Understanding the Relationship Between Brain Gene Expression and Social Behavior: Lessons from the Honey Bee

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Abstract

Behavior is a complex phenotype that is plastic and evolutionarily labile. The advent of genomics has revolutionized the field of behavioral genetics by providing tools to quantify the dynamic nature of brain gene expression in relation to behavioral output. The honey bee *Apis mellifera* provides an excellent platform for investigating the relationship between brain gene expression and behavior given both the remarkable behavioral repertoire expressed by members of its intricate society and the degree to which behavior is influenced by heredity and the social environment. Here, we review a linked series of studies that assayed changes in honey bee brain transcriptomes associated with natural and experimentally induced changes in behavioral state. These experiments demonstrate that brain gene expression is closely linked with behavior, that changes in brain gene expression mediate changes in behavior, and that the association between specific genes and behavior exists over multiple timescales, from physiological to evolutionary.

INTRODUCTION

Behavioral state: performance of a distinct and quantifiable behavior or a set of related behaviors for a measurable period of time

Brain gene expression: whole-brain transcriptome, assayed in the BeeSpace Project by microarray

Neurogenomic state: a distinct pattern of gene expression in the brain revealed by contrasting brain transcriptomes of individuals across different behavioral states

Eusocial: a society with reproductive division of labor, overlapping generations, and cooperative brood care

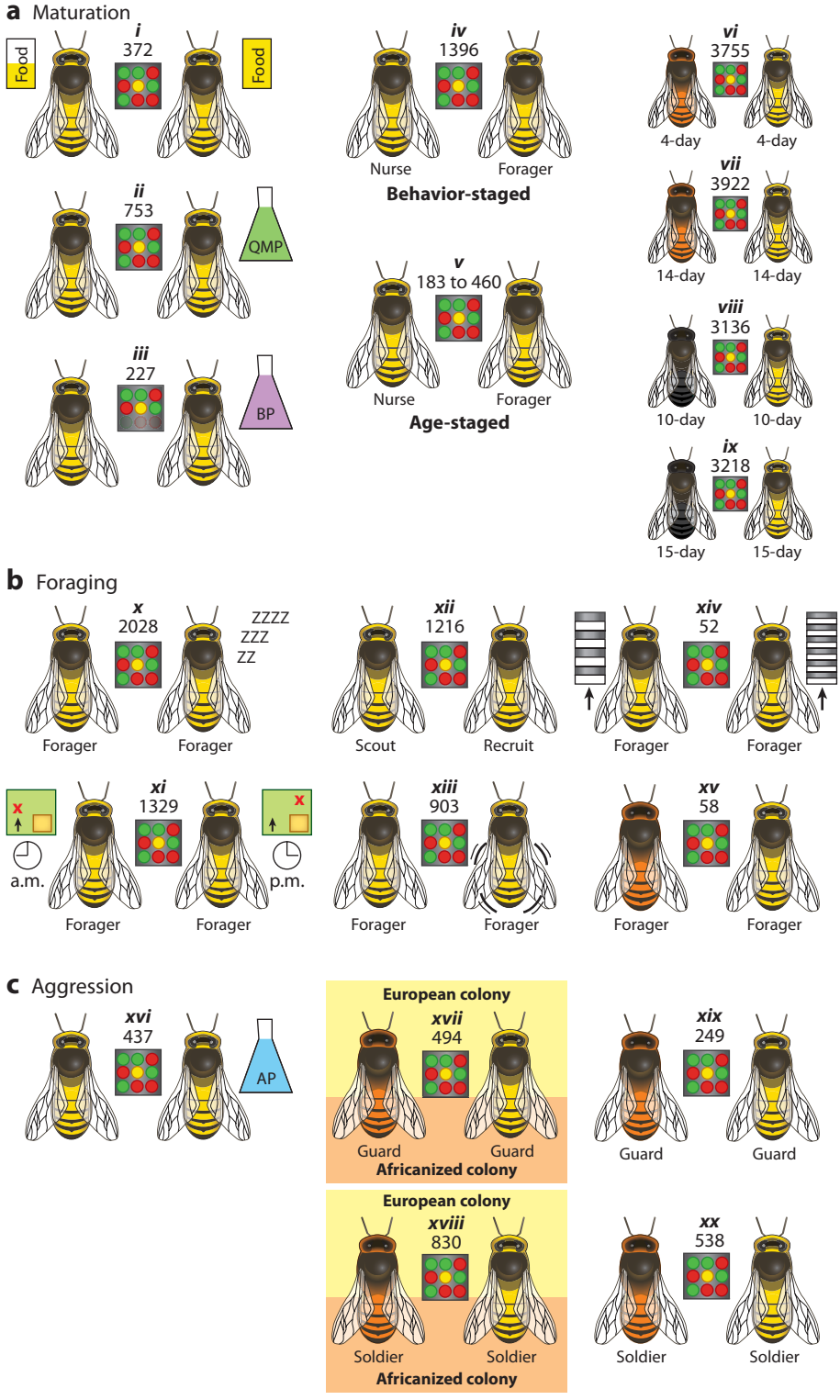
Over their lifetimes, animals perform different types of behavior related to feeding, reproduction, and care of offspring. Relative to morphological traits, behavior exhibits a great deal of plasticity in real time and lability over evolutionary time (125, 126). Behavior is enigmatic because it is both stable and flexible; animals can occupy a specific behavioral state at any given time but can transition into different behavioral states if needed. What molecular processes are responsible for the stability and flexibility of behavior? Although it is well known that both hereditary and environmental factors interact to influence behavior (16, 90, 91), we still lack a comprehensive understanding of how genes and the environment act on the brain to orchestrate changes in behavior.

Behavioral plasticity is coordinated, at least in part, by dynamic genomic processes, such as transcription, and a growing body of evidence demonstrates that changes in the expression of specific genes in the brain affect behavior (24, 90, 91). With increased availability of genomic resources, it is now possible to quantify the dynamic nature of brain gene expression in relation to its behavioral output in a broader range of species than ever before, including model social species (91) selected for their compelling and experimentally accessible social behavior. This portrait of a brain's neurogenomic state provides an introduction to understanding the relationship between genes, the brain, and behavior (91). Development of a deeper understanding of the general paradigms that govern behavioral plasticity and shape the evolution of behavioral traits is one of the frontiers in the study of neurobiology and behavior.

Here, we review a large-scale series of experiments that assayed global gene expression in the brain of the eusocial honey bee (*Apis mellifera*) in association with naturally occurring behavioral states. We draw primarily upon the BeeSpace Project, a series of linked studies that assayed the brain transcriptomes of nearly 1,000 individuals, using the same microarray platform, laboratory, and statistical techniques (**Figure 1**). The BeeSpace experiments utilized an oligonucleotide microarray targeting 12,777 known and predicted transcripts in the honey bee genome, including 10,206 targeted predictions from version 1.0 of the honey bee genome project's official gene

Figure 1

The BeeSpace experiments examined environmental and hereditary influences on brain gene expression and behavior in the honey bee. Hereditary influences were examined by comparing brain gene expression and behavior among the following honey bee subspecies: Africanized honey bees in the New World (predominantly *Apis mellifera scutellata*), the German bee *A. mellifera mellifera*, and the Italian bee *A. mellifera ligustica*, which are represented here by bees with orange, black, and yellow abdomens, respectively. Some experiments utilized managed European bees, which are predominately derived from *A. mellifera ligustica* or *A. mellifera carnica* (*yellow abdomens*). The number of differentially expressed genes is indicated for each contrast. (*a*) Maturation: Experiments assessed the effects of nutrition, queen mandibular pheromone (QMP), brood pheromone (BP), and maturation state on brain gene expression (contrasts *i* to *v*). Hereditary influences on maturation were examined by comparing brain gene expression among genotypes with intermediate rates of behavioral maturation (*A. mellifera ligustica*) relative to genotypes with fast (Africanized) or slow (*A. mellifera mellifera*) rates of maturation (*vi* to *ix*). Behavioral maturation is commonly measured in honey bees by determining the age at which bees start foraging. (*b*) Foraging: Experiments examined the relationship between brain gene expression and activity state (anticipating food or inactive), distinct spatiotemporal memories, the propensity of foragers to scout or send vibration signals, and distance perception (*x* to *xiv*). Hereditary foraging preferences were examined by comparing *A. mellifera ligustica* foragers with Africanized foragers; the latter exhibit a preference for pollen and lower sucrose response thresholds (*xv*). (*c*) Aggression: Manipulative experiments examined the effect of alarm pheromone (AP) and colony environment on brain gene expression and behavior (*xvi* to *xviii*). Hereditary and environmental influences on aggression were examined in parallel by comparing brain expression between aggressive Africanized bees and docile European bees across different behavioral states (*xix* to *xx*). For brevity, not all BeeSpace experiments are illustrated.



set (49). The experiments studied 48 distinct behavioral states related to one or more of three ecologically important behavioral categories: aggression (hive defense), maturation (from working in the hive to foraging outside), and foraging predisposition or type of experience (36). We also include earlier studies utilizing a bee brain cDNA microarray that targeted 7,329 transcripts (127). The massive scope of the BeeSpace Project provides unprecedented power (5) to study the relationship between brain gene expression and naturally occurring social behavior.

Through synthesis of the BeeSpace results, we deduced three general insights that shape the relationship between brain gene expression and social behavior. First, differences in behavior are closely linked with changes in the expression of many genes in the brain. Second, changes in brain gene expression are caused by both hereditary and environmental factors, and both result in changes in behavior. Third, there are parallels in the effects of some genes over physiological, developmental, and evolutionary timescales.

ATTRIBUTES OF HONEY BEES FOR STUDIES OF GENES AND BEHAVIOR

Honey bee societies represent one of the pinnacles of animal behavior (75, 104, 112, 132). A honey bee colony is composed of reproductive (queens and male drones) and nonreproductive (workers) castes, each with a remarkably distinct behavioral repertoire. Worker bees in particular exhibit striking patterns of division of labor and behavioral maturation that are crucial for colony survival and growth (29, 88) and are the subject of this review.

After emergence as adults, worker bees typically spend a period of approximately two to three weeks performing in-hive tasks, such as feeding larvae (nursing) and tending the queen, as well as tasks related to building honeycombs and storing and processing food. Bees then transition to working outside the hive, primarily as foragers for nectar and pollen. Foragers themselves exhibit an extensive range of behavioral traits and specializations, including one of the most fascinating behaviors in animals—the dance language (104, 121). The older (forager-age) bees also defend the hive; guard bees warn their nestmates of intruders by releasing alarm pheromone, and a subset of older individuals (soldiers) are the first to sting intruders, sacrificing themselves in the process (132).

Honey bee behavior is remarkable because it exhibits both extreme specialization and flexibility. Because of division of labor, each individual bee performs only a limited portion of its repertoire at any one time; for example, a nurse bee engages only in brood care activities and not foraging, and vice versa for a forager, despite the fact that both nurses and foragers are exposed to stimuli associated with both activities. However, despite the strength of these behavioral states, bees show flexibility and can drastically shift their state in response to cues present in their social environment (89, 104, 132). This flexibility allows bees to adaptively shift their behavior to best fulfill colony needs (99, 104, 131). These strong, yet flexible, behavioral states are well described and in some cases analyzed at the physiological level (104, 121, 132), making the honey bee a good experimental model for studying the relationship between brain gene expression and behavior.

The honey bee brain has been well characterized, and it has approximately one million neurons and several functionally distinct regions (75); it can be readily dissected, allowing for molecular biology analyses of hundreds to thousands of individual brains. Early bee microarray studies demonstrating extensive differences in whole-brain gene expression as a function of behavioral state (35, 46, 130) suggested that brain gene expression profiles can provide reasonable reflections of behaviorally related transcriptomic activity, thus facilitating the BeeSpace analyses, which were mostly done at the whole-brain level.

Honey bee colonies can be easily manipulated to expose individuals to different social conditions, such as changes in population demography, size, and genetic structure. For

Caste: a group of individuals in a eusocial society that specialize in performing specific tasks

Division of labor: workers show age-related tendencies to specialize in performing sets of activities related to colony growth, development, or reproduction

Behavioral maturation: a worker bee's transition from in-hive tasks (e.g., nursing) to foraging outside

Dance language: highly stereotyped movements that symbolically convey information on the distance, direction, and quality of a profitable food resource

Social environment: the colony's environment, which encompasses both physical and biological attributes (i.e., genotype, physiology, demography, behavior, and pheromones of nestmates)

example, bees can be readily cross- and cofostered in different colonies in the field, allowing classic common garden paradigms to be used to study effects of heredity and environment on brain gene expression and behavior. Other genetic attributes include (*a*) many genetically and behaviorally distinct subspecies (98, 128, 137), which provide ample opportunities to examine naturally occurring hereditary differences in behavior; (*b*) a sequenced genome enabling transcriptomic and proteomic studies (49) as well as manipulative experiments involving RNA interference (RNAi) (80) or targeted pharmacology (28, 110); (*c*) a haplodiploid genetic system, which allows closely related bees (average coefficient of relatedness for full sisters = 0.75) to be studied to minimize variation; and (*d*) instrumental insemination for controlled breeding. Together, these useful attributes facilitate experimental genetic studies of complex and ecologically relevant behaviors under natural conditions.

CLOSE RELATIONSHIP BETWEEN BRAIN GENE EXPRESSION AND BEHAVIORAL STATE

Gene expression in the brain provides the first measure of the interaction between the genome and the environment—the first phenotype (90). It also is the phenotype most distal from behavior, considering the layers of complexity separating the two, including posttranslational modification, neuronal development, neurophysiology, and neurochemistry. Despite earlier studies of learning, memory, and circadian rhythms in laboratory paradigms (25, 76), it is not known whether there is a close relationship between behavior and brain gene expression for naturally occurring behavior. However, previous studies showing such a relationship for a few genes in vertebrates and invertebrates (24, 76, 90–92) motivated investigation into the relationship between brain transcriptomes and naturally occurring behaviors in the honey bee (**Figure 2*a***).

Behavioral Maturation

Honey bees specialize in different behaviors as they get older, but their maturation also is flexible. Manipulation of the social environment can hasten, delay, or even reverse the pace of maturation, causing precocious foraging, overage nursing, or reversals from foraging to nursing (88). Behavioral maturation in honey bees provided the first glimpse of the surprisingly close relationship between neurogenomic state and behavioral state in an animal. Whitfield et al. (130) reported that 2,670 genes (39%) on a cDNA microarray were differentially expressed in the brains of nurses and foragers (130).

Were the observed shifts in brain gene expression associated more with age or behavioral state? Separating the effects of age and behavior is typically very difficult in animals but can be done under controlled experimental conditions with honey bees by studying single-cohort colonies experimentally composed initially of approximately 1,500 one-day-old bees and a laying queen. The absence of older bees in single-cohort colonies results in a proportion of the young bees becoming precocious foragers. Similarly, an eventual delay in the emergence of young adult bees in such colonies due to reduced egg laying and brood rearing necessitates that some bees continue nurse duties for several weeks past the norm (i.e., overage nurses). Whitfield et al. (130) showed that young and old foragers shared similar brain profiles distinct from those of young and old nurses, thereby demonstrating that behavior, and not age, was the major driver in patterns of brain gene expression. The nurse and forager neurogenomic states were so different that an unsupervised clustering algorithm was able to predict the behavioral state of individual workers with 95% accuracy given their brain profiles (130).

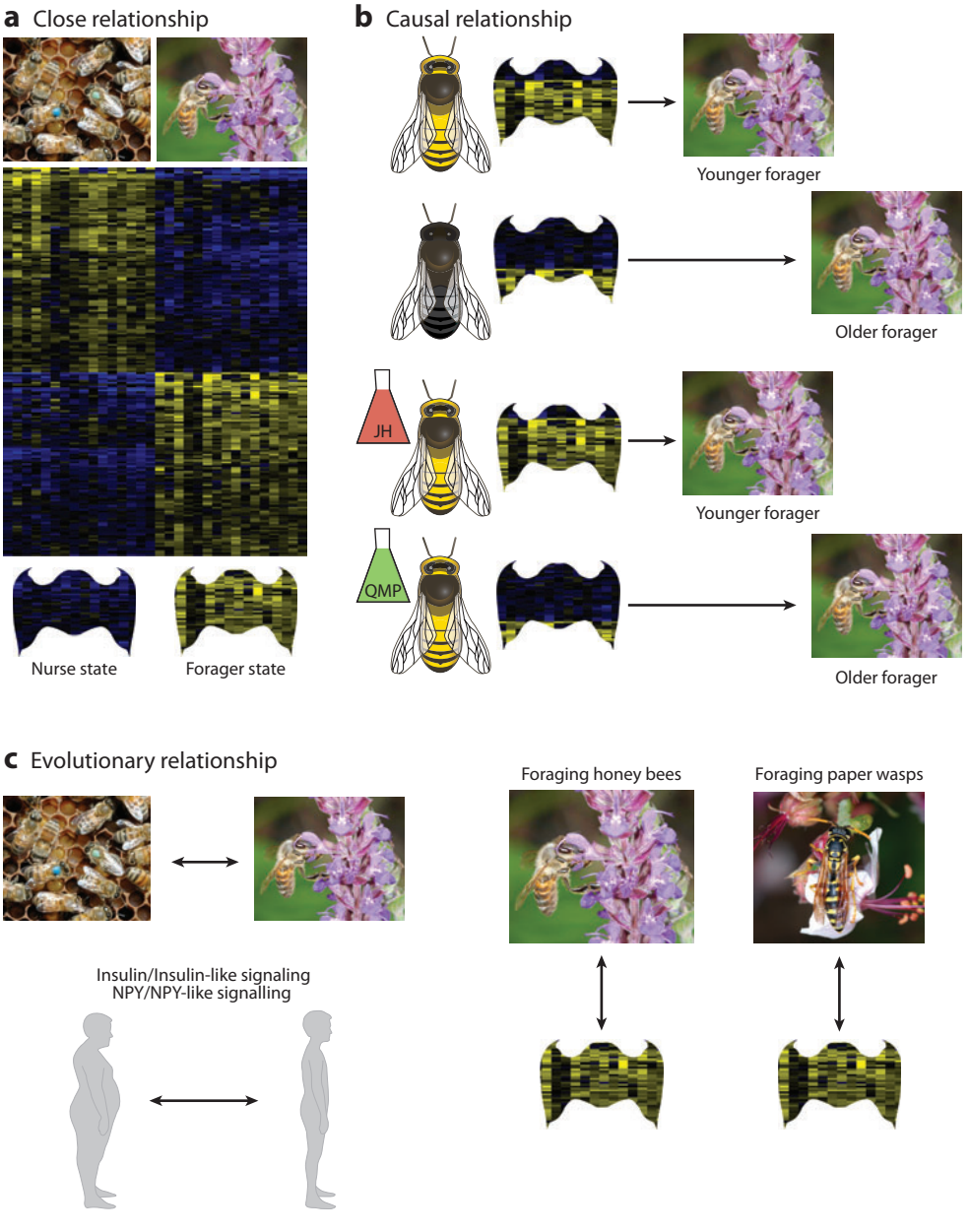
This profound shift in brain gene expression accompanying behavioral maturation was independently validated by several BeeSpace experiments using the oligo microarray platform (2, 36, 136). It was also confirmed by treating bees

Haplodiploid: females are diploid and develop from fertilized eggs, whereas males are haploid and develop from unfertilized eggs

with substances (pheromones, hormones, and intracellular signaling molecules) known to either speed up or slow down behavioral maturation, which induced brain gene expression profiles that were either more forager-like or nurse-like, respectively (129).

Aggression

Honey bee colony defense involves a complex blend of social, maturational, and genetic factors. Bees defend their colonies by releasing alarm pheromone and stinging intruders (132). Guard bees, which are approximately



two to three weeks old (132), patrol the hive entrance and release alarm pheromone upon encountering intruders. All older bees are more likely to respond to alarm pheromone when compared with hive-age bees, but a subset of the oldest bees, soldiers, are the first to respond by stinging intruders. The most famous hereditary difference in bee aggression involves the African subspecies *A. mellifera scutellata* and its derivative in the new world (i.e., Africanized "killer" bees), which are far more aggressive relative to European subspecies (51, 53).

Alaux et al. (4) reported hundreds of differences in brain gene expression associated with aggression in honey bees. They found many differences in brain gene expression between Africanized and European bees, and they also found that the magnitude of these differences was a function of involvement in nest defense; soldier bees exhibited the largest differences in brain gene expression between Africanized and European bees, followed by guards and then foragers. In addition to this correlative experiment, Alaux et al. (4) also determined the effects of alarm pheromone on brain gene expression, after first showing that alarm pheromone causes an aroused behavioral state that persists for several hours, in addition to its well-known acute effects on behavior (3). Alarm pheromone causes changes in the expression of hundreds of genes (4), which shows that even behavioral states of shorter duration can be characterized by distinct neurogenomic states. Alaux et al. (4) also found a significant overlap in the list of differentially expressed genes generated from the Africanized versus European bee comparisons and the genes induced by exposure to alarm pheromone, suggesting that at least some of these genes are involved in causing bees to become more aroused and more prone to aggression.

Gene Ontology (GO) analysis (19) revealed that differentially expressed genes associated with aggression were enriched for the well-fitting GO terms "response to stimulus" and "visual perception" (4). The neurogenomic state associated with aggression was also enriched for molecular functions involved in oxidative phosphorylation: Aggressive bees have relatively lower brain expression of oxidative phosphorylation genes (4). This counterintuitive result was validated via in vitro assays of mitochondrial activity from brain preparations (4). Several genes identified in this study were previously associated with aggression in vertebrates (*NMDA receptor* 2, *metabotropic*

Figure 2

(*a*) The close relationship between behavior and neurogenomic state. Changes in behavioral state in the honey bee are associated with shifts in brain gene expression defined by changes in the expression of hundreds to thousands of genes in the brain. The heat map represents only a few hundred (out of thousands) genes that shift from high expression (*yellow*) in brood care nurse bees to low expression in foragers (*blue*), and vice versa (136). Using transcriptomic data, distinct brain neurogenomic states associated with nursing and foraging can be defined. (*b*) Changes in brain gene expression are caused by both hereditary and environmental factors, and both result in changes in behavior. Italian honey bee workers (*yellow*) manifest a forager-like neurogenomic state at a much earlier age relative to German honey bee workers (*black*), concordant with heritable differences observed for the ontogeny of foraging between the two subspecies. Treatments that accelerate the ontogeny of foraging [e.g., juvenile hormone (JH) analog] also induce a forager-like neurogenomic state, whereas treatments that inhibit the ontogeny of foraging [e.g., queen mandibular pheromone (QMP)], induce a nurse-like neurogenomic state. The evidence suggests that hereditary and environmental influences on behavioral state are mediated through changes in neurogenomic state. (*c*) The close relationship between behavior and neurogenomic state over evolutionary time. Recent findings have indicated that the genes and pathways controlling feeding behavior and energy balance in vertebrates are also causally involved in the behavioral maturation of worker honey bees; nurses have ample abdominal lipid stores, whereas foragers are nutritionally deprived. Despite independent origins of social behavior, the neurogenomic state associated with foraging in honey bee workers is similar to that of foragers from the primitively eusocial paper wasp.

Quantitative trait loci: genomic regions that causally affect a quantitative phenotypic trait

glutamate receptor B, *moody*) and invertebrates (*Cyp6Q1*) (40, 108, 119). The expression of *14-3-3ε* is upregulated in brains of self-sacrificing soldier bees as well as in the brains of human suicide victims (135). Other differentially expressed genes had no previous associations with aggression in other species (as with all other behaviorally related lists of differentially expressed genes mentioned in this paper).

Foraging Specialists

Exploiting the behavioral richness of the honey bee foraging system, the BeeSpace experiments uncovered distinct neurogenomic states associated with several different types of foraging specialists.

Scouts. A relatively small fraction of a colony's foragers (5% to 25%) act as scouts by independently searching for novel food sources even when plentiful flower patches have been found. Recruits do not search for novel food sources and instead rely on information from scouts, communicated via dance language, to guide their foraging. By constantly discovering new flower patches, scouts help ensure that their colony is able to exploit profitable food sources whenever possible, despite the ephemeral nature of each patch. After training bees to forage on a feeder for several days in a large enclosure in the field, Liang et al. (67) introduced a novel feeder, marked with unique scent, while keeping available the original training feeder. Repeating this procedure, the authors were able to identify scout bees (i.e., those who repeatedly discovered novel feeders during the course of the experiments after being trained initially to the training feeder) and to contrast their brain gene expression with recruits. Scouts and recruits differed in brain expression for a substantial (16%, 1,216) portion of the genes represented on the microarray (67), including several catecholamine and glutamate receptors known to be involved in novelty-seeking behaviors in vertebrates (22). Pharmacological experiments revealed a causal relationship between individual differences in neurotransmitter signaling and the likelihood of becoming a scout (67).

Vibration signalers. These are another group of specialized foragers that generally make up less than 14% of a colony's foraging force (102). They perform a stereotypical vibratory signal that modulates the behavior of recipients. Vibration signalers hold other nestmates and vibrate dorsoventrally, which often results in increased task performance of recipient bees (1, 102). Alaux et al. (1) found changes in brain expression for 903 genes associated with vibration signaling after examining age-matched foragers with high or low affinity for performing this behavior. Genes underlying the distinct neurogenomic state associated with vibration signaling were enriched for locomotory behaviors, and several genes associated with vibration signaling in bees were orthologous to genes involved in Parkinson's Disease in humans (e.g., *DJ-1*, *SUMO-1*, *UBC7*, *EAAT2*) (1, 31, 71).

Nectar and pollen specialists. Another well-known form of foraging specialization involves the tendency to collect pollen or nectar. High pollen– and high nectar–collecting strains of bees have been produced by artificial selection (82), which demonstrates the presence of strong additive genetic variation for these behaviors. There has been extensive quantitative genetic analysis of these strains for more than 20 years, resulting in the identification of several major quantitative trait loci and candidate genes (51, 54, 82–85, 95–97). A detailed transcriptomic analysis of these strains has not yet been done, but a proteomic study revealed differences in brain abundance of several neuropeptides (33), and expression differences in a few genes have been reported (13). These results suggest that, as with scouts and vibration signalers, nectar and pollen foragers are characterized by distinct neurogenomic states.

Effects of Foraging Experience on Brain Gene Expression

Foraging-related behavior also was used to show the striking effects of experience on brain gene expression that, similar to the alarm pheromone study described above (4),

demonstrate that neurogenomic states can be associated with behavioral states of shorter duration. Evidence for this comes from the following two additional foraging-related BeeSpace experiments.

Spatiotemporal memories. Honey bees can learn to associate floral cues with food rewards over space and time, which allows them to remember both the location of profitable flowers and the time of day at which floral resources are most abundant. Naeger et al. (78) trained bees from the same colony to forage in either the morning or the afternoon and found extensive differences in brain gene expression. Of the 1,329 genes differentially expressed between the two groups with different spatiotemporal memories, 352 genes were not affected by time of collection/training or state of food anticipation, revealing a unique transcriptional signal that reflects the existence of spatiotemporal memories. Particularly prominent in these 352 genes were those involved in synaptogenesis, highlighting this neurobiological process in the formation of short-term memory-related foraging specializations. Naeger et al. (78) found 624 genes that varied in expression between the morning and afternoon, hinting at an influence of circadian rhythm on brain gene expression. Using a time-course experiment, Rodriguez-Zas et al. (94) found that the expression of 541 genes exhibited circadian oscillations in the brains of foragers, but only 160 genes showed oscillations in nurses; the latter group is characterized by attenuated circadian rhythms in behavior (30). These two studies suggest that, similar to mammals, changes in the expression of clock genes and their targets in the brains of foragers are related to their circadian food anticipatory behavior (78).

Distance measurement. Implicit in the ability of bees to form spatiotemporal floral memories is the ability to measure the distance of a floral resource from the hive and retain this information in the brain long enough to communicate it to nestmates via dance language. Honey bees measure distance using optic flow, i.e., the extent to which images of the world move on a bee's eyes during flight (43). Taking advantage of an elegant behavioral manipulation developed earlier (44, 109), Sen Sarma et al. (105) tricked two groups of bees into perceiving that they had flown either a short or a long distance even though both groups flew exactly the same distance. Specific brain regions were analyzed because the behavioral differences are more subtle than in most BeeSpace studies, suggesting that a whole-brain analysis would have been too coarse. Because perception of distance is likely coordinated between the optic lobes, which process visual information from the eye, and the mushroom bodies (MBs), which are involved in higher-order processing and integration of sensory information and learning and memory, these two brain regions were targeted. Sen Sarma et al. (105) reported that 52 genes (0.5%) were differentially expressed in association with perceiving either short or long distances in either the MBs, the optic lobes, or both. The majority of these genes showed consistent changes in both the MBs and the optic lobes (105), suggesting the possibility of coordinated changes in both of these regions.

General foraging experience and mushroom body gene expression. More evidence that short-term behavioral states bring about distinct patterns of brain gene expression is provided by a BeeSpace study that examined the relationship between foraging experience and gene expression in the MBs. Lutz et al. (72) quantified gene expression in the MBs of bees with 4, 8, 12, or 16 days of foraging experience. Nearly 500 genes were differentially expressed in association with foraging experience. The authors also found massive MB differences (i.e., 5,839 genes) between hive bees and foragers, echoing the previously discussed differences found at the whole-brain level. It is not known what aspects of foraging caused these changes; possibilities include effects of individual experience while foraging or social effects associated with returning to the hive and sharing food and information. This study foreshadows the potent effects of experience

and the social environment on brain gene expression, which we explore more fully below.

Meta-Analyses

The above studies demonstrate that different behavioral states are associated with distinct patterns of brain gene expression. Results from the following two meta-analyses of honey bee brain gene expression studies extend this insight by demonstrating that this relationship is based on a tight connection between transcriptional regulation and behavior.

Chandrasekaran et al. (36) performed a clustering of most of the brain gene expression profiles from the BeeSpace Project involving 853 bees sampled across 48 distinct behavioral states. They found that the profiles fit into three distinct clusters corresponding to maturation, aggression, and foraging. These were the original three behavioral categories that framed the BeeSpace Project, but what is noteworthy is that Chandrasekaran et al. (36) used an unsupervised clustering algorithm to achieve this result.

Chandrasekaran et al. (36) went on to use the same aggregated data set to reconstruct a brain transcriptional regulatory network (TRN), which aimed to predict large-scale patterns of gene expression in the brain given knowledge of the expression of transcription factors (TFs). It was possible to predict the expression of more than 2,000 genes (approximately 25% of the genes tested) as modules each tied to a single TF. Only four TFs (*broad*, *lilli*, *dl*, and *GB13780*) were globally active, meaning that their expression was able to predict the expression of a module of genes across all three behavioral categories, whereas other TFs were active only in one behavioral category and not the other two. Other TFs playing key roles in this TRN include well-known regulators of neural and behavioral plasticity, e.g., *creb*, as well as TFs better known in other biological contexts, e.g., *NF-κB* (immunity). These results demonstrate that the neurogenomic states underlying different behaviors rely upon both shared and distinct transcriptional modules, and despite the complexity of the brain, simple linear relationships between individual TFs and their putative target genes are a surprisingly prominent feature of the networks underlying behavior (36).

Although the brain TRN emphasized the effects of individual TFs on behaviorally relevant brain gene expression, results of another meta-analysis revealed more complex relationships among TFs in behavioral regulation while still highlighting the strong connection between transcriptional regulation and behavior. Ament et al. (11) used BeeSpace brain transcriptome profiles to scan promoter regions for *cis* regulatory motifs. They focused on profiles from approximately 400 individuals from experiments related to behavioral maturation. They showed bioinformatically that 11 different known determinants of behavioral maturation, some hereditary and some environmental, rely on specific, shared combinations of TFs to exert their effects. Similarly, in other honey bee studies, binding sites for a relatively small number of TFs were enriched in upstream sequences of differentially expressed genes (2, 4, 106), suggesting that differentially expressed genes are coherently regulated by the actions of TFs.

Summary

These results clearly demonstrate that behavioral states in the honey bee are associated with distinct brain gene expression profiles, that shifts in neurogenomic state involve changes in the expression of functional gene groups, and that these shifts are coherently regulated by the actions of a relatively small number of TFs. The close association between brain gene expression and behavioral state is especially remarkable given that transcription represents the most basic form of gene regulation. The honey bee has an active CpG methylation system (123), and ongoing research has demonstrated links among methylation, brain gene expression, and behavior (see sidebar, Methylation, Alternative Splicing, and Behavior in the Bee). The causes and ramifications of this intimate relationship between brain gene expression and behavior are discussed below.

NEUROGENOMIC STATES MEDIATE BEHAVIORAL STATES

The close association between brain gene expression and behavioral state does not in itself suggest a causal relationship between the two; distinct neurogenomic states may be either a cause or a consequence of behavior. Experimental manipulations of honey bee genetics, physiology, brain gene expression, behavior, and the social environment have been conducted to directly examine the degree of causality between brain gene expression and behavioral state. We review studies that show that both heredity and the environment influence behavior by affecting brain gene expression (**Figure 2*b***).

Genes Affect Neurogenomic State to Influence Social Behavior

Evidence for the idea that changes in expression of candidate genes bring about causal shifts in behavior is treated briefly here because the idea is so well established for animals and prior studies in honey bees have been reviewed elsewhere (107). The honey bee studies have largely focused on behavioral maturation, using both pharmacological and RNAi manipulations, and include studies of *foraging* (27, 28), *malvolio* (26), *vitellogenin* (80), *ultraspiracle* (14), and insulin-signaling genes (12, 124). In addition to these experiments, the above-mentioned BeeSpace study on scouts also led to pharmacological manipulations that established causal relationships between glutamate and catecholamine signaling and the probability of becoming a scout (67).

It is relatively easy to demonstrate causal relationships between brain gene expression and behavior for individual candidate genes, but how can we determine if changes in the neurogenomic state—which is defined by changes in the expression of hundreds to thousands of genes in the brain—causally affect behavior? Several lines of evidence suggest that much of the observed changes in brain gene expression are actively and coherently regulated by factors that are known to regulate behavior and that shifts in brain gene expression precede shifts in behavioral state.

One study that shows that shifts in brain gene expression precede shifts in behavioral state involved microarray analysis of bees treated with juvenile hormone (JH), a key insect developmental hormone that plays a major causal role in honey bee behavioral maturation. Foragers have higher circulating blood JH titers, hive bees treated with a JH analog forage precociously (50, 89), and surgical removal of the glands that produce JH results in delayed maturation, a deficit that is rescued by exogenous hormone treatment (110). Hive bees treated with a JH analog manifest a brain gene expression profile very similar to that of foragers even when kept in the laboratory and denied the opportunity to forage (129). Similarly, hive bees treated with cGMP and manganese—two treatments that increase the onset of foraging owing to effects on *foraging* and *malvolio*, respectively—also manifest a forager-like neurogenomic state in the absence of foraging

METHYLATION, ALTERNATIVE SPLICING, AND BEHAVIOR IN THE BEE

The honey bee was the first insect found to possess a functional CpG methylation system (123). DNA methylation is important for the regulation of gene expression (111), and bioinformatic and molecular analyses have implicated methylation as an important process in caste determination in the honey bee (41, 64). Functional studies have uncovered a great deal of differentially methylated genes in the brains of queens, workers, and drone bees (73). A candidate gene study found differences in methylation in the brains of nurses and foragers (70), suggesting that dynamic patterns of methylation can contribute to shifts in brain gene expression and the behavioral state of workers. The location of methylated CpGs within honey bee genes suggests that methylation plays an important role in alternative splicing (73). Jarosch et al. (58) provide a strong example of how alternative splicing of a single gene can affect major phenotypic traits. They found that alternative splicing of the transcription factor *gemini* underlies selfish reproductive behaviors of honey bee workers by controlling both ovary activation and thelytokous parthenogenesis.

Heritability: the proportion of phenotypic variance that is genetically determined

experience (129). In addition, bees treated with RNAi to knock down the expression of the JH-related TF *ultraspiracle* show a delay in behavioral maturation, and this manipulation affects the expression of many genes (14). Similar effects on behavioral maturation and gene expression have been obtained for nutritional manipulations (15). These results indicate that physiology alters brain gene expression, which in turn brings about shifts in behavior.

Additional evidence for the idea that shifts in brain gene expression precede shifts in behavioral state comes from microarray studies that compare different subspecies of honey bees with known differences in both behavioral maturation and aggression. There are genotypic differences in the rate of behavioral maturation in honey bees (51, 85, 96, 97). For example, bees of the Italian *A. mellifera ligustica* shift from hive work to foraging at a younger age than do bees of the German *A. mellifera mellifera*, even when the two are cofostered in the same hive (129). Differences in brain gene expression of *A. mellifera mellifera* and *A. mellifera ligustica* recapitulate those observed at the behavioral level. Bees from both subspecies exhibit large differences in brain gene expression when cofostered in the same colony, even at ages prior to the onset of foraging. These results suggest that brain gene expression is partially heritable; up to 30% of the genes in the honey bee genome showed significant differences in expression between the two subspecies in common garden experiments. Further, *A. mellifera ligustica* hive bees exhibited a forager-like neurogenomic state at an earlier (preforaging) age than *A. mellifera mellifera*, which is consistent with an earlier onset of foraging in the former (129).

There are also genotypic differences in aggressive behavior that are measured in terms of the speed and extent to which a colony will defend itself against an intruder, with Africanized bees much more aggressive than European subspecies (17, 18, 47, 52, 69). Again, bees from both subspecies show clear differences in behavior and brain gene expression when cofostered in the same environment (4). Differences in individual bee genotype (i.e., Africanized versus European) accounted for 30% of the variation in gene expression profiles across the experiment. Furthermore, 18 genes that showed differential brain expression in association with behavior were localized within previously identified quantitative trait loci affecting aggression in an Africanized × European honey bee cross (51), suggesting that heritability of aggression is partly caused by heritability of brain gene expression. Studies of several model genetic organisms have demonstrated a great deal of heritability in patterns of gene expression (34, 37, 42, 93, 101). The above two examples indicate that different honey bee genotypes exhibit heritable differences in both brain gene expression and behavior; future studies need to find the specific allelic differences to show how these hereditary influences on behavior are mediated through changes in brain gene expression.

The Social Environment Affects Neurogenomic State to Influence Social Behavior

The genome was once thought to be a relatively passive blueprint guiding organismal development. Studies of single genes revealed that genomes in fact remain highly responsive throughout life to a variety of stimuli associated with social behavior. Microarray analyses of honey bees were the first to demonstrate that brain responses to social stimuli can be massive, involving hundreds or thousands of genes (91). Social information can lead to changes in brain gene expression and behavior.

Honey bee behavior is tightly regulated by the social environment. BeeSpace experiments have demonstrated that the social environment plays a major role in regulating brain gene expression to alter behavior, as evidenced by the degree to which exposure to pheromones and different colony environments brings about causal shifts in neurogenomic and behavioral states.

Queen mandibular pheromone (QMP) has a strong inhibitory effect on worker behavioral maturation (60, 86, 132) and the development

of the antennal lobes (120). In cage experiments, the chronic exposure of young bees to QMP for several days caused a large shift in brain gene expression involving several hundred genes (36, 46). QMP treatments induced a nurse-like neurogenomic state consistent with its inhibitory effects on age at onset of foraging behavior (46). Brood pheromone (BP), secreted by bee larvae, also affects behavioral maturation but in an age-dependent manner. BP delays foraging in young bees but stimulates foraging in older bees (66). As with QMP, BP treatments caused distinct neurogenomic states in young and old bees as well as nurse-like changes in brain gene expression in young bees (2). Acting on a shorter timescale, exposure to alarm pheromone also brings about changes in the expression of several hundred genes in the bee brain, as discussed above (4). These results suggest that pheromone regulation of behavior is mediated in part by changes in brain gene expression.

Honey bee colonies have distinct behavioral personalities arising from the collective behavioral norms of their individual colony members (134). For example, there are striking and consistent differences between colonies in terms of foraging activity, aggressive behavior, corpse removal behavior, and comb repair (134). Many BeeSpace experiments have documented very strong colony differences in brain gene expression (4, 129, 136), and cross-fostering experiments related to aggression have revealed that these colony differences are due, in part, to the effects of the social environment on brain gene expression and behavior. Although, as stated above, a bee's genotype (Africanized versus European) has strong effects on brain gene expression, the effect of colony genotype is also massive, accounting for approximately 25% of the variance across all individuals (4). European bees cross-fostered in Africanized colonies exhibit an Africanized-like neurogenomic state and are more aggressive, whereas Africanized bees cross-fostered in European colonies exhibit a European-like neurogenomic state and become less aggressive (4, 53). Large colony effects on whole-body gene expression were also discovered in the social fire ant *Solenopsis invicta* (122). It is clear that extrinsic factors present in the social environment can alter brain gene expression to affect worker behavior.

Comparing the Imprints of Nature and Nurture on Neurogenomic State

The BeeSpace experiments demonstrate that distinct behavioral states are associated with distinct neurogenomic states and that hereditary and environmental influences on behavior are mediated through shifts in brain gene expression. But do nature and nurture affect the same brain transcriptional networks to alter behavior? The BeeSpace Project is uniquely positioned to address this question because it employed experimental designs that emphasized both intrinsic and extrinsic factors that relate to common behaviors.

Intrinsic and extrinsic determinants of behavior can influence some of the same genes, although often they act on different gene sets. For example, as discussed above, aggression and behavioral maturation are heritable traits that are also influenced in the short term by pheromones present in the social environment. The BeeSpace experiment (4) dissecting aggressive behavior clearly showed some overlap between hereditary and environmental influences on brain gene expression and behavior. For example, 5% to 10% of the genes that were significantly differentially regulated by alarm pheromone exposure also exhibited significant differences in expression between Africanized and European bees. There was also a moderate degree of correlation between changes in brain gene expression caused by alarm pheromone treatments and those affected by genotype across all assayed genes (4). Similar patterns were observed for behavioral maturation. Heritable differences in rate of behavioral maturation between *A. mellifera ligustica* and *A. mellifera mellifera* were associated with large differences in brain gene expression (129), and these differences were correlated with differences in the brain profiles of nurses and foragers as well

Endophenotypes: simple phenotypes that constitute a complex phenotype

as with differences caused by JH treatment (129). Similarly, the *cis*-regulatory meta-analysis described above (11) showed bioinformatically that 11 different determinants of behavioral maturation, some hereditary and some environmental, rely on specific, shared combinations of TFs to exert their effects. These results demonstrate that hereditary and social influences can sometimes result in common changes of brain gene expression.

Hereditary and environmental factors also affect distinct sets of genes. Chandrasekaran et al.'s (36) unsupervised clustering of the gene expression profiles not only demonstrated strong similarities within the behavioral categories of maturation, aggression, and foraging but also revealed the influence of timescales. Experiments with short environmental influences (i.e., hours to days) clustered separately from those with long environmental influences (i.e., weeks to months) (36). Experiments with environmental influences also clustered separately from those with hereditary influences (36). These findings indicate that environmental and hereditary influences on brain gene expression act on a mixture of common and distinct populations of genes.

We speculate that this may result in better integration of long-term hereditary influences and short-term environmental influences on the brain. Heredity may set a specific neurogenomic state and a behavioral tendency over the lifetime of an individual, which can then be fine-tuned by social influences in the short-term. This hypothesis is consistent with the observation that the social environment can modulate hereditary influences but does not completely override heredity; aggressive Africanized bees move toward a more docile neurogenomic and behavioral state when fostered in colonies of European bees, but they still exhibit aggressive behavior and large differences in brain gene expression because of genotype.

Summary

The neurogenomic state acts to mediate hereditary and environmental influences on the behavioral state. Manipulative experiments provide strong evidence that intrinsic and extrinsic factors bring about coherent shifts in brain gene expression prior to shifts in behavior, and shifts in brain gene expression are regulated by hierarchical and modular transcription regulatory networks. These results demonstrate the prominent role played by brain transcriptomes in regulating behavioral plasticity.

RELATIONSHIP BETWEEN BRAIN GENE EXPRESSION AND BEHAVIORAL EVOLUTION

The BeeSpace experiments demonstrate that brain gene expression is closely associated with behavioral state over diverse timescales. Responses to some pheromones reveal associations over short, physiologically relevant timescales, i.e., hours to days. Studies of plasticity in behavioral maturation reveal associations over developmentally relevant timescales of an intermediate nature, i.e., weeks to months. Genotypic differences in brain gene expression, i.e., between subspecies or strains of bees, reflect associations over longer, evolutionary timescales. An emerging insight is that these associations involve changes in expression of the same genes acting over different timescales.

The deep conservation of most gene families suggests the hypothesis that honey bee social behavior evolved, in part, through the co-option of conserved genes for novel functions. Studies in bees have identified several such conserved genes that have evolved novel functions in social behavior and characterized many more that have undergone accelerated sequence evolution in lineages of related eusocial insects. We review these findings and their implications for understanding the evolution of social behavior (**Figure 2*c***).

Common Molecular Underpinning of Behavior Across Social and Solitary Organisms

Most of the constituent endophenotypes underlying social behavior in honey bees, such as nest construction, foraging, associative

learning, and aggression (65, 77), are present in solitary species, but the motivations are different. For example, worker honey bees forage for their whole colony, which consists primarily of siblings; they determine the needs of their colony in addition to their own and in some cases communicate with each other. By contrast, a solitary insect forages for itself or its offspring. Similarly, relative to worker honey bees, the pace of male honey bee maturation appears to be more stereotyped and not sensitive to changes in colony environment. We review findings from experiments that exploit some of these differences to provide evidence for evolutionary commonalities for gene action, brain gene expression, and behavior.

Analysis of the *foraging* gene in honey bees clearly shows that the relationship between genes and behavior spans multiple timescales. *foraging*, which encodes a cyclic G-dependent protein kinase, affects feeding and food gathering–related activities in both honey bees and *Drosophila melanogaster* (27, 28, 38, 61, 81), demonstrating conserved gene action over approximately 300 million years. The same is true for *malvolio*, which encodes a manganese transporter active in neurons (26). *foraging* and several other genes involved with division of labor in honey bees are also associated with division of labor in other insects with independent origins of eusociality (56, 57, 63, 113, 117, 118). The results of these comparative studies indicate that the independently derived behaviors in different insect societies share common molecular and neurogenomic underpinnings.

A BeeSpace experiment that compared worker and male brain gene expression as a function of behavioral maturation (136) showed that most of the transcriptional changes observed during worker behavioral maturation are also observed during male behavioral maturation, despite the differences noted above. This finding suggests that worker social behavior was built upon a common platform for behavioral maturation in insects (136). There is a growing body of literature documenting common molecular underpinnings of homologous or analogous behavioral traits (87) in animals.

The BeeSpace experiments provide a wealth of new evidence demonstrating deep conservation for genes, gene networks, and behavior. For example, both Neuropeptide Y–like signaling and insulin-like signaling, which regulate food searching and intake in vertebrates and invertebrates, have been found to be associated with foraging behavior and maturation in honey bees (12, 13, 33). Nutrition is a strong determinant of behavioral maturation in honey bees; hive bees lose half of their abdominal lipids prior to the onset of foraging, and experimental inhibition of fatty acid synthesis leads to precocious foraging (114). Additional studies have uncovered common molecular underpinnings for novelty seeking in honey bees, nematodes, and humans (67); aggression in honey bees, other invertebrates, and vertebrates (4); and vibratory communication in both honey bees and fruit flies (1). These commonalities extend to the level of transcriptional regulation. Both the target genes and the regulatory sites involved in the *ultraspiracle* regulatory network are conserved in both honey bees and fruit flies (14). In addition, many key TFs in the bee brain TRN described above are well-known regulators of neuronal and behavioral plasticity in invertebrates and vertebrates (36). The BeeSpace studies indicate that the molecular machinery that produces behavior can be similar across taxa, lending support to the concept that particular genes and networks represent a basic toolkit that has been used repeatedly in the evolution of behavior (115, 116).

Modularity of Neurogenomic States and the Evolution of Social Behavior

How can we account for the highly derived behavior of honey bees if the molecular underpinnings of behavior are conserved across animals? Also, how can the behavior of the worker bee caste evolve without compromising that of its reproductive kin (e.g., queens and males)? As discussed above, many of the

behavioral endophenotypes displayed by honey bees are present in their solitary ancestors as well as in their reproductive conspecifics. We suggest that the uniqueness of worker honey bee behavior is based on the coexpression of specific endophenotypes and their regulation by the social environment. Within this framework, it has been possible to envision how co-option of preexisting genes and gene networks along with novel regulatory coupling (or decoupling) of behavioral modules in a caste-specific manner can give rise to the complex behavioral phenotypes exhibited by bees while still retaining the basic behavioral repertoire displayed by the reproductive castes (6, 55, 68, 115, 116, 126).

The BeeSpace experiments provide strong support for the existence of transcriptional modules (**Figure 3**) that influence behavior by altering brain gene expression (36), and the hierarchical nature of such modules (14, 15, 36) can theoretically account for both behavioral plasticity and evolutionary lability. For example, results from the bee brain TRN described above led to the prediction that changes in the expression of one or several TFs can influence local or global aspects of bee behavioral state, depending on their position in the TRN (36). It will be important in the future to develop an approach that allows for large-scale testing of predictions of this type under natural conditions. If these predictions prove to be correct, then we might be able to deduce that evolutionary changes in the regulation of a few key TFs (by pheromones or hormones, for example) lead to evolutionary changes in behavioral regulation.

Neurogenomic State, Behavior, and Adaptation

The honey bee has more than 20 different subspecies that differ at the genetic (48, 128), neurogenomic (4, 129), and behavioral levels (98, 132), providing ample opportunities for genetic analyses of naturally occurring variation in brain gene expression and behavior and of its role in facilitating adaptation in social organisms. For example, despite their geographic proximity, the German *A. mellifera mellifera* and Italian *A. mellifera ligustica* are the two most divergent honey bee subspecies, as they were derived from independent expansions out of the ancestral African population (128). Patterns of genetic differentiation across the honey bee genome suggest that these out-of-Africa expansions were associated with bouts of adaptive evolution at coding or nearby regulatory sequences (137). Indeed, *A. mellifera mellifera* and *A. mellifera ligustica* differ in several aspects of behavior, including aggression and behavioral maturation (8, 32, 132), in a presumably adaptive way. The two subspecies also exhibit distinct neurogenomic states when cofostered in the same colony. Is local adaptation in the honey bee mediated through genetic polymorphisms that affect brain gene expression and behavior?

The apparent heritability of brain gene expression in itself does not imply that a large number of *cis*-acting polymorphisms affecting brain gene expression are fixed between *A. mellifera mellifera* and *A. mellifera ligustica*. Alternatively, this heritability can be the result of pleiotropic effects upstream of TRNs in the brain. Cofostered bees of *A. mellifera mellifera* and *A. mellifera ligustica* also show significant differences in JH titers in a manner that is consistent with their known differences in behavioral maturation (32, 129). Given that JH can drastically affect brain gene expression and behavior (129), heritability of brain gene expression may be caused by polymorphisms that regulate aspects of hormone signaling that then act on the brain.

This suggestion is consistent with results from a molecular evolution analysis of the egg yolk protein vitellogenin (Vg), which has taken on novel functions in honey bee workers related to immunity, brood feeding, and behavioral maturation (7, 10, 80). Vg exhibits a mutually repressive relationship with JH and causally affects behavioral maturation (6, 9, 80); *vg* RNAi leads to precocious foraging, as predicted by

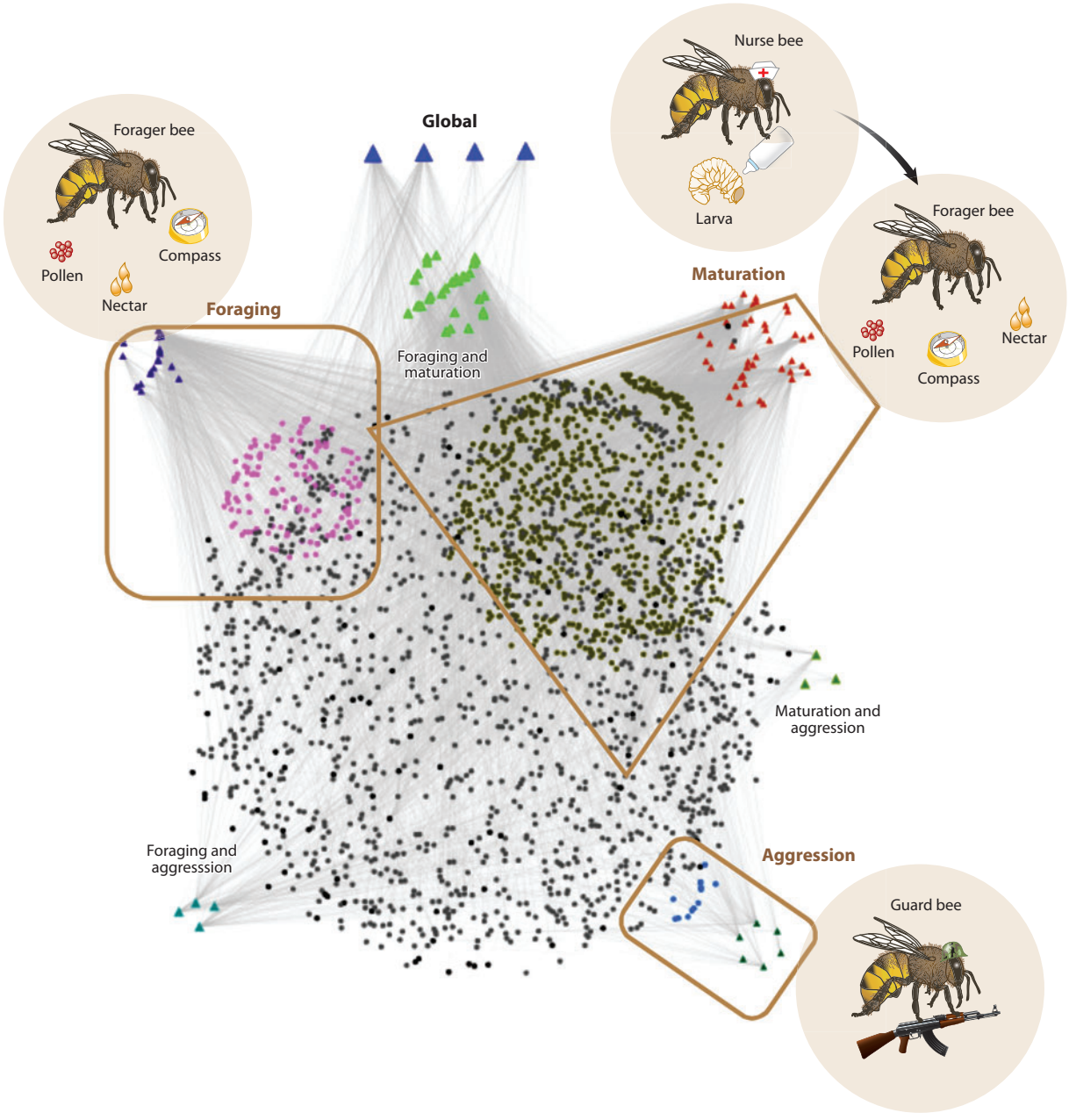


Figure 3

Brain transcriptional regulatory network (TRN) associated with behavior in the honey bee (36). Expression data from the BeeSpace experiments were used to reconstruct a TRN that predicts large-scale patterns of gene expression in the brain (*squares*) given knowledge of the expression of transcription factors (TFs, *triangles*). This TRN successfully predicted the expression patterns of 2,176 genes (approximately 25% of the genes on the microarray) that were differentially expressed in the honey bee brain in association with behavioral state given knowledge of the expression of approximately 190 TFs. The TRN is modular, with most TFs affecting behavior-specific gene subnetworks associated with behavioral maturation, foraging, or aggression. Several TFs affect subnetworks across two behavioral categories, and only four TFs act globally by affecting targets across all three behavioral categories.

Vg's and JH's dual repressive action (9). Up to 88% of the functional (i.e., nonsynonymous) mutations in *vg* have been driven to fixation by positive selection in the genus *Apis* (62). Further, there is an excess of amino acid–replacing mutations with outlier levels of genetic differentiation within *Apis mellifera* subspecies—a hallmark of local adaptation (62). These results suggest the possibility that functional mutations at *vg* affect fitness in honey bee populations by modulating division of labor through its interactions with JH.

Another gene implicated in behavioral maturation, the mitogen-activated protein kinase *erk7*, also shows strong signs of positive selection in honey bees. Brain expression of *erk7* represents one of the best biomarkers of foraging in honey bees: *erk7* is expressed in the brain at higher levels in foragers versus nurses and in *A. mellifera ligustica* versus *A. mellifera mellifera* (129, 130). ERK7 has remarkably high rates of adaptive protein evolution in honey bees, and the selection coefficient estimated for *erk7* exceeds that of *vg* by 50% (62).

A broad molecular evolution analysis also suggests that the evolution of eusociality in insects involves adaptive evolution of genes implicated in honey bee behavioral regulation in transcriptomic studies. Woodard et al. (133) compared the brain transcriptomes of ten bee species that encompass solitary, primitively eusocial, and advanced eusocial lifestyles and represent three independent origins of eusociality. They discovered several hundred genes with patterns of rapid and likely adaptive molecular evolution associated with the rise of eusociality. Genes with signs of positive selection were enriched for roles associated with neuronal development and metabolism in primitively eusocial and advanced eusocial bees, respectively. Genes in both of these categories are commonly found to be differentially expressed in the honey bee brain as a function of behavior (2, 4, 136).

Summary

These studies demonstrate that the molecular bases of social behavior share many common elements with those affecting simpler behaviors in solitary animals. The modularity of brain TRNs suggests that rewiring conserved modules may provide a general mechanism for the evolution of novel behavior through coupling of specific behavioral phenotypes under new or different physiological or environmental regulators. These studies also establish a link between neurogenomic and behavioral state and adaptation in social insects and set the stage for more direct tests of adaptive significance.

CONCLUSIONS

The studies reviewed here have helped provide an initial framework for understanding how heritable and environmental factors exert their effects on brain gene expression and behavior. These studies demonstrate that brain gene expression is closely linked with behavior, that changes in brain gene expression mediate changes in behavior, and that the association between specific genes and behavior exists over multiple timescales, from physiological to evolutionary. We still know little about how social factors are transduced to ultimately affect gene expression in cells in the brain, and this information is necessary to begin to develop a broad synthesis of behavior, which will involve both the social and life sciences and be imbued with evolutionary insight. Bees are excellent models to contribute to such a synthesis because of their rich behavioral repertoire, amenability to social manipulation, and diversity of related species with different kinds of social and solitary lifestyles.

SUMMARY POINTS

1. Brain gene expression profile is closely linked with behavioral state, suggesting that distinct behaviors are facilitated by distinct neurogenomic states.

2. Heredity, social environment, and physiology act on the brain by modulating brain gene expression and behavior.
3. The close relationship between brain gene expression and behavior spans physiological, developmental, and evolutionary timescales.
4. Complex behaviors of social insects likely evolved by using behavioral modules and genetic toolkits present in solitary ancestors.
5. Several genes underlying neurogenomic and behavioral state in the honey bee and other social insects evolve adaptively.

FUTURE ISSUES

1. How do changes in brain gene expression interact with brain circuits to influence behavior? It is not clear why whole-brain analyses provided such robust results given the strong functional differences that exist in all brains. Future studies need to measure gene expression at ever finer levels of neural organization.
2. What are the roles of epigenetics, alternative splicing, and miRNA in regulating brain gene expression and bee behavior?
3. What is the relative contribution of *cis*- versus *trans*-acting polymorphisms to heritability of brain gene expression in the honey bee?
4. What is the degree to which changes in gene regulatory and protein-coding sequences facilitate adaptive changes in behavior?
5. How do changes upstream of TRNs affect behavior, and how can these changes result in behavioral shifts during the evolution of sociality?
6. How do behaviorally related conserved and novel genes interact in mechanistic and evolutionary contexts? We focused on conserved genes because they are easier to identify, but as more genomes are sequenced and orthology analyses improve, it should be possible to identify novel genes associated with social behavior (59).
7. Is a close relationship between neurogenomic state and behavior a general phenomenon? A burgeoning literature has linked changes in brain gene expression to changes in behavior in several species (20, 21, 23, 24, 39, 45, 74, 79, 91, 100, 103, 117, 122), but further work is needed.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Alaux C, Duong N, Schneider SS, Southey BR, Rodriguez-Zas S, et al. 2009. Modulatory communication signal performance is associated with a distinct neurogenomic state in honey bees. *PLoS ONE* 4:e6694
2. Alaux C, Le Conte Y, Adams HA, Rodriguez-Zas S, Grozinger CM, et al. 2009. Regulation of brain gene expression in honey bees by brood pheromone. *Genes Brain Behav.* 8:309–19
3. Alaux C, Robinson GE. 2007. Alarm pheromone induces immediate-early gene expression and slow behavioral response in honey bees. *J. Chem. Ecol.* 33:1346–50
4. Alaux C, Sinha S, Hasadsri L, Hunt GJ, Guzman-Novoa E, et al. 2009. Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proc. Natl. Acad. Sci. USA* 106:15400–5
5. Allison DB, Cui XQ, Page GP, Sabripour M. 2006. Microarray data analysis: from disarray to consolidation and consensus. *Nat. Rev. Genet.* 7:55–65
6. Amdam GV, Norberg K, Fondrk MK, Page RE Jr. 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. USA* 101:11350–55
7. Amdam GV, Norberg K, Hagen A, Omholt SW. 2003. Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. USA* 100:1799–802
8. Amdam GV, Norberg K, Omholt SW, Kryger P, Lourenco AP, et al. 2005. Higher vitellogenin concentrations in honey bee workers may be an adaptation to life in temperate climates. *Insectes Soc.* 52:316–19
9. Amdam GV, Omholt SW. 2003. The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. *J. Theor. Biol.* 223:451–64
10. Amdam GV, Simoes ZLP, Hagen A, Norberg K, Schroder K, et al. 2004. Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. *Exp. Gerontol.* 39:767–73
11. Ament SA, Blatti CA, Alaux C, Wheeler MM, Toth AL, et al. 2012. New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. *Proc. Natl. Acad. Sci. USA* 109:E1801–10
12. Ament SA, Corona M, Pollock HS, Robinson GE. 2008. Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proc. Natl. Acad. Sci. USA* 105:4226–31
13. Ament SA, Velarde RA, Kolodkin MH, Moyse D, Robinson GE. 2011. Neuropeptide Y–like signalling and nutritionally mediated gene expression and behaviour in the honey bee. *Insect Mol. Biol.* 20:335–45
14. Ament SA, Wang Y, Chen C, Blatti CA, Hong F, et al. 2012. The transcription factor *ultraspiracle* influences honey bee social behavior and behavior-related gene expression. *PLoS Genet.* 8:e1002596
15. Ament SA, Wang Y, Robinson GE. 2010. Nutritional regulation of division of labor in honey bees: toward a systems biology perspective. *WIREs Syst. Biol. Med.* 2:566–76
16. Anholt RRH, Mackay TFC. 2004. Quantitative genetic analyses of complex behaviours in *Drosophila*. *Nat. Rev. Genet.* 5:838–49
17. Arechavaleta-Velasco ME, Hunt GJ. 2004. Binary trait loci that influence honey bee (Hymenoptera: Apidae) guarding behavior. *Ann. Entomol. Soc. Am.* 97:177–83
18. Arechavaleta-Velasco ME, Hunt GJ, Emore C. 2003. Quantitative trait loci that influence the expression of guarding and stinging behaviors of individual honey bees. *Behav. Genet.* 33:357–64
19. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, et al. 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25:25–29
20. Aubin-Horth N, Landry CR, Letcher BH, Hofmann HA. 2005. Alternative life histories shape brain gene expression profiles in males of the same population. *Proc. R. Soc. Lond. Ser. B* 272:1655–62
21. Badisco L, Ott SR, Rogers SM, Matheson T, Knapen D, et al. 2011. Microarray-based transcriptomic analysis of differences between long-term gregarious and solitarious desert locusts. *PLoS ONE* 6:e28110

22. Bardo MT, Donohew RL, Harrington NG. 1996. Psychobiology of novelty seeking and drug seeking behavior. *Behav. Brain Res.* 77:23–43
23. Bell AM, Aubin-Horth N. 2010. What can whole genome expression data tell us about the ecology and evolution of personality? *Phil. Trans. R. Soc. B* 365:4001–12
24. Bell AM, Robinson GE. 2011. Behavior and the dynamic genome. *Science* 332:1161–62
25. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, et al. 2005. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat. Rev. Genet.* 6:544–56
26. Ben-Shahar Y, Dudek NL, Robinson GE. 2004. Phenotypic deconstruction reveals involvement of manganese transporter *malvolio* in honey bee division of labor. *J. Exp. Biol.* 207:3281–88
27. Ben-Shahar Y, Leung H-T, 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. *J. Exp. Biol.* 206:2507–15
28. Ben-Shahar Y, Robichon A, Sokolowski MB, Robinson GE. 2002. Influence of gene action across different time scales on behavior. *Science* 296:741–44
29. Beshers SN, Fewell JH. 2001. Models of division of labor in social insects. *Annu. Rev. Entomol.* 46:413–40
30. Bloch G. 2010. The social clock of the honeybee. *J. Biol. Rhythms* 25:307–17
31. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, et al. 2003. Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* 299:256–59
32. Brillet C, Robinson GE, Bues R, LeConte Y. 2002. Racial differences in division of labor in colonies of the honey bee. *Ethology* 108:115–26
33. Brockmann A, Annangudi SP, Richmond TA, Ament SA, Xie F, et al. 2009. Quantitative peptidomics reveal brain peptide signatures of behavior. *Proc. Natl. Acad. Sci. USA* 106:2383–88
34. Bystrykh L, Weersing E, Dontje B, Sutton S, Pletcher MT, et al. 2005. Uncovering regulatory pathways that affect hematopoietic stem cell function using "genetical genomics." *Nat. Genet.* 37:225–32
35. Cash A, Whitfield CW, Ismail N, Robinson GE. 2005. Behavior and the limits of genomic plasticity: power and replicability in microarray analysis of honey bee brains. *Genes Brain Behav.* 4:267–71
36. Chandrasekaran S, Ament SA, Eddy JA, Rodriguez-Zas SL, Schatz BR, et al. 2011. Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. *Proc. Natl. Acad. Sci. USA* 108:18020–25
37. Chesler EJ, Lu L, Shou S, Qu Y, Gu J, et al. 2005. Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. *Nat. Genet.* 37:233–42
38. Dawson-Scully K, Armstrong GA, Kent C, Robertson RM, Sokolowski MB. 2007. Natural variation in the thermotolerance of neural function and behavior due to a cGMP-dependent protein kinase. *PLoS ONE* 2:e773
39. Dong S, Replogle KL, Hasadsri L, Imai BS, Yau PM, et al. 2009. Discrete molecular states in the brain accompany changing responses to a vocal signal. *Proc. Natl. Acad. Sci. USA* 106:11364–69
40. Drnevich JM, Reedy MM, Ruedi EA, Rodriguez-Zas S, Hughes KA. 2004. Quantitative evolutionary genomics: differential gene expression and male reproductive success in *Drosophila melanogaster*. *Proc. R. Soc. Lond. Ser. B* 271:2267–73
41. Elango N, Hunt BG, Goodisman MAD, Yi SV. 2009. DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. *Proc. Natl. Acad. Sci. USA* 106:11206–11
42. Emilsson V, Thorleifsson G, Zhang B, Leonardson AS, Zink F, et al. 2008. Genetics of gene expression and its effect on disease. *Nature* 452:423–28
43. Esch B, Burns JE. 1995. Honeybees use optic flow to measure the distance of a food source. *Naturwissenschaften* 82:38–40
44. Esch HE, Zhang SW, Srinivasan MV, Tautz J. 2001. Honeybee dances communicate distances measured by optic flow. *Nature* 411:581–83
45. Gan L, Liu XL, Xiang ZH, He NJ. 2011. Microarray-based gene expression profiles of silkworm brains. *BMC Neurosci.* 12:8
46. Grozinger CM, Sharabash NM, Whitfield CW, Robinson GE. 2003. Pheromone-mediated gene expression in the honey bee brain. *Proc. Natl. Acad. Sci. USA* 100(Suppl. 2):14519–25

47. Guzman-Novoa E, Hunt GJ, Uribe JL, Smith C, Arechavaleta-Velasco ME. 2002. Confirmation of QTL effects and evidence of genetic dominance of honeybee defensive behavior: results of colony and individual behavioral assays. *Behav. Genet.* 32:95–102
48. Harpur BA, Minaei S, Kent CF, Zayed A. 2012. Management increases genetic diversity of honey bees via admixture. *Mol. Ecol.* 21:4414–21
49. The Honeybee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443:931–49
50. Huang ZY, Robinson GE, Borst DW. 1994. Physiological correlates of division of labor among similarly aged honey bees. *J. Comp. Physiol. A* 174:731–39
51. Hunt GJ, Amdam GV, Schlipalius D, Emore C, Sardesai N, et al. 2007. Behavioral genomics of honeybee foraging and nest defense. *Naturwissenschaften* 94:247–67
52. Hunt GJ, Guzman-Novoa E, Fondrk MK, Page RE. 1998. Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* 148:1203–13
53. Hunt GJ, Guzman-Novoa E, Uribe-Rubio JL, Prieto-Merlos D. 2003. Genotype-environment interactions in honeybee guarding behaviour. *Anim. Behav.* 66:459–67
54. Hunt GJ, Page REJ, Fondrk MK, Dullum CJ. 1995. Major quantitative trait loci affecting honey bee foraging behavior. *Genetics* 141:1537–45
55. Hunt JH, Amdam GV. 2005. Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* 308:264–67
56. Ingram KK, Kleeman L, Peteru S. 2011. Differential regulation of the *foraging* gene associated with task behaviors in harvester ants. *BMC Ecol.* 11:19
57. Ingram KK, Oefner P, Gordon DM. 2005. Task-specific expression of the *foraging* gene in harvester ants. *Mol. Ecol.* 14:813–18
58. Jarosch A, Stolle E, Crewe RM, Moritz RFA. 2011. Alternative splicing of a single transcription factor drives selfish reproductive behavior in honeybee workers (*Apis mellifera*). *Proc. Natl. Acad. Sci. USA* 108:15282–87
59. Johnson BR, Tsutsui ND. 2011. Taxonomically restricted genes are associated with the evolution of sociality in the honey bee. *BMC Genomics* 12:164
60. Keeling CI, Slessor KN, Higo HA, Winston ML. 2003. New components of the honey bee (*Apis mellifera* L.) queen retinue pheromone. *Proc. Natl. Acad. Sci. USA* 100:4486–91
61. Kent CF, Daskalchuk T, Cook L, Sokolowski MB, Greenspan RJ. 2009. The *Drosophila foraging* gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation. *PLoS Genet.* 5:e1000609
62. Kent CF, Issa A, Bunting AC, Zayed A. 2011. Adaptive evolution of a key gene affecting queen and worker traits in the honey bee, *Apis mellifera*. *Mol. Ecol.* 20:5226–35
63. Kodaira Y, Ohtsuki H, Yokoyama J, Kawata M. 2009. Size-dependent *foraging* gene expression and behavioral caste differentiation in *Bombus ignitus*. *BMC Res. Notes* 2:184
64. Kucharski R, Maleszka J, Foret S, Maleszka R. 2008. Nutritional control of reproductive status in honeybees via DNA methylation. *Science* 319:1827–30
65. Leadbeater E, Chittka L. 2007. The dynamics of social learning in an insect model, the bumblebee (*Bombus terrestris*). *Behav. Ecol. Sociobiol.* 61:1789–96
66. Le Conte Y, Mohammedi A, Robinson GE. 2001. Primer effects of a brood pheromone on honeybee behavioural development. *Proc. R. Soc. Lond. Ser. B* 268:163–68
67. Liang ZS, Nguyen T, Mattila HR, Rodriguez-Zas SL, Seeley TD, et al. 2012. Molecular determinants of scouting behavior in honey bees. *Science* 335:1225–28
68. Linksvayer TA, Wade MJ. 2005. The evolutionary origin and elaboration of sociality in the aculeate Hymenoptera: maternal effects, sib-social effects, and heterochrony. *Q. Rev. Biol.* 80:317–36
69. Lobo NF, Ton LQ, Hill CA, Emore C, Romero-Severson J, et al. 2003. Genomic analysis in the sting-2 quantitative trait locus for defensive behavior in the honey bee, *Apis mellifera*. *Genome Res.* 13:2588–93
70. Lockett GA, Kucharski R, Maleszka R. 2012. DNA methylation changes elicited by social stimuli in the brains of worker honey bees. *Genes Brain Behav.* 11:235–42
71. Lotharius J, Brundin P. 2002. Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein. *Nat. Rev. Neurosci.* 3:932–42

72. Lutz CC, Rodriguez-Zas SL, Fahrbach SE, Robinson GE. 2011. Transcriptional response to foraging experience in the honey bee mushroom bodies. *Dev. Neurobiol.* 72:153–66
73. Lyko F, Foret S, Kucharski R, Wolf S, Falckenhayn C, et al. 2010. The honey bee epigenomes: differential methylation of brain DNA in queens and workers. *PLoS Biol.* 8:e1000506
74. Mello CV, Vicario DS, Clayton DF. 1992. Song presentation induces gene expression in the songbird forebrain. *Proc. Natl. Acad. Sci. USA* 89:6818–22
75. Menzel R, Giurfa M. 2001. Cognitive architecture of a mini-brain: the honeybee. *Trends Cogn. Sci.* 5:62–71
76. Merrow M, Spoelstra K, Roenneberg T. 2005. The circadian cycle: daily rhythms from behaviour to genes. *EMBO Rep.* 6:930–35
77. Michener CD. 2000. *The Bees of the World*. Baltimore, MD: Johns Hopkins Univ. Press
78. Naeger NL, Van Nest BN, Johnson JN, Boyd SD, Southey BR, et al. 2011. Neurogenomic signatures of spatiotemporal memories in time-trained forager honey bees. *J. Exp. Biol.* 214:979–87
79. Natt D, Lindqvist N, Stranneheim H, Lundeberg J, Torjesen PA, et al. 2009. Inheritance of acquired behaviour adaptations and brain gene expression in chickens. *PLoS ONE* 4:e6405
80. Nelson CM, Ihle KE, Fondrk MK, Page REJ, Amdam GV. 2007. The gene *vitellogenin* has multiple coordinating effects on social organization. *PLoS Biol.* 5:e62
81. Osborne KA, Robichon A, Burgess E, Butland S, Shaw RA, et al. 1997. Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* 277:834–36
82. Page RE, Fondrk MK. 1995. The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. *Behav. Ecol. Sociobiol.* 36:135–44
83. Page RE, Fondrk MK, Hunt GJ, Guzman-Novoa E, Humphries MA, et al. 2000. Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. *J. Hered.* 91:474–79
84. Page REJ, Scheiner R, Erber J, Amdam GV. 2006. The development and evolution of division of labor and foraging specialization in a social insect (*Apis mellifera* L.). *Curr. Top. Dev. Biol.* 74:253–86
85. Page REJ, Waddington KD, Hunt GJ, Fondrk MK. 1995. Genetic determinants of honey bee foraging behaviour. *Anim. Behav.* 50:1617–25
86. Pankiw T, Huang ZY, Robinson GE, Winston ML. 1998. Queen mandibular pheromone influences worker honey bee (*Apis mellifera*) foraging ontogeny and juvenile hormone titers. *J. Insect Physiol.* 44:685–92
87. Reaume CJ, Sokolowski MB. 2011. Conservation of gene function in behaviour. *Phil. Trans. R. Soc. B* 366:2100–10
88. Robinson GE. 1992. Regulation of division-of-labor in insect societies. *Annu. Rev. Entomol.* 37:637–65
89. Robinson GE. 2002. Genomics and integrative analyses of division of labor in honeybee colonies. *Am. Nat.* 160:160–71
90. Robinson GE. 2004. Beyond nature and nurture. *Science* 304:397–99
91. Robinson GE, Fernald RD, Clayton DF. 2008. Genes and social behavior. *Science* 322:896–900
92. Robinson GE, Grozinger CM, Whitfield CW. 2005. Sociogenomics: social life in molecular terms. *Nat. Rev. Genet.* 6:257–70
93. Rockman MV, Kruglyak L. 2006. Genetics of global gene expression. *Nat. Rev. Genet.* 7:862–72
94. Rodriguez-Zas SL, Southey BR, Shemesh Y, Rubin EB, Cohen M, et al. 2012. Microarray analysis of natural socially regulated plasticity in circadian rhythms of honey bees. *J. Biol. Rhythms* 27:12–24
95. Rüppell O, Chandra SBC, Pankiw T, Fondrk MK, Beye M, et al. 2006. The genetic architecture of sucrose responsiveness in the honeybee (*Apis mellifera* L.). *Genetics* 172:243–51
96. Rüppell O, Pankiw T, Nielsen DI, Fondrk MK, Beye M, et al. 2004. The genetic architecture of the behavioral ontogeny of foraging in honeybee workers. *Genetics* 167:1767–79
97. Rüppell O, Pankiw T, Page REJ. 2004. Pleiotropy, epistasis and new QTL: the genetic architecture of honey bee foraging behavior. *J. Hered.* 95:481–91
98. Ruttner F. 1988. *Biogeography and Taxonomy of Honeybees*. New York: Springer
99. Sagili RR, Pankiw T, Metz BN. 2011. Division of labor associated with brood rearing in the honey bee: How does it translate to colony fitness? *PLoS ONE* 6:e16785
100. Sanogo YO, Hankison S, Band M, Obregon A, Bell AM. 2011. Brain transcriptomic response of three-spine sticklebacks to cues of a predator. *Brain Behav. Evol.* 77:270–85

101. Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, et al. 2003. Genetics of gene expression surveyed in maize, mouse and man. *Nature* 422:297–302
102. Schneider SS, Lewis LA. 2004. The vibration signal, modulatory communication and the organization of labor in honey bees, *Apis mellifera*. *Apidologie* 35:117–31
103. Schumer M, Krishnakant K, Renn SCP. 2011. Comparative gene expression profiles for highly similar aggressive phenotypes in male and female cichlid fishes (*Julidochromis*). *J. Exp. Biol.* 214:3269–78
104. Seeley TD. 1996. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Cambridge, MA: Harvard Univ. Press
105. Sen Sarma M, Rodriguez-Zas SL, Gernat T, Nguyen T, Newman T, et al. 2010. Distance-responsive genes found in dancing honey bees. *Genes Brain Behav.* 9:825–30
106. Sinha S, Ling X, Whitfield CW, Zhai CX, Robinson GE. 2006. Genome scan for *cis*-regulatory DNA motifs associated with social behavior in honey bees. *Proc. Natl. Acad. Sci. USA* 103:16352–57
107. Smith CR, Toth AL, Suarez AV, Robinson GE. 2008. Genetic and genomic analyses of the division of labour in insect societies. *Nat. Rev. Genet.* 9:735–48
108. Soma KK, Scotti MA, Newman AE, Charlier TD, Demas GE. 2008. Novel mechanisms for neuroendocrine regulation of aggression. *Front. Neuroendocrinol.* 29:476–89
109. Srinivasan MV, Zhang SW, Altwein M, Tautz J. 2000. Honeybee navigation: nature and calibration of the "odometer." *Science* 287:851–53
110. Sullivan JP, Jassim O, Fahrbach SE, Robinson GE. 2000. Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* 37:1–14
111. Suzuki MM, Bird A. 2008. DNA methylation landscapes: provocative insights from epigenomics. *Nat. Rev. Genet.* 9:465–76
112. Tautz J. 2008. *The Buzz About Bees: Biology of a Superorganism*. Berlin: Springer Verlag
113. Tobback J, Mommaerts V, Vandersmissen HP, Smagghe G, Huybrechts R. 2011. Age- and task-dependent *foraging* gene expression in the bumblebee *Bombus terrestris*. *Arch. Insect Biochem. Physiol.* 76:30–42
114. Toth AL, Kantarovich S, Meisel AF, Robinson GE. 2005. Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* 208:4641–49
115. Toth AL, Robinson GE. 2007. Evo-devo and the evolution of social behavior. *Trends Genet.* 23:334–41
116. Toth AL, Robinson GE. 2009. Evo-devo and the evolution of social behavior: brain gene expression analyses in social insects. *Cold Spring Harb. Symp. Quant. Biol.* 74:419–26
117. Toth AL, Varala K, Henshaw MT, Rodriguez-Zas SL, Hudson ME, et al. 2010. Brain transcriptomic analysis in paper wasps identifies genes associated with behaviour across social insect lineages. *Proc. R. Soc. Lond. Ser. B* 277:2139–48
118. Toth AL, Varala K, Newman TC, Miguez FE, Hutchison SK, et al. 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science* 318:441–44
119. Vekovischeva OY, Aitta-Aho T, Verbitskaya E, Sandnabba K, Korpi ER. 2007. Acute effects of AMPA-type glutamate receptor antagonists on intermale social behavior in two mouse lines bidirectionally selected for offensive aggression. *Pharmacol. Biochem. Behav.* 87:241–49
120. Vergoz V, McQuillan HJ, Geddes LH, Pullar K, Nicholson BJ, et al. 2009. Peripheral modulation of worker bee responses to queen mandibular pheromone. *Proc. Natl. Acad. Sci. USA* 106:20930–35
121. von Frisch K. 1967. *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard Univ. Press
122. Wang J, Ross KG, Keller L. 2008. Genome-wide expression patterns and the genetic architecture of a fundamental social trait. *PLoS Genet.* 4:e1000127
123. Wang Y, Jorda M, Jones PL, Maleszka R, Ling X, et al. 2006. Functional CpG methylation system in a social insect. *Science* 314:645–47
124. Wang Y, Mutti NS, Ihle KE, Siegel A, Dolezal AG, et al. 2010. Down-regulation of honey bee *IRS* gene biases behavior toward food rich in protein. *PLoS Genet.* 6:e1000896
125. Wcislo WT. 1989. Behavioral environments and evolutionary change. *Annu. Rev. Ecol. Syst.* 20:137–69
126. West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20:249–78
127. Whitfield CW, Band MR, Bonaldo MF, Kumar CG, Liu L, et al. 2002. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Res.* 12:555–66

128. Whitfield CW, Behura SK, Berlocher SH, Clark AG, Johnston JS, et al. 2006. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* 314:642–45
129. Whitfield CW, Ben-Shahar Y, Brillet C, Leoncini I, Crauser D, et al. 2006. Genomic dissection of behavioral maturation in the honey bee. *Proc. Natl. Acad. Sci. USA* 103:16068–75
130. Whitfield CW, Cziko AM, Robinson GE. 2003. Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302:296–99
131. Wilson EO. 1985. The sociogenesis of insect colonies. *Science* 228:1489–95
132. Winston ML. 1987. *The Biology of the Honey Bee*. Cambridge, MA: Harvard Univ. Press
133. Woodard SH, Fischman BJ, Venkat A, Hudson ME, Varala K, et al. 2011. Genes involved in convergent evolution of eusociality in bees. *Proc. Natl. Acad. Sci. USA* 108:7472–77
134. Wray MK, Mattila HR, Seeley TD. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim. Behav.* 81:559–68
135. Yanagi M, Shirakawa O, Kitamura N, Okamura K, Sakurai K, et al. 2005. Association of 14-3-3 epsilon gene haplotype with completed suicide in Japanese. *J. Hum. Genet.* 50:210–16
136. Zayed A, Neager NL, Rodriguezzas SL, Robinson GE. 2011. Common and novel transcriptional routes to behavioral maturation in worker and male honey bees. *Genes Brain Behav.* 11:253–61
137. Zayed A, Whitfield CW. 2008. A genome-wide signature of positive selection in ancient and recent invasive expansions of the honey bee *Apis mellifera*. *Proc. Natl. Acad. Sci. USA* 105:3421–26

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Errata

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