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Review

What can whole genome expression data tell us about the ecology and evolution of personality?

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Consistent individual differences in behaviour, aka personality, pose several evolutionary questions. For example, it is difficult to explain within-individual consistency in behaviour because behavioural plasticity is often advantageous. In addition, selection erodes heritable behavioural variation that is related to fitness, therefore we wish to know the mechanisms that can maintain between-individual variation in behaviour. In this paper, we argue that whole genome expression data can reveal new insights into the proximate mechanisms underlying personality, as well as its evolutionary consequences. After introducing the basics of whole genome expression analysis, we show how whole genome expression data can be used to understand whether behaviours in different contexts are affected by the same molecular mechanisms. We suggest strategies for using the power of genomics to understand what maintains behavioural variation, to study the evolution of behavioural correlations and to compare personality traits across diverse organisms.

Keywords: gene expression; consistency; individual variation; genetics; behavioural syndrome

1. INTRODUCTION

Growing evidence for consistent individual differences in behaviour, or personality, prompts several evolutionary questions. First, it is difficult to explain why individuals should behave consistently through time and across situations. In an ideal world, animals would be infinitely plastic in their behaviour and modify their behaviour in response to changes in the environment. An animal that is consistently bold and aggressive, for example, might reap benefits during competition for resources, but if that individual cannot adjust its behavioural tendency, then it might end up with low fitness when boldness is not favoured, such as when confronted by a predator (see Sih *et al.* 2004). If behavioural consistency within and across contexts reflects a genetic correlation, i.e. a positive genetic correlation between boldness and aggressiveness, then selection favouring one behaviour can produce a correlated response to selection on another behaviour (Falconer & Mackay 1996). Therefore, genetic correlations between traits (including different behavioural traits) might constrain the ability of a population to reach an adaptive peak over short periods of evolutionary time (Lande & Arnold 1983; Schluter 1996).

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Second, between-individual behavioural variation within populations is also a puzzle. Over time, we expect natural selection to favour some behavioural phenotypes over others. Provided there is a heritable basis to the phenotypic variation, we expect to see a reduction in variation over generations. A major problem within all of evolutionary biology is to understand why we observe heritable phenotypic variation within populations, including between-individual variation in behaviour (Wilson 1998).

Finally, consistent individual differences in behaviour have been documented in a wide range of organisms, from molluscs to fish to birds and mammals, including humans. Decades of research into personality in human psychology have organized human personality traits around the Big Five (McCrae & Costa 1997)—extraversion, agreeableness, conscientiousness, openness to experience and neuroticism. Is there an equivalent or comparable system for non-human animals and, if so, how do we study personality traits across diverse species (Gosling 2001)?

Interest in correlations between behaviours and inconsistent individual differences in behaviour is prompting revived interest in the genetic mechanisms underlying behaviour (van Oers *et al.* 2005). This is because although the three issues presented above operate over different timescales—within an organism's lifetime, between generations, over longer evolutionary periods—all of them are either implicitly

Table 1. How whole genome expression data can be used to address evolutionary questions about personality.

evolutionary question	genetic mechanism	whole genome expression approach
why do individuals behave consistently?	pleiotropy; cost of plasticity	overlap between transcripts expressed in different contexts, or between different behavioural types; overall transcriptomic response to different challenges
what maintains heritable variation in personality traits?	additive genetic variation underlying fitness-related personality traits	identify polymorphic genes using whole genome expression data combined with another approach; detect selection on polymorphic genes related to personality traits; measure gene expression in different environments
can we compare personality axes across species?	homologous genes/pathways underlying personality traits	compare gene expression in response to similar challenges in different species

or explicitly concerned with *genetic mechanisms* (table 1). For example, *pleiotropy* (when a single locus affects multiple traits) is the textbook cause of limited plasticity: female fishing spiders might engage in maladaptive precopulatory sexual cannibalism because high levels of voracity towards prey is favoured in juveniles, and this voracity 'spills over' to cause females to eat their mates prior to mating with them (Johnson 2001). In other words, the same genes influence different behaviours that together form a behavioural syndrome. Second, researchers studying the evolution of animal personality are interested in individual behavioural variation that is *heritable* (Dingemanse & Réale 2005). In other words, we wish to know whether there is additive genetic variation underlying variation in a behaviour of interest, and whether the behaviour can respond to selection. Finally, one standard of evidence for the homology of personality traits across species is if the same *genes* influence them.

However, until recently, most studies interested in natural variation in ecologically relevant, fitness-related traits (including personality) have had to treat the genetic and molecular mechanisms underlying their phenotypes of interest as a black box (van Oers *et al.* 2005). Although traditional quantitative genetic approaches have helped us to understand the genetic architecture underlying natural variation in personality traits (Dingemanse *et al.* 2002; Drent *et al.* 2003; Réale & Festa-Bianchet 2003; Bell 2005; Dochtermann & Roff 2010), such approaches necessarily measure phenotypes, rather than the genes that underlie behavioural variation, and therefore have limited use for understanding specific molecular and genetic mechanisms. When studies have tried to relate specific individual genes to personality traits in humans, model laboratory animals or domesticated animals (Champoux *et al.* 2002; Geller *et al.* 2002; Adamec *et al.* 2006; Fidler *et al.* 2007), they have found that specific candidate genes, such as the dopamine receptor 4 (DRD4), the serotonin transporter (SERT) and monoamine oxidase (MAO), only explain a small fraction of the total genetic variation (reviewed in Reif & Lesch 2003), indicating that we are yet to learn the identity of most of the important genes (or that all of the genes are of very small effect). Moreover, epistatic interactions between loci make it difficult to study the effects of single genes one-at-a-time (Mackay 2009).

Another challenge of studying the genetic mechanisms underlying personality traits is that although there is a genetic component to personality traits, it cannot be denied that early experience affects personality traits in both humans (Farrington 2005) and non-human animals (Caldji *et al.* 2000; Meaney 2001) and there is mounting evidence that the environment can influence behaviour in a genotype-specific way (genotype by environment interaction, GxE; Eaves *et al.* 2003; Caspi & Moffitt 2006). Arguably, the ubiquity (and effect size, more than 75% of the phenotypic variation in some cases) of GxE interactions (Cadoret *et al.* 1995; Caspi & Moffitt 2006; Kaufman *et al.* 2006), as well as the possibility of GxE correlations, where certain genotypes are associated with certain environments (Stamps & Groothuis 2010) is an indication that studies will have the biggest impact if they simultaneously consider both genetic and environmental factors.

In this paper, we argue that whole-genome expression data can give us new insights into the evolution of personality. Whole genome expression profiling involves studying variation in expression in a large number of genes, and is an attractive approach for studying personality because behavioural traits are often polygenic, sensitive to the environment and subject to epistatic interactions. Furthermore, a growing number of studies are showing that gene regulation is often correlated with phenotypic variation (Abzhanov *et al.* 2004; Shapiro *et al.* 2004; McGregor *et al.* 2007). Also, the pleiotropy hypothesis for limited plasticity can be tested by examining whether the same molecular mechanisms and pathways are involved in behaviours expressed in different functional contexts (Bell 2007). Whole genome expression profiling provides us with a large-scale perspective on the molecular mechanisms that are associated with behavioural variation. Large-scale transcription surveys are efficient, in that they allow us to study many candidate genes simultaneously. In addition, such surveys provide information about which genes have similar expression patterns and which type of biological processes are repeatedly implicated with behavioural variation. Expression differences can point us in the direction of the genetic differences that may drive

Box 1. Experimental designs to study gene expression and personality.

Measures of gene expression levels are often comparative. For example, two samples that differ in phenotype are compared. A classic example would be to compare a tissue in a disease state with a healthy, control tissue. Another example would be to compare two time points during development, or two tissues of the same individual.

To study the molecular pathways involved in behavioural variation, one approach is to compare gene expression between behaviourally divergent individuals, for example, bold and shy individuals, in a relevant tissue such as the brain or a specific region of the brain. The genes that differ in expression between the two types of individuals are said to be associated with behavioural variation.

The expression of genes in behaviourally divergent groups can also be compared. For example, lines that had been artificially selected for behavioural traits presumably differ in the frequency of alleles related to the behavioural variation (e.g. mouse selected for aggression (van Oortmerssen & Bakker 1981), great tits selected for exploratory behaviour (Drent *et al.* 2003), rainbow trout selected for stress responsiveness (Pottinger & Carrick 1999), trout selected for growth that also differ in behaviour (Biro & Post 2008), and chickens selected for feather pecking (Buitenhuis *et al.* 2009)). Comparing expression differences between such lines could potentially tell us the identity of those genes that differ between them. The same principle also applies to natural populations that differ in personality traits, such as more bold versus less bold populations of sticklebacks, or more aggressive versus less aggressive populations as in the Africanized and European honeybees (Alaux *et al.* 2009).

Rather than comparing behaviourally divergent individuals or groups, another approach for identifying genes associated with behaviour is to compare gene expression in individuals facing different situations. We could compare the gene expression in the brain of animals that were exposed to cues of predators versus those in a control neutral condition, or individuals that were confronted by an intruder and those that were not (Mukai *et al.* 2009). For example, Cummings *et al.* compared the brain genomic response of a female swordtail fish when presented with different social stimuli: another female, a preferred male or a non-preferred male (Cummings *et al.* 2008). If we observe that the same sets of genes or pathways are expressed in response to different stimuli, e.g. a potential mate or a potential threat, this suggests that the same molecular mechanisms could be involved in different behavioural responses, and might be underlying a behavioural syndrome.

expression differences, especially when combined with other approaches (Gibson 2003). Furthermore, whole genome expression profiling has a distinct advantage over other approaches: it can show us how the genome is responsive to the environment. Possible experimental designs for whole genome expression experiments to study personality are in box 1.

After introducing the basics of whole genome expression analysis, in this paper we focus in particular on what whole genome expression data can tell us about the mechanistic basis of limited plasticity. We suspect that whole genome expression data are well suited for revealing some mechanistic constraints on behavioural plasticity. As a result, this paper emphasizes proximate constraints. However, we do not mean to imply that personality variation is always non-adaptive (Wilson 1998; Dall *et al.* 2004; Wolf & Weissing 2010). Along the way, we describe other ways in which whole genome expression data can be used to address other evolutionary questions about personality, including the question of homologous personality traits across species.

2. WHAT IS GENE EXPRESSION?

Gene expression is the transcription of gene sequence into a mature mRNA message that is then usually translated into a protein. All cells have the same genomic sequence information but only particular cells express certain genes of the genome at a given point in time. Therefore, measuring gene expression gives a 'snapshot' approximation of the quantity of proteins being made at a given point in time and in a particular location (box 2).

Gene expression is a phenotype. As such, it is affected by both genetic and environmental factors. That is, differences between two individuals in levels of expression of a particular gene could reflect either inherited, genetically based differences between the two individuals, or it could reflect environmental effects, or their interaction (G × E, see Landry *et al.* 2006, for an example). For example, the serotonin transporter gene is polymorphic, and its expression is also plastic, i.e. sensitive to the environment (Champoux *et al.* 2002). Therefore differences between two individuals in the expression of the serotonin transporter gene could reflect a direct effect of the environment on the gene, or of gene sequence variation (e.g. single nucleotide polymorphisms; SNPs) in the gene's regulatory or control region, or both. Whole genome expression data, combined with other approaches such as linkage mapping or association studies, help us to identify the specific genetic variants that influence variation in gene expression and ultimately in the organismic phenotype such as behaviour (Schadt *et al.* 2003; Emilsson *et al.* 2008; Ayroles *et al.* 2009), reviewed in Gilad *et al.* (2008). The advantage of measuring gene expression over behaviour is that the genes that are associated with variation in behaviour gives us direct information about the molecular mechanisms, networks and pathways involved.

The fact that gene expression is sensitive to the environment makes it an attractive approach for studying the molecular basis underlying personality traits, because the environment also influences personality traits. That is, generally less than 35 per cent of the variation in personality traits is owing to genetic variation (Stirling *et al.* 2002; van Oers *et al.* 2005), and there is an important environmental component to personality traits. The advantage of measuring gene expression, as opposed to strictly concentrating on fixed genetic (sequence) differences between genotypes, is that we can simultaneously study genes that are responsive to the environment and which might

Box 2. Caveats: things to keep in mind when studying gene expression.

The fact that gene expression is a phenotype and represents an instantaneous snapshot of the proteins that are being made in that specific tissue at that particular time means that great care must be taken when selecting the time and location of sampling. Studying the molecular basis of behavioural traits, as opposed to morphological traits, poses unique challenges (Toth & Robinson 2009). For most of us interested in behaviour, we are primarily interested in the brain, but the brain is a heterogeneous organ (Greenwood *et al.* 2008). Studying the whole brain may help to detect genes of importance that were previously unknown without knowing *a priori* which brain regions are important (Aubin-Horth *et al.* 2005, 2007; Renn *et al.* 2008). However, studying specific brain regions may provide better resolution because the same gene might be upregulated in some brain regions, and downregulated in other regions (Overli *et al.* 2005).

In addition, the timing of sampling is critical. Without *a priori* knowledge about the time course of the transcriptomic response, it can be difficult to know when, following an experimental treatment, to sample for gene expression. Because gene expression is dynamic, one might get very different results from sampling individuals 30 min after a stimulus, for example, compared with 1 day after a stimulus (Dong *et al.* 2009). Different genes are likely to be involved in the initiation of the plastic response, the response itself, and the maintenance of it, and all of those could be interesting and relevant to understanding behavioural plasticity (Aubin-Horth & Renn 2009). Preliminary studies measuring the expression of immediate early genes (Mello *et al.* 1992) in brain sections at different points in time following a stimulus could help identify the important regions and time points for further study.

The same applies to the period that is studied during development. Gene expression differences between behavioural types that were measured during the organization of brain differences early in development will differ from gene expression measured in behaviourally divergent adults. Arguably, measuring later periods of development will tell us more about the consequences of behavioural differences, rather than its causes.

Finally, there are a few important things to keep in mind about the fact that we are measuring mRNA. For example, the abundance of mRNA in a sample does not strictly predict the abundance of protein, and many of the differentially expressed genes are likely to be unimportant because of buffering mechanisms or alternative pathways, or are redundant because of gene duplication (Feder & Walser 2005).

be genetically variable among groups. Different behavioural types that are caused by differences in early life experience can still show large molecular differences, for example in the brain, which can be uncovered with gene expression measurements.

3. WHY IS IT ESPECIALLY USEFUL TO STUDY WHOLE GENOME EXPRESSION?

While in the past it was only feasible to measure the expression of a small number of genes at a time, the 'genomic revolution' has introduced high-throughput technologies such as microarrays and transcriptome sequencing that allow researchers to measure the expression of the entire genome simultaneously. These tools are increasingly becoming available for non-traditional model organisms (Ellegren 2008; Mortazavi *et al.* 2008; Nagalakshmi *et al.* 2008; Aubin-Horth & Renn 2009; Zhang *et al.* 2009; Fontanillas *et al.* 2010; Wilhelm *et al.* 2010).

Collecting expression data for a large number of genes simultaneously has many advantages. For one, it is probable that the genetic basis of personality is polygenic, so it makes sense to simultaneously study many genes. In addition, gene products rarely act alone. Instead, they perform their function by interacting together in pathways and networks. As a result, the molecular changes that characterize a phenotype are frequently not based on a single marker or gene, but rather on an entire pathway. Therefore, studying covariation among genes within a pathway might give a more complete picture of the causal systems underlying behavioural variation (box 3). For example, it has been shown that some of the genes that show expression differences in the brain between male salmon exhibiting alternative reproductive behaviours also differ in expression between different migratory types of salmon (Aubin-Horth *et al.* 2009). Some of the differentially expressed genes include candidate genes for behaviour (gonadotropins, prolactin, proopiomelanocortin, somatolactin, somatotropin, rod-opsin). Seventy per cent of these genes are not only co-regulated in the same direction in the comparison of both alternative reproductive behaviours and between different migratory types, but also show the same magnitude of expression differences between types. This gene module seems to be involved in several different behavioural transitions—between sneaking and courting, and between migrating and staying in freshwater for another year. The covariation could be interpreted as the sign that there is a master regulator that is involved in life-history transitions generally, although this remains to be tested.

An unbiased survey of a large number of genes also opens the possibility that genes that had not been previously related to the phenotype of interest can be uncovered (Villeneuve *et al.* 2007). Given that we probably do not know the identity of many of the genes related to personality variation, whole genome expression profiling therefore has the potential to reveal new candidates genes and pathways.

Another advantage of measuring the expression of large numbers of genes simultaneously is that it opens up new possibilities for analysis. For example, one of the first things to do with a list of differentially expressed genes is a gene ontology (GO) analysis, which asks whether genes with specific biological functions are over-represented in our list of differentially expressed genes compared with the total list of genes studied (Ashburner *et al.* 2000). One of the advantages of GO analysis is that it allows us to compare results across species without the need to study exactly the same homologues in each species (Roelofs *et al.* 2008).

Box 3. What happens next?

The result of a whole genome expression analysis is a list of genes that are differentially expressed between different samples. Once we have such a list in hand, what happens next?

After higher-order analyses of the gene list such as GO analyses (see text), an obvious next step is to determine whether differences in gene expression are the cause or consequence of the behavioural difference, or if both traits are modulated by a third variable. For example, a male cichlid that rises in social dominance and becomes territorial, which will give him the opportunity to court females and to breed, shows changes in gene expression in his brain in less than an hour after the change in dominance behaviour (Burmeister *et al.* 2005). In such a case, behaviour is driving gene expression rather than vice versa.

Disentangling cause and effect often means going to a single gene approach. Directly manipulating the expression level of a gene and observing a behavioural change implicates that gene directly in the behaviour and shows that the observed gene expression difference was the cause of the behavioural variation. One strategy is to manipulate the gene product or other components of the interaction network, such as a receptor and components upstream of the gene, using pharmaceutical drugs, RNAi and (if studying a model organism) knock outs, knock ins and gene silencing. The observed concomitant changes in traits of interest can be used to link functionally the gene to the phenotype in a causal rather than a correlative manner.

For example, 5HT (serotonin) transporter deficiency is known to be associated with anxiety behaviour in mammals. Pharmacologically blocking its action during mouse development revealed how 5HT transporter deficiency organizes response behaviours. Moreover, the manipulation recapitulated the effect of genetic defects that affect expression of this transporter (Ansorge *et al.* 2004).

Once candidate genes or modules have been identified, a great deal of time can be spent at the bench and at the computer. One can look for common motifs upstream of the candidate genes to look for transcription factor binding sites (Alaux *et al.* 2009). If common motifs and their transcription factors are identified, those transcription factors are interesting candidate master switches that might regulate a suite of gene expression changes.

It is important to recognize that expression data alone will not reveal the loci responsible for variation in quantitative traits without being employed in tandem with other approaches such as linkage mapping, association studies or functional genetics. However, a list of candidate genes from a whole genome expression study can help point us in the right direction to identify genetic variants. By sequencing coding regions of the candidate genes and regions upstream of each gene, one can begin to look for differences in the DNA sequence between behaviourally divergent individuals. If polymorphisms (differences in the sequence of different behavioural types) are found, one could ask whether those polymorphisms reflect coding rather than silent mutations, where the polymorphism occurs within the gene (in the coding region, or promoter, or other regulatory regions), and if a signature of selection on the gene can be detected. If allelic differences are not found, that would suggest that the differential expression is either owing to genetic variation upstream, or to an environmental effect.

A common criticism of whole genome expression profiling is that it is a 'fishing expedition'. However, statistical analyses of whole genome expression data are getting increasingly sophisticated, allowing more targeted, hypothesis-driven approaches. In many cases, individual genes that are part of an important pathway that is related to the phenotype are not statistically differentially expressed. The statistical probability that several components of the pathway change in expression owing to chance alone can be estimated, allowing researchers to detect significant changes at the pathway level (for examples in model systems, see Grosu *et al.* (2002) and Draghici *et al.* (2007)). Furthermore, hypotheses can be made *a priori* for specific pathways based on knowledge of that pathway's function (Villeneuve *et al.* 2007).

Microarray platforms are not available for all species, but the situation is rapidly improving. New technologies such as RNA-seq can be exploited to develop expressed sequence tags (ESTs), which can be used to construct a microarray (Vera *et al.* 2008), or can be used to directly estimate transcript abundance and sequence variation. The cost of such technologies is decreasing rapidly, making it within the budget of researchers who do not study traditional model genetic organisms, e.g. *Drosophila*, mouse, *Caenorhabditis elegans*. Furthermore, if a microarray platform is available for a closely related species, heterologous DNA hybridization can be used. With this method, DNA sequence similarity allows a researcher to hybridize mRNA from one species to a microarray built using genomic sequence from another species. For further information, see Renn *et al.* (2004) and Buckley (2007).

4. WHAT CAN WHOLE GENOME EXPRESSION DATA TELL US ABOUT LIMITED PLASTICITY?

Animals are renowned for their behavioural flexibility—within their lifetime, an individual bird, for example, goes from hunting for prey, to finding mates, to caring for young to avoiding predators, and can be confronted simultaneously with conflicting demands, such as foraging while still remaining vigilant to detect predators (Krebs & Davies 1997). However, when individuals behave consistently across contexts, this opens the possibility that individuals do not optimally change their behaviour as much as they ideally should (Sih *et al.* 2004). In other words, limited plasticity can result in maladaptive behaviour. Note that limited plasticity (within individual consistency) does not imply that individuals do not change their behaviour, or that they are not plastic; instead, the rank order differences between individuals is maintained across contexts (a behavioural syndrome). In that case, it is possible that no single individual behaves optimally in both contexts.

Within-individual consistency in behaviour can result from several different processes (reviewed in Sih & Bell 2008), but the textbook cause of limited plasticity is a proximate, physiological or genetic

constraint. For example, high levels of testosterone in response to a territorial challenge in male birds can carry-over to influence parenting behaviour (Ketterson & Nolan 1999). In general, understanding the proximate mechanisms causing limited plasticity can help explain why individuals behave consistently. Studying the mechanisms underlying plasticity can help us to understand why adaptive plasticity is not more universal (van Kleunen & Fisher 2005).

We can use whole genome expression data in several ways to study the proximate mechanisms underlying behavioural plasticity. For example, whole genome expression data can reveal whether the same genes that are differentially expressed in response to a predator are also differentially expressed in response to a conspecific or to a novel object, etc. By looking for overlap among the lists, we can ask whether the same genes are expressed in different behavioural contexts to see if the behaviours are regulated by the same mechanisms. If the same genes are expressed in different behavioural contexts (e.g. around a conspecific and a predator), then that suggests that the two behaviours are not entirely mechanistically independent of one another.

Our understanding of the evolution of behavioural syndromes could be improved if we knew the identity of the genes related to the behaviours. While traditional quantitative genetic approaches can be used to estimate the degree to which two traits share common genetic control, an estimate of the genetic correlation does not tell us anything about the actual genes that are shared between the two traits, or the molecular mechanisms underlying pleiotropy. However, studying the genetic and molecular mechanisms underlying a behavioural correlation can reveal some surprising insights. For example, a study on horse personality that assessed genetic variation at a candidate gene found that curiosity and vigilance were, respectively, positively and negatively associated with a SNP causing an amino-acid change in the D4 dopamine receptor (Momosawa *et al.* 2005), suggesting that these two traits are not free to evolve independently.

In general, knowing the mechanism that underlies a behavioural syndrome is important because the fate of a correlation over time will depend on the mechanism linking the traits together. A correlation that reflects the pleiotropic effects of shared genes is difficult to break apart over short periods of evolutionary time, even if it is favoured. However, if selection persists, the constraint could be overcome (Mezey & Houle 2005). On the other hand, a genetic correlation generated by linkage disequilibrium can be uncoupled in subsequent generations if random mating occurs. In contrast, a correlation that reflects a plastic response to the environment can be modified within an organism's lifetime (Stearns *et al.* 1991; Sgro & Hoffmann 2004).

For further reading, the interested reader is referred to the growing literature on the molecular basis for life-history tradeoffs and antagonistic pleiotropy (Stearns & Magwene 2003; Bochdanovits & de Jong 2004; Roff 2007; Hughes 2010). Many of the insights emerging from studying life-history tradeoffs can readily be applied to tradeoffs between behavioural traits (Sih *et al.* 2004).

Another way in which whole genome expression data can provide insights into limited plasticity is to compare the overall transcriptomic response that is required to move between different behavioural contexts. In other words, how much needs to change within an individual in order to switch from behaving aggressively, for example, to avoiding predators? If a large number of genes are differentially expressed between these different behavioural states, this could imply that the animal has to undergo a dramatic change, which could be costly and time consuming (DeWitt *et al.* 1998). However, if fewer genes are differentially expressed between different behavioural states, then it might not be as difficult to move between behavioural tasks. For example, individual honeybees change from being nurses (caring for brood) to foragers (foraging for nectar) as they mature. In an elegant experiment in which they controlled for the effect of age on the behavioural transition, Whitfield *et al.* (2003) found that the transition between nurse and forager, which are relatively stable occupations, was associated with changes in 39 per cent of the transcriptome. In contrast, less stable occupations, such as the transition between guarding and undertaking, were associated with fewer transcriptional changes (Cash *et al.* 2005).

These findings prompt the hypothesis that long-term, stable behavioural changes within an individual are associated with substantial physiological remodelling, whereas less dramatic behavioural changes are associated with fewer physiological changes. If we can use the number of genes that change when moving between different behaviours as an index of the cost of behavioural plasticity, then we might predict that limited plasticity (behavioural consistency) is more likely to occur when dramatic transcriptional changes are required in order to switch behaviours. This hypothesis could be tested by measuring gene expression at a particular interval after a behavioural response to dramatically different stimuli, e.g. a predator or a conspecific, compared with a control group that is not confronted by either stimuli. If a larger fraction of the transcriptome is expressed in response to a predator versus a conspecific, this suggests that more physiological remodelling is required to respond to predator threats versus a challenge by a conspecific. In that case, we would expect to observe greater within-individual consistency in antipredator behaviour compared with aggressive behaviour.

Finally, whole genome expression data can also be used to test hypotheses about the evolutionary history of limited plasticity. One proximate explanation for the evolution of limited plasticity is that genes related to the behaviour are constitutively 'turned on', and no longer responsive to the environment (West-Eberhard 2003). Because the genome can be responsive to the environment, we can test this hypothesis by comparing gene expression in different populations that have adapted to different environments and have lost plasticity in gene expression. We could ask whether genes whose expression levels exhibit plasticity in response to the environment also show the greatest among-population (or species) difference in gene expression (Bochdanovits *et al.* 2003; Swindell *et al.* 2007).

This hypothesis was tested recently in a study on honeybees. In general, Africanized honeybees are more aggressive than European honeybees. Alaux *et al.* (2009) compared brain gene expression in these two subspecies, and also compared the molecular response to an alarm pheromone, which triggers aggression, in the European honeybees. They found that some of the genes that were involved in the behavioural response to alarm pheromone were also differentially expressed between the two subspecies, in the absence of alarm pheromone. The authors suggested that the plastic aggressive response to alarm pheromone could be at the origin of population differences in baseline levels of aggression (Alaux *et al.* 2009). It would be interesting to know if individual Africanized honeybees behave more consistently than individuals from the non-aggressive populations. If so, one hypothesis to explain limited plasticity in the Africanized honeybees is that genes related to aggressiveness are constitutively 'turned on' in those populations.

Another series of studies has also shown that the same gene that is involved in plastic responses to the environment is also polymorphic between genotypes (populations or species). Differences in activity between *Drosophila* larvae (rovers and sitters) are caused by differences in the expression of the *foraging* gene (*for*), which codes for a cyclic guanosine monophosphate (cGMP)-dependent protein kinase (Osborne *et al.* 1997). Rovers have higher protein kinase activity in their heads than sitters. The difference is caused by mutations in the *for* gene. In honeybees, the homologue of the *Drosophila for* gene is differentially expressed in nurses and foragers, which, like the rovers and sitters, differ in locomotor activity. However, in the honeybee case, the different locomotory behavioural types reflect a plastic, ontogenetic change that the bees undergo during behavioural development. That is, the difference in expression of the foraging gene in the brain of nurses and foragers is not owing to genetic variation at the *for* locus. Instead, both nurses and foragers have the same allele, but the allele is upregulated when the bee gets older and becomes a forager (Whitfield *et al.* 2003).

Both of the two aforementioned examples suggest that molecular mechanisms involved in plastic responses to the environment are also involved in evolutionary divergence. Understanding the generality of this pattern, and whether the same molecular mechanisms lead to limited plasticity in different species, is an obvious fascinating question for further work.

5. WHAT CAN WHOLE GENOME DATA TELL US ABOUT THE EXTENT OF SIMILARITY IN PERSONALITY AMONG SPECIES?

It has recently been proposed that the study of personality in non-human animals be organized along five different axes: sociability, boldness, aggressiveness, exploration and activity (Réale *et al.* 2007). Are these axes universal, or comparable across species? One criterion for comparability is if they reflect the same molecular mechanisms. A comparative approach to the study of the molecular basis of personality allows us to establish if the same proximate mechanisms underlie personality in different species, and thus to determine if it has evolved once or several times. The same question can be asked within species, by comparing different populations that are behaviourally similar.

For example, genetic variation in a candidate gene, the D4 dopamine receptor, *DRD4*, has been related to consistent individual differences in exploratory behaviour in a wild bird, the great tit (Fidler *et al.* 2007), selected lines of a domesticated bird, laying hens (Flisikowski *et al.* 2009), horses (Momozawa *et al.* 2005) and humans (Munafo *et al.* 2008), suggesting that exploratory behaviour in this diverse array of vertebrate species shares a common molecular basis (but see Korsten *et al.* 2010). It is important to stress that in these cases, a polymorphism has been found in this receptor, but the functional significance of this polymorphism has not been tested in all species.

As a parallel, whole genome expression profiling could tell us if specific functional differences at the molecular level are associated with a particular personality trait in different species. For example, in certain populations of whitefish, there are both normal and dwarf ecotypes. The ecotypes differ in growth and swimming activity and co-occur. Derome and Bernatchez compared gene expression between normal and dwarf ecotypes within and between different populations. They found similar patterns of gene expression between normal and dwarf ecotypes in both populations and the genes had functions (energy metabolism, muscle contraction) that seemed plausibly related to the organismal differences between the 'normal' and 'dwarf' ecotypes (Derome & Bernatchez 2006; Derome *et al.* 2006). Another species (the cisco, *Coregonus artedi*) that inhabits the same functional niche as the dwarf whitefish ecotype showed a similar transcriptomic profile as the dwarf whitefish ecotype (Derome *et al.* 2006). In the same way, whole genome expression profiling of individuals with different behavioural types (Sih *et al.* 2004) in different species could be analysed to explore whether the same genes are consistently differentially expressed between behavioural types.

However, whether the *same* molecular mechanisms are the cause of the same phenotypes is a trickier question than it might appear at first glance. The problem is that it is not entirely clear what we mean by 'the same'. For example, parallelism and convergence are terms that originally distinguished phenotypic similarities that evolved independently in closely related and distantly related species, respectively, although these terms have also been used to distinguish phenotypes that result from the same genetic changes (parallelism) or from different genetic mechanisms (convergence) without considering phylogeny (Arendt & Reznick 2008). Until recently, whether the same molecular mechanisms were the cause of these similar phenotypes at the organismic level was unknown. Now that these mechanisms can be uncovered in more and more cases, it has been proposed that the term convergent evolution be used in all cases and that the specific genetic change involved be stated explicitly when known (Arendt & Reznick

2008). For example, two species might have inherited an allele related to a personality trait from a common ancestor (homology), or the two species might have independently converged on the same allele (convergence). Similarly, it is possible that the same gene is related to personality variation in two different species, but different specific genetic changes are present in the two species, i.e. a polymorphism in the promoter versus a polymorphism in the coding region. On the other hand, considering that genes do not work in isolation of one another (as described above), perhaps our criteria for similarity should be broader and include the same molecular pathways, rather than the same specific genes. However, comparing pathways has the same problem, because different mutations along the same pathway might result in the same phenotype. Our point in going through these complications is that a genetic criterion for comparing personality traits across species might be more complicated than it appears at first glance.

6. PROMISING STRATEGIES FOR USING WHOLE GENOME EXPRESSION DATA TO ADDRESS OTHER EVOLUTIONARY QUESTIONS ABOUT PERSONALITY

An outstanding challenge within evolutionary biology is to understand the processes that can maintain genetic variation within populations. With respect to personality, why do multiple behavioural types coexist within the same population? Natural selection by predators, for example, will remove individuals that do not express the appropriate antipredator behaviour from the population. Provided that there is a heritable basis to antipredator behaviour, over evolutionary time we would expect all individuals within the population to express the same antipredator behaviour. However, we often see heritable variation in antipredator behaviour within a population (Bell 2005), which prompts the question: what is maintaining this behavioural variation? It is important to note that this is an outstanding question in all of evolutionary biology and is not restricted to behavioural traits.

There are several mechanisms that might maintain genetic variation within populations (variation in selection pressures, negative frequency dependence, mutation, antagonistic pleiotropy, overdominance; Hedrick 2006). Despite the promise of using genomic data for understanding the mechanisms of natural selection, to our knowledge, there are only a few examples of using whole genome expression data to test whether any of these mechanisms are maintaining genetic variation in natural populations (Whitehead & Crawford 2005). Therefore, this section of the paper is largely speculative; our aim is to encourage future research in this area.

One approach for gleaning insights into the mechanisms that might be maintaining genetic variation in personality traits is to use whole genome expression data combined with other approaches (linkage mapping, association studies) to identify genes that might be polymorphic (van Oers & Mueller 2010). Once genes related to the personality traits have been found and sequenced (box 3), we can look for a signature of balancing selection on the genes (for a worked example see de Luca *et al.* 2003; Carbone *et al.* 2006). If we detect a signature of balancing selection, then that could be direct evidence that negative frequency dependence, or variable selection pressures, or overdominance is maintaining inherited behavioural variation.

Spatial or temporal variation in selection pressures is one process that can, in some circumstances (Hedrick 2006), maintain genetic variation. If certain genotypes do well in some years, while other genotypes have higher fitness in other years, then both genotypes can be maintained within the population. For example, 'fast' exploring great tits have higher fitness than 'slow' explorers in some years, but not in others (Dingemanse *et al.* 2004). Measuring whole genome expression in different environments might reveal insights into the molecular mechanisms that are involved in response to variable selection pressures. For example, one could use whole genome expression data, combined with genotypic data, to understand whether different behaviours exhibited in different years or in different environments reflect the actions of different alleles at the same locus, or different loci, or allelic sensitivity to the environment (Schlichting & Smith 2002).

This could be useful because while there have been many studies on spatial and temporal variation in selection on heritable phenotypes, including personality traits (Réale & Festa-Bianchet 2003; Dingemanse *et al.* 2004; Dingemanse & Réale 2005), rarely do we know the specific genotypes that underlie the phenotype. Moreover, our models and data rely on a relatively simple genotype–phenotype relationship (single locus, no environmental effect; van Oers & Sinn 2010). Whole genome expression data have the potential to reveal more about the specific mechanisms underlying complex traits that are influenced by many genes, and which are responsive to the environment. As it is probable that whole genome approaches will become accessible and routine for any organism in the near future (Robinson *et al.* 2008), the next generation of animal behaviourists will be equipped with powerful tools for understanding the causes and consequences of behavioural variation in ecologically relevant situations.

7. SUMMING UP AND FUTURE DIRECTIONS

Our objective in this paper has been to stimulate the reader's interest in genomics. We have argued that studies that measure whole genome expression can help address several unanswered evolutionary questions about personality, especially about limited plasticity. Another objective of this paper is to encourage researchers studying personality in natural populations to consider measuring gene expression in their organism; powerful tools for measuring whole genome expression are no longer just for traditional model organisms.

Finally, there is a great deal of promise for using gene expression data to answer some deep, long-standing issues in animal behaviour. Indeed, the 'genomic revolution' has been hailed as an opportunity to finally integrate genes and environment (Fitzpatrick *et al.*

2005; Robinson *et al*. 2008). We foresee the imminent appearance of studies providing proximate and ultimate answers to questions such as the following. Why are certain behaviours influenced by few genes, while others are more polygenic? Why are certain behaviours stable through time, while others are not? Why do certain behaviours cluster together into a suite of correlated traits, while others do not? And finally, why are certain behaviours more or less environmentally sensitive?

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