Special Issue

Genetic accommodation and behavioural evolution: insights from genomic studies

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Many behaviours vary in response to the environment (biotic or abiotic) and therefore represent an interesting form of phenotypic plasticity. Behavioural plasticity, like other plastic traits, can evolve through genetic assimilation or accommodation. However, little is known about the nature of changes in gene expression plasticity that accompanies these evolutionary changes in phenotypic plasticity. We know that variation in gene expression level, a first-order phenotype, underlies much behavioural variation. Several studies have begun to document which genes show expression-level variation related to plastic changes in behaviour as well as evolved changes in behaviour. Advances in sequencing technology allow us to address these questions on a genomic scale. By characterizing changes in gene expression according to the concept of a norm of reaction one can describe the evolved patterns of gene expression that accompany the evolution of behavioural plasticity. Here, we describe how genomic approaches can help us understand changes in gene expression that accompany or underlie the evolution of behavioural plasticity. To do this, we provide a framework of classification for the evolved patterns of gene expression plasticity that could underlie genetic assimilation or accommodation of behaviour. We provide examples of genetic assimilation from the animal behaviour and animal physiology literature that have been, or can be, studied at a genomic level. We then describe the characteristics of an appropriate study system and briefly address experimental design using the available genomic tools in a comparative context. Studying the patterns of gene expression associated with genetic assimilation will elucidate processes by which behavioural plasticity has evolved.

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Because the development and expression of behaviour can be remarkably flexible and often environmentally dependent, behavioural plasticity should be considered a potentially important form of adaptive phenotypic plasticity, in which the reprogramming of the functional genome in response to the environment allows an animal to maintain high fitness. Phenotypic plasticity implies that two or more distinct phenotypes can be produced from a single genome through differential regulation of gene expression for single genes or networks of genes. While a plastic response to environmental change is not always adaptive, there are a number of examples of behavioural plasticity that show clear adaptive function, including novel foraging behaviour (Price et al. 2003), learning (Dukas 2013), sex-role (Forsgren et al. 2004), parental care behaviour (Itzkowitz et al. 2001), mate preference (Rodríguez et al. 2013) and integrated phenotypes (Kasumovic 2013).

There is great interest in understanding how phenotypic plasticity influences evolutionary processes, either by promoting diversification or by buffering against it (Schlichting & Pigliucci 1998; Huey et al. 2003; Price et al. 2003; West-Eberhard 2003; Schlichting 2004; Wund et al. 2008; Pfennig et al. 2010). Behavioural plasticity has an unusual and especially intriguing potential role in evolution in that behavioural modification provides a means by which an organism can manipulate its own environment and thereby alter the selective pressures to which it is subjected (e.g. Odling-Smee 1988; Wcislo 1989; West-Eberhard 2003; Palmer 2011). Although behavioural plasticity has historically been studied on the phenotypic level, investigating the underlying molecular mechanisms at the level of gene expression has the potential to offer key insights into the processes by which behavioural plasticity has evolved. In this review, we present a framework with which to consider the evolution of gene expression plasticity to more fully understand the evolution of behavioural plasticity. Recent advances in genomic techniques allow us to survey virtually all expressed

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genes, even in organisms for which few prior genomic and genetic tools were available. Such genomic approaches provide powerful comparative data sets with which to investigate the evolutionary processes that accompany changes in phenotypic plasticity (e.g. Aubin-Horth & Renn 2009; Beldade et al. 2011).

The degree of behavioural plasticity, just like developmental, morphological or physiological plasticity, can evolve. Behavioural plasticity can be represented through the concept of a norm of reaction (Schlichting & Pigliucci 1998; Carroll & Corneli 1999; Pigliucci & Murren 2003; West-Eberhard 2003; Dingemanse et al. 2010), in which the level or value of a behaviour produced by a particular genotype can be depicted as it varies across environments such that the slope of the norm of reaction reflects the magnitude of plasticity of that genotype. In other words, for a simple norm of reaction evaluated in two environments, a steeper slope reflects greater plasticity. When this slope is correlated with fitness and there is genetic variation for plasticity, the degree of behavioural plasticity can evolve adaptively. Quantitative genetic models illustrate how plasticity (i.e. the norm of reaction) can evolve under different selective pressures in different environments (e.g. Via & Lande 1985; Price et al. 2003). Mechanisms through which this could be achieved include changes in neural gene expression, as well as altered gene expression related to hormone release, target sites and actions.

Here, we ask whether one should expect to see the evolution of decreased plasticity in gene expression when decreased behavioural plasticity is observed and, conversely, whether one should expect to see the evolution of increased plasticity in gene expression when increased behavioural plasticity is observed. In this review we establish a framework within which to consider changes in gene expression that can accompany the evolutionary change of behavioural plasticity. Although behavioural plasticity may increase or decrease through evolution, we focus much of our discussion of gene expression plasticity to examples of evolutionary change through a process known as genetic assimilation (discussed below) because this process has been well characterized in the literature and provides clear parallels that can be addressed at the level of gene expression. None the less, the framework that we present is sufficiently general to be applied to contexts including both an increased and decreased degree of behavioural plasticity.

Background on Genetic Assimilation and the Study of Behaviour

The concept termed 'genetic assimilation' provides a structure to describe divergent evolution of behavioural phenotypes accompanied by loss of plasticity (canalization) for that phenotype. Genetic assimilation is the process by which a phenotype, initially produced in response to a specific environmental stimulus becomes, over evolutionary time, constitutively expressed and thus, independent of the original evoking stimulus (Pigliucci et al. 2006) (Fig. 1). The concept, as described in the 19th century (Spalding 1873; Morgan 1896; Osborn 1897), initially proposed plasticity as a positive driving force of evolution, particularly in relation to behaviour and learning (Baldwin 1896, 1902). However, during its formalization as a genetic concept in the 1940s, Waddington (1942, 1952, 1953, 1961) and Schmalhausen (1949) moved the focus to developmental plasticity rather than behaviour and learning. Empirically, Waddington demonstrated genetic assimilation with the morphological cross-veinless trait in *Drosophila melanogaster*. Initially, this trait occurred at low frequency in response to a certain developmental heat shock regime. After just a few generations of artificial selection, this initially environmentally induced phenotype became more prevalent in the population, even in the absence of heat shock. A modern interpretation of these results is that the stimulus exposed cryptic genetic variation, allowing selection for
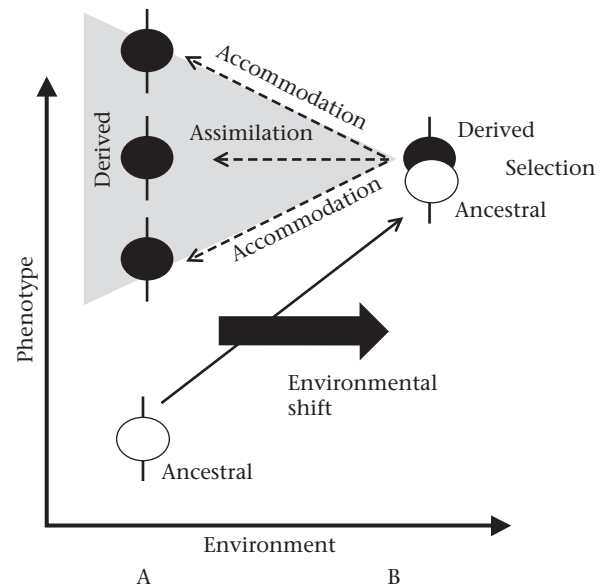

Figure 1. The concept of genetic accommodation depicted as norms of reaction. The ancestral population (open symbol) initially inhabits one environment, A. Following a shift in the environment to B (heavy black arrow), previously unrealized plasticity now results in an alternate behavioural phenotype. The ancestral norm of reaction (thin solid arrow) describes the phenotypic plasticity for this population. If the population remains in environment B, allowing natural selection to act on existing or novel genetic variation, the resulting derived genotype (black circle) will continue to produce the alternate phenotype. Due to this natural selection, the phenotype produced by the derived genotype may be different when the environment shifts back to A. The new derived norm of reaction (dashed arrows) reveals altered plasticity. The alternative phenotype may be fixed (assimilation) regardless of environment or may show altered environmentally sensitivity (accommodation).

the constitutive expression of this trait (Rutherford & Lindquist 1998; Gibson & Dworkin 2004; Schlichting 2008). Waddington's findings have inspired further research on genetic assimilation in natural populations, also leading to the more broadly encompassing concept of 'genetic accommodation' (West-Eberhard 2003). The term 'genetic accommodation' includes both genetic assimilation and instances in which the evolutionary outcome for the plastic phenotype, initially induced by either the environment or by genetic mutation, may include either increased or fine-tuned plasticity through accumulated genetic changes (for further discussion of the distinction see Crispo 2007). Studies have confirmed these concepts in many systems. Examples include adaptation to different elevations in plants (summarized in Pigliucci & Murren 2003), plant–insect interactions (Heil et al. 2004; Linsenmair & Boland 2004), body size in snakes (Keogh et al. 2005), colour polymorphism in the tobacco hornworm (Suzuki & Nihout 2006), among others (e.g. Chapman et al. 2000; West-Eberhard 2003; Ghalambor et al. 2007; Aubret & Shine 2009; Snell-Rood et al. 2010).

Recent studies have investigated the role of genetic accommodation and assimilation in the evolution of behaviour (Badyaev 2005, 2009; Duckworth 2009; Ghalambor et al. 2010; Foster 2013). One such study demonstrated that plasticity in oviposition preference in *Drosophila* can become heritable when selected over multiple generations (Mery & Kawecki 2004). Another demonstrated that *Caenorhabditis elegans* can imprint on its olfactory environment and this odorant preference can become genetically based (Remy 2010). While many such experiments rely on artificial selection and laboratory-based model organisms, the questions driving the research stem from observations of natural variation and adaptive phenotypes. Other studies have capitalized on variation among natural populations by comparing ancestral-type groups that show plasticity in a focal trait to derived groups for

which that trait is fixed. The comparison of these two types of populations (one plastic and one canalized) provides a model with which to study of the genetic assimilation of behaviour. For example, males of ocean-dwelling (ancestral) stickleback fish perform a zigzag dance in response to females approaching their nest. Unlike the situation in the oceanic form, the dance is no longer inhibited by cannibalistic foraging groups in derived freshwater limnetic fish, suggesting genetic assimilation (Shaw et al. 2007; Foster 2013). These past studies demonstrate that genetic assimilation and accommodation can play an important role in phenotypic evolution, including the evolution of gene expression underlying the behavioural shifts (Bell & Robinson 2011).

Gene Expression Profile of Genetic Accommodation

Although genetic assimilation and accommodation may be major mechanisms through which behaviour evolves (Carroll & Corneli 1999; West-Eberhard 2003; Ghalambor et al. 2010; Bell & Robinson 2011), we know next to nothing about the molecular changes associated with these processes. The majority of studies of these processes have addressed outwardly observable phenotypes, referred to here as 'higher-order phenotypes'. However, in order to understand how genetic accommodation has occurred one must also ask about changes in the molecular mechanisms that regulate these phenotypes. According to this approach, gene expression can be considered a 'first-order phenotype' and a norm of reaction can depict the level of expression for each gene under different environments that induce plasticity of the higher-order phenotype (e.g. behaviour, morphology or physiology). Just as plastic phenotypes can evolve increased or decreased levels of plasticity in response to selection, the regulation of gene expression associated with a plastic phenotype may also evolve. It is therefore interesting to determine the extent to which the evolved patterns of gene expression mirror the evolved phenotypic plasticity. In other words, while one might observe genetic assimilation of the first-order phenotype, gene expression, such that the expression level of a gene that was initially sensitive to the environment becomes fixed at one extreme or the other, that is not the only possible evolved pattern of gene expression.

Although not directly related to behavioural plasticity, an example from recent research in *Daphnia melanica* provides evidence for genetic assimilation of gene expression as a molecular mechanism for genetic assimilation of the higher-order phenotype. These freshwater crustaceans respond plastically to the presence of UV radiation by increasing pigmentation through down-regulation of the *dopa decarboxylase* (*ddc*) gene and the interacting gene *ebony*. In certain populations, even in the presence of UV radiation, *D. melanica* remains unpigmented (Scoville & Pfrender 2010), which is a key adaptation for this lineage in response to the recent introduction of a predator that hunts visually. Candidate gene studies revealed that the environmentally insensitive phenotype is realized through invariant up-regulation of the both *ddc* gene and *ebony* gene. This case of adaptation through canalization is particularly interesting because the genetic assimilation observed at the level of the higher-order phenotype (constitutively reduced melanization) involves genetic assimilation of gene expression (constitutive up-regulation of *ddc*). This result demonstrates one case in which evolutionary change has targeted the same molecular mechanisms that orchestrated phenotypic plasticity of the ancestral phenotype.

While, as in the case described for *Daphnia*, it may not be surprising to find that canalization of the molecular mechanism can underlie genetic assimilation of the phenotype, it is also possible that novel patterns of gene expression could be co-opted in this process. Although we currently know little about the genome-wide patterns of gene expression associated with genetic accommodation of behaviour, we are now well poised to study these complex mechanisms. Advances in modern genomics allow us to survey gene expression patterns in nonmodel organisms in a comparative way (Ekblom & Galindo 2011). Because genetic assimilation or accommodation of a higher-order phenotype is likely accompanied by changes in gene expression plasticity across numerous loci (e.g. Hodgins-Davis et al. 2012), a genome-wide approach is best suited to answering questions about the mechanisms involved. Borrowing terminology from ecological studies of phenotypic plasticity and evolution (e.g. Latta et al. 2012) we provide a framework of classification for the types of evolved changes in gene expression that could be associated with genetic accommodation of behaviour.

EVOLVED PATTERNS OF GENE EXPRESSION PLASTICITY ASSOCIATED WITH GENETIC ACCOMMODATION

Given that many genetic mechanisms often underlie the production of a single behavioural trait, genetic accommodation of a behavioural phenotype is likely to involve many different patterns of evolved changes in gene expression plasticity. By describing the possible patterns or categories of evolved gene expression plasticity we can ask to what extent plasticity of the first-order phenotype, gene expression, mirrors evolved behavioural plasticity. While evolution of behavioural plasticity can clearly result in increased or decreased plasticity (i.e. genetic accommodation), we focus the discussion of gene expression categories on example of reduced phenotypic plasticity (i.e. genetic assimilation) because this concept, in addition to being well studied at the phenotype level, provides clear parallels that can be described at the level of gene expression. When a higher-order trait becomes canalized, a number of possible changes in gene expression plasticity could be at play. These patterns of gene expression range from a strict canalization of gene expression level to the evolution of completely novel expression patterns. The different patterns of evolved gene expression plasticity can be categorized according to the relationship between the slope of the norm of reaction for the ancestral genotype and the slope of the norm of reaction for the derived genotype (Fig. 2). It should be emphasized that the overall set of evolved patterns for gene expression plasticity that underlie the genetic assimilation of a complex trait (like certain behaviours) is bound to involve multiple patterns of change at different loci.

Assimilated Gene Expression Plasticity

In the most extreme case, genes expressed plastically in response to the environment may become fixed in their expression, being constitutively expressed at a level equal to that previously induced by one environment or the other (Fig. 2a). In this case, the first-order phenotype, gene expression, appears to have itself undergone genetic assimilation. Clearly fixation of previously plastic gene expression could result in constitutive expression of the previously plastic phenotype. In a broader definition, evolved constitutive gene expression may be canalized at an intermediate level, an increased level or a decreased level not previously associated with induced ancestral phenotypic state.

Accommodated Gene Expression Plasticity

Gene expression plasticity may evolve such that the level of gene expression is responsive to the environment in both ancestral and derived genotypes, but the slopes of the norms of reactions are different (Fig. 2b), having been enhanced or fine-tuned by natural selection. Accommodation of gene expression plasticity may underlie assimilation at the level of the behavioural phenotype in that
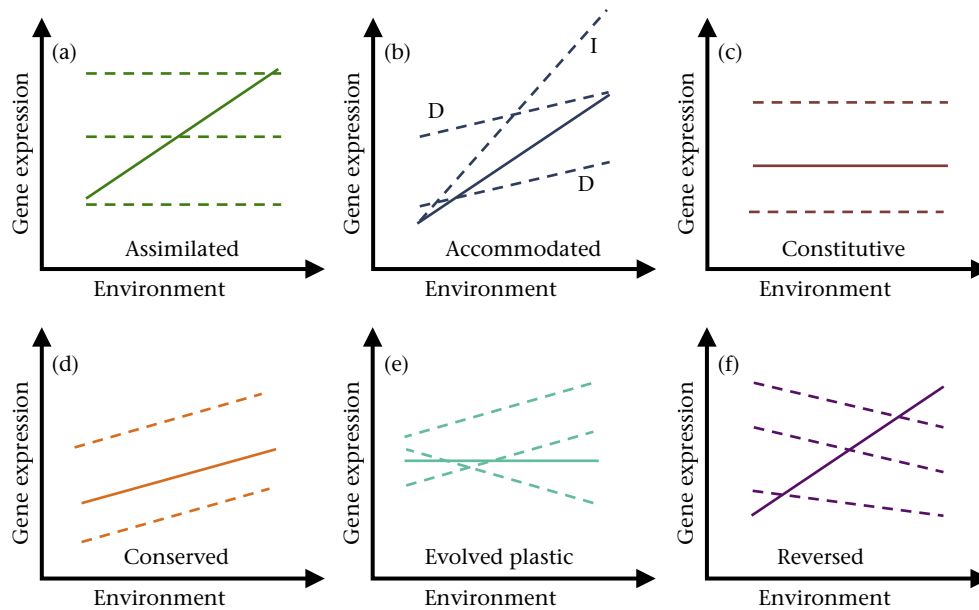
Figure 2. Hypothetical patterns of gene regulatory evolution that may contribute to genetic accommodation of behavioural plasticity. (a–f) Norms of reaction for gene expression in the ancestral genotype (solid line) and derived genotypes (dashed lines). (b, c, e, f) Instances of up-regulation by environment (a positive norm of reaction) in the ancestral genotype. A second set of individual plots could be drawn to represent a negative norm of reaction for the ancestral genotype. (a) Assimilated gene expression plasticity. These genes are environmentally insensitive in the derived population, fixed at one extreme, or mid-level compared to the plastic ancestral expression. (b) Accommodated gene expression plasticity. These genes are environmentally responsive in both genotypes. Generally one would expect these genes to show decreased responsivity (D) in the derived genotype but increased responsivity (I), perhaps for negative regulators, should not be ruled out. (c) Novel constitutive gene expression in both ancestral and derived genotypes. These genes are not responsive to the environment in either genotype. While the constitutive character (environmental insensitivity) of these genes is conserved, the absolute level of expression has evolved to be different. Therefore, these evolved constitutive genes may contribute to a novel behavioral phenotype. (d) Conserved gene expression plasticity. The level of expression of these genes is similarly sensitive to the environment in both genotypes. While plasticity is conserved, the overall level of expression may have evolved to be different and may underlie a novel phenotype. (e) Evolved gene expression plasticity. These genes are sensitive to environment only in the derived genotype and not in the ancestral phenotype. Here a novel molecular mechanism can responsible for establishing a novel or a canalized phenotype. (f) Reversed gene expression plasticity. The level of expression of these genes is sensitive to the environment in both genotypes, but the direction of regulation is reversed.

genes previously regulated by the environment may still respond to the environment, but the degree of responsiveness is altered and does not translate into plasticity in the higher-order phenotype, thus producing a canalized behavioural phenotype.

Novel Constitutive Gene Expression

Genes that are not associated with producing the plastic trait in the ancestral population may be co-opted to constitutively produce the higher-order phenotype. The derived higher-order phenotype is produced through a different molecular mechanism than the outwardly similar ancestral phenotype (Fig. 2c). A new constitutive level of gene expression may be necessary to buffer against, or reduce sensitivity to, environmental induction. Some functional classes of genes may be expected to evolve increased expression level (buffering) while others might be expected to evolve reduced expression (decreased sensitivity).

Conserved Gene Expression Plasticity

Genes for which the level of expression is similarly sensitive to the environment in both ancestral and derived genotypes are said to show conserved plasticity. However, for this category of genes, while the slopes for the norms of reaction for the gene in the ancestral and derived population are parallel, the absolute level of expression (the Y intercept) may be different (Fig. 2d). In such cases, this change in gene expression plasticity may contribute to genetic assimilation at the level of the phenotype if the level of gene expression now falls above or below some threshold necessary to produce behavioural plasticity.

Evolved Gene Expression Plasticity

While the behavioural phenotype may show genetic assimilation in that it no longer varies when the environment changes, there may be novel biased gene expression induced by the environment (Fig. 2e). This environmentally inducible gene expression may be required to produce the canalized higher-order phenotype in order to compensate for other evolved changes in components of the gene expression network.

Reversed Gene Expression Plasticity

A more extreme case of evolved plasticity might manifest as a complete reversal of gene expression regulation such that the environment that previously caused up-regulation of a gene associated with the plastic phenotype now causes down-regulation of the gene in order to produce the invariant phenotype under these conditions (Fig. 2f). Again, this pattern would indicate a compensatory mechanism and would imply additional changes elsewhere in the gene network.

Potential Patterns

Within this framework, we can describe the evolutionary change in gene expression plasticity during genetic accommodation or assimilation of a behavioural phenotype. We can address how many and which genes have evolved expression changes that mirror the observed pattern of change in phenotype and to what extent various novel patterns of gene expression plasticity are recruited when behavioural plasticity evolves. It is important to keep in mind, however, that determining which of the above

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categories appropriately describes the evolved plasticity of each individual gene may rely on a statistical threshold for significance. Therefore, appropriate statistical tests of equivalence (Qiu & Cui 2010), or a hierarchical analysis (e.g. McManus et al. 2010) must also be employed.

The categories described above provide guidelines for interpreting evolved gene expression patterns that are associated with genetic accommodation of a plastic behavioural phenotype (see below, for examples from bees, cichlids, killifish and fruit flies). Currently, the relative contribution of each category of evolved gene expression is unknown for most cases of evolved behavioural plasticity. In a case of genetic assimilation, if the assimilated behavioural phenotype was produced in large part by the same molecular mechanisms as the induced ancestral phenotype, the category of assimilated and/or accommodated gene expression plasticity would predominate (Fig. 3a). Alternatively, if the assimilated phenotype was produced in large part by novel molecular mechanisms, the categories of novel constitutive or evolved plasticity of gene expression would predominate (Fig. 3b). It is possible that the relative importance of the different categories will differ depending on the phenotype in question. For example, the evolution of plastic social behaviours may rely more heavily on one evolved pattern, while the evolution of plastic foraging behaviours may rely more frequently on another. Similarly, whether the ancestral phenotype is developmentally irreversible, restricted to a particular developmental stage, or reversibly plastic even in adults, may favour evolution according one or the other patterns described above. In addition, we may find that different functional types of genes (e.g. secreted molecules versus cell surface receptors) or genes that occupy different gene network positions (e.g. central versus terminal nodes or pleiotropic versus single function genes) will differ in their tendency to undergo specific categories of regulatory evolution. Finally, such tendencies may also be influenced by structural characteristics of the genome. Here, the field of gene regulatory evolvability (Landry et al. 2007; Tirosh et al. 2009; Rosin et al. 2012) should contribute to the studies of evolution of behavioural plasticity.

EXAMPLES FROM THE LITERATURE

Several comparative studies in honeybees have examined the molecular mechanisms for behaviours such as dance (Sen Sarma et al. 2009), sociality (Johnson & Tsutsui 2011), eusociality (Toth et al. 2007; Bloch & Grozinger 2011) and aggression (Alaux et al. 2009). This last example explicitly looked for evidence of genetic assimilation for gene expression plasticity. It compared two species, one that showed a plastic aggressive phenotype and one with a constitutive aggressive phenotype (Alaux et al. 2009). By first identifying gene expression plasticity associated with a plastic increase in aggression in European bees (either age-related or pheromone-induced), Robinson and colleagues were able to then ask whether canalized gene expression was seen in the constitutively more aggressive Africanized bees. Based on principle component analysis, the authors found strong evidence for genetic assimilation at the level of gene expression plasticity. However, additional analyses addressing the norm of reaction for individual genes in this data set would be necessary to determine the relative proportion of changes in gene expression regulation that fall into each category described above. Instead the authors took a different approach, analysing putative cis-regulatory motifs (for methods see Ament et al. 2012), to suggest that evolved patterns of gene expression regulation may occur at the transcriptional network level. Based on their results, the authors suggested that the plastic aggressive response to alarm pheromone could be an evolutionary antecedent to an increased baseline level of aggression in Africanized bees (Alaux et al. 2009). This is an example of genetic assimilation for behavioural plasticity mirrored in part by assimilation of gene expression plasticity.

Similarly, although not specifically framed in the terminology of genetic accommodation, recent comparative studies have addressed gene expression changes that accompany the evolution of behaviour in cichlids, such as mating strategy (Machado et al. 2009) and sex-biased behaviours (Schumer et al. 2011). *Julidochromis marlieri* and *Julidochromis transcriptus* have evolved different sex-biased behaviour patterns. While *J. transcriptus* predominantly follows the ancestral pattern of male dominance, male-biased sexual size dimorphism and territoriality, a minority of pairs in the wild express a reversal of this sex-biased pattern. For *J. marlieri*, the predominant sex-biased pattern is naturally reversed, such that females show these male-typical behaviours and morphology (Barlow & Lee 2005); these aggressive females are less plastic than *J. transcriptus* individuals. Comparing gene expression patterns associated with plastically aggressive
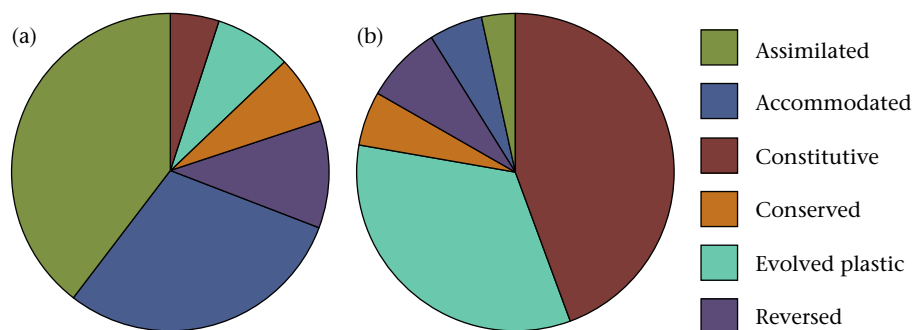

Figure 3. Representation of the potential relative contribution for each category of evolved gene expression plasticity. While each gene whose expression is associated with plasticity in either the ancestral or derived phenotype can be categorized according to the framework presented in Fig. 2, a complex set of these changes in gene expression plasticity will likely underlie a single case of behavioural genetic assimilation. The full set of evolved changes may include several (possibly all) of the described categories of evolved gene expression plasticity. However, the relative contribution of each category may differ for different instances evolved phenotypic plasticity. When the predominant categories of evolved gene expression plasticity include assimilated and/or accommodated gene expression plasticity (a), the derived canalized phenotype is orchestrates through largely the same mechanisms as the previously plastic ancestral phenotype. However, when the categories of novel constitutive or evolved plasticity of gene expression predominate (b), it is clear that the derived canalized phenotype is produced through novel molecular mechanisms. Different forms of behavioural phenotypic plasticity may rely more heavily on the different categories of regulatory evolution; for example, assimilated gene expression plasticity may be the predominant pattern in irreversible plasticity (a) while constitutive gene expression plasticity may be the predominant pattern for reversible plasticity (b). Furthermore, it is possible that different functional categories of genes are predisposed to undergo different types of regulatory evolution; for example, constitutive gene expression plasticity may be the predominant pattern for transcription factors (b) while assimilated gene expression plasticity may be the predominant pattern for metabolic enzymes (a). These are hypothetical distinctions, but empirical research should inform us about the true patterns.

J. transcriptus females to stably aggressive *J. marlieri* females suggests that genetic assimilation of gene expression patterns that were previously plastic may have contributed to this behavioural shift (Schumer et al. 2011; M. Schumer & S. Renn, unpublished data). *Julidochromis marlieri* females naturally exhibit gene expression patterns highly similar to *J. transcriptus* males, and *J. transcriptus* females exhibit male-like expression patterns when they are induced to plastically increase aggressive behaviour.

Other examples from the literature show recruitment of genes not previously induced by the environment and novel patterns of gene expression during evolutionary change in phenotypic plasticity. Killifish have become a model system for plasticity in ecological physiology (Whitehead 2012). While individuals of some killifish species are highly flexible (e.g. salinity temperature, hypoxia or pollutants), closely related species vary in the extent of plasticity (e.g. Whitehead et al. 2011, 2012a). By using replicate pairs of populations (Whitehead et al. 2012b) or multiple populations along an environmental gradient (Whitehead et al. 2011), researchers have been able to study the patterns of gene expression that evolved to produce increased or decreased plasticity. One such study focused on pollution tolerance (Whitehead et al. 2012a) and showed that individuals from three polluted populations showed dissimilar plastic gene expression responses to pollution, suggesting independent evolutionary solutions. While the environmental pollutants may also have differed among the three sites, this result suggests that even for similar instances of genetic assimilation of a plastic phenotype, the co-opted genes and their evolved plasticity may differ.

An ecological physiology approach has also been applied to study host specialization in the cactophilic fly *Drosophila mojavensis*, a North American desert species composed of four recently diverged populations. Among these populations, each has specialized with regard to detoxification (physiology) and host preference (behaviour). A pair of recent studies quantified gene expression for two populations, one endemic to the ancestral host (Matzkin et al. 2006) and one endemic to a derived host (Matzkin 2012). Due to advances in technology, a new microarray was used in the second study, making it impossible to directly relate absolute gene expression level across the two studies. None the less, 23% of host-induced gene expression plasticity was shared between populations, suggesting either conserved or accommodated gene expression plasticity. Further quantification of gene expression plasticity in the ancestral population is necessary to accurately categorize the evolved patterns of gene expression plasticity.

While some of these studies provide strong support for a role for genetic assimilation of gene expression plasticity, they also demonstrate the importance of novel gene expression patterns in the process of genetic assimilation for behavioural plasticity. Each genome-wide study reveals a complex pattern of gene expression evolution that can be addressed by the framework provided above. With the study of additional systems, and a consistent framework to classify the evolution of gene expression plasticity, a clearer picture should emerge as to how many and which types of genes have evolved plasticity changes that mirror the observed patterns of change at the level of behaviour.

REQUIRED CHARACTERISTICS FOR APPROPRIATE STUDY SYSTEMS

A consensus has yet to be reached about whether certain evolved changes in gene expression are more or less likely to accompany genetic accommodation, although some theoretical studies suggest that assimilation of gene regulation may be common (Espinosa-Soto et al. 2011). To study the mechanistic changes associated with genetic accommodation of the higher-order phenotype, an appropriate behavioural and genomic system must

be selected. Outlined here are four conceptual and practical issues to be considered in selecting an appropriate study system, followed by a summary of current techniques that can be applied.

Ancestral and Derived Populations with Differing Degree of Plasticity

To address the process of genetic accommodation of behaviour, multiple species with the appropriate phenotypic and phylogenetic relationships must be identified to establish a comparative framework. The most basic studies of genetic accommodation require an ancestral population/species with significant phenotypic plasticity and at least one derived population with altered plasticity in that trait. For many study systems, the true ancestral population is no longer extant and a contemporary population most similar to the hypothesized ancestor in the trait of interest must be used. More complex comparisons including multiple parallel evolutionary events (e.g. copepod: Lee et al. 2011; freshwater invasion killifish: Whitehead et al. 2012a), multiple lineages with varying degrees of phenotypic plasticity (e.g. Khaitovich et al. 2004) or multiple lineages/populations of known phylogenetic relationships, would provide further power to eliminate spurious correlations as well as to differentiate between neutral and adaptive changes in gene expression regulation.

Quantifiable Phenotype

To identify the evolved patterns of gene expression that account for adaptive evolutionary change in behavioural plasticity, the behavioural phenotype(s) should be directly quantifiable, related to the organism's fitness and amenable to comparison across environmental conditions and populations. Behavioural measures must be developed that encompass the range of phenotypes for the entire study (although not all populations will exhibit the full range of phenotypes). Furthermore, when physiological measures, for example hormone titre, are used as a proxy for behavioural measures, it is necessary to verify the validity of this measure across all environmental conditions and populations included in the study. Although seemingly obvious, this constraint on experimental design makes meta-analysis of existing behavioural data sets difficult.

Ability to Induce Plasticity in a Controlled Setting

Given the goal to understand mechanisms that underlie plasticity or loss of plasticity, one must control, to the extent possible, additional developmental or maternal effects. Transplantation experiments (Cheviron et al. 2008) and natural experiments in the field (great tit, *Parus major*: Santure et al. 2011) are likely to be technically more difficult than those in a controlled laboratory setting due to greater variation. While individual variation can be informative, gene expression studies should be designed to minimize sampling and environmental variation.

Genomic Resources for Multispecies Gene Expression Studies

To investigate the mechanisms of genetic accommodation, researchers face the challenge of not only establishing genomic resources for a single species but also establishing tools that allow comparison among multiple species or diverged populations. A robust genomic study requires the ability to perform four comparisons. The first and second compare samples collected from two environmental conditions, one within the ancestral population and the other within the derived population, to quantify gene expression plasticity in each population. These two comparisons establish a norm of reaction for each gene in each population. The third and

the fourth comparisons must quantify gene expression differences between the ancestral and derived populations within each set of environmental conditions. These two comparisons quantify evolutionary change in gene expression level for each gene and place the ancestral and derived norms of reaction relative to each other. These four comparisons are necessary to identify whether genes that are plastically expressed in response to the environment in the ancestral phenotype show altered expression in the derived phenotype. Until recently, the availability of genomic resources greatly constrained the choice of experimental systems, and comparative studies present additional challenges in terms of orthologue identification and sequence divergence.

Promising Systems for Future Study

While genome-wide approaches are now commonly applied to address the genomic basis of organism–environment interaction (Aubin-Horth & Renn 2009; Renn & Siemens 2010; Whitehead 2012), few behavioural studies have addressed plasticity in a comparative context. None the less, there are several promising systems that fulfil the first three criteria above and that are poised to capitalize on the fourth. By applying the proposed framework to promising systems such as these, we will advance our understanding of the evolutionary processes that underlie evolved changes in behavioural plasticity.

The postglacial radiation of the threespine stickleback fish offers an excellent opportunity to study the evolution of behavioural plasticity and its genomic underpinnings (McKinnon & Rundle 2002; Kitano et al. 2010; Wark et al. 2011). Here, behavioural phenotypes can be evaluated in the oceanic threespine stickleback fish, which represent the ancestral condition, as well as for the derived freshwater populations (Hohenlohe et al. 2010). Among these fish, clear associations have been established between ecological contexts and phenotypic attributes such as foraging (McPhail 1994), antipredator behaviour (Foster 1994; Huntingford et al. 1994), boldness (Huntingford 1982; Alvarez & Bell 2007; Dingemanse et al. 2007), schooling (Wark et al. 2011) and courtship (Foster 1995; Foster et al. 1998). Many of these ecologically correlated behaviours (reviewed in Bell & Foster 1994) are retained under controlled experimental conditions (e.g. Lacasse & Aubin-Horth 2012). Furthermore, a wealth of recently developed molecular tools has made it possible to address genetic mechanisms of adaptive differentiation in this system (Cresko et al. 2007; Kingsley & Peichel 2007; Miller et al. 2007; Jones et al. 2012). Although the evolution of behavioural norms of reaction have been examined only to a limited degree in the species group (Shaw et al. 2007; Foster & Wund 2011), there exists potential to explore the patterns of gene expression plasticity that have evolved in response to repeated, parallel patterns of selection (Bell & Stamps 2004; Bell & Sih 2007; Bell & Robinson 2011; Yibayiri et al. 2011; Sanogo et al. 2012).

Among the swordtails and platyfishes (genus *Xiphophorus*) there are many well studied species that show plasticity in male reproductive strategies (Rios-Cardenas & Morris 2011). This plasticity is either developmental (irreversible) or context dependent (reversible). Plasticity appears to be ancestral, and there are species in which male behavioural plasticity has been lost or reduced (Ryan & Wagner 1987; Rauchenberger et al. 1990; Morris et al. 2001, 2005). Researchers are currently examining the hypothesis that during the evolution of this clade (including *Xiphophorus pygmaeus* and *Xiphophorus continens*), genetic assimilation of a previously plastic reproductive strategy has occurred (M. Morris, personal communication). In combination with related studies that identify (Cummings et al. 2008) and localize (Lynch et al. 2012) gene expression differences in female brains during mate choice, this promises to be an exciting system for mechanistic studies of genetic assimilation.

In a more general sense, urbanization represents a major shift of environment and is likely to favour behavioural phenotypes that represent the extremes of a previously plastic behavioural response. The effects of urbanization on behaviour have been examined in birds in the context of foraging (e.g. Thomas et al. 2003), personality (e.g. Evans et al. 2010; Scales et al. 2011), stress (e.g. Atwell et al. 2012) and communication (e.g. Slabbekoorn & Ripmeester 2008; Slabbekoorn 2013), and some cases of altered behavioural plasticity have been identified. For example, recent research has found evolved changes in plastic communication characteristics in some bird species (Hanna et al. 2011). Combined with years of research on the neural circuitry involved in avian song (Brenowitz et al. 1997) and recent genomic studies of this process (London & Clayton 2010), a investigation of genetic accommodation for plastic communication may be possible, especially as genomic resources are further developed for certain avian species (e.g. finch: Warren et al. 2010; great tit: Santure et al. 2011; junco: Peterson et al. 2012).

These examples represent but a few of the many exciting possibilities. As genomic tools become available for emerging model organism, we will be able to address questions about the evolution of behavioural plasticity in a wide variety of taxa. For example, in addition to those described above, genomic resources are being developed for a range of taxa that have been previously studied at the level of behavioural, developmental, physiological or ecological plasticity, such as dung beetles (Snell-Rood et al. 2011), pea aphids (Simon et al. 2011), butterfly groups (Beldade et al. 2008) and pipefish (Mobley et al. 2011) to name a few.

TOOLS FOR EXPERIMENTAL GENOMICS

A number of techniques exist for characterizing gene expression associated with plastic and constitutive behaviours. These techniques allow us to consider the full transcriptome, the set of expressed genes, for an individual. The expression level for each gene in the transcriptome results from the interaction of the genotype and the environment. Most current techniques available to quantify gene expression provide a measure of the expression level of mRNA transcripts that will be translated into protein (but see Hackenberg 2012 for microRNA profiling) that ultimately influence an organism's phenotype.

Microarray analysis, although first developed in model organisms, has provided a wealth of gene expression data for nonmodel species, ranging from fish (Oleksiak et al. 2002; Cossins & Crawford 2005) to bees (Whitfield et al. 2002) to crustaceans (Stillman et al. 2006) to birds (Replogle et al. 2008), many of which were designed explicitly for comparative studies (Bar-Or et al. 2007; Buckley 2007). Microarrays provided the first opportunity for researchers to evaluate gene expression on a genome-wide scale in nonmodel organisms. Because microarray platforms developed in one species can be used in closely related species ('heterologous hybridization'; Renn et al. 2004) with appropriate controls (Machado et al. 2009; Renn et al. 2010), these techniques greatly expanded the number of species that could be investigated on a genomic scale and have been successfully used to investigate diverse phenotypes such as thermal tolerance in fish (Buckley et al. 2005), the molecular mechanisms of complex social structure (Aubin-Horth et al. 2007; Schumer et al. 2011), numerous studies in salmonids (e.g. Aubin-Horth et al. 2005; Pavey et al. 2010; Hutchings 2011) and the molecular mechanisms involved in mating tactics (Machado et al. 2009).

While gene expression data on a genome level has, for the past 20 years, relied primarily on microarray hybridization techniques,

advances in DNA sequencing technology have introduced RNA sequencing as a technique that is quickly replacing microarrays in many fields. Several excellent reviews cover the application of these techniques to nonmodel organisms (Gibbons et al. 2009; Ekblom & Galindo 2011; Ward et al. 2012).

The term 'RNA-seq' describes a variety of methods based on deep sequencing and quantitative analysis (Wang et al. 2009; Wilhelm & Landry 2009). RNA-seq involves direct sequencing to produce short (50–100 base pairs) reads from a cDNA template (reverse-transcribed from the RNA sample of interest) and expression can be quantified by counting the number of reads produced for each gene (Costa et al. 2010). This technique has many advantages over microarray hybridization because it generates large amounts of sequence information in addition to gene expression information (Robertson et al. 2010; Grabherr et al. 2011). RNA-seq also offers a greater dynamic range (Wang et al. 2009) and thus the ability to quantify even genes with very low expression level, as may be the case for behaviourally relevant genes. However, some of these advantages come at a financial and computational cost. While full transcriptome information has become available for many nonmodel species (Fraser et al. 2011; Santure et al. 2011), few studies have used RNA-seq to identify condition-dependent or population-level expression differences (but see: white fish: Jeukens et al. 2010; Wolf et al. 2010). Because sequencing multiple individuals dramatically increases costs, microarrays may still present a cost-effective option for population-level studies. Another limitation is that some analysis pipelines rely on a well assembled and annotated reference genome to which the reads can be aligned (Labaj et al. 2011). Alternate pipelines do not require a fully assembled genome but instead use as reference a transcriptome (Li & Dewey 2011) that can be assembled de novo from the RNA-seq data collected for the particular experiment (Zerbino & Birney 2008; Grabherr et al. 2011), While sequencing approaches such as RNA-seq have many benefits, developing a transcriptome without a genome sequence as a resource can be challenging, particularly for diverged populations, due to ortho-logues and paralogues, as well as the high level of polymorphism found in some natural populations (Robertson et al. 2010; Harrison 2012; Yandell & Ence 2012).

In addition to microarrays and RNA-seq, researchers are using combinations of tools to obtain comparative gene expression data at low costs. Matz and colleagues (Meyer et al. 2011) used a restriction digest-based procedure to reduce sequencing costs, but they still provided counts of genes and used only a transcriptome assembly as a reference. In another creative experiment investigating whether genes with environmentally sensitive expression experience more rapid sequence divergence, Pfennig and colleagues (Leichty et al. 2012) characterized expression bias and sequence divergence in four species of frog using a creative combination of heterologous hybridization to generate a candidate list, and transcriptome sequencing to both characterize divergence and facilitate PCR-based techniques to validate the evolution of gene expression. In this way they revealed that increased rates of sequence divergence predated the evolution of plasticity of gene expression level.

Of particular concern to researchers investigating the gene expression mechanisms associated with behaviour is the total amount of sequence data that may be required to obtain sufficient information about genes that are expressed at low levels. For some samples, over 75% of all RNA-seq data accounts for less than 7% of the known transcriptome (Labaj et al. 2011); more sequence information (or 'depth') is required to accurately detect genes with low expression levels. Often the most highly expressed genes will not be the genes of interest in behaviour studies. Because of the high cost of this technique, many early studies include inadequate biological and technical replication or flawed experimental design,

limiting the biological inference that has been possible. However, the increased sequencing depth required to investigate genes with low levels of expression will become more feasible as sequencing costs continue to decrease.

CONCLUSIONS

Understanding the gene expression plasticity associated with behavioural plasticity and how it evolves relative to the behavioural phenotype itself is a lofty but feasible goal. In this review, we describe a framework including six categories for the gene expression norms of reaction that could be responsible for genetic accommodation at the level of behavioural phenotype. While genetic assimilation of gene expression plasticity is one category that could underlie canalization of behavioural plasticity, we emphasize that many other categories, including those in which the evolved gene expression is more plastic (i.e. evolved gene expression plasticity or accommodated gene expression plasticity), may be involved in producing an environmentally invariant behavioural phenotype. Using modern genomic techniques and a carefully chosen study system (e.g. case studies discussed above), it will be possible to determine the relative importance of each of these norm of reaction categories during the evolution of behavioural plasticity. Based on theoretical models suggesting that co-option of complex polygenic traits is a more likely evolutionary pathway than is the evolution of new mechanisms to produce the same phenotype, genetic assimilation of gene expression plasticity is predicted to be prevalent (Espinosa-Soto et al. 2011). However, it will be interesting to learn the degree to which this prediction holds for different phenotypes or different functional and structural classes of genes. For example, Snell-Rood (2013) differentiates between two types of behavioural plasticity, 'context-dependent behavioural plasticity' and 'developmental behavioural plasticity,' the distinction being that of timescale. Regardless of whether these definitions create a dichotomous distinction or represent a timescale gradient, it is interesting to ask to what extent the evolved patterns of gene expression associated with these different types of plasticity are shared versus independent.

The categories of evolved gene expression plasticity that we present in relation to genetic accommodation of behavioural plasticity apply broadly to any evolutionary change in behavioural plasticity. We emphasize that the evolved patterns of gene expression plasticity may or may not mirror the reaction norms that can be drawn for the evolved plasticity of behavioural phenotype. However, by determining the overall set of evolved patterns of gene expression we can begin to elucidate the molecular processes that accompany the evolution of behavioural plasticity. These categories are not novel but rather are borrowed from theoretical and empirical studies on the evolution of plasticity and the evolution of gene expression. Here we have explained how these categories pertain to the evolution of behaviour, and we have outlined approaches and methodological issues. Although next-generation sequencing techniques make such questions approachable, new model organisms must be selected carefully, and, cost still presents a limitation for experimental designs that require multiple comparisons and both biological and technical replication. As animal behaviourists and behavioural ecologists move to genomic techniques, it is essential to retain rigour in experimental design and keep these methodological concerns in mind.

participants in the symposium and other attendees at the meeting with whom conversations helped clarify and solidify these ideas. Several of the cited authors provided clarification and context of their work and Leigh Latta offered comments on earlier versions of the manuscript. The project has been enjoyable and eye opening.

References

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzmán-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J. L., Southey, B. R., Rodriguez-Zas, S. & Robinson, G. E. 2009. Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proceedings of the National Academy of Sciences, U.S.A.*, **106**, 15400–15405.

Alvarez, D. & Bell, A. M. 2007. Sticklebacks from streams are more bold than sticklebacks from ponds. *Behavioural Processes*, **76**, 215–217.

Ament, S. A., Blatti, C. A., Alaux, C., Wheeler, M. M., Toth, A. L., Le Conte, Y., Hunte, G. J., Guzmán-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J. L., et al. 2012. New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. *Proceedings of the National Academy of Sciences, U.S.A.*, **109**, E1801–E1810.

Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W. & Ketterson, E. D. 2012. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, **23**, 960–969.

Aubin-Horth, N. & Renn, S. C. P. 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, **18**, 3763–3780.

Aubin-Horth, N., Landry, C. R., Letcher, B. H. & Hofmann, H. A. 2005. Alternative life histories shape brain gene expression profiles in males of the same population. *Proceedings of the Royal Society B*, **272**, 1655–1662.

Aubin-Horth, N., Desjardins, J. K., Martei, Y. M., Balshine, S. & Hofmann, H. A. 2007. Masculinized dominant females in a cooperatively breeding species. *Molecular Ecology*, **16**, 1349–1358.

Aubret, F. & Shine, R. 2009. Genetic assimilation and the post-colonisation erosion of phenotypic plasticity in island tiger snakes. *Current Biology*, **19**, 1932–1936.

Badyaev, A. V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society B*, **272**, 877–886.

Badyaev, A. V. 2009. Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Philosophical Transactions of the Royal Society B*, **364**, 1125–1141.

Baldwin, J. 1896. A new factor in evolution. *Science*, **4**, 139.

Baldwin, J. M. 1902. *Development and Evolution*. New York: Macmillan.

Barlow, G. W. & Lee, J. S. F. 2005. Sex-reversed dominance and aggression in the cichlid fish *Julidochromis marlieri*. *Annales Zoologici Fennici*, **42**, 477–483.

Bar-Or, C., Novikov, E., Reiner, A., Czosnek, H. & Koltai, H. 2007. Utilizing microarray spot characteristics to improve cross-species hybridization results. *Genomics*, **90**, 636–645.

Beldade, P., McMillan, W. O. & Papanicolaou, A. 2008. Butterfly genomics eclosing. *Heredity*, **100**, 150–157.

Beldade, P., Mateus, A. R. & Keller, R. A. 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, **20**, 1347–1363.

Bell, M. A. & Foster, S. A. (Eds). 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press.

Bell, A. M. & Robinson, G. E. 2011. Behavior and the dynamic genome. *Science*, **332**, 1161–1162.

Bell, A. M. & Sih, A. 2007. Exposure to predation generates personality in threespine sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, **10**, 828–834.

Bell, A. M. & Stamps, J. A. 2004. Development of behavioural differences between individuals and populations of sticklebacks, *Gasteseus aculeatus*. *Animal Behaviour*, **68**, 1339–1348.

Bloch, G. & Grozinger, C. M. 2011. Social molecular pathways and the evolution of bee societies. *Philosophical Transactions of the Royal Society B*, **366**, 2155–2170.

Brenowitz, E. A., Margoliash, D. & Nordeen, K. W. 1997. An introduction to birdsong and the avian song system. *Journal of Neurobiology*, **33**, 495–500.

Buckley, B. A. 2007. Comparative environmental genomics in non-model species: using heterologous hybridization to DNA-based microarrays. *Journal of Experimental Biology*, **210**, 1602–1606.

Buckley, B. A., Gracey, A. Y. & Somero, G. N. 2005. Gene expression profiling in a eurythermic goby exposed to an elevated temperature cycle. *Comparative Biochemistry and Physiology A, Supplement*, **141**, S349–S357.

Carroll, S. P. & Corneli, P. S. 1999. The evolution of behavioral norms of reaction as a problem in ecological genetics: theory, methods and data. In: *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms* (Ed. by S. A. Foster & J. A. Endler), pp. 52–68. New York: Oxford University Press.

Chapman, L. J., Galis, F. & Shinn, J. 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters*, **3**, 387–393.

Cheviron, Z. A., Whitehead, A. & Brumfield, R. T. 2008. Transcriptomic variation and plasticity in rufous-collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient. *Molecular Ecology*, **17**, 4556–4569.

Cossins, A. R. & Crawford, D. L. 2005. Opinion: fish as models for environmental genomics. *Nature Reviews Genetics*, **6**, 324–333.

Costa, V., Angelini, C., De Feis, I. & Ciccodicola, A. 2010. Uncovering the complexity of transcriptomes with RNA-seq. *Journal of Biomedicine and Biotechnology*, **2010**, Article ID 853916, http://dx.doi.org/10.1155/2010/853916.

Cresko, W., McGuigan, K., Phillips, P. & Postlethwait, J. 2007. Studies of threespine stickleback developmental evolution: progress and promise. *Genetica*, **129**, 105–126.

Crispo, E. 2007. The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*, **61**, 2469–2479.

Cummings, M. E., Larkins-Ford, J., Reilly, C. R. L., Wong, R. Y., Ramsey, M. & Hofmann, H. A. 2008. Sexual and social stimuli elicit rapid and contrasting genomic responses. *Proceedings of the Royal Society B*, **275**, 393–402.

Dingemanse, N. J., Wright, J., Kazem, A. J. N., Thomas, D. K., Hickling, R. & Dawnay, N. 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology*, **76**, 1128–1138.

Dingemanse, N. J., Kazem, A. J. N., Reale, D. & Wright, J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, **25**, 81–89.

Duckworth, R. A. 2009. The role of behavior in evolution: a search for mechanism. *Evolutionary Ecology*, **23**, 513–531.

Dukas, R. 2013. Effects of learning on evolution: robustness, innovation and speciation. *Animal Behaviour*, **85**, 1023–1030.

Ekblom, R. & Galindo, J. 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, **107**, 1–15.

Espinosa-Soto, C., Martin, O. C. & Wagner, A. 2011. Phenotypic plasticity can facilitate adaptive evolution in gene regulatory circuits. *BMC Evolutionary Biology*, **11**, 5, http://dx.doi.org/10.1186/1471-2148-11-5.

Evans, J., Boudreau, K. & Hyman, J. 2010. Behavioural syndromes in urban and rural populations of song sparrows. *Ethology*, **116**, 588–595.

Forsgren, E., Amundsen, T., Borg, A. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. *Nature*, **429**, 551–554.

Foster, S. A. 1994. Inference of evolutionary pattern: diversionary displays of threespine stickleback. *Behavioral Ecology*, **5**, 114–121.

Foster, S. A. 1995. Understanding the evolution of behaviour in threespine stickleback: the value of geographic variation. *Behaviour*, **132**, 1107–1129.

Foster, S. A. 2013. Evolution of behavioural phenotypes: influences of ancestry and expression. *Animal Behaviour*, **85**, 1061–1075.

Foster, S. A. & Wund, M. A. 2011. Plasticity and evolution in the adaptive radiation of the threespine stickleback. In: *Epigenetics: Linking Genotype and Phenotype in Development and Evolution* (Ed. by B. Hallgrimsson & B. K. Hall), pp. 317–336. Berkeley: University of California Press.

Foster, S. A., Scott, R. J. & Cresko, W. A. 1998. Nested biological variation and speciation. *Philosophical Transactions of the Royal Society of London, Series B*, **353**, 207–218.

Fraser, B. A., Weadick, C. J., Janowitz, I., Rodd, F. H. & Hughes, K. A. 2011. Sequencing and characterization of the guppy (*Poecilia reticulata*) transcriptome. *BMC Genomics*, **12**, 202, http://dx.doi.org/10.1186/1471-2164-12-202.

Ghalambor, C. K., McKay, J. K., Carrol, S. P. & Reznick, D. N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Ghalambor, C. K., Angeloni, L. M. & Carroll, S. P. 2010. Behavior as phenotypic plasticity. In: *Evolutionary Behavioral Ecology* (Ed. by D. F. Westneat & C. F. Fox), pp. 90–107. Chicago: Oxford University Press.

Gibbons, J. G., Janson, E. M., Hittinger, C. T., Johnston, M., Abbot, P. & Rokas, A. 2009. Benchmarking next-generation transcriptome sequencing for functional and evolutionary genomics. *Molecular Biology and Evolution*, **26**, 2731–2744.

Gibson, G. & Dworkin, I. 2004. Uncovering cryptic genetic variation. *Nature Reviews Genetics*, **5**, 681–690.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. 2011. Full-length transcriptome assembly from RNA-seq data without a reference genome. *Nature Biotechnology*, **29**, 644–652.

Hackenberg, M. 2012. MicroRNA expression profiling and discovery. In: *Bioinformatics for High Throughput Sequencing* (Ed. by M. Hackenberg), pp. 191–208. New York: Springer.

Hanna, D., Blouin-Demers, G., Wilson, D. R. & Mennill, D. J. 2011. Anthropogenic noise affects song structure in red-winged blackbirds (*Agelaius phoeniceus*). *Journal of Experimental Biology*, **214**, 3549–3556.

Harrison, R. J. 2012. Understanding genetic variation and function: the applications of next generation sequencing. *Seminars in Cell and Developmental Biology*, **2**, 230–236.

Heil, M., Greiner, S., Meimberg, H., Kruger, R., Noyer, J. L., Heubl, G., Linsenmair, K. E. & Boland, W. 2004. Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature*, **430**, 205–208.

Hodgins-Davis, A., Adomas, A. B., Warringer, J. & Townsend, J. P. 2012. Abundant gene-by-environment interactions in gene expression reaction norms to copper within *Saccharomyces cerevisiae*. *Genome Biology and Evolution*, **4**, 1061–1079.

Hohenlohe, P. A., Bassham, S., Ettern, P. D., Stiffler, N., Johnson, E. A. & Cresko, W. A. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genetics*, **6**, e1000862.

Huey, R. B., Hertz, P. E. & Sinervo, B. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. *American Naturalist*, **161**, 357–366.

Huntingford, F. A. 1982. Do interspecific and intraspecific aggression vary in relation to predation pressure in sticklebacks? *Animal Behaviour*, **30**, 909–916.

Huntingford, F. A., Wright, P. J. & Tierney, J. F. 1994. Adaptive variation in anti-predator behaviour in threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M. A. Bell & S. A. Foster), pp. 277–296. Oxford: Oxford University Press.

Hutchings, J. A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. *Heredity*, **106**, 421–437.

Itzkowitz, M., Santangelo, N. & Richter, M. 2001. Parental division of labour and the shift from minimal to maximal role specializations: an examination using a biparental fish. *Animal Behaviour*, **61**, 1237–1245.

Linsenmair, K. E. & Boland, W. 2004. Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature*, **430**, 205–208.

Jeukens, J., Renaut, S., St-Cyr, J., Nolte, A. W. & Bernatchez, L. 2010. The transcriptomics of sympatric dwarf and normal lake whitefish (*Coregonus clupeaformis* spp., Salmonidae) divergence as revealed by next-generation sequencing. *Molecular Ecology*, **19**, 5389–5403.

Johnson, B. R. & Tsutsui, N. D. 2011. Taxonomically restricted genes are associated with the evolution of sociality in the honeybee. *BMC Genomics*, **12**, 164, http://dx.doi.org/10.1186/1471-2164-12-164.

Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., Swofford, R., Pirun, M., Zody, M. C., White, S., et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, **484**, 55–61.

Kasumovic, A. A. 2013. The multidimensional consequences of the juvenile environment: towards an integrative view of the adult phenotype. *Animal Behaviour*, **85**, 1049–1059.

Keogh, J. S., Scott, I. A. W. & Hayes, C. 2005. Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. *Evolution*, **59**, 226–233.

Khaitovich, P., Weiss, G., Lachmann, M., Hellmann, I., Enard, W., Muetzel, B., Wirkner, U., Ansorge, W. & Paabo, S. 2004. A neutral model of transcriptome evolution. *PLoS Biology*, **2**, 682–689.

Kingsley, D. M. & Peichel, C. L. 2007. The molecular genetics of evolutionary change in sticklebacks. In: *Biology of the Three-spined Stickleback* (Ed. by S. Östlund-Nilsson, I. Mayer & F. A. Huntingford), pp. 41–81. Boca Raton, Florida: CRC Press.

Kitano, J., Lema, S. C., Luckenbach, J. A., Mori, S., Kawagishi, Y., Kusakabe, M., Swanson, P. & Peichel, C. L. 2010. Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. *Current Biology*, **20**, 2124–2130.

Labaj, P. P., Leparc, G. G., Linggi, B. E., Markillie, L. M., Wiley, H. S. & Kreil, D. P. 2011. Characterization and improvement of RNA-seq precision in quantitative transcript expression profiling. *Bioinformatics*, **27**, I383–I391.

Lacasse, J. & Aubin-Horth, N. 2012. A test of the coupling of predator defense morphology and behavior variation in two threespine stickleback populations. *Current Zoology*, **58**, 53–65.

Landry, C. R., Lemos, B., Rifkin, S. A., Dickinson, W. J. & Hartl, D. L. 2007. Genetic properties influencing the evolvability of gene expression. *Science*, **317**, 118–121.

Latta, L. C., Weider, L. J., Colbourne, J. K. & Pfrender, M. E. 2012. The evolution of salinity tolerance in *Daphnia*: a functional genomics approach. *Ecology Letters*, **15**, 794–802.

Lee, G., Kiergaard, M., Gelembiuk, G. W., Eads, B. D. & Posavi, M. 2011. Pumping ions: rapid parallel evolution of ionic regulation following habitat invasions. *Evolution*, **65**, 2229–2244.

Leichty, A. R., Pfennig, D. W., Jones, C. D. & Pfennig, K. S. 2012. Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. *Integrative and Comparative Biology*, **52**, 16–30.

Li, B. & Dewey, C. N. 2011. RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinformatics*, **12**, 323, http://dx.doi.org/10.1186/1471-2105-12-323.

London, S. E. & Clayton, D. F. 2010. The neurobiology of zebra finch song: insights from gene expression studies. *Emu*, **110**, 219–232.

Lynch, K. S., Ramsey, M. E. & Cummings, M. E. 2012. The mate choice brain: comparing gene profiles between female choice and male coercive poeciliids. *Genes Brain and Behavior*, **11**, 222–229.

Machado, H. E., Pollen, A. A., Hofmann, H. A. & Renn, S. C. P. 2009. Interspecific profiling of gene expression informed by comparative genomic hybridization: a review and a novel approach in African cichlid fishes. *Integrative and Comparative Biology*, **49**, 644–659.

McKinnon, J. S. & Rundle, H. D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution*, **17**, 480–488.

McManus, C. J., Coolon, J. D., Duff, M. O., Eipper-Mains, J., Graveley, B. R. & Wittkopp, P. J. 2010. Regulatory divergence in *Drosophila* revealed by mRNA-seq. *Genome Research*, **20**, 816–825.

McPhail, J. D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M. A. Bell & S. A. Foster), pp. 399–437. Oxford: Oxford University Press.

Matzkin, L. M. 2012. Population transcriptomics of cactus host shifts in *Drosophila mojavensis*. *Molecular Ecology*, **21**, 2428–2439.

Matzkin, L. M., Watts, T. D., Bitler, B. G., Machado, C. A. & Markow, T. A. 2006. Functional genomics of cactus host shifts in *Drosophila mojavensis*. *Molecular Ecology*, **15**, 4635–4643.

Mery, F. & Kawecki, T. J. 2004. The effect of learning on experimental evolution of resource preference in *Drosophila melanogaster*. *Evolution*, **58**, 757–767.

Meyer, E., Aglyamova, G. V. & Matz, M. V. 2011. Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-seq procedure. *Molecular Ecology*, **20**, 3599–3616.

Miller, M. R., Dunham, J. P., Amores, A., Cresko, W. A. & Johnson, E. A. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Research*, **17**, 240–248.

Mobley, K. B., Small, C. M. & Jones, A. G. 2011. The genetics and genomics of Syngnathidae: pipefishes, seahorses and seadragons. *Journal of Fish Biology*, **78**, 1624–1646.

Morgan, C. L. 1896. *Habit and Instinct*. London: Arnold.

Morris, M. R., de Queiroz, K. & Morizot, D. C. 2001. Phylogenetic relationships among populations of northern swordtails (*Xiphophorus*) as inferred from allozyme data. *Copeia*, **2001**, 65–81.

Morris, M. R., Moretz, J. A., Farley, K. & Nicoletto, P. 2005. The role of sexual selection in the loss of sexually selected traits in the swordtail fish *Xiphophorus continens*. *Animal Behaviour*, **69**, 1415–1424.

Odling-Smee, F. J. 1988. Niche constructing phenotypes. In: *The Role of Behavior in Evolution* (Ed. by H. C. Plotkin), pp. 1–17. Cambridge, Massachusetts: MIT Press.

Oleksiak, M. F., Churchill, G. A. & Crawford, D. L. 2002. Variation in gene expression within and among natural populations. *Nature Genetics*, **32**, 261–266.

Osborn, H. F. 1897. Organic selection. *Science*, **15**, 583–587.

Palmer, A. R. 2011. Developmental plasticity and the origin of novel forms: unveiling cryptic genetic variation via 'use and disuse'. *Journal of Experimental Zoology (Molecular Development and Evolution)*, **318B**, 466–479.

Pavey, S. A., Collin, H., Nosil, P. & Rogers, S. M. 2010. The role of gene expression in ecological speciation. *Annals of the New York Academy of Sciences*, **1206**, 110–129.

Peterson, M. P., Whittaker, D. J., Ambreth, S., Sureshchandra, S., Mockatis, K., Buechlein, A., Podicheti, R., Choi, J. H., Lai, Z., Colbourne, J. K., et al. 2012. De novo transcriptome sequencing in a songbird, the dark-eyed junco (*Junco hyemalis*): genomic tools for an ecological model system. *BMC Genomics*, **13**, 305, http://dx.doi.org/10.1186/1471-2164-13-305.

Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D. & Moczek, A. P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, **26**, 459–467.

Pigliucci, M. & Murren, C. J. 2003. Genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? *Evolution*, **57**, 1455–1464.

Pigliucci, M., Murren, C. J. & Schlichting, C. D. 2006. Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.

Price, T. D., Qvarnstrom, A. & Irwin, D. E. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B*, **270**, 1433–1440.

Qiu, J. & Cui, X. 2010. Evaluation of a statistical equivalence test applied to microarray data. *Journal of Biopharmaceutical Statistics*, **20**, 240–266.

Rauchenberger, M., Kallman, K. D. & Morizot, D. C. 1990. Monophyly and geography of the Río Pánuco Basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *American Museum Novitates*, **2975**, 1–41.

Remy, J.-J. 2010. Stable inheritance of an acquired behavior in *Caenorhabditis elegans*. *Current Biology*, **20**, R877–R878.

Renn, S. C. P. & Siemens, D. H. 2010. Ecological genomics-changing perspectives on Darwin's basic concerns. *Molecular Ecology*, **19**, 3025–3030.

Renn, S. C. P., Aubin-Horth, N. & Hofmann, H. A. 2004. Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. *BMC Genomics*, **5**, 42, http://dx.doi.org/10.1186/1471-2164-5-42.

Renn, S. C. P., Machado, H. E., Jones, A., Soneji, K., Kulathinal, R. J. & Hofmann, H. A. 2010. Using comparative genomic hybridization to survey genomic sequence divergence across species: a proof-of-concept from *Drosophila*. *BMC Genomics*, **11**, 271, http://dx.doi.org/10.1186/1471-2164-11-271.

Replogle, K., Arnold, A. P., Ball, G. F., Band, M., Bensch, S., Brenowitz, E. A., Dong, S., Drnevich, J., Ferris, M., George, J. M., et al. 2008. The songbird neurogenomics (SoNG) initiative: community-based tools and strategies for study of brain gene function and evolution. *BMC Genomics*, **9**, 131, http://dx.doi.org/10.1186/1471-2164-9-131.

Rios-Cardenas, O. & Morris, M. R. 2011. Precopulatory sexual selection in Poeciliidae. In: *Ecology and Evolution of Poeciliid Fishes* (Ed. by J. Evans, A. Pilastro & I. Schlupp), pp. 187–196. Chicago: University of Chicago Press.

Robertson, G., Schein, J., Chiu, R., Corbett, R., Field, M., Jackman, S. D., Mungall, K., Lee, S., Okada, H. M., Qian, J. Q., et al. 2010. De novo assembly and analysis of RNA-seq data. *Nature Methods*, **7**, 909–912.

Rodríguez, R. L., Rebar, D. & Fowler-Finn, K. D. 2013. The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour*, **85**, 1041–1047.

Rosin, D., Hornung, G., Tirosh, I., Gispan, A. & Barkai, N. 2012. Promoter nucleosome organization shapes the evolution of gene expression. *PLoS Genetics*, **8**, e1002579.

Rutherford, S. L. & Lindquist, S. 1998. Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.

Ryan, M. J. & Wagner, W. E. 1987. Asymmetries in mating preferences between species: female swordtails prefer heterospecific males. *Science*, **236**, 595–597.

Sanogo, Y. O., Band, M. A., Blatti, C., Sinha, S. & Bell, A. M. 2012. Transcriptional regulation of brain gene expression in response to a territorial intrusion. *Proceedings of the Royal Society B*, **279**, 4929–4938.

Santure, A. W., Gratten, J., Mossman, J. A., Sheldon, B. C. & Slate, J. 2011. Characterisation of the transcriptome of a wild great tit *Parus major* population by

next generation sequencing. *BMC Genomics*, **12**, 283, http://dx.doi.org/10.1186/1471-2164-12-283.

Scales, J., Hyman, J. & Hughes, M. 2011. Behavioral syndromes break down in urban song sparrow populations. *Ethology*, **117**, 887–895.

Schlichting, C. D. 2004. The role of phenotypic plasticity in diversification. In: *Phenotypic Plasticity: Functional and Conceptual Approaches* (Ed. by T. J. DeWitt & S. M. Scheiner), pp. 191–200. Oxford: Oxford University Press.

Schlichting, C. D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences*, **1133**, 187–203.

Schlichting, C. D. & Pigliucci, M. 1998. *Phenotypic Evolution: a Reaction Norm Perspective.* Sunderland, Massachusetts: Sinauer.

Schmalhausen, I. I. 1949. *Factors of Evolution.* Philadelphia: Blakiston.

Schumer, M. E., Krishnakant, K. & Renn, S. C. P. 2011. Comparative gene expression profiles for highly similar aggressive phenotypes in male and female cichlid fishes (*Julidochromis*). *Journal of Experimental Biology*, **214**, 3269–3278.

Scoville, A. G. & Pfrender, M. E. 2010. Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proceedings of the National Academy of Sciences, U.S.A.*, **107**, 4260–4263.

Sen Sarma, M., Rodriguez-Zas, S. L., Hong, F., Zhong, S. & Robinson, G. E. 2009. Transcriptomic profiling of central nervous system regions in three species of honey bee during dance communication behavior. *PLoS One*, **4**, e6408.

Shaw, K. A., Scotti, M. L. & Foster, S. A. 2007. Ancestral plasticity and the evolutionary diversification of courtship behaviour in threespine stickleback. *Animal Behaviour*, **73**, 415–422.

Simon, J. C., Pfrender, M. E., Tollrian, R., Tagu, D. & Colbourne, J. K. 2011. Genomics of environmentally induced phenotypes in 2 extremely plastic arthropods. *Journal of Heredity*, **102**, 512–525.

Slabbekoorn, H. 2013. Songs of the city; noise-dependent plasticity in the acoustic phenotype of urban birds. *Animal Behaviour*, **85**, 1089–1099.

Slabbekoorn, H. & Ripmeester, E. A. P. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. *Molecular Ecology*, **17**, 72–83.

Snell-Rood, E. C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour*, **85**, 1004–1011.

Snell-Rood, E. C., Van Dyken, J. D., Cruickshank, T., Wade, M. J. & Moczek, A. P. 2010. Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *Bioessays*, **32**, 71–81.

Snell-Rood, E. C., Cash, A., Han, M. V., Kijimoto, T., Andrews, J. & Moczek, A. P. 2011. Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn-polyphenic beetles. *Evolution*, **65**, 231–245.

Spalding, D. 1873. Instinct with original observations on young animals. *Macmillan's Magazine*, **27**, 282–293.

Stillman, J. H., Teranishi, K. S., Tagmount, A., Lindquist, E. A. & Brokstein, P. B. 2006. Construction and characterization of EST libraries from the porcelain crab, *Petrolisthes cinctipes*. *Integrative and Comparative Biology*, **46**, 919–930.

Suzuki, Y. & Nihout, H. F. 2006. Evolution of a polyphenism by genetic accommodation. *Science*, **311**, 650–652.

Thomas, K., Kvitek, R. G. & Bretz, C. 2003. Effects of human activity on the foraging behavior of sanderlings *Calidris alba*. *Biological Conservation*, **109**, 67–71.

Tirosh, I., Barkai, N. & Verstrepen, K. J. 2009. Promoter architecture and the evolvability of gene expression. *Journal of Biology*, **8**, 95.

Toth, A. L., Varala, K., Newman, T. C., Miguez, F. E., Hutchison, S. K., Willoughby, D. A., Simons, J. F., Egholm, M., Hunt, J. H., Hudson, M. E., et al. 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science*, **318**, 441–444.

Via, S. & Lande, R. 1985. Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution*, **39**, 505–522.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. *Nature*, **150**, 563–565.

Waddington, C. H. 1952. Selection of the genetic basis for an acquired character. *Nature*, **169**, 278.

Waddington, C. H. 1953. Genetic assimilation of an acquired character. *Evolution*, **7**, 118–126.

Waddington, C. H. 1961. Genetic assimilation. *Advances in Genetics*, **10**, 257–293.

Wang, Z., Gerstein, M. & Snyder, M. 2009. RNA-seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, **10**, 57–63.

Ward, J. A., Ponnala, L. & Weber, C. A. 2012. Strategies for transcriptome analysis in nonmodel plants. *American Journal of Botany*, **99**, 267–276.

Wark, A. R., Greenwood, A. K., Taylor, E. M., Yoshida, K. & Peichel, C. L. 2011. Heritable differences in schooling behavior among threespine stickleback populations revealed by a novel assay. *PLoS One*, **6**, e18316.

Warren, W. C., Clayton, D. F., Ellegren, H., Arnold, A. P., Hillier, L. W., Kunstner, A., Searle, S., White, S., Vilella, A. J., Fairley, S., et al. 2010. The genome of a songbird. *Nature*, **464**, 757–762.

Wcislo, W. T. 1989. Behavioral environments and evolutionary change. *Annual Review of Ecology and Systematics*, **20**, 137–169.

West-Eberhard, M. J. 2003. *Developmental Plasticity and Evolution.* New York: Oxford University Press.

Whitehead, A. 2012. Comparative genomics in ecological physiology: toward a more nuanced understanding of acclimation and adaptation. *Journal of Experimental Biology*, **215**, 884–891.

Whitehead, A., Roach, J. L., Zhang, S. & Galvez, F. 2011. Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. *Proceedings of the National Academy of Sciences, U.S.A.*, **108**, 6193–6198.

Whitehead, A., Roach, J. L., Zhang, S. & Galvez, F. 2012a. Salinity- and population-dependent genome regulatory response during osmotic acclimation in the killifish (*Fundulus heteroclitus*) gill. *Journal of Experimental Biology*, **215**, 1293–1305.

Whitehead, A., Pilcher, W., Champlin, D. & Nacci, D. 2012b. Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings of the Royal Society B*, **279**, 427–433.

Whitfield, C. W., Band, M. R., Bonaldo, M. F., Kumar, C. G., Liu, L., Pardinas, J. R., Robertson, H. M., Soares, M. B. & Robinson, G. E. 2002. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Research*, **12**, 555–566.

Wilhelm, B. T. & Landry, J.-R. 2009. RNA-seq-quantitative measurement of expression through massively parallel RNA-sequencing. *Methods*, **48**, 249–257.

Wolf, J. B. W., Lindell, J. & Backstrom, N. 2010. Speciation genetics: current status and evolving approaches. *Philosophical Transactions of the Royal Society B*, **365**, 1717–1733.

Wund, M. A., Baker, J. A., Clancy, B., Golub, J. L. & Foster, S. A. 2008. A test of the 'flexible stem' model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *American Naturalist*, **172**, 449–462.

Yandell, M. & Ence, D. 2012. A beginner's guide to eukaryotic genome annotation. *Nature Reviews Genetics*, **13**, 329–342.

Yibayiri, O. S., Hankison, S., Band, M., Obregon, A. & Bell, A. M. 2011. Brain transcriptomic response of threespine sticklebacks to cues of a predator. *Brain, Behavior and Evolution*, **77**, 270–285.

Zerbino, D. R. & Birney, E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research*, **18**, 821–829.