure; however, gene interaction networks and heritable changes in chromosome methylation and histone modification patterns that underlie behaviours also might be conserved across species.

to design paradigms that are relevant to the natural histories of each species but at the same time can be compared functionally across species [12]. This is more straightforward for some categories of behaviour than others (e.g. characteristics of rest/wake cycles compared with courtship behaviour). Considerations of the social context of behaviours should be incorporated into analyses as this information is inseparable from the phenotypes. Finally, the extent of plasticity of the behaviour is also important as it tells us whether and how much the phenotype varies across environments. These latter considerations introduce considerable analytical difficulties when comparing divergent species.

3. CONSERVATION OF GENE FUNCTION IN BEHAVIOUR

Information from neurogenetics and genomics helps determine which behaviours are homologous. Investigations of gene function homology in behaviour can be approached through interspecific comparisons of the various components that affect the behavioural phenotype in question [5,13,14]. The implicated genes, their sequence variation and the relevant signalling pathways and tissues (cells, organs, circuits) are all informative (see case studies below). In this sense, we define 'gene function in behaviour' as a hierarchical phenomenon that includes not only sequence identity and transcriptional events but also the position and role of the gene product in a signalling pathway that acts in defined cells and circuits in the expression of the behavioural phenotype (figure 1). Evo-devo studies have found that homologous morphological phenotypes may result from genetic and developmental mechanisms that are not themselves necessarily homologous [3,5]. This suggests that questions related to gene function homology should focus on a single hierarchical level of gene function (as it relates to behaviour) at a time. This is discussed further below.

We mentioned above the need for comparable and unbiased behavioural tests and well-known phylogenies for the species in question (see [12] for further discussion). Examples of other pertinent investigations include (i) genome or gene sequencing to test for sequence homology, (ii) transcriptional studies to assess gene expression, (iii) functional studies examining post-translational and signalling pathways, (iv) comparative histological studies examining developmental and physiological aspects of the tissues and structures involved in gene function and execution of the behaviour in question, and (v) comparative studies examining gene–environment interactions of the gene(s) and behaviour(s) under investigation. This list is not exhaustive but provides several perspectives from which a gene's function in behaviour may be investigated. We suggest that the question of homology needs to be addressed and considered at each relevant level of gene function (cell, circuit, neural substrate, transcription, translation, signalling pathway, behaviour) independently (see case studies below).

Conservation of a gene's function can occur at the levels of molecular pathways, plasticity or gene–environment interactions, neural circuitry, developmental

functions and through its pleiotropy (figure 1). What is meant here by conservation of a gene's pleiotropic function is when a gene affects the same suite of behaviours in two different species, suggesting shared pleiotropic functions of the gene in these species. Some scientists interested in genes and behaviour use the candidate gene approach to facilitate the identification of genes involved in the behaviours of a variety of species [15]. In this respect, candidate genes are those that are defined in one organism (often in well-defined genetic models like *D. melanogaster* and *Mus musculus*) and then investigated for similar effects in organisms without a genetic tool-box. Mutations in the genes of the former group of organisms are available or can be generated along with transgenic animals that can be used to increase or decrease expression of a gene and to target the expression of that gene in time and space. Numerous behaviours are studied in this way including courtship and mating, circadian rhythms, sleep, learning and memory, aggression, maternal behaviour and food-related behaviours [16,17]. The importance of olfaction, audition, taste, touch and other stimuli to these behaviours is also under investigation. In some cases, the neural substrates important to these behaviours have been identified and manipulated. Many genes that influence these behaviours have been discovered using analyses of genetic mutants. Natural genetic variants in behaviours have also been studied and genes that affect normal individual differences in behaviour have been uncovered (e.g. *foraging* in *Drosophila* [18]; *npr1* in *C. elegans* [19,20]; vasopressin receptor in mice and voles [21]).

An important challenge to identifying homology of gene function in behaviour is that well-resolved phylogenies are lacking for many species, making it difficult to test alternative hypotheses [12]. Below, we examine several well-known and extensively studied examples of gene function that influence behaviour across species. It is difficult to decipher what definition of 'homology' remains once a system-based approach to gene function in behaviour is adopted. The case studies presented below show that there is no singular approach to assess homology, because examinations at different levels of the system can lead to different conclusions. This is not an exhaustive review of all pertinent examples but a selection of useful studies for illustrative purposes.

4. CASE STUDIES IN THE CONSERVATION OF GENE FUNCTION IN BEHAVIOUR

(a) *Gene-by-environment interactions and the serotonin transporter gene*

Behaviour as a phenotype is highly responsive to the environment. This plasticity makes it particularly challenging for studies of homology. Despite the plasticity that emerges from the abiotic and biotic factors experienced by organisms during development and adulthood, it is still possible to find common patterns in their responses to environments. One way to investigate this is to use genetic variation to ask whether different genotypes differ in their sensitivity to the environment. One of the best examples of gene-by-environment interactions that apply across species involves allelic variation in the serotonin transporter gene (*5-HTT*) and its interaction with early experience [22]. *5-HTT* encodes for a protein involved in serotonin re-uptake. Studies of this gene in non-human primates and in human populations have identified a promoter-linked polymorphic region that interacts with early experience to affect behaviours in the young and adults [23]. The long and short alleles result from a 43 bp insertion/deletion in the promoter region of the *5-HTT* gene. In humans, the short allele has approximately three times less *in vitro* basal transcription of 5-HTT mRNA when compared with the long allele [24]. The allelic variants are differently associated with depression and other related behaviours when an individual has a history of adversity early in life [22]. In general, the short allele is thought to confer risk to early adversity while the long allele confers protection (but this is not always the case [25]). Although rats do not have the long–short polymorphism in their *5-HTT* promoter region, polymorphisms in the rat *5-HTT* gene have also been found to interact with early experience to affect similar behaviours to those reported in rhesus monkeys and humans [26].

(b) *Gene pathways and the biological clock*

Genetic analyses of the functions of biological clocks help us to understand the conservation of molecular pathways in behaviour. The molecular pathways involved in circadian phenotypes, such as sleep/wake cycles, have been well-described in diverse organisms. Additionally, as mentioned previously, the behavioural outputs of the clock (e.g. activity, sleep/wake cycles) are relatively easily compared between divergent species (figure 2). First, we provide some background on the biological clock in *Drosophila*, where the genetic underpinnings of the clock were first discovered [28,29].

The *period* (*per*) gene, which affects circadian rhythm in *D. melanogaster*, has been used as a candidate gene to examine *per* homologues involved in other insects [28] and mammals [30]; *per* was the first gene discovered to affect circadian behaviour [31]. Three mutations called long (per^{l}), short (per^{s}) and arrhythmic (per^{0}) alter eclosion rhythms and circadian patterns of locomotor activity. A second clock gene called *timeless* (*tim*) affects circadian rhythmicity and *per* expression [32–34]. Genes *per* and *tim* are transcriptionally regulated in a cyclic manner. Transcripts of both genes are present early in the day but the highest levels are found late in the day and at the beginning of the night [35–37]. PER and TIM proteins accumulate during the night and form a heterodimer that moves into the nucleus to bind to transcription factors Clock (CLK) and Cycle (CYC). This prevents CLK and CYC binding to the *per* and *tim* promoters which results in the transcriptional repression of *per* and *tim*. In early morning hours, TIM and PER degrade and allow for the rise in *tim* and *per* transcripts. This negative effect of PER and TIM on their own transcription creates the negative feedback loop that has been the central theme of clocks in many species [38]. Natural variants in *per* and *tim* are also known [39] and provide fertile ground for exploring gene function homology in behaviour. In fact, detailed comparisons among a number of different insect orders have already commenced [40]. There is general agreement that the function of *per* in biological rhythms is conserved across a broad range of species

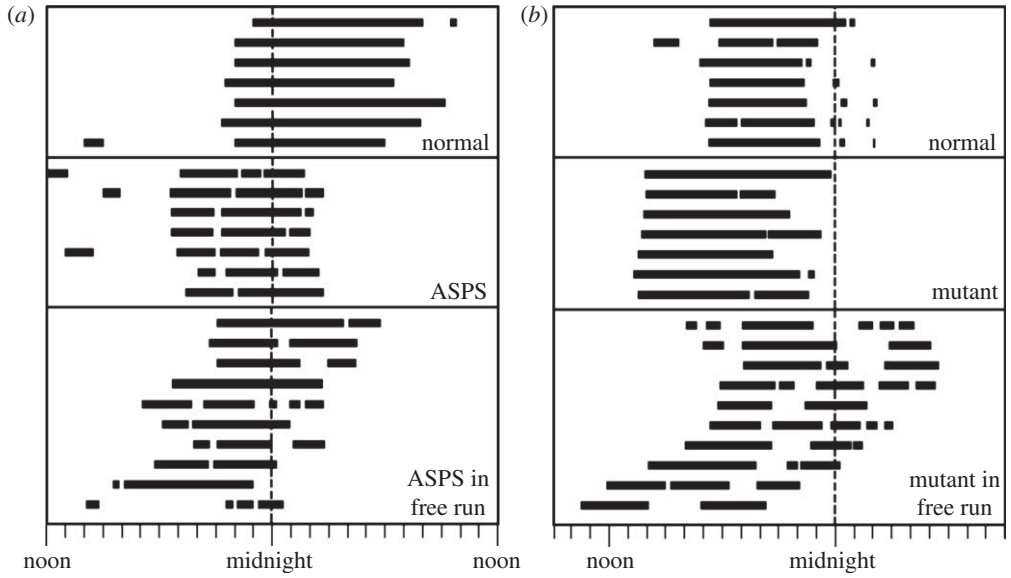

Figure 2. Actograms of behavioural rhythms in (*a*) humans and (*b*) rodents. Activity is measured as an episode of sleep (dark line) in the human and wheel running (dark line) in the rodent. Episodes of behaviour are shown relative to midnight with dark lines. Consecutive days are plotted top to bottom for each organism. The upper panels show behaviour rhythms of individuals maintained on light : dark cycles. The middle panels show a human with a sleep disorder called advanced sleep phase syndrome (ASPS) and a hamster with a mutation in the double-time gene which affects the biological clock. In the bottom panel the individuals are kept in constant darkness without any exposure to temporal cues. In both cases, the human with the sleep disorder and the mouse with a mutation in the period gene *Per2* show drift (from the normal 24 h period) in their rhythms. This drift is indicative of shortened periods. This figure illustrates the similarities in the circadian phenotypes of humans and rodents (figure adapted from [27]).

from insects to humans; however, *tim's* role in the biological clock is not. Gene and genome duplication events have produced four paralogues of *per* genes in mammals known as *mPer1* to *mPer4* [41]. The *mPer1* and *mPer2* genes appear to have a functionally similar role in the signalling pathways of flies and mammals. Circadian genes are thought to function in a number of human disorders [42].

Figure 3 shows the molecular pathways involved in clock function in the fly and the mouse. What appears to be conserved is the structure of the clock molecular mechanism but not necessarily each particular gene or gene product. (See also a discussion of relationship of the cyanobacterial clock to eukaryote clocks [43]). While some genes play the same roles in insects and mammals (e.g. *period)*, others do not (*timeless*), and still others are found in some but not all species. Nevertheless, similar to well-known examples in evo-devo, we can conclude that the structure of the molecular mechanism underlying clocks is similar in both groups. The genes involved are highly conserved at the DNA level, and some genes function in the same way and position in the clock molecular mechanism. Qualitative comparisons of figure 3 suggest that the raw material from the fly clock may have been 'tinkered with', in an evolutionary sense, to 'build' the mammalian clocks. If we were to ask whether there is conservation of a specific gene's function in behaviour, we would conclude that this is true for some genes and not others. For example, one could ask when and why the function of the *timeless* gene has changed over the course of vertebrate evolution. Or how genomic evolution has allowed for the potential conservation of the structure of clock molecular mechanisms while the body plan and organs in which it acts have changed dramatically. In general, it is valuable to focus on divergences and convergences found between species at any level of organization as these cases will be informative from an evolutionary perspective and may suggest novel hypotheses.

To summarize this example, circadian rhythms are found in organisms from bacteria to humans, and these seem to be controlled by a 'clock' mechanism. Interestingly, while there is broad overlap between the 'genes' involved, they are not always the same. The evo-devo approach for circadian behaviour works well, and candidate gene approaches allowed us to make significant headway in understanding the genetic basis of circadian behaviour. However, the precise details of clock function require the study of clock mechanisms in specific organisms.

(c) *Regulation of the* foraging *gene across species*

Even when sequence homology is found, as is required for the candidate gene approach, the details of gene function may differ at any or all hierarchical levels. The candidate gene approach [14] has been successfully used for studies of the *D. melanogaster foraging* (*for*) gene, investigated in *C. elegans*, *Apis mellifera* and the ants *Pogonomyrmex barbatus* and *Pheidole pallidula* (see below). The *for* gene encodes a cGMP-dependent protein kinase (PKG) in *D. melanogaster* and affects a large array of behaviours including food-related behaviours, responses to stress and learning and memory [44]. Foraging behaviour in insects has been a major focus of research on the behavioural effects of PKG. In nature, larval and adult flies behave as rovers or sitters [45]. Well-fed rovers exhibit significantly more locomotion in the presence of food than

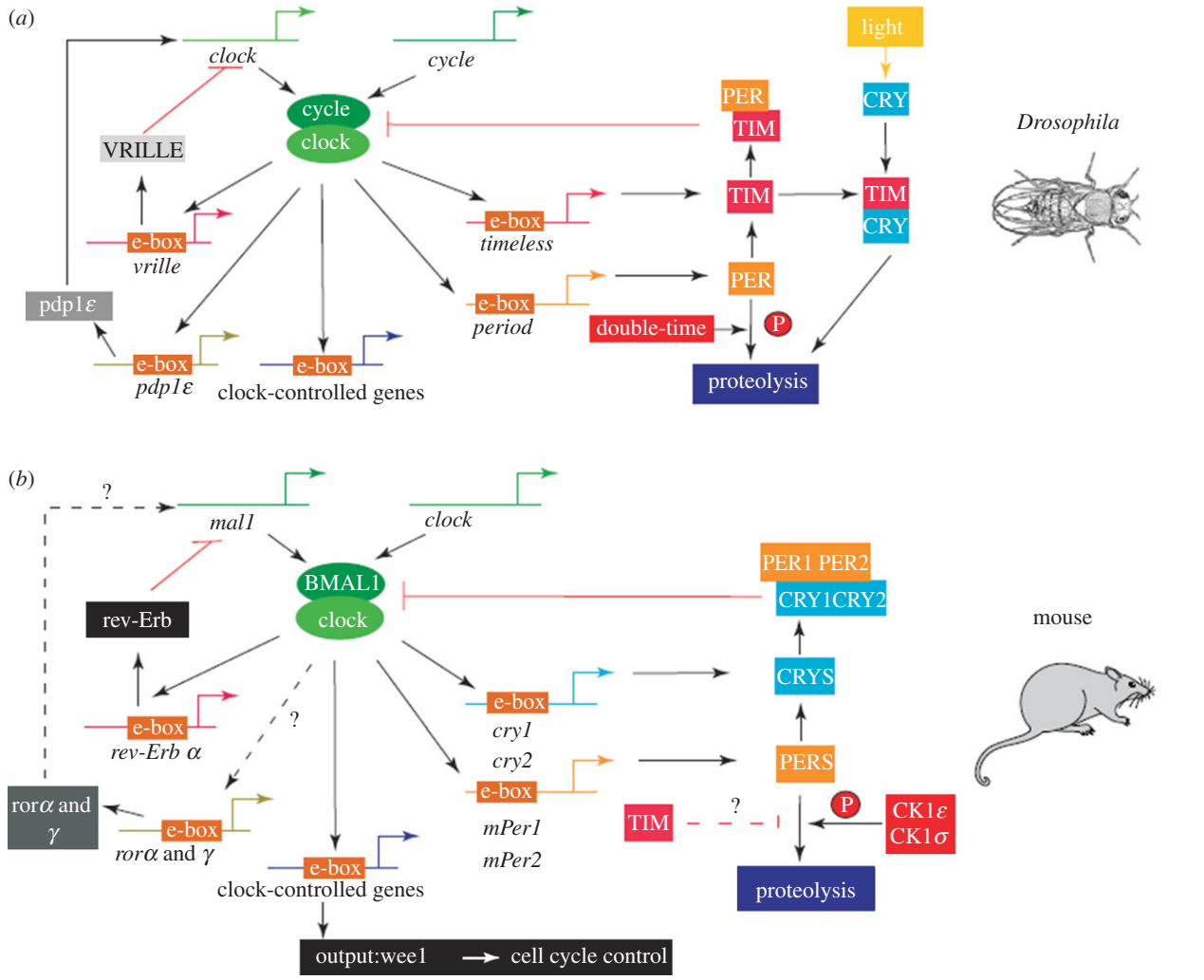

Figure 3. The molecular mechanism of the (*a*) *Drosophila* and (*b*) mammalian clocks. The genes that show sequence homology are shown in the same colour, those that are not homologous are shown in shades of grey. Proteins that form dimerization partners are presented as a single complex (figure adapted from [41]).

sitters. When food is patchy, rovers have a greater tendency to leave a patch in search of new food patches compared with sitters [15]. Food deprived rover larvae behave as sitters and exhibit concomitant reductions in their PKG enzyme activity resembling well-fed sitters [46,47]. In addition, well-fed rover larvae have lower food intake than sitters. Adult rovers and sitters show flexible responses to food deprivation as reflected in behavioural, metabolomic and microarray data [48,49]. As mentioned above, the *for* gene also plays a role in food-related behaviours in *C. elegans*, *D. melanogaster*, *A. mellifera*, *P. barbatus* and *P. pallidula* [50–53]. However, the particular functions of *for* and the signalling pathways involved must differ between species in significant ways. In *D. melanogaster* and *A. mellifera*, high levels of *for* result in rover and forager flies and bees, respectively. In *C. elegans* and *P. pallidula*, high levels of *for* result in dweller (not roamer) worms, and forager (not defender) ants, respectively. Furthermore, *for*'s contribution to the plasticity of behaviour differs between these species. In *D. melanogaster*, both chronic food deprivation and short-term acute deprivation changes *for* expression and behaviour [46,47]. In *A. mellifera* and *P. barbatus*, *for* is involved in long-term plastic changes in behaviour tied to maturation (temporal polyphenism). In *P. pallidula*, *for* is involved in a worker ant's rapid switch from defending the nest to foraging. Finally, only in *P. pallidula* has a difference in the spatial localization of FOR protein been reported. The brains of defender worker ants have five more cells expressing FOR than do the brains of the forager worker ants [53]. Together, these differences suggest that in a very broad sense, *for* is involved in the plasticity of food-related behaviours, but the actual time scale and mechanisms involved in the various species probably differ. It seems clear, however, that *for* modulates many phenotypes. It is not known whether *for* affects the same suite of phenotypes in other species and, if so, whether the multiple functions of a given gene (i.e. its pleiotropy) could be useful for between species comparisons. Further research should shed light on these issues.

The *for* gene example shows that a given gene or set of genes can contribute to phenotypic plasticity in behaviour across a diverse range of species [54]. This suggests, as in the biological clock example above, that the gene–gene interactions may also show conservation. The gene *for* and genes in the insulin signalling pathways are involved in both rover/sitter foraging

[48] and the transition from nurse to forager in *A. mellifera* where the behavioural phenotypes and patterns of gene expression are nutritionally regulated.

(d) *Genetic analyses of learning and memory*

Another field of research, learning and memory, helps shed light on the conservation of gene function in behaviours across divergent species. The many types of learning and memory will not be discussed here nor will their relevance for the species' life histories. There are many paradigms for testing learning and memory, both non-associative and associative, that are informative for interspecific comparisons. One non-associative paradigm is habituation distinguished by decreases in a response to a stimulus following repeated stimulation. Another is sensitization, an increase in response to a neutral stimulus following exposure to a stronger stimulus. Habituation and sensitization are observed in species as diverse as *C. elegans* [55], *D. melanogaster* [56], *Rattus norvegicus* [57] and humans [58]. Associative learning has also been observed in many species [59,60].

Associative learning paradigms can be separated into two categories: (i) operant and (ii) classical (Pavlovian) conditioning [60]. In operant conditioning, an animal acts on its environment to establish associations between unrelated stimuli. The concept can easily be demonstrated by imagining an operant conditioning chamber ('Skinner box'). In one of its most basic forms, the operant conditioning chamber contains a lever that an animal learns to press in order to acquire a food reward. Classical conditioning usually involves the pairing of two stimuli. There is a reflexive stimulus that elicits a response (e.g. food reward, electric shock) and a 'conditioned stimulus' (e.g. an odour). The animal learns to associate the conditioned stimulus with the reflexive stimulus resulting in a conditioned response. These and other forms of non-associative and associative learning can be used to address questions of homology of gene function. However, some forms will be more tractable than others. For example, in operant conditioning, there is less control over the timing, duration and strength of the stimuli when compared with classical conditioning. Another consideration is the divergence of the organisms in question. Simpler learning paradigms such as habituation and sensitization are more suitable for addressing questions about the conservation of gene function.

Similarities in the behavioural properties of classical conditioning are found in a wide array of species from molluscs to insects, fish, rodents and humans. Many of the genetic pathways underlying memory formation are shared in these species [61,62]. For example, the cAMP-dependent signalling pathway has been implicated in memory formation in *Aplysia* [63], honey bees [64], flies [65] and rodents [66] as well as other species. More specifically, CREB (cAMP responsive element binding protein) is thought to be necessary for the formation of protein synthesis-dependent long-term memory in flies and rodents [67–70]. The importance of the cAMP signalling pathway for learning and memory is well established [71]. Some recent investigations in *Drosophila* and mice show that PKG (called cGKI in mammals) also functions in learning and memory. In the mouse, PKG plays a role in fear conditioning in the amygdala [72]. In *Drosophila*, PKG is encoded by *for* with the rover and sitter natural variants described above. *for* is the fly homologue of mammalian cGK1 and is known to affect Pavlovian associative olfactory aversive learning [73,74], appetitive learning [46] and operant visual learning [75].

Many of the genes and signalling pathways associated with memory formation are conserved across a wide range of species. *Drosophila* genes involved in cAMP signalling, ras/MAP kinase signalling, Staufen RNA binding protein, and genes involved in human neurocognitive disorders all play a role in memory formation [61,62]. Furthermore, structural elements of the neural circuitry underlying associative learning are postulated to be homologous in insects and mammals [61]. Imaging analyses have advanced to the point where a common origin between the annelid mushroom bodies and the vertebrate pallium has been suggested [76]. Technological advancements in functional neuroanatomy and real-time imaging improve along with our understanding of the cellular mechanisms underlying learning and memory, and will facilitate further interspecific studies of learning and memory [71].

(e) *Sleep*

Sleep is another good model to examine homology of gene function in behaviour [77–80]. A number of behavioural criteria can be used to compare sleep between species [81]. They were designed to distinguish sleep from other states of quiescence and include: (i) a quiescent period, (ii) a reduction in the response to external stimuli (increased arousal threshold), (iii) increased rest after prolonged waking, and (iv) reversibility. These criteria led to the discovery of sleep states in a number of non-mammalian species including *C. elegans* [82], *Leucophea maderae* [83], *A. mellifera* [84], *D. melanogster* [85,86] and *Danio rerio* [87].

A cAMP–CREB pathway has been implicated in sleep, providing examples of various components of hierarchical gene function homology in flies, worms and mammals [78–80]. In *Drosophila*, mutants with increased cAMP levels have reduced sleep, while mutants with reduced cAMP levels have increased sleep [88]. Additionally, manipulations of CREB activity demonstrated its role in wakefulness. Further studies showed mice lacking either one or two CREB isoforms exhibited reduced wakefulness [89]. In worms, the mutants *pde-4* (reduced-function cyclic nucleotide phosphodiesterase) and *acy-1* (gain-of-function adenelyte cyclase) result in increased cAMP levels, and show increase sensory responsiveness during lethargus [82]. These data suggest that the role of cAMP signalling in sleep behaviour may be homologous at multiple levels in very diverse organisms.

Interestingly, the cGMP pathway, involving PKG, has been implicated in sleep-like behaviour. Raizen *et al.* [82] showed that PKG regulates sleep-like behaviours in flies and worms (*for* and *egl-4*, respectively). The authors compared gain- and loss-of-function

egl-4 mutants and demonstrated that PKG is associated with the extent of behavioural quiescence as well as its time-dependence. They then used rover and sitter fly lines to ask whether the behavioural effect of PKG on sleep is evolutionarily conserved. They found that rovers with higher PKG activity slept more than the sitters. These preliminary data suggest that PKG activity is positively associated with the amount of sleep that an animal displays in both species and appears to be conserved. In mice, a conditional knock-out of mammalian PKG called cGKI results in increased sleep fragmentation, exaggerated delta rebound following deprivation and reduced rapid-eye movement sleep [90]. The cAMP and cGMP signalling pathways are important to sleep in diverse animals.

(f) *Dopamine and reward*

A given gene, or set of genes, may play a role in development and/or functioning of the neural circuitry of a behaviour phenotype. If this neural circuitry shows some conservation between species, then this circuitry can be investigated for conservation of behavioural function. Dopamine signalling regulates a variety of complex behaviours in a wide range of organisms [91]. The dopamine system is of interest because it functions in reward which is intimately linked with many behaviours such as feeding, mothering, sex, learning and addictive behaviours. Dopamine neurons express dopamine pathway genes whose products are involved in dopamine synthesis and transport in most organisms. All dopamine neurons share a small number of genes that code for enzymes and transporters important for the synthesis, packaging and re-uptake of dopamine. How these genes are regulated in diverse species is poorly understood. Flames & Hobert [91] recently found that the function of a dopamine *cis* regulatory motif called DA is conserved (and interchangeable) in *C. elegans* and *M. musculus*. These and other findings will open the door towards understanding the evolution of structures and neural circuits in animal brains [92].

(g) *Neuropeptides and social behaviour*

As discussed, questions about the conservation of behavioural phenotypes across distantly related species are difficult to answer. Donaldson & Young [93] discuss how vasopressin and its receptors play a role in the modulation of social and reproductive behaviours, a broad class of behaviours found in many organisms. However, the actual effects of this neuropeptide on components of these behaviours are highly species-specific. In closely related vole species, species-specific differences in the social bonding result from differences in the expression of the arginine vasopressin V1a receptor (V1aR). Monogamous prairie vole males have a higher number of V1a receptors than polygamous meadow vole males [21]. These voles differ in sequence variation at the 5′ region of this gene. Genetic polymorphisms in this gene have also been associated with variation in sociobehavioral traits in humans, including autism spectrum disorders. However, the evolution of the 5′ region of this vasopressin receptor gene did not directly contribute to variation in social behaviour investigated in 13 species of primates [94]. It is difficult to determine whether the similarities in the behavioural functions of the vasopressin molecule and its receptors across distantly related species are due to homology. A category of behaviour that includes social and reproductive behaviours may be too broad to be used to address questions of homology. This suggests that the level of behavioural analysis (e.g. endophenotypes, behavioural components) that is chosen for investigations of homology can affect the conclusions.

When vasopressin is investigated as a class of molecules, a great deal of conservation of the neural expression of these genes is found [93]. DNA sequence homologues of this neuropeptide found in animals from hydra to vertebrates existed hundreds of millions of years ago. Examination of tissue-specific expression patterns shows that in mammals, vasopressin is found in the hypothalamic brain regions and then in the pituitary where it travels to affect the brain and is also released into the periphery. Interestingly, genetic homologues of vasopressin are found in related neurosecretory structures in the brains of other organisms such as worms and fish. So at the tissue level, this neuropeptide is found in functionally related regions and tissue types in mammals, fish and worms.

One way to ask whether the function of a gene has been conserved is to perform transgenic experiments between species. Transgenic experiments, using another neuropeptide called oxytocin, were performed to ask whether genes expressed in evolutionarily conserved neural tissue exhibit similar functions across species. Indeed transgenic rats carrying a blowfish oxytocin homologue were able to express oxytocin in neurons of the rat hypothalamus; this suggested consistency between the regulatory features of the blowfish and rat genes [95]. Additionally, the fish gene in transgenic rats exhibited normal physiological functions. While the behaviours investigated are highly species-specific, the levels of DNA sequence, tissue-specific expression, regulation and physiological function appear to be conserved in these neuropeptides. This example demonstrates that different levels of investigation can provide different insights into questions of gene function homology across diverse species.

(h) *Epigenetics, genomic responses to early adversity*

A relatively new area of research that can potentially be scrutinized for gene function conservation in behaviour is epigenetics. For instance, specific environmental factors that alter methylation patterns or histone modification of conserved genes that affect behaviour might be homologous across diverse taxa. Meaney, Szyf and colleagues have characterized an epigenetic mechanism which results in 'maternal programming' that has been shown to affect stress responses in rats [96–98]. This response is mediated by glucocorticoid receptors in the hippocampus. Maternal licking and grooming provided by the mothers during the first week of life change the levels of RNA expression of the glucocorticoid receptors. High licking and grooming mothers produce offspring with higher levels of

glucocorticoid receptor mRNA, while low licking and grooming mothers produce offspring with lower levels of this RNA. Individuals that received higher maternal stimulation showed less behavioural and neuroendocrine reactivity to stressful stimuli. These changes in gene activity in response to stress are controlled by patterns of methylation that define an epigenetic response to mothering. The glucocorticoid receptor is affected by patterns of DNA methylation within the promotor region of the gene. In the case of low licking and grooming mothers, the offspring's promoter of the glucocorticoid receptor is methylated resulting in a decrease in the expression of this gene. This does not occur in offspring of high licking and grooming mothers. The patterns of methylation are maintained into later stages of life. There is some evidence that the stress response-related effects of maternal licking and grooming are passed on to female offspring. The female offspring mother their offspring according to how they themselves were reared (with high or low licking and grooming). The transmission of this maternal behaviour across generations is related to methylation of the oestrogen receptor gene passed from mother to daughter [98]. In a related human study, McGowan *et al.* [99] showed that methylation patterns and RNA expression levels of the glucocorticoid receptor in the hippocampus of the brains of suicide victims were altered when the victims had a history of abuse. This suggested that analogous to low licking and grooming in mice, early adversity in humans causes a downregulation of the glucocorticoid receptor gene. Thus, this gene-by-early environment adversity interaction is found in rats and humans.

5. SUMMARY

Early on, the field of behaviour genetics focused on organisms that could be genetically manipulated. However, this limited the breadth of species and behaviours that could be studied using a genetic approach. Advances in DNA sequencing and the 'omics sciences as well as the use of candidate genes has allowed for a broader focus that includes new model species studied from ecological and neurobiological perspectives. Indeed, it has been argued that an understanding of how genes evolve to affect phenotypes should include a comparative approach, and should consider many species and collaborations between evolutionary and molecular biologists [100,101]. While relatively little is known about how evolutionary processes shape intra- and interspecific variation in behavioural phenotypes and the genes that underlie them [7,8], one intriguing theme is that genes which show homology at the level of DNA sequence appear to influence similar categories of behaviours across taxa.

What can functional genomics, systems biology and the plethora of data provided [102] tell us about conservation of a gene's function in behaviour? As the sophistication of genome databases rapidly improve, we will learn more about molecular pathways involved in specific brain functions and how these pathways translate to species-specific differences in behaviour. By making comparisons across genomes, we can better understand how sequence variation, genetic architecture and expression patterns associate with conserved phenotypes. Evo-devo has already used genome-wide linkage mapping and transcriptional profiling for interspecific comparisons. Such genome-wide comparisons can be measured dynamically and in parallel between multiple species of interest. Combined with information on nervous system function, neural circuitry and plasticity, we will be able to compare and contrast molecular pathways and their neural substrates for well-defined behavioural phenotypes across species in different environments. This system-level approach will provide data to address issues of conservation of the molecular and neural pathways that underlie specific behavioural variation. Rapid developments should soon make it possible to link levels of organization on a genome and nervous system-wide scale, making it possible to address issues of conservation across all levels of organization in more quantitative ways.

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