MOLECULAR ECOLOGY

Molecular Ecology (2009) doi: 10.1111/j.1365-294X.2009.04313.x

INVITED REVIEW

Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity

NADIA AUBIN-HORTH* and SUSAN C. P. RENN†

*Département de Sciences biologiques, Université de Montréal, Québec, Canada, H2V259, †Department of Biology Reed College, OR, USA 97202, *Present address: Département de Biologie et Institut de biologie intégrative et des systèmes, Université Laval, Québec, Canada, G1V OA6*

Abstract

Phenotypic plasticity is the development of different phenotypes from a single genotype, depending on the environment. Such plasticity is a pervasive feature of life, is observed for various traits and is often argued to be the result of natural selection. A thorough study of phenotypic plasticity should thus include an ecological and an evolutionary perspective. Recent advances in large-scale gene expression technology make it possible to also study plasticity from a molecular perspective, and the addition of these data will help answer long-standing questions about this widespread phenomenon. In this review, we present examples of integrative studies that illustrate the molecular and cellular mechanisms underlying plastic traits, and show how new techniques will grow in importance in the study of these plastic molecular processes. These techniques include: (i) heterologous hybridization to DNA microarrays; (ii) next generation sequencing technologies applied to transcriptomics; (iii) techniques for studying the function of noncoding small RNAs; and (iv) proteomic tools. We also present recent studies on genetic model systems that uncover how environmental cues triggering different plastic responses are sensed and integrated by the organism. Finally, we describe recent work on changes in gene expression in response to an environmental cue that persist after the cue is removed. Such long-term responses are made possible by epigenetic molecular mechanisms, including DNA methylation. The results of these current studies help us outline future avenues for the study of plasticity.

Keywords: DNA methylation, ecological annotation, heterologous hybridization, microarrays, modules, phenotypic plasticity, phosphoproteome, transcriptomics

Received 23 February 2009; revised 26 June 2009; accepted 30 June 2009

Phenotypic plasticity: how ecology modulates the genome

Introduction

Phenotypic plasticity can be defined as re-programming of the genome in response to the environment. A trait is plastic when the same genotype results in the development of a different phenotype depending on the environment the organism faces (Pigliucci 2001; West-Eberhard 2003). Plastic responses range from morphological modifications to drastic changes in physiology, life history and behaviour, and often involve changes in a suite of traits (Pigliucci 2001; West-Eberhard 2003). In many cases, it has been shown that not only is a plastic response adaptive and allows individuals to meet the challenges brought about by a variable environment, but that it can also be costly compared to the constitutive expression of the trait (Pigliucci 2001, 2005). Phenotypic plasticity can be illustrated with the concept of a reaction norm, which is the representation of values that a trait takes in each environment (Fig. 1). Different genotypes may have different reaction norms, meaning

Correspondence: Nadia Aubin-Horth, Fax: 514 343 2293;
E-mail: n.aubin-horth@umontreal.ca

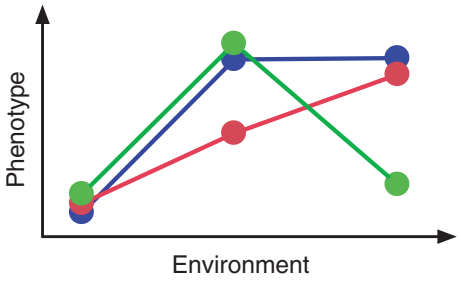

Fig. 1 A reaction norm, the representation of trait values in relation to the environment, is used to define the level of plasticity of that trait. The *y*-axis can also represent three time steps in the development of a plastic phenotype. The red reaction norm shows a progression in phenotype value from environments 1 to 3. The green reaction norm shows a difference in a phenotype only in the intermediate environment. This could also represent a phenotype such as a gene expression level that is higher only during the transition phase of development of a plastic phenotype. The blue reaction norm shows a phenotype that changes during the transition and keeps the same value in the endpoint step of development.

that they respond differently to the same environmental conditions. Indeed, it is possible that one genotype responds to a change in an environmental variable while another does not change, or changes in another direction (Cote *et al.* 2007). The presence of genetic variation for a trait, along with the force of selection, determines whether selection acting on that trait will result in an evolutionary response. Therefore, if different genotypes in a population produce different reaction norms, and if the slope of the reaction norm is positively correlated with fitness, increased plasticity should evolve in that population for that particular trait (Schlichting & Pigliucci 1998). The amount of plasticity can thus evolve differently in distinct populations and species, depending on the selection pressures they each face (for an experimental evolution example, see Suzuki & Nijhout 2006). Furthermore, if certain genotypes are plastic within a population and others are not, it is possible that genetic variation for the trait will be measured as null in one environment (all genotypes develop the same phenotype) and high in another (where plasticity is expressed and genotypes differ in phenotypes, Fig. 1, and see Landry *et al.* 2006). Therefore, the response to selection on the same trait can vary among environments, for the same set of genotypes (Schlichting & Pigliucci 1998). Finally, it has been proposed that plasticity can accelerate evolution by allowing individuals to exploit a novel environment if they possess the capacity to develop a new phenotype when faced with that novel environment (Price *et al.* 2003; Yeh & Price 2004). Implicit in this hypothesis is the idea that plastic individuals will be favoured over nonplastic ones by natural selection. Eventually, the new phenotype may become fixed through genetic assimilation, or there may be an evolutionary shift in the elevation or the slope of the reaction norm, a process referred to as genetic accommodation (West-Eberhard 2003; Pigliucci *et al.* 2006; Suzuki & Nijhout 2006; Crispo 2007).

Determining how plastic developmental changes that occur in response to environmental conditions are coordinated at the molecular and cellular levels is a challenge that combines ecology with developmental biology (Gilbert 2005). Perhaps unsurprisingly, the historic lack of interaction between these fields means that the mechanisms that underlie the development of the alternative phenotypes are still largely unknown for many systems. In the few systems in which they have been studied, the identified mechanisms include changes in gene expression as well as changes in protein and hormone activity (Gilbert 2005). The mechanisms by which an environmental cue is detected and triggers a plastic response at the molecular and cellular level are also largely unknown, especially in animals. Although understanding which changes in gene expression are related to a plastic response would clearly enlighten our comprehension of plasticity and its evolution, these changes are partially or completely unknown in many cases.

Fortunately, novel genome-level molecular approaches that have been developed in genetic model organisms are increasingly available for nonmodel species. These approaches come at an opportune time to address many long-standing questions regarding the processes and mechanisms of phenotypic plasticity. These questions can be broadly categorized into three groups: (i) determining the genomic make-up of plastic traits; (ii) understanding the higher-level biological processes involved and their conservation across species; and (iii) determining the molecular machinery that interfaces the genotype and the environment. For many of these questions, the answers require molecular studies of species that have served as models for specific questions, but have not been the focus of past genetic analysis. These will be referred to as 'nonmodel' species.

Integrative biology

Until recently, there was a partition between the study of plasticity from an ecological and an evolutionary perspective (using nonmodel species) and the study of plasticity from a molecular and genetic angle (using genetic model species). Recently, however, these disparate fields have been united in the new discipline of integrative biology. This discipline examines the molecular and cellular mechanisms underlying traits of

interest in ecology and evolution, particularly in species previously unstudied with molecular and genetic tools. It also takes genetic model species out of the laboratory and extends knowledge of these systems to include the effects of their natural habitat. Integrative biology employs techniques and approaches to study several different levels of biological organization within a single research programme. It often requires the collaboration of several complementary researchers, each with specific disciplinary expertise, to tackle a unified problem from many angles (Wake 2003). Several integrative biology studies published in recent years have provided insights on the molecular basis of interspecific variation in phenotypes by borrowing concepts and tools from the fields of functional genomics and systems biology (Carleton & Kocher 2001; Toma *et al.* 2002; Reed & Serfas 2004; Abzhanov *et al.* 2006; Derome *et al.* 2006; Diz & Skibinski 2007; Jordan *et al.* 2007; Landry *et al.* 2007). In this review, we present the implementation of this strategy for the study of plastic variation in phenotypes. This discovery-driven approach that employs high-throughput molecular tools to study genomic reaction norms has proven to be a powerful approach for uncovering hundreds of new candidate genes and new biological processes.

Ecological annotation of genes

The integrative biology approach associates molecular results with traits of ecological and evolutionary interest. It gives us a better understanding of the function of genes in relevant environments and therefore results in ecological annotation for genes and processes. Furthermore, the relationship between molecular and ecological knowledge is a two-way interaction. On the one hand, molecular techniques promise to further our understanding of gene functions and molecular mechanisms underlying ecologically important traits. On the other hand, ecological studies are necessary for a comprehensive understanding of functional genomics, as many genes in genetic model species show no phenotype when knocked out or manipulated in the laboratory (Carroll & Potts 2006; Pena-Castillo & Hughes 2007). For example, some genes may not have effects on survival in the laboratory, yet might be essential when mounting a plastic stress response to an environmental variable. Quantifying the contribution of genes to a phenotype in ecological (as opposed to laboratory) environments will allow an 'ecological annotation of genes and genomes' (Landry & Aubin-Horth 2007) and will thus fill gaps in our knowledge of gene function. Indeed, studying how ecology modulates genome activity will not only enhance our understanding of this pervasive process, but also document the molecular functions and biological processes with which a gene is associated (Fig. 2).

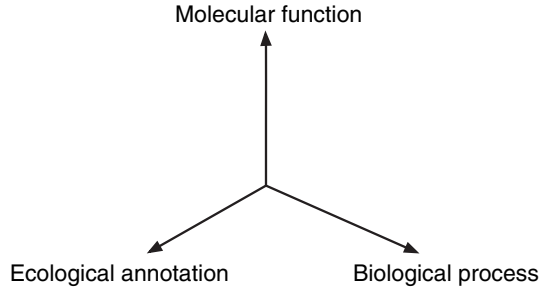

Fig. 2 The three axes of information on a gene: molecular functions, biological processes and ecological annotation.

However, to distinguish causation from consequence, one must eventually go beyond simply correlating phenotypes with gene expression patterns. Indeed, gene expression is itself a trait that can be plastic (it is affected by the genotype and by the environment) and can be the result of a response to a change in the environment (Cote *et al.* 2007). It is thus possible to represent the different levels of expression of a gene in different environments as a reaction norm. Furthermore, it has been shown that different genotypes can have different reaction norms of gene expression (Landry *et al.* 2006; Li *et al.* 2006). Distinguishing transcriptional changes that are the cause rather than the consequence of plastic development can be achieved using time series that track the development of the plastic trait after manipulating the internal and/or external environment. This time series approach reveals the progression of transcriptional variation and allows the identification of genes whose transcription changes early and potentially transitively. Such early, transient changes are the most probable triggers of the plastic phenotypic changes. Below, we present examples of this type of developmental series in the study of plasticity in animals.

The comparative method

Another means by which to study the relationship between gene expression and phenotype is the comparative method. For example, techniques to examine expression profile changes have been developed for the medical field and applied to comparative studies of environmental stress (Gasch *et al.* 2000; Cossins *et al.* 2006; Gasch 2007; Reinius *et al.* 2008; Kassahn *et al.* 2009). The comparison may examine a given tissue for several species, a response to different environmental cues in the same species or a response to the same environmental cue but in different tissues. Beyond understanding the molecular mechanisms of a particular instance of plastic development, comparative studies

collectively draw a more complete picture of which molecular networks are rearranged (and how they are rearranged), which molecular networks are reused in different species and how ecology modulates genome-wide processes. We further discuss the comparative approach in the second part of this review.

We also present how integrative biology has answered, or has the potential to answer, the above questions about phenotypic plasticity. We reveal how newer techniques will grow in importance for the study of these molecular processes. These techniques include (i) heterologous hybridization on DNA microarrays; (ii) new sequencing technologies applied to transcriptomics; (iii) techniques for the study of small RNAs for gene regulation and epigenetic effects; as well as (iv) proteomic techniques. We also review recent studies in genetic model systems that have uncovered mechanisms by which the environmental cues that trigger different plastic responses are sensed and integrated by the organism and result in a plastic change. Finally, we showcase a subset of the recent studies in order to demonstrate that plastic changes in gene expression in response to an environmental cue can persist after this cue is removed and that this long-term response is made possible by several epigenetic molecular mechanisms, including DNA methylation. We finally outline future advances that may arise through the study of plasticity using the above approaches.

Genomic make-up of plasticity

Understanding plasticity requires first and foremost the understanding of its genomic make-up. Can we define genomic reaction norms of plastic traits (Fig. 1)? How many and which types of genes vary in expression between the different phenotypes of a plastic trait? Can we determine which genes are up-regulated at each step of development of the plastic trait and which genes are only found up-regulated in the final phenotype? Which genes are the 'cause' and which are the 'consequence' of plastic development?

A genomic reaction norm defines the number and nature of genes that vary in expression

Understanding the genomic make-up of plastic genotypes requires establishing how the developmental or physiological programme of an organism is modified as it changes in response to the environmental cue. By determining the differences in gene expression among environments, we define a genomic reaction norm (Fig. 1). The transcriptomes of various nonmodel organisms have recently been probed using new molecular tools such as custom-made DNA microarrays. By studying a large portion of the genome, it is possible to estimate how many genes are associated with plastic responses to the environment. By incorporating gene annotation based on studies in genetic model systems, such as *Drosophila*, rodents and humans, it is also possible to know the molecular function, related cellular component and associated biological process for the genes involved. Finally, understanding the molecular changes that are associated with different versions of a plastic trait will direct research in novel directions by allowing the manipulation of these genes for testing ecological and evolutionary hypotheses, such as examining the adaptive value of phenotypic plasticity (Schmitt *et al.* 1999).

Ontogeny in honeybees. Decades of research on the behaviour of social insects have shown that an individual honeybee (*Apis mellifera*) faces distinct environments and novel tasks at each of its life stages. Therefore, honeybees have different behavioural stages, posing the interesting question: 'What happens to a honeybee's brain during its life?'. Whitfield *et al.* (2003, 2006) have answered part of this question by studying variation in gene expression in the brain of a honeybee in the nurse and the forager life stage. The nurse stage is characterized by life within the hive and the task of brood caring, while the forager stage is spent outside the hive, flying to find nectar and pollen (Whitfield *et al.* 2003). In each life stage, the relative expression level for 5500 genes was determined. They defined genomic reaction norms, showing that 39% of the genome was differentially expressed between the nurse and forager stage. This result suggests that a large rearrangement of genome activity is necessary to create important phenotypic changes in physiology, behaviour and flight activity that help an individual to face different environmental challenges.

This pioneering study of individual gene expression profiles in an insect that is already well characterized at the behavioural, ecological and evolutionary level can also help explain changes in behaviour. For example, Whitfield *et al*. found differentially regulated candidate genes for neural and behavioural plasticity based on the function of the homologue found in genetic model systems such as the fruit fly (*Drosophila*). These genes were involved in changes in brain structure (genes *BM-40-SPARC* and *fax*) and onset of foraging behaviour (gene *foraging*). The foraging honeybees leave the hive to collect pollen, while nurses never leave the hive. Therefore, the demands on the honeybees' cognitive ability and orchestration of behaviour are very much altered throughout life. Linking these genes with ecologically relevant phenotypes adds a new component to the annotation of genes first studied in *Drosophila*.

In nature, the nurse and forager life stages typically occur at different ages (Whitfield *et al.* 2003). Could it be that age is solely responsible for the difference in expression between nurses and foragers? Whitfield *et al.* eliminated this confounding factor of age by artificially selecting honeybees that were foragers at the same age at which they are usually in the nurse stage in nature. These results showed that the developmental stage (nurse or forager) is responsible for a much larger source of variation in gene expression than age. Whitfield *et al.* (2003, 2006) also showed that individual brain expression patterns are so dramatically different between life stages that they can be used to classify an individual honeybee as a nurse or as a forager with a very high assignation rate. These experimental studies on honeybees, combined with those of social behaviours in other organisms, will allow for the construction of conceptual models that describe the complex relationships that connect genes, the brain and plastic behaviour on physiological, developmental and evolutionary timescales (Robinson *et al.* 2008).

Alternative mating tactics in Atlantic salmon. Plasticity in the development of reproductive phenotypes is found in many species. Examples include numerous invertebrates and vertebrates (Taborsky 2001; Knapp 2003; Tomkins & Hazel 2007). In Atlantic salmon, *Salmo salar*, the life cycle of typical males includes hatching in a river, followed by a seaward migration, growth in saltwater and a homing journey to the birthplace to reproduce as a large fighting male. However, these large males co-exist with males that develop as sneakers. Sneaker males remain in freshwater and use an alternative reproductive tactic by 'sneaking' into the nest of a migrating female. The mating tactic of males is plastic; the development into one type of male or the other depends on size attained and energy reserves accumulated during a specific critical period before reproduction (Rowe & Thorpe 1990; Hutchings & Myers 1994; Thorpe 1994; Thorpe *et al.* 1998; Aubin-Horth & Dodson 2004). The threshold level of energy and size that must be surpassed for the development of the sneaker phenotype is genetically determined and varies among genotypes and populations (Hutchings & Myers 1994; Aubin-Horth & Dodson 2004; Aubin-Horth *et al.* 2006; Piche *et al.* 2008). Thus, any male can become a sneaker if the environment and internal conditions are right, but some males are more likely to develop into sneakers than others.

In a recent study of the molecular basis of this plastic trait, males of the same age expressing one of the two different tactics (sneaker males and immature males of the same age that will eventually become large fighting males) were compared in the first brain gene expression study done in wild vertebrates (Aubin-Horth *et al.* 2005a, b). The genomic reaction norm for these alternative reproductive tactics was determined by quantifying the gene expression levels for each male type. Aubin-Horth *et al.* found that hundreds of genes vary in expression between the two male types, equal to about 15% of the portion of the genome that was examined. These include genes involved in the endocrine reproductive pathway (gonadotropins) necessary for protein synthesis and maturation, and also genes known to function in processes of learning and memory. In accordance with differences observed at the organismic level, genes related to cognition (learning and memory) and reproduction were up-regulated in sneaker males, while genes related to cellular growth were up-regulated in immature fighter males. Indeed, the sexually mature sneaker phenotype is already fully developed by autumn, and immature males grow faster than sneaker males. However, the implicated differences in learning and memory were unexpected, and suggest that specific learning abilities become important when a male becomes reproductive. This result thus provides a new set of hypotheses to investigate. Currently, it is not known whether this change in learning ability is related to the sneaking reproductive tactic or to spawning-readiness in general. However, this hypothesis could be addressed using complementary experimental approaches in the laboratory, such as pharmacological manipulations. It also remains to be tested if and how the same genes could be involved in other salmonid life stages, such as saltwater migration or reproduction as a large anadromous individual.

Aubin-Horth *et al.* (2005a, b) found that immature males have not merely reduced the expression of 'sneaker' genes, but have in fact up-regulated a suite of genes related to many different biological functions. These genes could be interpreted as genes that have to be activated in order to repress maturation and its associated changes in behaviour and morphology, and/or as genes that are shut down in sneaker males to allow the development of mature gonads. Prior to this genomic study, models for the evolution of the salmonid life cycle have proposed that development of a plastic alternative reproductive tactic in this species is the result of an active repression of maturation (Thorpe 1994). Probing the genome at the mRNA level on a large scale has therefore provided molecular evidence for this hypothesis. This work also showed that samples collected from natural populations showed individual expression profiles that were more similar among males of the same tactics.

Larval development in fruit flies. In the cactophilic fly *Drosophila mojavensis*, larvae develop on cactuses, and

different populations use different cactus species as hosts. Matzkin *et al.* (2006) raised larvae of an agria-living population on two different cactus hosts to study plasticity of transcription under the different rearing environments. Flies descended from a population usually living on agria cactus were raised on their original host and on an organpipe cactus, which is normally used by another population of *D. mojavensis*. They found that larvae showed extensive variation in gene expression (almost 7%) between rearing environments, and the genes involved mainly related to metabolism, detoxification and defence, perhaps reflecting the very different energy sources and microfauna environments they face on either cactus. Matzkin *et al.* (2006) also uncovered tens of genes that were differentially expressed between the agria-raised and organpipe-raised flies that have no homologues in *D. melanogaster*, suggesting that they are specific to *D. mojavensis* and potentially important for the evolution of adaptations related to its cactophilic lifestyle. This type of study identifies how organisms adapt to life in environments that necessitate very specific responses, and also point to the environmental cues and internal mechanisms used to build the plastic molecular response appropriate for its host plant. Furthermore, studying a species with a unique lifestyle provides information regarding molecular mechanisms related to plasticity that cannot be obtained from studying a genetic model organism in the laboratory.

The above work on these three different species suggests that we can measure robust differences in gene expression between alternative phenotypes resulting from plastic development and thus define genomic reaction norms. It also suggests that many genes are involved in the development of plastic traits, either as a cause of the plastic change or as a consequence. As these identified genes have molecular functions and are related to known biological processes, their identification enlightens our knowledge of the plastic trait at the organismic level. Studies from both honeybees and salmon show that precise and repeatable measurements of gene expression levels can be made on free-living individuals, even when they are the result of phenotypically plastic development.

Molecular time series of the development of a plastic trait

In each of the three previous examples, differential gene expression was estimated at only one point in time. We do not know the time point at which the differential expression was induced, nor whether the same genes were differentially regulated at various developmental steps between the two end points. It is possible that some genes are only differentially expressed during the initial transition period when developmental change is initiated, and not during the stable 'maintenance' phase once the phenotypes become established (Fig. 1). Furthermore, when focusing on the final phenotypes, it is not possible to know whether the observed differential expression is a cause of the plastic change in phenotype or only a consequence of the new phenotype. Studying the onset of the plastic development process, through the use of a time series, such as those used in developmental or cell cycle studies (Spellman *et al.* 1998), can address these questions. Time series experiments have a unique set of challenges, but they offer an amazing tool with which to uncover the genes that trigger plastic development.

Social dominance in males of an African cichlid fish. The African cichlid, *Astatotilapia burtoni*, offers a good model system with which to study plastic change. In this species, males can be found in two states: (i) dominant, territorial and reproductive, with bright colouration; or (ii) subordinate, nonterritorial and sexually inactive, with a dull coloration (Fernald & Hirata 1977). The plastic change of a male from a subordinate, nonreproductive phenotype into a dominant, territorial and sexually active phenotype is the result of phenotypic plasticity that depends on the social environment. Males can be induced to become dominant by removing all competing territorial males in the tank of a subordinate male. Alternatively, they can also be induced to become subordinate and nonterritorial by surrounding them in a social environment of larger territorial males (Francis *et al.* 1993; White *et al.* 2002). This effect makes it a good system to study changes at different organisation levels. Accordingly, the numerous studies on *A. burtoni* over the last 30 years have sketched the nature and timing of the changes at the behavioural, morphological, physiological and hormonal levels. Dominant, territorial and reproductive behaviours are more common in dominant males than in subordinate males. Dominant males also show greater gonad size and higher plasma levels of androgens. In the brain, dominant males show increased *GnRH* (gonadotropins-releasing hormone, a neurohormone involved in the reproductive axis) *AR-alpha*, *AR-beta*, *ER-beta-a* and *ER-beta-b* (androgen and oestrogen steroid hormone receptors) expression, as well as *somatostatin* and one of its receptor, *sstR3* (traditionally known to be involved in the growth axis but also related to aggressive behaviour). In the pituitary there is increased expression of the GnRH receptor. On the other hand, growth is higher in subordinate individuals (Francis *et al.* 1993; Hofmann *et al.* 1999; White *et al.* 2002; Au *et al.* 2006; Parikh *et al.* 2006; Burmeister *et al.* 2007; Trainor & Hofmann 2007).

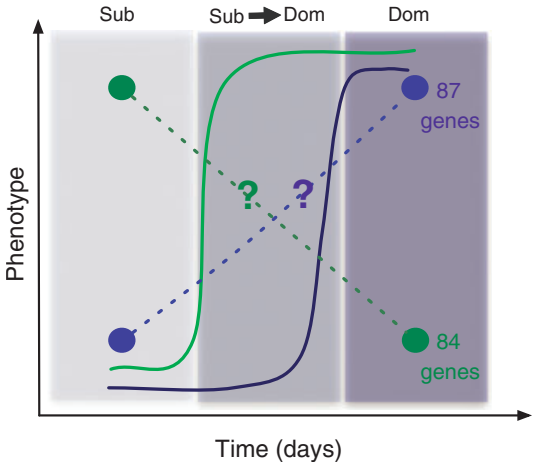

Fig. 3 Reaction norms of behaviour, physiology, hormones and gene expression varying with the social environment and dominance status development in *Astatotilapia burtoni*. Subordinate males (Sub) develop into dominant males (Dom) and go through a transition phase (Sub-Dom) in which the various components of the dominant phenotype develop at different rates. Solid green curve: dominance behaviour (White *et al.* 2002; Burmeister *et al.* 2005); solid blue curve: gonad development (White *et al.* 2002), dotted green and blue lines: gene expression (Renn *et al.* 2008).

These studies allow the definition of reaction norms for behaviour, physiology, hormones and gene expression according to changes in the social environment (dominance and subordinance) (Fig. 3). Recently, a genomic picture was added to this wealth of information when the two types of males of these plastic traits were studied using a species-specific microarray containing more than 2000 genes (Renn *et al.* 2008). In that single study, 171 new genomic reaction norms were defined by studying the genes varying significantly in expression between dominant and subordinate males (Fig. 3, see Gene modules of plastic traits section for details) (Renn *et al.* 2008). However, all of the important components of dominance may change at different rates. For example, behavioural changes are much more rapid than physiological ones (Fig. 3). The relative time-course for each of these complex transitions is related to specific set of underlying molecular changes. In the first hours of becoming dominant, an immediate early gene (*egr-1*, also known as *zenk*) is expressed specifically in the neurons that trigger the hormonal reproductive axis, thus pointing to a link between the perceived change in the environment (the male is freed of challenges from the dominant opponents) and the development of the dominant and reproductive phenotype (Burmeister *et al.* 2005). Importantly, this higher expression of *egr-1* is not found in stable dominant or subordinate males (similar to the green reaction norm in Fig. 1) and is thus specifically related to social ascension. In future studies featuring genome-wide analysis of molecular changes, the time-course of change for genes associated with the transition phase and maintenance phase will provide clues on the characteristics of the gene networks involved. The identity and connectivity of these networks will vary according to the type of plastic trait under study, but common features may also emerge. These commonalities will aid in detecting candidate genes for the initial development of the phenotype in other species.

Higher-order processes of the mechanisms of phenotypic plasticity

Large-scale gene expression studies have given biologists the power to uncover patterns that can only emerge from looking simultaneously at a large set of genes that are related to different biological processes (Ihmels *et al.* 2005). Indeed, there is often no single gene whose change in expression has the power to define a biological state. However, identifying several genes that are up-regulated or down-regulated often results in a unique gene expression pattern that reflects the biological phenotype. Therefore, one of the advantages of using large-scale functional genomics tools to study plasticity is that expression levels of thousands of genes can be studied simultaneously, and the genes can be classified according to their responses to specific environmental variables. Genes that respond in the same way to the environmental cue can be identified, and their biological and genomic characteristics studied, resulting in the analysis of higher-order biological processes linked to phenotypic plasticity. Addressing higher-order processes that comprise the mechanisms of plasticity and identifying, its genomic make-up may precede a thorough understanding of specific mechanisms. Are there modules that are co-opted, re-deployed, combined and/or deconstructed as a phenotype is altered? Does evolution conserve higher-order mechanisms across species? What techniques and approaches will allow us to quantify these patterns within and between species?

Gene modules of plastic traits

The consideration of biological systems according to the concept of modularity has gained popularity in the past 10 years. These concepts consider the functional and structural heterogeneity of an organism at many different levels. Analysis of gene expression data according to modules can be used to identify functional enrichment, to model gene regulatory networks, to reconstruct metabolic networks (Ravasz *et al.* 2002), to study

evolution of gene expression (e.g. Singh *et al.* 2008), to screen for potentially useful biomarkers (Horvath *et al.* 2006; Wong *et al.* 2008) or to annotate novel genes (e.g. Shiga *et al.* 2007). These useful modules may be defined *a priori* by information external to the particular experiment or may be defined by observed patterns and associations internal to the particular study.

Modules provide information on the function of the genes that compose them. We have long been aware that genes interact to produce a phenotype. Even when a single gene is responsible for a phenotype, it interacts with the genomic background in which it is found. Therefore, focusing a study on the combination of gene expression patterns uncovers the varying molecular interactions that lead to the observed phenotypes. It also informs how the function of a gene is modulated by the other genes that are co-expressed. This set of co-regulated genes constitutes a molecular module (Segal *et al.* 2004) that can be defined by information internal to the particular experiment. Modules can be useful in the study of plasticity in nonmodel species because we can characterize the function of genes within a module and thus annotate genes using 'guilt by association' (Shiga *et al.* 2007). The most basic internally defined gene module from gene expression data is a gene list that is based on a threshold applied to the rank list of differential expression between two phenotypes. These gross gene modules can then be further refined through examination of the data according to additional phenotypes. In the *A. burtoni* example reviewed above, females are subordinate to the dominant males but unlike subordinate males, the females do reproduce. Including data on these females thus allowed us to subdivide the genes that were up-regulated in dominant males into distinct modules, a super-male-dominance module and a reproductive module (Renn *et al.* 2008). Therefore, a potential confounding factor (whether an individual reproduces) on the focal factor (dominance or subordination) was removed by using this module approach. This approach allowed genes associated solely with plasticity of dominance behaviour to be separated from those involved in reproduction. Gene ontology (Ashburner *et al.* 2000) classifies and annotates genes according to molecular function, biological process and cellular location (Rhee *et al.* 2008), such that coordinated changes in expression can identify modules in disease (Segal *et al.* 2004), ageing (McCarroll *et al.* 2004) and development (Arbeitman *et al.* 2002). Associating these externally defined gene modules with a particular phenotype provides a framework for assigning gene function (Wolfe *et al.* 2005) through guilt by association to identify novel functions for orthologs in diverse species. Therefore, the genes in the cichlid social phenotype modules that were annotated to 'unknown' or to 'predicted protein' now obtain functional annotation within this species by using their expression pattern (e.g. expressed only in dominant individuals) as an annotation.

Data reduction and the study of the molecular basis of life history trade-offs. Modules also allow a reduced dimensionality of data obtained in high-throughput methods. This reduction is useful in order to relate gene expression information to phenotype information on other levels (Bochdanovits & de Jong 2004; Subramanian *et al.* 2005; Zahn *et al.* 2006; Zahn & Kim 2007). This use of modules can be extremely successful in the study of plasticity and trade-offs. A trade-off is manifested by investment in one trait at the expense of another. For example, a bird may have many offspring with low survival or a few offspring with a high survival probability, or a fish may either invest in growth or invest in gonads for reproduction. These life history trade-offs are thought to arise at the phenotypic level because there is a limited energetic resource available. Uncovering the molecular basis of these trade-offs allows the identification of gene expression that co-varies positively with some traits and negatively with other traits (Stearns & Magwene 2003). In one of the few studies to tackle this topic, correlations between modules of co-expressed genes and life history traits that show a trade-off at the organismic level (adult body mass and larval survival) have been identified in *Drosophila*. Bochdanovits & de Jong (2004) applied principal component analysis to reduce a set of 34 co-expressed genes to a single vector and correlated this vector with the life history traits that show specific relationships between survival and growth in wild populations of *Drosophila melanogaster* (low juvenile survival and high adult weight or vice versa). They found that the function of these genes was in accordance with their expression patterns, e.g. genes with biological functions likely to be involved in enhanced larval survival but detrimental to adult body size were highly expressed in populations with high larvae survival and low adult body size and vice versa. They concluded from the annotated components of the gene expression modules that the observed organismic trade-off between survival and growth is the result of adaptive resource allocation resulting from shifts in cellular metabolism, which in turn is controlled by signalling at the molecular level (Bochdanovits & de Jong 2004).

Comparative approach to studying plasticity. Modules that are defined *a priori* based on external biological information on genes [Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Protein

family (Pfam)] can be used to relate function across large phylogenetic distance. This approach has been applied most successfully to relate function between genetic model organisms for functional analysis of stem cells (Sun *et al.* 2007) and ageing (McCarroll *et al.* 2004; Zahn *et al.* 2006). Sequence similarity and predicted protein structure allow annotation across orthologs in nonmodel organisms (e.g. Whitfield *et al.* 2002; Paschall *et al.* 2004; Danley *et al.* 2007; Salzburger *et al.* 2008) using available tools (Khatri & Draghici 2005; Schmid & Blaxter 2008). A drawback to the definition of functional modules in nonmodel organisms based upon sequence similarity is that the potential for the evolution of novel gene function is obscured. In an ecological context, this approach can nevertheless be profitable for the identification of functional modules across species with common life history strategies. For example, functional modules related to temperature adaptation are possible across a range of fish species (e.g. carp: Gracey *et al.* 2004; Williams *et al.* 2008; catfish: Ju *et al.* 2002; reef fish: Kassahn *et al.* 2007; bluefin tuna: Castilho *et al.* 2007; goby: Buckley *et al.* 2006). Interestingly, GO analysis of gene expression in Antarctic fish (Chen *et al.* 2008) suggests that evolutionary adaptation in this clade has co-opted some of the mechanisms that underlie physiological response to cold in temperate fish (Gracey *et al.* 2004). Furthermore, one preliminary study (Williams *et al.* 2004) contrasted the cold response in carp to expression changes in the squirrel liver during hibernation, through the comparison of GO terms. The species differences in the identified modules matched differences in the overall cold-response strategy of the two species. Carp sustain biological activity, while mammals suppress biological activity during winter periods. To carry the cross-species comparison farther, Hudson *et al.* (2008) compared the metabolic depression of aestivation in green-striped burrowing frogs to the reduced oxygen consumption during hibernation and showed a similar transcriptional silencing of skeletal muscle bioenergetic genes. Comparisons of this sort are not possible on a gene-by-gene basis and a module-based approach is clearly critical.

Comparative microarray analysis allows validating differentially expressed genes by combining results from many studies, rather than by applying alternate techniques to the same study. Furthermore, using comparative microarray analysis, new hypotheses can be tested and fundamental patterns of gene regulation can be discovered, such as modules that are common to many species. Most studies of the molecular basis of plastic traits focus on transcript levels. However, gene expression measurement is a proxy for the quantification of protein abundance in a cell or tissue. Being able to directly determine variation in protein quantity depending on the environment faced by an organism is thus highly desirable, but often not feasible. Lefevre *et al.* (2007b) used proteomics to understand the underlying mechanisms that cause infected vector-hosts to act in ways that benefit transmission of the parasite to the final host. They found differences in metabolism, heat-shock, transmitter signalling and other proteins in brains of mosquitoes infected with *Plasmodium*, which is responsible for malaria, compared to individuals that were not infected. Similarly, the same group (Lefevre *et al.* 2007a) has addressed proteome changes in the tsetse fly when infected with *Trypanosoma brucei brucei*, the trypanosome responsible for sleeping sickness in humans. Interestingly, they identified proteins similarly involved in apoptosis, molecular chaperones and neurotransmitter systems. The similarity in results between changes due to infection in mosquito and tsetse fly presents the testable hypothesis that phylogenetically distant parasites use the same proximate mechanism to alter the behaviour of their host.

What methods can we use to study plasticity in a large array of ecological model species?

Using heterologous hybridization to study the molecular mechanisms of plasticity. We are faced with the problem that most species with plastic traits of interest do not have tools available to investigate gene expression patterns. Fortunately, both technical reports and biological findings support the feasibility of 'heterologous hybridization' on DNA microarray as a means to obtain gene expression profiles for nonmodel species. Heterologous hybridization is the study of changes in gene expression for a species using a platform constructed with sequences from another species (Renn *et al.* 2004). Renn *et al.* demonstrated the feasibility of this technique for spotted cDNA arrays, by identifying similar brain-specific and muscle-specific gene expression in fish species ranging from the cichlids, guppies (poecilids) and even salmon (salmonids) and zebrafish (cyprinids). As expected, successful gene identification decreased with phylogenetic distance. The same approach has produced biologically meaningful results for questions concerning alternate life history tactics in salmon (Aubin-Horth *et al.* 2005b), social dominance in a cooperatively breeding cichlid (Aubin-Horth *et al.* 2007), early heat stress response in a coral reef fish (Kassahn *et al.* 2007) and mate choice in female swordtails (Cummings *et al.* 2008). There are advantages and disadvantages to using a given type of array for heterologous hybridization (Buckley *et al.* 2006) depending upon the degree of sequence divergence between the species of interest and the platform species. For example, while the high-density oligonucleotide arrays

provide greater genome coverage, their shorter probes are more sensitive to single base-pair changes than cDNA arrays. As microarrays become available for a greater number of species, the possibility to identify the appropriate tool for sister species of ecological interest becomes a reality.

Using new sequencing techniques to study the molecular mechanisms of plasticity. Throughout the last decade, a number of advances have been made in high-throughput technologies for DNA sequencing. The increase in throughput has come at the cost of read length, which at first, threatened to limit the applications to resequencing projects focused on organisms for which full genome sequence was available. While some techniques, such as nanopore, single molecule sequencing and sequencing by ligation, are still in the development and testing stage (Ryan *et al.* 2007; Harris *et al.* 2008; Pihlak *et al.* 2008; Shendure *et al.* 2008; Eid *et al.* 2009), other techniques are finding application. Currently, the array-based hybridization techniques for sequencing appear to be most appropriate for resequencing a focused set of genomic positions for projects such as the assessment of single nucleotide polymorphisms (Borevitz & Nordborg 2003). Pyrosequencing and multiple variations of sequencing-by-synthesis (Margulies *et al.* 2005; Turner *et al.* 2009) are finding utility in both applied and basic research that may be applicable to the study of phenotypic plasticity.

The novelty and benefit of most next-generation sequencing strategies lies in the massively parallel nature of the process, which produces hundreds of thousands of individual sequencing reactions simultaneously. For example, pyrosequencing is a fundamentally different sequencing methodology than traditional Sanger sequencing. Standard cDNA library construction and normalization provides an appropriate template for transcriptome characterization using a heterologous genomic sequence as a reference for assembly. When 100 000 *Manduca sexta* expressed sequence tags, generated by pyrosequencing, were assembled and compared to *Drosophila* genome, 1178 homologues could be identified (Zou *et al.* 2008). Furthermore, EST analysis via pyrosequencing was directly compared to traditional sequencing techniques for RNA transcripts from venom glands dissected from the parasitoid wasp *Microctonus hyperodae* (Crawford *et al.* 2008). In this study, a single pyrosequencing run provided data similar to that obtained by the traditional methods in a fraction of the time and at a fraction of the cost. The application of sequencing-by-synthesis promises to provide similar advantages for transcript sequencing (Rokas & Abbot 2009), though few of these studies have been published for nonmodel organisms.

The drawbacks and limitations of most next-generation sequencing strategies lies in the reduced length for each read from the individual sequencing reactions. While the sequences generated by pyrosequencing are considered short (<500 bp), through the use of new algorithms and bionformatic tools, assembly of a full genome sequence has been possible (Chaisson *et al.* 2004; Parker & Parker 2008), as demonstrated for the barley genome effort that employed a combined strategy (Wicker *et al.* 2006). Even more challenging, from a bioinformatic standpoint, is the *de novo* assembly of shorter individual sequences (25–75 bp) such as those currently produced through sequencing-by-synthesis and sequencing-by-ligation strategies. While algorithms exist that perform well with small genomes (reviewed by Rokas & Abbot 2009; Turner *et al.* 2009), either longer sequence read length, better algorithms or closely related references genomes will be necessary to tackle larger complex eukaryotic genomes with these new technologies. Nonetheless, through ongoing advances and the ever-falling cost of DNA sequencing, genomic tools and even full genome sequencing may become available for many of the species we wish to study. Until then, these technologies can be used to generate useful genomic information short of full genome sequence.

The quality and coverage of sequence information obtained by these next-generation sequencing techniques provides sufficient information for the development of high-density oligonucleotide microarrays. A custom microarray was generated for the largemouth bass (*Micropterus salmoides*) based upon cDNA library sequence obtained by pyrosequencing, and then validated with an ecotoxicological study (Garcia-Reyero *et al.* 2008). In a similar study, a custom microarray was designed for the Glanville fritillary butterfly, *Melitaea cinxia*, for a population biology model. In this study, the sequence information was obtained from reverse transcribed RNA rather than a cDNA library. While there was no previous genomic data for this species, the 600 000 reads (~100 bp each) could be assembled and compared to the predicted proteins from the *Bombyx mori* genome, predicting 9000 unique genes, the majority of which were then validated through the construction of the high-density microarray (Vera *et al.* 2008).

Because the relative number of sequence reads provides an accurate and direct measure of the number of transcript templates that were present in the sample pool, next-generation sequencing techniques can be used for the analysis of a complex unnormalized RNA sample from a tissue or treatment of interest to provide a gene expression profile without the construction of a microarray. This technique is now known as RNA-seq. When applied to nonmodel species, RNA-seq still

requires the use of a heterologous reference genome in order to assemble reads and identify genes. For the analysis of protein-coding genes, however, the reference genome may be that of a somewhat more distantly related species. Robinson *et al*. have used this heterologous RNA-seq technique to explore the evolution of behaviour in a primitively eusocial wasp species, *Polistes metricus*, by comparing gene expression patterns in the brain of workers, queens and gynes. Because little genomic information existed for this species, the genome of the honeybee was used as the reference genome. This heterologous reference enabled the identification of more than 3000 genes (Toth *et al.* 2007). Rather than relying on gene sequence counts with this heterologous strategy, quantitative-reverse-transcription PCR was used to assay gene expression for 32 candidate genes in this new species. A robust association between individual gene expression patterns and behavioral differences among particular groups supported the hypothesis that the worker phenotype has evolved from a maternal phenotype, thus illuminating the evolutionary history of eusociality. This example supports the feasibility of using high-throughput sequencing techniques to obtain transcriptome information for nonmodel organisms but also highlights the technical difficulties introduced through the mandatory use of a heterologous genome that restricts the accurate quantification of gene expression profiles.

The fact that next-generation sequencing technology does not require the samples to be cloned into a library and propagated through phage or plasmid allows researcher to avoid technical biases and overcome some of the problems associated with metagenomics, the sequencing of environmental samples without the prior identification of the organisms. This high-throughput approach to metagenomics has, for example, revealed a more comprehensive metabolic and taxonomic snapshot of microbes associated with reef-building coral (Wegley *et al.* 2007); highlighted important differences in metabolic potential in different deep mine environments (Edwards *et al.* 2006); and quantified the relative occurrence of metabolic variation across nine biomes representing soil, water and animal associated communities (Dinsdale *et al.* 2008). These entire communities can now be investigated for plasticity.

The degree to which next-generation sequencing techniques will supplant the microarray technique and heterologous hybridization for expression profiling is still unknown, even for research using genetic model organisms. However, it is clear that advances in sequencing techniques will make possible functional genomic studies in a much wider array of organisms (Ellegren 2008) and a broader range of molecular levels. Beyond its use in expression profiling of protein-coding genes, next-generation sequencing, in creative combination with additional emerging genomics tools, will allow investigation into the control of gene expression by micro-RNA (Liu 2008), genome-wide DNA methylation patterns (Tost & Gut 2007; Reinders *et al.* 2008; Pomraning *et al.* 2009), chromatin structure (Owen-Hughes & Engeholm 2007; Schones & Zhao 2008), DNA-binding sites (Johnson *et al.* 2007) and allele-specific expression (Doostzadeh *et al.* 2008). At this time, however, the majority of the studies that have successfully applied these techniques rely upon genetic model organisms, although their potential application in other organisms is recognized (reviewed in Rokas & Abbot 2009).

Future directions

Plastic changes are mediated by different mechanisms, including signalling by neuroendocrine pathways that affect transcription, or direct effects of the environmental variable on hormonal axes and enzymes. Other epigenetic changes come through alteration of transcription capacity by mechanisms such as DNA methylation, histone modification and interference by small RNAs, or through changes in protein level and activity by post-translational modifications such as ubiquination and phosphorylation (Gilbert 2005; Garland & Kelly 2006; Suzuki & Nijhout 2006; Gilbert & Epel 2009). Uncovering the molecular machinery that interfaces the genotype and the environment will help us understand how phenotypic plasticity is possible. But several questions still stand regarding the molecular mechanisms of plasticity. By which mechanisms is the change in environment sensed, integrated and transformed into a response? How are these response maintained in the long term?

Sensing, integrating and signalling environmental changes that result in a plastic response

The environmental cues that trigger different plastic responses must be sensed, integrated and then translated by the organism to result in the activation of the different molecular pathways associated with the plastic changes at the organismic level. There is still a great deal to learn regarding the mechanisms that underlie environmental signalling in the context of a plastic response, especially in animals, as more recent progress has been made for plants (Schmitt *et al.* 1999). While the mechanisms that transform environmental cues into organismic responses have not been often studied in the framework of phenotypic plasticity, the sensing and signalling machinery has been studied for genetic model systems within the context of a variable environment. Methods used and signalling circuits studied in these genetic model systems can help us outline future lines

of research in the study of plasticity in ecologically relevant species. These signals are varied and include, among others, triggering of a neural response and activation of proteins by kinases. For example, in the fruit fly, *Drosophila melanogaster*, rise in ambient temperature to noxious levels is directly translated into a cellular response by ion channels of the transient receptor potential family, which then trigger an increase of intracellular Ca^{2+} levels and membrane depolarization (Sokabe *et al.* 2008). These ion channels are located in sensory neurons, and thus this change in polarization activates other neurons and allows monitoring of the thermal environment (Hamada *et al.* 2008). As another example, the nematode, *Pristionchus pacificus*, finds live beetles to use as hosts and continues its life cycle after the host dies. Host detection via the presence of host pheromones is crucial to this species. Populations in different geographical regions are attracted to different host species and thus vary in their attraction to specific chemicals. This variation for attraction is associated with genetic variation in a cGMP-dependent protein kinase, *Ppa*-EGL-4, found in neurons (Hong *et al.* 2008). Hong *et al.* (2008) used exogenous addition of cGMP to specifically enhance the activity of EGL-4, in order to demonstrate that EGL-4 directly triggers a higher attraction to pheromones in the treated worms. Therefore, it was possible to directly manipulate the magnitude of the plastic behavioural response by using an artificial signal that mimicked the molecular change necessary for a worm to detect a change in the external environment. The gene coding for the EGL-4 kinase is the homologue of *foraging*, found to be up-regulated two types of foraging tactics in *Drosophila* (rovers and sitters) (Osborne *et al.* 1997) and in the brain of honeybee moving from the nurse to the forager life stage (Ben-Shahar *et al.* 2003; Whitfield *et al.* 2003; Ben-Shahar *et al.* 2005); see section on Genomic make-up of plasticity).

Protein kinases function to transfer a phosphate group to other proteins (i.e. to phosphorylate), a process that usually modifies their function by different means. Phosphorylation change triggers cell signalling networks and a cascade of cellular processes, which eventually lead to an organismic-level phenotype (Cohen 1992, 2000). This dynamic post-translational modification of proteins is thus involved in the development and maintenance of plastic traits. What the above studies exemplify is that it is not only essential to measure differences in gene expression and in protein levels between phenotypes that are the result of plasticity, but that eventually it will become vital to study whether proteins are in an active or an inactive state. The description of the state of phosphorylation of all the proteins in a cell is called the phosphoproteome or phosphorylome (Nita-Lazar *et al.* 2008). The techniques allowing such surveys are very recent additions to the molecular toolbox and no truly quantitative study of phosphoproteome variation with environmental changes is yet available. One study gives insight as to what the future may hold. A study of the phosphoproteome in the wine yeast (*Saccharomyces cerevisiae*) in response to variation in pheromone levels in the environment, a very-well studied signalling pathway in this species, showed increased phosphorylation levels of proteins related to the mitogen-activated protein kinase signalling pathway and to transcriptional and cell cycle regulation (Gruhler *et al.* 2005). This exemplifies the type of experiment that could be used to study the phosphorylome of plastic traits.

Beyond expression profiling for protein-coding genes

The additional applications available through high-throughput sequencing should translate to novel organisms well. When RNA-seq was first applied to, and validated for, genetic model organisms (Liu *et al.* 2007; Marioni *et al.* 2008), a large proportion of the transcribed genome was revealed to be derived from regions for which no predicted genes exist (*e.g.* mouse: (Carninci *et al.* 2005); Arabidopsis: (Weber *et al.* 2007); nematode: (Shin *et al.* 2008), suggesting that noncoding RNAs are more widespread than previously appreciated. This finding provides corroborating evidence in support of the high number of ESTs in a study of a butterfly species for which there was no predicted gene homologue in *Bombyx mori* (Vera *et al.* 2008). In combination with the suggestion that phenotypic diversity is highly reliant upon noncoding RNAs, this evidence suggests that ecological genomics must not overlook these unconventional 'genes'. Recent computational work suggests the adaptive evolution of newly emerged miRNA genes in *Drosophila* (Lu *et al.* 2008). Furthermore, recent experimental work demonstrates that adaptation to salt tolerance involves regulation by miRNAs for metabolic, morphological and physiological adaptations of maize seedlings (Ding *et al.* 2009). Such regulatory, noncoding RNA molecules are but one of the emerging areas of research regarding the mechanisms and molecular make-up of phenotypic plasticity allowed by advancing molecular technologies.

DNA methylation and long-term maintenance of plastic response

The studies presented above demonstrate that the environment changes genomic expression. Ongoing work provides insights into the mechanisms by which this signal is transferred between the environment and

genome interface. In some cases these changes in gene expression can persist even after the environmental cue is removed, through molecular mechanisms that are independent of changes in gene sequences. These 'epigenetic molecular processes' that leave sequences intact have been shown to be important for ecologically relevant traits, such as behaviour in rodents, life history determination in honeybees and environmental stress response in plants (Meaney & Szyf 2005; Dalmay 2006; Beck & Rakyan 2008; Bossdorf *et al.* 2008; Kucharski *et al.* 2008). One of these epigenetic mechanisms is cytosine DNA methylation, which is important in regulating gene expression. In most cases, methylation of a DNA site upstream of a coding sequence such as in the promoter region of a gene alters the regulation of that gene through various mechanisms, for instance by reducing gene expression. This methylation is highly responsive to environmental variations, and can thus be seen as a long-term mediator of plasticity at the phenotypic level. Again, advancing technologies will continue to open new avenues of research in the area of epi-genetic regulation (Pomraning *et al.* 2009).

Maternal care as a social environment. Variation in stress responsiveness in adult rats has been linked to their social (maternal) environment as pups. Rats with high-caring mother that frequently licked and groomed her pups have a less responsive hormonal stress response as adults and vice versa (Liu *et al.* 1997). Pups from a low-care mother cross-fostered to a high-care mother have low stress responsiveness as adults, showing that this trait is plastic and dependent on the early environment. The stress response includes a rise in the plasma level of cortisol, a glucocorticoid hormone that mediates its effect by binding to its glucocorticoid receptors that are found in different tissues. By binding to its receptor in the brain, cortisol activates a negative feedback loop that results in a hormonal signal through the pituitary to the adrenal glands to stop production of cortisol. It has been shown that the 5′-noncoding variable exon 1 region of the rat hippocampal glucocorticoid receptor gene contains several potential alternate sequences, including exon 1_7, a brain-specific promoter. Methylation of this specific alternate exon results in lower expression of the glucocorticoid receptor. It has been shown that this methylation pattern is absent in individuals with caring mothers, while it stays untouched in rats with low-care mothers that are highly responsive to stress as adults (Meaney & Szyf 2005). Having a low-care mother as a pup results in a long-term lowering of expression of the glucocorticoid receptor in the hippocampus, which in turn results in a faulty negative feedback loop and a highly reactive stress response. The different social environments have carry-over effects, mediated in the long term by DNA methylation, at the molecular, hormonal and behavioural levels, well into adulthood of the cared-for or neglected rats.

Queens and workers in honeybees. For honeybees, it has been shown that workers and queens result from plastic development following exposure to a particular environment. The developmental trajectory of a larvae is dramatically affected by food supplementation to the 'future queen' larvae and by the subsequent change in levels of juvenile hormone (Hartfelder & Engels 1998). The queen development pathway can be triggered with pharmacological manipulations of juvenile hormone levels (Elekonich *et al.* 2003). The two social castes exhibit differential gene expression, and fully developed workers have similar expression profiles to undetermined earlier life stages, while queens show suppression of the expression of certain specific genes and up-regulation of others (Evans & Wheeler 2000). This expression profiling suggests that the default developmental pathway of a honeybee larva is to develop into a worker. To become a fast-growing, reproductive queen, large changes in gene expression are necessary, which is in accordance with the differences in juvenile hormone levels that are necessary to trigger the development of a larva into a queen. A specific environmental cue thus results in the differential regulation of gene networks leading to the queen phenotype. More recently, it has been suggested that this environmental cue elicits a molecular response and the methylation of specific genes in future workers (Kucharski *et al.* 2008). When the gene responsible for adding methylation patterns was shut down in honeybee larvae using RNAi techniques, a majority of larvae became queen-like. Recently, analysis of gene methylation has been expanded to the genome-wide level, revealing what has been dubbed the 'epigenome' of an organism. With such a genome-wide approach, the overall level of methylation in the genome of honeybees has been shown to also change during development, from the larval to the adult stage (Kronforst *et al.* 2008). It is clear from these reports, and other yet unpublished studies, that the role that methylation plays in variation in ecologically relevant plastic traits will become increasingly apparent in the coming years. Advancing technology to reveal epigenetic regulation will allow new discoveries about how ecology modulates genome activity.

Acknowledgements

We thank Christian Landry for reading many earlier versions of this manuscript. Members of the Aubin-Horth and Renn labs, as well as Sophie St-Cyr, Erika Crispo and Martin Cuddy,

gave helpful comments. This work was funded by NSERC discovery and FQRNT nouveau chercheur grants to NAH and by NIH (R15GM080727) and NSF (081895) awards to SCPR.

References

Abzhanov A, Kuo WP, Hartmann C *et al.* (2006) The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature*, **442**, 563–567.

Arbeitman MN, Furlong EEM, Imam F *et al.* (2002) Gene expression during the life cycle of *Drosophila melanogaster*. *Science*, **297**, 2270–2275.

Ashburner M, Ball CA, Blake JA *et al.* (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics*, **25**, 25–29.

Au TM, Greenwood AK, Fernald RD (2006) Differential social regulation of two pituitary gonadotropin-releasing hormone receptors. *Behavioural Brain Research*, **170**, 342–346.

Aubin-Horth N, Dodson JJ (2004) Influence of individual body size and variable thresholds on the incidence of a sneaker male reproductive tactic in Atlantic salmon. *Evolution*, **58**, 136–144.

Aubin-Horth N, Landry CR, Letcher BH, Hofmann HA (2005a) Alternative life histories shape brain gene expression profiles in males of the same population. *Proceedings. Biological Sciences*, **272**, 1655–1662.

Aubin-Horth N, Letcher BH, Hofmann HA (2005b) Interaction of rearing environment and reproductive tactic on gene expression profiles in Atlantic Salmon. *Journal of Heredity*, **96**, 261–278.

Aubin-Horth N, Bourque J-F, Daigle G, Hedger R, Dodson JJ (2006) Longitudinal gradients in threshold sizes for alternative male life history tactics in a population of Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 2067–2075.

Aubin-Horth N, Desjardins JK, Martei YM, Balshine S, Hofmann HA (2007) Masculinized dominant females in a cooperatively breeding species. *Molecular Ecology*, **16**, 1349–1358.

Beck S, Rakyan VK (2008) The methylome: approaches for global DNA methylation profiling. *Trends in Genetics*, **24**, 231–237.

Ben-Shahar Y (2005) The foraging gene, behavioral plasticity, and honeybee division of labor. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, **191**, 987–994.

Ben-Shahar Y, Leung HT, Pak WL, Sokolowski MB, Robinson GE (2003) cGMP-dependent changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. *Journal of Experimental Biology*, **206**, 2507–2515.

Bochdanovits Z, de Jong G (2004) Antagonistic pleiotropy for life-history traits at the gene expression level. *Proceedings. Biological Sciences*, **271**(Suppl. 3), S75–S78.

Borevitz JO, Nordborg M (2003) The impact of genomics on the study of natural variation in Arabidopsis. *Plant Physiology*, **132**, 718–725.

Bossdorf O, Richards CL, Pigliucci M (2008) Epigenetics for ecologists. *Ecological Letters*, **11**, 106–115.

Buckley BA, Gracey AY, Somero GN (2006) The cellular response to heat stress in the goby *Gillichthys mirabilis*: a cDNA microarray and protein-level analysis. *Journal of Experimental Biology*, **209**, 2660–2677.

Burmeister SS, Jarvis ED, Fernald RD (2005) Rapid behavioral and genomic responses to social opportunity. *PLoS Biology*, **3**, e363.

Burmeister SS, Kailasanath V, Fernald RD (2007) Social dominance regulates androgen and estrogen receptor gene expression. *Hormones and Behavior*, **51**, 164–170.

Carleton KL, Kocher TD (2001) Cone opsin genes of african cichlid fishes: tuning spectral sensitivity by differential gene expression. *Molecular Biology and Evolution*, **18**, 1540–1550.

Carninci P, Kasukawa T, Katayama S *et al.* (2005) The transcriptional landscape of the mammalian genome. *Science*, **309**, 1559–1563.

Carroll LS, Potts WK (2006) Functional genomics requires ecology. *Advances in the Study of Behavior*, **36**, 173–215.

Castilho PC, Buckley BA, Gracey AY, Block BA (2007) Gene expression profiling using heterologous hybridization to a cDNA microarray in cold-acclimated bluefin tuna. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, **148**, S151.

Chaisson M, Pevzner P, Tang HX (2004) Fragment assembly with short reads. *Bioinformatics*, **20**, 2067–2074.

Chen Z, Cheng CH, Zhang J *et al.* (2008) Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 12944–12949.

Cohen P (1992) Signal integration at the level of protein kinases, protein phosphatases and their substrates. *Trends in Biochemical Sciences*, **17**, 408–413.

Cohen P (2000) The regulation of protein function by multisite phosphorylation – a 25 year update. *Trends in Biochemical Sciences*, **25**, 596–601.

Cossins A, Fraser J, Hughes M, Gracey A (2006) Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *Journal of Experimental Biology*, **209**, 2328–2336.

Cote G, Perry G, Blier P, Bernatchez L (2007) The influence of gene-environment interactions on GHR and IGF-1 expression and their association with growth in brook charr, *Salvelinus fontinalis* (Mitchill). *BMC Genetics*, **8**, 87.

Crawford AM, Brauning R, Smolenski G *et al.* (2008) The constituents of *Microctonus* sp. parasitoid venoms. *Insect Molecular Biology*, **17**, 313–324.

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

Cummings ME, Larkins-Ford J, Reilly CR *et al.* (2008) Sexual and social stimuli elicit rapid and contrasting genomic responses. *Proceedings. Biological Sciences*, **275**, 393–402.

Dalmay T (2006) Short RNAs in environmental adaptation. *Proceedings. Biological Sciences*, **273**, 1579–1585.

Danley PD, Mullen SP, Liu F *et al.* (2007) A cricket Gene Index: a genomic resource for studying neurobiology, speciation, and molecular evolution. *BMC Genomics*, **8**, 109.

Derome N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Molecular Ecology*, **15**, 1239–1249.

Ding D, Zhang L, Wang H *et al.* (2009) Differential expression of miRNAs in response to salt stress in maize roots. *Annals of Botany (London)*, **103**, 29–38.

Dinsdale EA, Edwards RA, Hall D *et al.* (2008) Functional metagenomic profiling of nine biomes. *Nature*, **452**, 629–632.

Diz AP, Skibinski DOF (2007) Evolution of 2-DE protein patterns in a mussel hybrid zone. *Proteomics*, **7**, 2111–2120.

Doostzadeh J, Shokralla S, Absalan F *et al.* (2008) High throughput automated allele frequency estimation by pyrosequencing. *PLoS ONE*, **3**, e2693.

Edwards RA, Rodriguez-Brito B, Wegley L *et al.* (2006) Using pyrosequencing to shed light on deep mine microbial ecology. *BMC Genomics*, **7**, 57.

Eid J, Fehr A, Gray J *et al.* (2009) Real-time DNA sequencing from single polymerase molecules. *Science*, **323**, 133–138.

Elekonich MM, Jez K, Ross AJ, Robinson GE (2003) Larval juvenile hormone treatment affects pre-adult development, but not adult age at onset of foraging in worker honey bees (*Apis mellifera*). *Journal of Insect Physiology*, **49**, 359–366.

Ellegren H (2008) Sequencing goes 454 and takes large-scale genomics into the wild. *Molecular Ecology*, **17**, 1629–1631.

Evans JD, Wheeler DE (2000) Expression profiles during honeybee caste determination. *Genome Biology*, **2**, RESEARCH0001.1-1-0001-6.

Fernald RD, Hirata NR (1977) Field study of *Haplochromis burtoni*: habitats and co-habitant. *Environmental Biology of Fishes*, **2**, 299–308.

Francis RC, Soma K, Fernald RD (1993) Social regulation of the brain-pituitary-gonadal axis. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 7794–7798.

Garcia-Reyero N, Griffitt RJ, Liu L *et al.* (2008) Construction of a robust microarray from a non-model species largemouth bass, *Micropterus salmoides* (Lacepede), using pyrosequencing technology. *Journal of Fish Biology*, **72**, 2354–2376.

Garland T Jr, Kelly SA (2006) Phenotypic plasticity and experimental evolution. *Journal of Experimental Biology*, **209**, 2344–2361.

Gasch AP (2007) Comparative genomics of the environmental stress response in ascomycete fungi. *Yeast*, **24**, 961–976.

Gasch AP, Spellman PT, Kao CM *et al.* (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell*, **11**, 4241–4257.

Gilbert SF (2005) Mechanisms for the environmental regulation of gene expression: ecological aspects of animal development. *Journal of Biosciences*, **30**, 65–74.

Gilbert SF, Epel D (2009) *Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution*. Sinauer Associates, Sunderland, MA, USA.

Gracey AY, Fraser EJ, Li WZ *et al.* (2004) Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 16970–16975.

Gruhler A, Olsen JV, Mohammed S *et al.* (2005) Quantitative phosphoproteomics applied to the yeast pheromone signaling pathway. *Molecular & Cellular Proteomics*, **4**, 310–327.

Hamada FN, Rosenzweig M, Kang K *et al.* (2008) An internal thermal sensor controlling temperature preference in *Drosophila*. *Nature*, **454**, 217–220.

Harris TD, Buzby PR, Babcock H *et al.* (2008) Single-molecule DNA sequencing of a viral genome. *Science*, **320**, 106–109.

Hartfelder K, Engels W (1998) Social insect polymorphism: hormonal regulation of plasticity in development and reproduction in the honeybee. *Current Topics in Developmental Biology*, **40**, 45–77.

Hofmann HA, Benson ME, Fernald RD (1999) Social status regulates growth rate: consequences for life-history strategies. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 14171–14176.

Hong RL, Witte H, Sommer RJ (2008) Natural variation in *Pristionchus pacificus* insect pheromone attraction involves the protein kinase EGL-4. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 7779–7784.

Horvath S, Zhang B, Carlson M *et al.* (2006) Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 17402–17407.

Hudson NJ, Lonhienne TGA, Franklin CE, Harper GS, Lehnert SA (2008) Epigenetic silencers are enriched in dormant desert frog muscle. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, **178**, 729–734.

Hutchings JA, Myers RA (1994) The evolution of alternative mating strategies in variable environments. *Evolutionary Ecology*, **8**, 256–268.

Ihmels J, Bergmann S, Berman J, Barkai N (2005) Comparative gene expression analysis by differential clustering approach: application to the *Candida albicans* transcription program. *PLoS Genetics*, **1**, e39.

Johnson DS, Mortazavi A, Myers RM, Wold B (2007) Genome-wide mapping of in vivo protein-DNA interactions. *Science*, **316**, 1497–1502.

Jordan KW, Carbone MA, Yamamoto A, Morgan TJ, Mackay TF (2007) Quantitative genomics of locomotor behavior in *Drosophila melanogaster*. *Genome Biology*, **8**, R172.

Ju Z, Dunham RA, Liu Z (2002) Differential gene expression in the brain of channel catfish (*Ictalurus punctatus*) in response to cold acclimation. *Molecular Genetics and Genomics*, **268**, 87–95.

Kassahn K, Caley MJ, Ward AC *et al.* (2007) Heterologous microarray experiments used to identify the early gene response to heat stress in a coral reef fish. *Molecular Ecology*, **16**, 1749–1763.

Kassahn KS, Crozier RH, Portner HO, Caley MJ (2009) Animal performance and stress: responses and tolerance limits at different levels of biological organisation. *Biological Reviews of the Cambridge Philosophical Society*, **84**, 277–292.

Khatri P, Draghici S (2005) Ontological analysis of gene expression data: current tools, limitations, and open problems. *Bioinformatics*, **21**, 3587–3595.

Knapp R (2003) Endocrine mediation of vertebrate male alternative reproductive tactics: the next generation of studies. *Integrative and Comparative Biology*, **43**, 658–668.

Kronforst MR, Gilley DC, Strassmann JE, Queller DC (2008) DNA methylation is widespread across social Hymenoptera. *Current Biology*, **18**, R287–R288.

Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in honeybees via DNA methylation. *Science*, **319**, 1827–1830.

Landry CR, Aubin-Horth N (2007) Ecological annotation of genes and genomes through ecological genomics. *Molecular Ecology*, **16**, 4419–4421.

Landry CR, Oh J, Hartl DL, Cavalieri D (2006) Genome-wide scan reveals that genetic variation for transcriptional plasticity in yeast is biased towards multi-copy and dispensable genes. *Gene*, **366**, 343–351.

Landry CR, Castillo-Davis CI, Ogura A, Liu JS, Hartl DL (2007) Systems-level analysis and evolution of the phototransduction network in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3283–3288.

Lefevre T, Thomas F, Ravel S *et al.* (2007a) *Trypanosoma brucei brucei* induces alteration in the head proteome of the tsetse fly vector Glossina palpalis gambiensis. *Insect Molecular Biology*, **16**, 651–660.

Lefevre T, Thomas F, Schwartz A *et al.* (2007b) Malaria *Plasmodium* agent induces alteration in the head proteome of their *Anopheles* mosquito host. *Proteomics*, **7**, 1908–1915.

Li Y, Alvarez OA, Gutteling EW *et al.* (2006) Mapping determinants of gene expression plasticity by genetical genomics in *C. elegans*. *PLoS Genetics*, **2**, e222.

Liu JD (2008) Control of protein synthesis and mRNA degradation by microRNAs. *Current Opinion in Cell Biology*, **20**, 214–221.

Liu D, Diorio J, Tannenbaum B *et al.* (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, **277**, 1659–1662.

Liu F, Jenssen TK, Trimarchi J *et al.* (2007) Comparison of hybridization-based and sequencing-based gene expression technologies on biological replicates. *BMC Genomics*, **8**, 153.

Lu J, Fu Y, Kumar S *et al.* (2008) Adaptive evolution of newly emerged micro-RNA genes in Drosophila. *Molecular Biology and Evolution*, **25**, 929–938.

Margulies M, Egholm M, Altman WE *et al.* (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376–380.

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Research*, **18**, 1509–1517.

Matzkin LM, Watts TD, Bitler BG, Machado CA, Markow TA (2006) Functional genomics of cactus host shifts in *Drosophila mojavensis*. *Molecular Ecology*, **15**, 4635–4643.

McCarroll SA, Murphy CT, Zou SG *et al.* (2004) Comparing genomic expression patterns across species identifies shared transcriptional profile in aging. *Nature Genetics*, **36**, 197–204.

Meaney MJ, Szyf M (2005) Maternal care as a model for experience-dependent chromatin plasticity? *Trends in Neurosciences*, **28**, 456–463.

Nita-Lazar A, Saito-Benz H, White FM (2008) Quantitative phosphoproteomics by mass spectrometry: past, present, and future. *Proteomics*, **8**, 4433–4443.

Osborne KA, Robichon A, Burgess E *et al.* (1997) Natural behavior polymorphism due to a cGMP-dependent protein kinase of Drosophila. *Science*, **277**, 834–836.

Owen-Hughes T, Engeholm M (2007) Pyrosequencing positions nucleosomes precisely. *Genome Biology*, **8**, 217.

Parikh VN, Clement TS, Fernald RD (2006) Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. *Behavioural Brain Research*, **166**, 291–295.

Parker NJ, Parker AG (2008) Simple tools for assembling and searching high-density picolitre pyrophosphate sequence data. *Source Code for Biology and Medicine*, **3**, 5.

Paschall JE, Oleksiak MF, VanWye JD *et al.* (2004) FunnyBase: a systems level functional annotation of *Fundulus* ESTs for the analysis of gene expression. *BMC Genomics*, **5**, 96.

Pena-Castillo L, Hughes TR (2007) Why are there still over 1000 uncharacterized yeast genes? *Genetics*, **176**, 7–14.

Piche J, Hutchings JA, Blanchard W (2008) Genetic variation in threshold reaction norms for alternative reproductive tactics in male Atlantic salmon, *Salmo salar*. *Proceedings. Biological Sciences*, **275**, 1571–1575.

Pigliucci M (2001) *Phenotypic Plasticity: Beyond Nature and Nurture*. John Hopkins University Press, Baltimore, MD.

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution*, **20**, 481–486.

Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.

Pihlak A, Bauren G, Hersoug E *et al.* (2008) Rapid genome sequencing with short universal tiling probes. *Nature Biotechnology*, **26**, 676–684.

Pomraning KR, Smith KM, Freitag M (2009) Genome-wide high throughput analysis of DNA methylation in eukaryotes. *Methods*, **47**, 142–150.

Price TD, Qvarnstrom A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proceedings. Biological Sciences*, **270**, 1433–1440.

Ravasz E, Somera AL, Mongru DA, Oltvai ZN, Barabasi AL (2002) Hierarchical organization of modularity in metabolic networks. *Science*, **297**, 1551–1555.

Reed RD, Serfas MS (2004) Butterfly wing pattern evolution is associated with changes in a Notch/Distal-less temporal pattern formation process. *Current Biology*, **14**, 1159–1166.

Reinders J, Vivier CD, Theiler G *et al.* (2008) Genome-wide, high-resolution DNA methylation profiling using bisulfate-mediated cytosine conversion. *Genome Research*, **18**, 469–476.

Reinius B, Saetre P, Leonard JA *et al.* (2008) An evolutionarily conserved sexual signature in the primate brain. *PLoS Genetics*, **4**, e1000100.

Renn SC, Aubin-Horth N, Hofmann HA (2004) Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. *BMC Genomics*, **5**, 42.

Renn SC, Aubin-Horth N, Hofmann HA (2008) Fish and chips: functional genomics of social plasticity in an African cichlid fish. *Journal of Experimental Biology*, **211**, 3041–3056.

Rhee SY, Wood V, Dolinski K, Draghici S (2008) Use and misuse of the gene ontology annotations. *Nature Reviews. Genetics*, **9**, 509–515.

Robinson GE, Fernald RD, Clayton DF (2008) Genes and social behavior. *Science*, **322**, 896–900.

Rokas A, Abbot P (2009) Harnessing genomics for evolutionary insights. *Trends in Ecology & Evolution*, **24**, 192–200.

Rowe DK, Thorpe JE (1990) Differences in growth between maturing and non-maturing male Atlantic salmon, *Salmo salar* L., parr. *Journal of Fish Biology*, **36**, 643–658.

Ryan D, Rahimi M, Lund J, Mehta R, Parviz BA (2007) Toward nanoscale genome sequencing. *Trends in Biotechnology*, **25**, 385–389.

Salzburger W, Renn SC, Steinke D *et al.* (2008) Annotation of expressed sequence tags for the East African cichlid fish *Astatotilapia burtoni* and evolutionary analyses of cichlid ORFs. *BMC Genomics*, **9**, 96.

Schlichting CD, Pigliucci M (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates, Sunderland, MA.

Schmid R, Blaxter ML (2008) annot8r: GO, EC and KEGG annotation of EST datasets. *BMC Bioinformatics*, **9**, 180.

Schmitt J, Dudley SA, Pigliucci M (1999) Manipulative approaches to testing adaptive plasticity: phytochrome mediated shade avoidance responses in plants. *The American Naturalist*, **154**, S43–S54.

Schones DE, Zhao K (2008) Genome-wide approaches to studying chromatin modifications. *Nature Reviews Genetics*, **9**, 179–191.

Segal E, Friedman N, Koller D, Regev A (2004) A module map showing conditional activity of expression modules in cancer. *Nature Genetics*, **36**, 1090–1098.

Shendure JA, Porreca GJ, Church GM (2008) Overview of DNA sequencing strategies. *Current Protocols in Molecular Biology*, **8**. Chapter 7, Unit 7.1.1.–7.1.11.

Shiga M, Takigawa I, Mamitsuka H (2007) Annotating gene function by combining expression data with a modular gene network. *Bioinformatics*, **23**, I468–I478.

Shin H, Hirst M, Bainbridge MN *et al.* (2008) Transcriptome analysis for *Caenorhabditis elegans* based on novel expressed sequence tags. *BMC Biology*, **6**, 30.

Singh AH, Wolf DM, Wang P, Arkin AP (2008) Modularity of stress response evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 7500–7505.

Sokabe T, Tsujiuchi S, Kadowaki T, Tominaga M (2008) *Drosophila* painless is a Ca^{2+}-requiring channel activated by noxious heat. *Journal of Neuroscience*, **28**, 9929–9938.

Spellman PT, Sherlock G, Zhang MQ *et al.* (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Molecular Biology of the Cell*, **9**, 3273–3297.

Stearns SC, Magwene P (2003) The naturalist in a world of genomics. *American Naturalist*, **161**, 171–180.

Subramanian A, Tamayo P, Mootha VK *et al.* (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 15545–15550.

Sun Y, Li H, Liu Y *et al.* (2007) Cross-species transcriptional profiles establish a functional portrait of embryonic stem cells. *Genomics*, **89**, 22–35.

Suzuki Y, Nijhout HF (2006) Evolution of a polyphenism by genetic accommodation. *Science*, **311**, 650–652.

Taborsky M (2001) The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *Journal of Heredity*, **92**, 100–110.

Thorpe J (1994) Reproductive strategies in Atlantic salmon, *Salmo salar* L. *Aquac. Res.*, **25**, 77–87.

Thorpe JE, Mangel M, Metcalfe NB, Huntingford FA (1998) Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. *Evolutionary Ecology*, **12**, 581–599.

Toma DP, White KP, Hirsch J, Greenspan RJ (2002) Identification of genes involved in *Drosophila melanogaster* geotaxis, a complex behavioral trait. *Nature Genetics*, **31**, 349–353.

Tomkins JL, Hazel W (2007) The status of the conditional evolutionarily stable strategy. *Trends in Ecology & Evolution*, **22**, 522–528.

Tost J, Gut IG (2007) DNA methylation analysis by pyrosequencing. *Nature Protocols*, **2**, 2265–2275.

Toth AL, Varala K, Newman TC *et al.* (2007) Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science*, **318**, 441–444.

Trainor BC, Hofmann HA (2007) Somatostatin and somatostatin receptor gene expression in dominant and subordinate males of an African cichlid fish. *Behavioural Brain Research*, **179**, 314–320.

Turner DJ, Keane TM, Sudbery I, Adams DJ (2009) Next-generation sequencing of vertebrate experimental organisms. *Mammalian Genome*, **20**, 327–333.

Vera JC, Wheat CW, Fescemyer HW *et al.* (2008) Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. *Molecular Ecology*, **17**, 1636–1647.

Wake M (2003) What is ''Integrative Biology''? *Integrative and Comparative Biology*, **43**, 239–241.

Weber APM, Weber KL, Carr K, Wilkerson C, Ohlrogge JB (2007) Sampling the *ARABIDOPSIS* transcriptome with massively parallel pyrosequencing. *Plant Physiology*, **144**, 32–42.

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environmental Microbiology*, **9**, 2707–2719.

West-Eberhard MJ (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.

White SA, Nguyen T, Fernald RD (2002) Social regulation of gonadotropin-releasing hormone. *Journal of Experimental Biology*, **205**, 2567–2581.

Whitfield CW, Band MR, Bonaldo MF *et al.* (2002) Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Research*, **12**, 555–566.

Whitfield CW, Cziko AM, Robinson GE (2003) Gene expression profiles in the brain predict behavior in individual honey bees. *Science*, **302**, 296–299.

Whitfield CW, Ben-Shahar Y, Brillet C *et al.* (2006) Genomic dissection of behavioral maturation in the honey bee. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 16068–16075.

Wicker T, Schlagenhauf E, Graner A *et al.* (2006) 454 sequencing put to the test using the complex genome of barley. *BMC Genomics*, **7**, 275.

Williams D, Epperson LE, Cossins AR *et al.* (2004) Post-genomic approaches to the mechanisms of cold response in fish and hibernating small mammals. *Biological Papers of the University of Alaska*, **27**, 467–475.

Williams DR, Li W, Hughes MA *et al.* (2008) Genomic resources and microarrays for the common carp *Cyprinus carpio*. *Journal of Fish Biology*, **72**, 2095–2117.

Wolfe CJ, Kohane IS, Butte AJ (2005) Systematic survey reveals general applicability of ''guilt-by-association'' within gene coexpression networks. *BMC Bioinformatics*, **6**, 227.

Wong DJ, Nuyten DSA, Regev A *et al.* (2008) Revealing targeted therapy for human cancer by gene module maps. *Cancer Research*, **68**, 369–378.

Yeh PJ, Price TD (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. *American Naturalist*, **164**, 531–542.

Zahn JM, Kim SK (2007) Systems biology of aging in four species. *Current Opinion in Biotechnology*, **18**, 355–359.

Zahn JM, Sonu R, Vogel H *et al.* (2006) Transcriptional profiling of aging in human muscle reveals a common aging signature. *PLoS Genetics*, **2**, e115.

Zou Z, Najar F, Wang Y, Roe B, Jiang HB (2008) Pyrosequence analysis of expressed sequence tags for *Manduca sexta* hemolymph proteins involved in immune responses. *Insect Biochemistry and Molecular Biology*, **38**, 677–682.

Nadia Aubin-Horth is an assistant professor at Université de Montréal. Members of her lab study the molecular and hormonal mechanisms that underlie inter-individual variation in social dominance, temperament and reproductive tactics in African cichlids and the threespine stickleback. They do so using a combination of behavioural biology, endocrinology, pharmacological and environmental perturbations, as well as functional genomics approaches. Suzy Renn is an assistant professor of Biology at Reed College. She aims to determine if similar behaviours result from conserved or converged processes of evolution in African Cichlid fishes. She does so by studying the molecular basis of species- and context-specific behaviours through the synergistic combination of functional genomics, behaviour, physiology and ecology.