Apidologie

DOI: 10.1007/s13592-013-0251-4

Review article

Honey bee sociogenomics: a genome-scale perspective on bee social behavior and health

Adam G. Dolezal[1], Amy L. Toth[1,2]

[1]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA
[2]Department of Entomology, Iowa State University, Ames, IA, USA

Received 3 July 2013 – Revised 9 October 2013 – Accepted 18 October 2013

Abstract – The biology of honey bees involves a host of developmental, behavioral, and physiological components that allow thousands of individual bees to form complex social units. Fueled by a wealth of information from new genomic technologies, a new approach, sociogenomics, uses a focus on the genome to integrate the molecular underpinnings and ultimate explanations of social life. This approach has resulted in a massive influx of data from the honey bee genome and transcriptome, a flurry of research activity, and new insights into honey bee biology. Here, we provide an up-to-date review describing how the honey bee has been successfully studied using this approach, highlighting how the integration of genomic information into honey bee research has provided insights into worker division of labor, communication, caste differences and development, evolution, and honey bee health. We also highlight how genomic studies in other eusocial insect species have provided insights into social evolution via comparative analyses. These data have led to several important new insights about how social behavior is organized on a genomic level, including (1) the fact that gene expression is highly dynamic and responsive to the social environment, (2) that large-scale changes in gene expression can contribute to caste and behavioral differences, (3) that transcriptional networks regulating these behaviors can be related to previously established hormonal mechanisms, and (4) that some genes and pathways retain conserved roles in behavior across contexts and social insect taxa.

genome / division of labor / behavioral maturation / caste / comparative genomics

1. INTRODUCTION

The social life of bees has been of intense interest to biologists and apiculturists for centuries. As such, there has been a wealth of studies on the evolution, behavior, colony organization, and development of honey bees and their societies. These studies have spanned across levels of analysis, providing insight into both the proximate and ultimate causes behind the social complexity of honey bee society. However, specific focus on integrating these approaches to bridge gaps between evolutionary and mechanistic approaches to studying animal societies began little more than a decade ago.

This new approach, dubbed sociogenomics (Robinson 1999), proposed that the **genome** can form a centerpiece for linking different levels of analysis, allowing researchers to integrate the proximate causes of behavior, like gene expression and physiology, with more ultimate analyses, like behavioral ecology. By using the genome as a focal point, sociogenomics seeks to provide a more comprehensive method for understanding social life, from its evolution to its genetic regulation—and everywhere in between (Robinson et al. 2005). Since studies on honey bees have historically run the gamut

Corresponding author: A. Dolezal, adolezal@gmail.com
Manuscript editor: Stan Schneider

across these levels of analysis, it is unsurprising that bees have become an important model for applying sociogenomic approaches. With the increased scope and availability of genomic tools (Table I), including the sequencing of the complete honey bee genome (Weinstock et al. 2006), studies grounded in a genomic perspective have been successful in helping to tease apart the different components that intersect to build complex social organisms (Smith et al. 2008).

The sociogenomic approach spans across a large swathe of applications and has been defined broadly (Robinson et al. 2005). Here, we restrict our definition to research focusing on large numbers of genes or on single genes that provide key insights into larger genetic pathways, and exclude a rich and informative literature on single genes (e.g., Ben-Shahar 2005; Amdam et al. 2010) that are beyond the scope of this review. We focus our review on large-scale genomic or **transcriptomic** analyses, **microarrays**, or targeted studies that explore or clarify genetic pathways consisting of multiple genes. Furthermore, while we focus specifically on how the rise of sociogenomics has helped us understand the social life of bees, this approach has been very successful in other **eusocial** insects (Smith et al. 2008). In fact, one important strength of sociogenomics is the capability to make comparisons: the power to search for homologies in genomes across taxa helps find clues to understand the evolution of bee societies (Fischman et al. 2011).

Here, we review how the use of sociogenomics has advanced knowledge of many facets of honey bee biology. We begin with worker temporal polyethism; the behavioral transition from in-hive to foraging tasks is arguably the best studied honey bee behavioral phenomenon using a sociogenomic approach, and we use it as a benchmark for comparison with other research foci. Then, we follow with descriptions of the progress made in understanding the evolution and regulation of caste differences, communication, and social immunity, and we also review how the approaches and methods pioneered with sociogenomics have been applied to honey bee disease and pathogen responses. Finally, while we focus specifically on how sociogenomics has improved our understanding of bee biology, throughout the review, we highlight how a sociogenomic-minded exploration across social insect taxa has fueled comparisons for a better understanding of the evolution of eusociality.

2. WORKER DIVISION OF LABOR

2.1. Foraging ontogeny

One of the most striking aspects of eusocial insect societies, and honey bees in particular, is the behavioral plasticity found within the worker caste. This flexibility takes the form of **temporal polyethism**, in which workers transition across different task repertoires as they age. After adult emergence, workers specialize on a variety of in-nest tasks, such as brood care, and then transition through stages of other tasks, such as nest maintenance and guarding, culminating in foraging behavior (Winston 1987). While this sequence of behavioral maturation occurs as a general pattern, workers exhibit a high level of flexibility in the rate of behavioral development, which allows individuals to respond to differing colonial demands (Robinson 1992).

At this point in time, sociogenomics has been more thoroughly applied to the study of worker behavioral maturation than any other facet of honey bee biology and, therefore, stands as the best example of how successful this approach can be. This is largely due to the strong background of literature and expertise spanning behavioral, genetic, neurobiological, and physiological studies on temporal polyethism, which has allowed researchers to build a more comprehensive understanding of this system centering on investigation of the genome (Robinson 2002; Robinson et al. 2005; Smith et al. 2008) (Figure 1).

Before sequencing of the honey bee genome, most of the studies described as sociogenomic were borne from the integration of behavioral, neuronal, and physiological mechanisms with genomic information generated from partial genome resources, which provided vastly more information than previous approaches that focused

Table I. How has the sequencing of the honey bee genome changed sociogenomic applications?

Application	Pre-genome	Post-genome	Gain from genome	References
Gene discovery	e.g., Degenerate polymerase chain reaction (PCR) and cloning on a gene-by-gene basis	Computational analysis of entire genome sequence	Whole genome instead of single gene, less labor-intensive, allows discovery of novel genes	Elsik, Mackey et al. (2007)
Identification of regulatory sequences	e.g., Rapid amplification of cDNA ends on a gene-by-gene basis	Computational analysis of entire genome sequence, chromatin immunoprecipitation and sequencing	Whole genome instead of single gene, less labor-intensive	Ament et al. (2012b)
Epigenetic profiling (e.g., identifying methylation)	e.g., Methylation sensitive amplified fragment length polymorphism of anonymous methylated sites	Computational analysis of CpG observed/expected content of genome, whole genome bisulfite sequencing	Ability to identify actual genes and nucleotides that are methylated, whole genome coverage instead of a small subset	Kronforst, Gilley et al. (2008); Herb et al. (2012)
Gene expression profiling	Quantitative PCR for select genes, EST-based microarrays, screening ESTs for a small number of differentially expressed genes	Whole-genome microarrays, RNA sequencing compared to whole genome	Whole genome instead of a fraction of the genome, RNA-Seq is less labor-intensive and has a greater dynamic range	Whitfield et al. (2002, 2003); Chen et al. (2012); Liang et al. (2012)
Sequence evolution	Complex sequence of molecular techniques, with the ability to target only a small number of genes	Bioinformatic comparisons across species that include numerous genes and entire gene families	Whole genome instead of select genes, enhanced ability to uncover gene losses and gains	Hunt et al. (2010); Johnson and Tsutsui (2011)

Many studies on honey bee sociogenomics occurred before the sequencing of the honey bee genome and provided significant insights. However, the existence of an annotated genome provides researchers with many powerful tools to investigate factors that would be much more difficult, or even impossible, without the ability to look at the whole genome. This table summarizes how different applications have changed with the advent of the genome

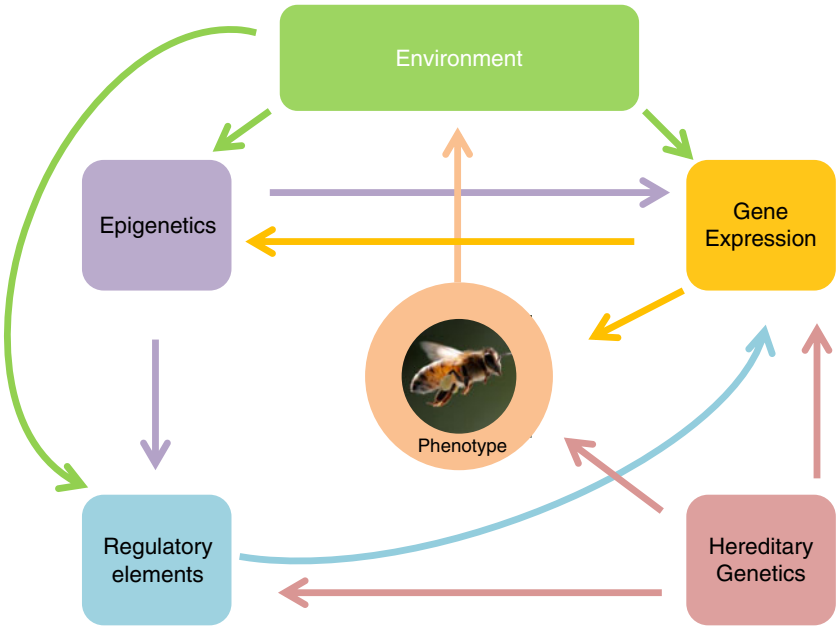

Figure 1. An integrative, sociogenomic approach as applied to the study of behavioral maturation into a forager in worker honey bees. A sociogenomic approach to social behaviors has the potential to integrate many different forms of genomic mechanisms that interact to affect phenotype. The best example of the use of sociogenomics is the investigation of the underlying regulation of honey bee worker behavioral maturation, or foraging onset. Integration of behavioral studies with a variety of genomic tools has provided insights into how the environment (both external and social) and allelic variation in individuals affects gene expression, regulatory elements, and epigenetics to form a network of effects that result in a specific behavioral phenotype. Investigation of other phenotypes with a sociogenomic approach promises to reveal similar networks. Image ©Alex Wild, used by permission.

on one or a few genes (Whitfield et al. 2002). These included **expressed sequence tags (ESTs)** and **microarrays**. For the honey bee, the combination of these techniques, which provided partial sequence information for close to 50 % of the genes in the genome, allowed researchers to identify thousands of genes of interest and then screen them for expression levels (Whitfield et al. 2002). Microarrays allowed for large-scale screenings for gene expression differences between behavioral groups, helping to identify how differences in gene expression are related to worker **division of labor**. Comparisons of the brains of young nurses to old foragers (Kucharski and Maleszka 2002; Whitfield et al. 2003) found clear differences in brain gene expression. Then, behavioral manipulations using **single-cohort colonies** were used to decouple the nurse-to-forager transition from chronological age, revealing changes in over 2,000 genes or about 40 % of genes assayed. Further, these gene expression profiles can be used to predict the behavior of individual bees (Whitfield et al. 2003). In fact, many of the same molecular processes involved in the nurse-to-forager transition appear to be conserved in the brain across species within the genus *Apis*, though others, such as those involved in carbohydrate metabolism, circadian rhythm, and colony defense, differ between species (Sen Sarma et al. 2007). In addition, a comparison of thousands of transcripts from the brain and abdomen, across nine bee species, representing three origins of sociality, showed that genes involved in carbohydrate metabolism are more rapidly evolving in eusocial

lineages, supporting arguments for the involvement of metabolic pathways in social evolution (Woodard et al. 2011).

After initial screenings identified correlations between behavioral state and gene expression differences, more in-depth investigations further fleshed out the relationship between worker behavioral maturation and brain gene expression. By integrating genomic analysis with experimental approaches, it has been possible to build a more complete picture of the interaction between physiology, environment, and genotype on worker behavioral maturation. The brain gene expression differences found between nurses and foragers appear to be influenced strongly by **queen mandibular pheromone** (**QMP**; Grozinger et al. 2003), which is produced by honey bee queens to regulate the behavior of workers (Winston and Slessor 1998). **Juvenile hormone** (**JH**) signaling, long known to be an important regulator of foraging onset (Robinson 1987; Sullivan et al. 2000) had very large effects on gene expression, leading to forager-like brain gene expression even in bees reared in cages with no prior foraging experience (Whitfield et al. 2006). Further investigation showed how nutrition and nutritional signaling pathways, specifically **insulin/insulin-like signaling** (**IIS**), are involved in behavior and how other factors are affected downstream. In insects, IIS acts as a key regulator of feeding behavior and metabolism and also interacts with **target of rapamycin** (**TOR**) (Edgar 2006), another important metabolic pathway regulator, and JH (Tu et al. 2005). Based on single-gene experimental studies (Ben-Shahar 2005; Nelson et al. 2007), a focus on the effects of these pathways showed that changes in IIS and TOR affect behavioral maturation, and a reanalysis of previous microarray data (Grozinger et al. 2003; Whitfield et al. 2006) showed differences in energy metabolism between nurses and foragers (Ament et al. 2008, 2010). In fact, experimental perturbation of IIS causes changes in the timing of foraging initiation, further showing how IIS and its interaction with nutritional and metabolic pathways are involved in worker behavioral maturation (Ament et al. 2008).

Studies using the honey bee genome also identified several **transcription factors** that differ in expression between worker behavioral groups. Genes such as *Creb*, involved in neural plasticity in many animals (McClung and Nestler 2008), and *dorsal*, which is involved in insect developmental patterning and immune response (Qiu et al. 1998), were identified as part of a transcriptional network that could predict the expression of many other behaviorally linked genes (Weinstock et al. 2006; Chandrasekaran et al. 2011). Further genomic analyses of the brain showed that transcription factors known to regulate development may be also involved in behavioral maturation in honey bee workers (Sinha et al. 2006). In particular, *ultraspiracle* (*usp*), a transcription factor linked to JH signaling, appears to interact with other transcription factors to help orchestrate a network of gene expression that occurs between the brain and peripheral tissues to regulate worker behavioral changes (Ament et al. 2012b). **DNA methylation**, an **epigenetic** modification to DNA (discussed in more detail in Section 4), also appears to have a role in worker behavioral change; not only do nurse and forager bees differ in brain gene methylation patterns, but the methylation patterns are also behaviorally reversible (Herb et al. 2012).

Sociogenomic studies on division of labor in other eusocial species have also provided information on how common **genetic toolkits** could be used to build convergent social behaviors. Microarray screening of the brains of *Polistes metricus* wasps showed that the gene expression profiles of foraging *P. metricus* had significant overlap with the profiles of foraging honey bees, especially genes related to heat stress, locomotion, and lipid metabolism (Toth et al. 2010). Further experiments showed that starved *P. metricus* workers had reduced lipid levels and increased foraging activity (Daugherty et al. 2011), similar to the nutritional regulation of foraging in the honey bee (Toth et al. 2005; Toth and Robinson 2005). These changes were accompanied by changes in brain gene expression that

significantly overlapped with changes found in honey bees, including genes involved in insulin and JH signaling. These two studies together provide some support for the idea that a common genetic toolkit, centering on nutritional responses, could have a key role in the evolution of division of labor across eusocial insect lineages (Daugherty et al. 2011).

As a whole, studies on behavioral maturation in workers form the most complete picture of how a sociogenomic approach can link many different factors to better explain a complex biological process. By integrating the wealth of behavioral, physiological, and genetic information with newly developed genomic tools, it has been possible to more thoroughly understand the molecular underpinnings of behavioral phenotypes. Even before sequencing of the honey bee genome, microarray screens were able to identify how patterns in gene expression differ as bees change behaviors (Whitfield et al. 2003) and in response to pheromonal stimuli (Grozinger and Robinson 2002). Sequencing of the honey bee genome has enabled more complete genomic analyses that include nearly all of the genes in the genome and has given researchers ready access to noncoding regions of the honey bee genome. Table I provides a summary of pre-genome and post-genome approaches to studying the genetic basis of honey bee biology. For example, full genome information has helped to clarify how transcription factors and regulatory elements (Ament et al. 2012a, b) and DNA methylation (Herb et al. 2012) are involved in driving a host of pathway changes that influence behavioral maturation (Figure 1).

2.2. Pollen-hoarding syndromes

Genomic approaches have also been used to study colony-level pollen-hoarding phenotypes and the individual physiological and behavioral differences that accompany them. Researchers used selective breeding to produce bee strains exhibiting opposite colony-level traits for pollen storage, either high or low levels of pollen hoarding (Page and Fondrk 1995). Thus, the selection regime produced divergent phenotypes that were highly amenable to genetic mapping studies. A series of analyses identified **quantitative trait loci** (**QTLs**) associated with pollen hoarding, individual forager preferences (Hunt et al. 1995), ovary size (Linksvayer et al. 2009; Graham et al. 2011), and JH responsiveness (Page et al. 2012) in these high-pollen-hoarding and low-pollen-hoarding strains. Furthermore, localization of these QTLs using the honey bee genome indicated that the QTLs contained several genes related to IIS signaling, highlighting the potential importance of IIS signaling on these behavioral phenotypes (Hunt et al. 2007). Microarray screening of the ovaries of high-pollen-hoarding and low-pollen-hoarding strains of bees further identified transcriptional differences related to these behavioral phenotypes, particularly tyramine receptor (*TYR*) and a putative ecdysteroid hormone receptor (*HR46*) (Wang et al. 2012). Combined with the strong body of experimental studies using single-gene and physiological approaches to bee behavior, particularly those focusing on the yolk precursor *vitellogenin*, these studies have provided many insights into the physiological regulation and possible evolutionary pathways to eusocial insect behaviors (reviewed by Page et al. 2012; for further review, see Rueppell 2013).

2.3. Guarding, undertaking, and scouting

While the sociogenomics of foraging onset is the most investigated, other worker honey bee behaviors have also been studied with genomic approaches. There are some important differences with respect to the aforementioned work on behavioral maturation in that some worker behaviors are more short-term responses to colony needs and do not appear to involve extensive shifts in gene expression. For example, microarray comparisons of brain tissue showed no significant differences in brain gene expression of bees exhibiting guarding and undertaking behaviors, which occur for short periods of time between nursing and foraging onset, even though these behaviors are clearly discernible, indicating that distinct behavioral

changes can occur even in the absence of large-scale transcriptional changes in the brain (Cash et al. 2005).

Another important behavior, which only some workers ever actually perform, is scouting behavior, which can take the form of worker bees scouting for new food sources or new nest sites after a swarming event (Seeley 1985). A whole-genome microarray comparison of the brains of food scouts and non-scouting forager bees revealed extensive differences in gene expression, most notably in genes involved in neurotransmitter systems known to be involved in novelty seeking behavior in other insect species and humans, like catecholamine, glutamate, and GABA signaling (Liang et al. 2012).

2.4. Aggression

The genetic basis of yet another worker behavior, defensiveness or aggressive behavior, has been a topic of active investigation. Crosses of high-defensive-response Africanized honey bees with low-defensive-response European honey bees revealed several QTLs linked to increased defensiveness (*sting-1*, *sting-2*, and *sting-3*; Hunt et al. 1998). The use of genomic sequencing and linkage mapping on these QTLs narrowed down the number of candidate genes associated with defensive responsiveness, identifying orthologs of genes involved in nervous system development and activity and sensory signaling (Hunt et al. 2007), though another study showed that potentially novel genes may also be involved (Lobo et al. 2003). The molecular basis for increased aggressiveness in different contexts appears to utilize some conserved mechanisms, whether due to heredity (i.e., Africanized vs. European strains), age (young vs. old bees), or environment (exposure to alarm pheromone). The fact that similar genes expressed in the brain influence aggressive response due to these different influences supports the argument that changes in the regulation of gene expression via ***cis*-regulatory** mechanisms are at the heart of some forms of behavioral diversity (Alaux et al. 2009c).

3. COMMUNICATION

3.1. Pheromones

The organization and maintenance of complex social colonies requires intricate systems of communication. In honey bees, the predominant method of communication is through chemicals, mostly in the form of pheromones, which act as chemical signals by members of the hive to prevent intracolonial conflict and regulate behavioral plasticity. Primer pheromones affect long-term physiological changes that result in delayed behavioral responses, while releaser pheromones act on more short-term processes to quickly change behavioral performance (Le Conte and Hefetz 2008). Queens produce QMP, a primer pheromone that prevents workers from developing active ovaries and foraging onset (Pankiw et al. 1998). Experimental manipulation, followed by microarray screening of brain gene expression, also showed that QMP treatment changes brain gene expression in over 2,500 genes of laboratory bees and around 700 genes in bees from field colonies, specifically activating genes associated with nursing behavior and repressing those associated with foraging. In addition, transcription factor genes were affected at a higher proportion than other genes, suggesting that QMP may act by targeting transcription factors to initiate downstream cascades of expression changes (Grozinger et al. 2003). Further, individual workers vary in their attraction to QMP, and their brain gene expression reflects these differences. Analysis showed 960 differentially expressed brain transcripts between high response and low response to QMP bees, with particular differences in gene networks related to neural network structure (Kocher et al. 2010a).

Alarm pheromone is a releaser pheromone that quickly stimulates an aggressive response in workers (Winston 1987). Even though response to alarm pheromone is very fast, gene expression changes still occur in the brain, particularly the immediate early gene *c-Jun*. In addition to affecting *c-Jun*, a transcription factor involved in neural circuits, alarm pheromone

also affects behavioral responses to subsequent exposure long after the initial aggressive response has ceased (Alaux and Robinson 2007). Furthermore, while alarm pheromone results in increased behavioral activity, it also causes downregulation of genes involved in brain metabolism, posing interesting questions regarding the relationship between brain metabolic activity and overall behavior. Genes modulated by alarm pheromone also show overlap with those that are upregulated in highly aggressive Africanized honey bees. The fact that the same genes have effects on aggressive behaviors in different contexts suggests that alarm pheromone-regulated genes were likely involved in the evolution of different aggressiveness phenotypes in honey bees (Alaux et al. 2009c).

Brood pheromone acts as both a primer pheromone, acting in the long term to delay foraging in young bees, and as a releaser pheromone, stimulating foraging in old bees (Le Conte et al. 2001). Brain gene expression profiling reflects these effects, as brood pheromone causes different effects on bees of different ages: in young workers, brood pheromone upregulates genes associated with nursing and downregulates genes associated with foraging, and does the inverse in old bees, supporting the argument that pheromones affect behavior by mediation of gene expression, even in different contexts (Alaux et al. 2009b).

Bees perceive pheromonal signals through an incredibly sensitive and well-developed olfactory system. In insects, pheromones and other odorants are carried to odorant receptors by odorant-binding proteins or chemosensory proteins (Pelosi et al. 2005). The sequencing of the honey bee genome afforded an opportunity to explore the full complement of ORs and OBPs in honey bees and indicated that there has been an evolutionary expansion of the number of olfactory proteins in honey bees compared to other, nonsocial insects (Foret and Maleszka 2006). Interestingly, even though the antennae are the site of odorant sensation, both odorant-binding proteins (Foret and Maleszka 2006) and chemosensory proteins (Foret et al. 2007) are commonly expressed in other tissue, indicating their possible role in other physiological functions.

In addition to studies in honey bees, the power of a genomic approach to understanding the molecular basis of chemical communication has been exemplified by findings made possible by the recent sequencing of the *Solenopsis invicta* fire ant genome. In fire ants, a single Mendelian factor in the form of different alleles at the *Gp-9* locus determines if workers accept one or many queens. *Gp-9* codes for an odorant-binding protein, so it has been suggested that its effects are due to modulation of pheromone responses (Gotzek and Ross 2007). However, its effects are much more diverse, influencing a number of different traits, including female size and fecundity (Keller and Ross 1999; Gotzek and Ross 2009). Facilitated by the sequencing of the genome (Wurm et al. 2011), a recent investigation of the genomic region where *Gp-9* is located found that the *Gp-9* allele is part of a heteromorphic chromosome, similar to a Y sex determination chromosome. Instead of determining sex, these chromosomal differences help maintain different intraspecific social phenotypes (Wang et al. 2013). While a similar social chromosome has not been identified in other social insects, similar systems, increased access to genomic tools can make novel discoveries such as this possible.

3.2. Dance language

In addition to a complex system of chemical communication, honey bees are well known for their dance language, in which returning foragers use mechanical signals (i.e., sound, vibration, and tactile interaction) to communicate the location of food in the environment to bees within the hive (Dyer 2002). Transcriptomic profiling of the nervous systems of dancing foragers identified gene expression changes linked to dancing, with differences particularly found in the mushroom bodies of the brain. Comparisons with dancing foragers from *Apis florea* and *Apis dorsata* identified species-specific and species-consistent genes related to dancing behavior. Further analysis of between-species differences, like those linked to motor control and metabolism, may

provide further insights into how genetic differences between these species underlie the differences in their dance language phenotypes (Sen Sarma et al. 2009). Furthermore, dancing bees that perceive the location of food to be further away have different gene expression profiles than those perceiving food to be nearby, particularly in the mushroom bodies and optic lobes, with notable differences in learning and memory systems (Sen Sarma et al. 2010). In addition, differences in mushroom body gene expression arise as bees accrue foraging experience (Lutz et al. 2012), further indicating the importance of genomic changes in the regulation of behavioral plasticity, especially in the brain.

Communication systems have also been implicated in the evolution and diversification of sociality in bees. By using next-generation sequencing for the rapid generation of transcriptomes of nine different bee species, spanning three independent eusocial origins, researchers conducted genome-scale comparative analyses to determine which genes show evidence of more rapid rates of protein evolution and how these relate to different levels of sociality. The results indicated that gland development genes were rapidly evolving in eusocial lineages including honey bees. This suggests that glandular structures and their chemical products, likely used for increased social communication, were targets of selection during eusocial evolution (Fischman et al. 2011; Woodard et al. 2011). In addition to the work on pheromones and dance, there has been some inquiry into the sociogenomics of honey bee vibrational signals. These signals are produced when some bees grasp a nestmate and rapidly vibrate, resulting in the recipient bee changing its behavior in a context-dependent manner (Schneider and Lewis 2004). Using a microarray, researchers showed that brain gene expression differs in over 900 genes, with around half upregulated and half downregulated, in bees that send these signals vs. those that do not. This is particularly notable because the number of differentially expressed genes linked to this vibrational signal is surprisingly substantial. For comparison, around 1,300 genes are differentially expressed between young nurses and old foragers, which differ in many more aspects than bees differentially performing vibrational signals. Interestingly, some of the genes differentially expressed in these signaling bees are those associated with motor activities like locomotion courtship (Alaux et al. 2009a).

4. CASTE POLYPHENISM

4.1. Queen–worker developmental differentiation

In honey bees, the reproductive division of labor between queens and workers is based on strict **caste** polyphenism, with extreme differences in physiology, morphology, and behavior between reproductive queens and functionally sterile workers. The differences between the castes are determined due to differential feeding at critical stages during larval development; workers are fed a restricted diet, while queens receive a diet richer in royal jelly (Winston 1987). Changes in diet cause a cascade of changes in gene expression and hormone signaling that result in the production of different caste phenotypes. Larval consumption of a diet rich in royal jelly, and specifically the protein royalactin (Kamakura 2011), results in increased JH levels (Rembold 1987; Rachinsky and Hartfelder 1990) which are involved in triggering queen development (Rembold et al. 1974). Screening of whole-body gene expression showed that many of the genes overexpressed in queen-destined larvae were linked to metabolism and hormone responsiveness (Evans and Wheeler 2001; Cristino et al. 2006; Barchuk et al. 2007). In particular, insulin receptor and insulin receptor substrate, components of the IIS pathway, were overexpressed in queen-destined larvae (Wheeler et al. 2006) during times where JH content also rises (Rembold 1987). The identification of metabolic genes as possible modulators of JH signaling and queen development led to further investigations, ultimately showing the importance the epidermal growth factor receptor pathway as a modulator of queen–worker differentiation, triggered by the ingestion of royalactin (Kamakura 2011). In addition to the differences found during development, genomic analyses have shown

significant differences in gene expression between adult queens and workers. Microarray screening of adult brains showed that approximately 2,000 genes are differentially expressed in the brains of queens and workers and over 200 of these are expressed in a more queen-like manner in reproductive workers, identifying a set of genes likely involved in reproductive activity, regardless of caste (Grozinger and Robinson 2007). Further investigation into the transcriptomic differences between queen-destined and worker-destined larvae used next-generation **RNA-Seq** technology to provide a more complete catalog of transcriptional differences than previously possible using ESTs or microarrays. These comparisons identified over 4,000 differentially expressed genes and clarified the dynamics of TOR expression, showing that differences are greatest between queens and workers during the fourth (of five) larval instar (Chen et al. 2012).

Another aspect of honey bee development that has been identified due to the expansion of genomic tools is the importance of epigenetic effects caste determination. Epigenetic modifications occur when chemical modifications to DNA take place in response to an environmental stimulus. Such modifications do not affect the DNA sequence, but cause structural changes in chromatin that can result in alterations in gene expression that may last across an individual's lifetime or even across generations. One form of epigenetic modification is DNA methylation, in which methyl groups are attached to nucleotides, usually CpG dinucleotides, and have the potential to affect the expression of methylated sequences (Bird 2007). In honey bees, dynamic "de novo" methylation is driven by DNA methyltransferase-3 (DNMT3). Experimental silencing of DNMT3 in developing larvae prevents the attenuation of gene expression and mimics the response to a diet rich in royal jelly (Kucharski et al. 2008). Furthermore, over 2,000 genes are differentially methylated in worker-destined larvae compared to queen-destined larvae, with the majority being up-methylated in worker-destined larvae (Foret et al. 2012). While not as drastic, there are also methylation differences in 550 genes in the brains of adult queens and workers (Lyko et al. 2010), even though brain methylation does not differ in newly emerged queens and workers (Herb et al. 2012). How does methylation affect expression? Methylation does not appear to be closely tied to differential upregulation or downregulation of genes. Rather, methylation is often clustered in areas of genes where splicing occurs, suggesting that methylation may be involved in the regulation of alternative splice variants (Flores et al. 2012; Foret et al. 2012). Also, methylation differences occurred on genes coding for some histones, proteins that are also epigenetically regulated and can affect chromatin structure and gene expression (Lyko et al. 2010) and which may have an important role in the regulation of bee development (Dickman et al. 2013). Methylation differences are also associated with caste differences of other eusocial insect species, including several ant species (Bonasio et al. 2012; Smith et al. 2012) and *Polistes* wasps (Weiner et al. 2013), indicating the possible importance of epigenetic modifications in the convergent evolution of eusocial societies.

4.2. Reproductive behavior

Genomic tools have also been used to investigate the molecular underpinnings of reproductive activation in both queens and workers. Since queen bees express extreme differences in behavior and physiology before and after mating, they are an excellent model in which to investigate the changes that occur with mating. Even though their mating biology is very different, changes in brain and ovary transcriptional profiles in honey bee queens overlapped with those observed in *Drosophila melanogaster* females, indicating that the regulation of post-mating behavior may be strongly conserved across insect taxa (Kocher et al. 2008, 2010b). Though both involve individual behavioral changes, there was no clear relationship between genes associated with queen mating behavior and worker behavioral maturation (Kocher et al. 2008).

"Anarchistic" bees are an unusual strain of honey bees where workers develop ovaries and lay viable eggs, even in the presence of a laying

queen. This cheating behavior is partially explained by four QTLs found in anarchistic workers (Oxley et al. 2008). Screening of the heads and abdomens showed that wild-type workers have more genes upregulated than anarchistic workers, and it has thereby been hypothesized that egg laying may be the default and that normal workers upregulate genes that "switch off" ovarian activation (Thompson et al. 2006). Anarchistic bees do show significant upregulation of some genes in the head and abdomen, particularly in *vitellogenin*, involved in ovarian activation, and *AdoHycase*, which is possibly involved in the regulation of DNA methylation (Thompson et al. 2008). Similarly, a genome-wide comparison of gene expression in the whole bodies of workers showed that over 1,200 genes are differentially expressed in normal workers vs. workers that became reproductive due to queenlessness. Reproductive workers overexpressed genes involved in reproductive activation, compared to nonreproductive workers, which exhibited increased expression of genes involved in flight metabolism and foraging. Therefore, gene expression comparisons indicated differences in reproductive activation, as would be expected, but also differences in overall activity levels and behavioral performance between these different phenotypes (Cardoen et al. 2011).

Comparisons of the genomics of caste and reproduction between honey bees and other eusocial insect species has also helped elucidate how different genomic components could be involved in eusocial evolution. Identification of gene expression profiles of whole bodies for adult queens and workers of the paper wasp *Polistes canadensis* allowed interspecies comparisons, identifying nine genes with conserved caste function across species from four different origins of eusociality, including bees, ants, and wasps (Sumner et al. 2006). A microarray study of *P. metricus* brains further showed that, while wasps had different gene expression profiles based on their reproductive status, there was no significant overlap between wasp reproductive genes and genes involved in honey bee caste differences. This suggests that different mechanisms are involved in reproductive division of labor in these species, while worker behavioral regulation shows more conservation (Toth et al. 2010). Similarly, transcriptomic profiling of *P. canadensis* queen and worker brains showed little overlap with honey bee caste-specific genes (Ferreira et al. 2013). A comparison of gene expression profiles between two species of adult and pupal fire ant that analyzed the whole bodies of queens, workers, and males showed that, while gene expression differences occurred between queens and workers, the greatest interspecific gene expression differences were found between adult workers (Ometto et al. 2011). *P. canadensis* RNA-Seq data suggest that genes that are worker-biased in their expression are more likely to be "novel," with no homology to known sequences, further suggesting that molecular evolution occurs more rapidly in genes of importance to the worker caste (Ferreira et al. 2013).

The use of comparative **bioinformatic** analysis on existing datasets has become a useful tool as genomic technologies have advanced and the amount of sequence data for honey bees and other insects has drastically increased. This approach can be exemplified by studies seeking to better understand rates of gene evolution in queens and workers. By honing in on previously identified genes with worker-biased or queen-biased expression, it was possible to compare rates of amino acid substitution of these genes across honey bees and various nonsocial insects for which genomic sequence data were available. A comparison of queen-biased genes with worker-biased or non-biased genes showed that proteins associated with the queen caste had evolved more rapidly than other proteins, suggesting that selective pressure acted strongly on queen caste genes (Hunt et al. 2010). Another study, however, predicted that novel genes would be necessary for the evolution of complex social behaviors and, given that the majority of these behaviors occur in workers, worker-biased genes should be more likely to be novel. Their analysis showed that, indeed, the worker caste expresses more genes specific to social insect taxa than the queen caste. Howev-

er, while novel worker behaviors may have arisen from novel genes, it is also possible that rapid evolutionary change still occurred in the queen caste, albeit acting upon ancestral genes (Johnson and Tsutsui 2011).

5. HONEY BEE HEALTH

While the primary goal of sociogenomic studies is to provide a fuller understanding of the evolution and maintenance of sociality, the tools and approaches that were spawned from sociogenomics have been applied to other questions in honey bee biology. In particular, increased applications of genome-level investigation has helped provide a better understanding of how a variety of factors influence honey bee health. Therefore, while the original intent of sociogenomics was to investigate basic questions in evolution, behavior, and physiology, this work has quickly provided key information for addressing applied questions. With increasing worldwide concerns regarding pollinator health (Gallai et al. 2009), a better understanding of how honey bees respond to health stresses on a genomic scale is of great utility. Stresses implicated in honey bee declines include pesticides (Mullin et al. 2010), nutrition (Naug 2009), and disease. Honey bees suffer from a number of diseases caused by bacteria, viruses, and fungi (Evans and Schwarz 2011) and are affected by a number of pests, most notably the *Varroa destructor* mite (Rosenkranz et al. 2010).

Given the highly social nature of honey bee colonies, response to disease stress occurs at both the individual and group levels. Individual bees respond to immune stress via cellular and humoral mechanisms, similar to other insects, but colonies also exhibit social mechanisms (Wilson-Rich et al. 2009), such as high levels of hygienic behavior (Rothenbuhler 1964), to prevent the spread of disease. Hygienic behavior, characterized by uncapping of pupal cells and the removal of diseased pupae, has been linked to several QTLs (Oxley et al. 2010). Interestingly, hygienic behavior is also affected by interactions between individual workers of different genotypes. When high-hygiene and low-hygiene worker genotypes are mixed within the same colony, indirect social effects cause behavioral changes. Specifically, low-hygiene bees increase their hygienic behavior and exhibit changes in brain gene expression when mixed with high-hygiene nestmates (Gempe et al. 2012). Therefore, while genotypic effects are clearly important, changes in the social environment have strong potential to increase bee hygiene.

With sequencing of the honey bee genome, broad-scale investigation of the genetic pathways involved in honey bee immune response became more tractable. Comparisons of the honey bee genome with that of *Drosophila* flies and *Anopheles* mosquitoes first indicated that honey bees, despite the higher pathogen risks associated with colonial living, actually possess substantially fewer immunity-associated genes. This suggests that, among other possibilities, selective pressure on disease prevention has acted predominantly on social behavioral responses to disease (such as hygienic behavior) and not individual innate immune responses (Evans et al. 2006). Despite this, the immune genes in honey bees show higher rates of evolution than those of *Drosophila* or nonimmune honey bee genes (Viljakainen et al. 2009). Further investigation showed that bacterial immunostimulation of honey bees results in changes in expression of hundreds of genes, many of which are not normally associated with immune response. Changes in some of these, particularly those related to chemical signaling, suggest that changes in expression of nonimmune response genes help to orchestrate behavioral changes, such as increased grooming (Wilson-Rich et al. 2009), that mitigate pathogen risks (Richard et al. 2012).

This hypothesis was further supported through investigation of the effects of *Varroa* infestation on gene expression. When *Varroa* infestation occurs, many gene expression changes occur, but bees with naturally higher tolerance to *Varroa* more highly express genes associated with olfaction and stimulus sensitivity, not immunity (Navajas et al. 2008). A

similar study comparing typical colonies with those from another lineage of *Varroa*-resistant bees, the *Varroa*-sensitive hygiene (VSH) strain, which have been selected for resistance to mite infestation via increased hygienic behavior, also did not implicate immune genes in the resistance to mite infestation. Furthermore, comparison of VSH bees with the *Varroa*-resistant bees from Navajas et al. (2008) showed little overlap in gene expression between the two sources of *Varroa*-resistant bees. Instead, VSH bees had a similar profile to bees stimulated with brood pheromone, indicating a possible connection to brood care phenotypes, and with Africanized honey bees, which are also very hygienic towards *Varroa* (Le Conte et al. 2011). Another investigation compared *Varroa* effects on gene expression in *Apis mellifera* and the mite-resistant congener *Apis cerana*, finding significant differences in genes associated with metabolism and nerve signaling (Zhang et al. 2010). These studies indicate that there may be several distinct genomic routes to behavioral mite resistance.

Further experimentation showed how genes that are upregulated by pollen consumption, like those involved in protein metabolism, are downregulated due to *Varroa* infestation (and the accompanying viruses that mites vector). This provides insight into how mites may stress bees nutritionally and thus, supplies clues that may be helpful in preventing some pest or pathogen effects (Alaux et al. 2011). Gut microarray analysis of bees suffering from colony collapse disorder (CCD) identified a list of 65 transcripts that may be markers for CCD, as well as the increased presence of ribosomal RNA fragments in CCD colonies, possibly due to increased viral infections (Johnson et al. 2009).

Genomic methods have also been applied to the pests, pathogens, and beneficial microorganisms that affect honey bees. While not using the honey bee genome itself, these studies still show how the expansion of genomic approaches are helping build a better understanding of honey bee biology. Genomic tools developed for *Varroa* provide useful methods for understanding host–parasite interactions (Cornman et al. 2010), and RNA deep sequencing has helped to identify novel strains of bee viruses (Cornman et al. 2012, 2013). Metagenomic analyses, where researchers screen diverse genetic material directly from the environment, have also been useful for understanding bee health. A metagenomic survey of honey bees from colonies suffering from CCD helped to identify pathogens, specifically Israeli acute paralysis virus, that were associated with that form of colony loss (Cox-Foster et al. 2007). Further, metagenomic screening of the microbiota of the honey bee gut suggests that a suite of different bacteria in healthy bee guts have a role in pathogen defense and nutrient utilization (Engel et al. 2012).

6. CONCLUSIONS

As genomic tools have become available to honey bee researchers, more facets of honey bee biology have been investigated using a sociogenomic approach. Worker behavioral maturation, the transition from nurse to forager, has received the most attention. Integration of genomic studies with the strong background knowledge of this system from behavioral, ecological, physiological, and genetic studies has provided the most comprehensive characterization of any component of honey bee biology (Figure 1). These investigations stand as the best examples of how fruitful a sociogenomic approach can be. In particular, the work exploring how transcription factors are involved in regulating large-scale gene expression changes has helped to focus in on key transcriptional networks; this information can easily be buried in the data deluge from large-scale transcriptomic analyses. By understanding how some transcription factors could act as genomic "hubs" to interact and control networks of gene expression, this approach has helped bring new understanding to how complex transcriptional networks regulate phenotypes and how transcription factors could be at the heart of a genetic toolkits that have been used by natural selection to build diverse phenotypes (Sinha et al. 2006; Chandrasekaran

et al. 2011; Ament et al. 2012a, b). It is worth noting, however, that most of these studies provide predominantly correlative data (i.e., between transcriptome and phenotype), and as such, functional analyses on these genes and pathways are still mostly lacking. Future work focusing on filling in these gaps will be vital for building a fuller understanding of the genomic aspects of honey bee biology.

Further genomic investigations will also likely identify other players in honey bee social organization. For example, the detection of **microRNAs** in the honey bee genome has only recently begun to reveal the importance of these small, noncoding regions of RNA that regulate gene expression. After computational identification (Weaver et al. 2007), experimental studies have begun to examine how microRNAs may have a role in behavioral maturation (Behura and Whitfield 2010; Greenberg et al. 2012; Liu et al. 2012), showing their potential importance in division of labor. Future studies on microRNAs will likely help to clarify their placement in the network of factors that affect honey bee behavior.

As genomic tools become more advanced and accessible, the study of more honey bee phenotypes at the genomic level become more tractable, providing a clearer vision of honey bee behavior, communication, development, and health. Future work will also likely provide a more complete picture of the chain from genome to phenotype. Though the honey bee genome provided new insights into bee proteomics (Wolschin and Amdam 2007), most studies assume that changes in mRNA expression reliably represent changes in protein expression, which lies closer to the actual phenotype. As proteomic techniques, like the ability to perform large-scale proteomic analyses (Hernandez et al. 2012), continue to improve, the link between genes, proteins, and organismal phenotype should become clearer.

In addition, genomic studies are becoming increasingly possible in solitary Hymenoptera and other social insects, like wasps, ants, and bees. Comparisons with these other species allow for insights for identification of both shared and novel genes and pathways that are involved in the convergent evolution of similar social traits and analyses can help identify genes that have evolved rapidly to build social phenotypes from solitary traits.

Glossary of terms: Words rendered in bold font in the body of the text are defined here.

Bioinformatics: The use of computational techniques to manage and analyze large quantities of information from biological systems, predominantly genomic and transcriptomic data (Hogeweg 2011).

Caste: Term used to describe a group of individuals in social insect colonies that specializes, to some extent, in specific occupations as a result of division of labor. Social insect castes can be associated with differences in age, anatomy, and morphology.

cis-regulatory elements: A sequence of DNA which, via the binding of transcription factors or other proteins, regulates the expression of a gene or genes on the same chromosome (Wray 2007).

Division of labor: A social system in which individuals specialize in specific occupations. In insect societies, queens mostly reproduce, whereas workers engage in all tasks related to colony growth and development. Young workers tend to work in the nest, whereas older individuals forage outside the nest.

DNA methylation: A form of epigenetic modification in which methyl groups are attached to nucleotides, usually CpG dinucleotides, have the potential to affect the expression of methylated sequences (Bird 2007).

Epigenetics: Environmental mediation of an individual's genome and/or its descendants, without changes in DNA sequence, via mechanisms like DNA methylation and histone modification (Crews 2008).

Eusocial: Traditionally defined as social species that show three features: extreme asymmetries in reproduction, with some individuals reproducing a great deal and others little or not at all; overlapping generations of adults in the nest; and cooperative care of offspring (Wilson 1971).

Expressed sequence tags (EST): ESTs are produced by sequencing many clones from cDNA libraries; since the sequences from these cDNA libraries are originally derived from mRNA from the organism of interest, ESTs provide important information regarding what genes are being expressed (Gerhold and Caskey 1996).

Genome: The complete genetic code for an organism.

Genetic toolkit: The concept that conserved genes and pathways have similar roles across a variety of taxa, helping to "build" different phenotypes from the same "tools," resulting in diverse phenotypes regulated by similar factors (Toth and Robinson 2007).

Insulin/insulin-like signaling (IIS): Metabolic pathway that acts as a key regulator of growth, feeding behavior, and metabolism; in insects, it also interacts with target of TOR and JH (Edgar 2006, Tu et al. 2005).

Juvenile hormone (JH): Insect hormone involved in many behavioral and developmental processes, including onset of foraging behavior in honey bees (Hartfelder 2000).

Microarray: Technology that allow for the quantification of gene expression via the hybridization of cDNA to complementary sequences on a chip (Schena et al. 1995), used in conjunction with ESTs to quantify known genes.

MicroRNA: A small section of noncoding RNA that has transcriptional and posttranslational effects on gene expression (Chen and Rajewsky 2007).

Quantitative trait loci (QTL): Sections of DNA sequence (loci) that contain or are linked to quantitative trait. QTLs can also be mapped to whole or partial genomes to further identify genes associated with the trait of interest (Erickson et al. 2004).

Queen mandibular pheromone (QMP): Pheromone produced by honey bee queens to regulate the behavior and reproductive physiology of workers (Winston and Slessor 1998).

RNA-Seq: A form of transcriptomic profiling where high-throughput sequencing of all the cDNA contained in a sample provides precise measurements of gene expression (Wang et al. 2009).

Single-cohort colonies: Behavioral manipulation in which hives are created solely from young workers. This modification of normal age demography results in newly formed colonies that lack foragers, and young workers subsequently transition to foraging behaviors earlier than normal, allowing researchers to compare same-aged individuals that perform different tasks (Nelson 1927; Robinson et al. 1989).

Target of rapamycin (TOR): An important metabolic regulator that interacts with the IIS pathway (Tu et al. 2005).

Transcription factor: A protein that binds to a regulatory DNA segment, regulating the transcription of specific target genes into mRNA.

Sociogénomique de l'abeille: une perspective à l'échelle génomique sur le comportement social et la santé de l'abeille

Génome / division du travail / maturation comportementale / caste / génomique comparative

Honigbienen-Soziogenomik: Eine genomweite Sicht auf das Sozialverhalten und die Gesundheit von Honigbienen

Genom / Arbeitsteilung / altersbedingte Verhaltensreifung / Kaste / vergleichende Genomik

REFERENCES

Alaux, C., Robinson, G.E. (2007) Alarm pheromone induces immediate-early gene expression and slow behavioral response in honey bees. J. Chem. Ecol. **33**(7), 1346–1350

Alaux, C., Duong, N., Schneider, S.S., Southey, B.R., Rodriguez-Zas, S., et al. (2009a) Modulatory communication signal performance is associated with a distinct neurogenomic state in honey bees. PloS ONE **4**(8), e6694. doi:10.1371/journal.pone.0006694

Alaux, C., Le Conte, Y., Adams, H.A., Rodriguez-Zas, S., Grozinger, C.M., et al. (2009b) Regulation of brain gene expression in honey bees by brood pheromone. Genes Brain Behav. **8**(3), 309–319

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G.J., Guzman-Novoa, E., et al. (2009c) Honey bee aggression supports a link between gene regulation and behavioral evolution. Proc. Natl. Acad. Sci. U. S. A. **106**(36), 15400–15405

Alaux, C., Dantec, C., Parrinello, H., Le Conte, Y. (2011) Nutrigenomics in honey bees: Digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. BMC Genomics **12**, 496. doi:10.1186/1471-2164-12-496

Amdam, G.V., Page Jr., R.E., Fondrk, M.K., Brent, C.S. (2010) Hormone response to bidirectional selection on social behavior. Evol. Dev. **12**(5), 428–436

Ament, S.A., Corona, M., Pollock, H.S., Robinson, G.E. (2008) Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. Proc. Natl. Acad. Sci. U. S. A. **105**(11), 4226–4231

Ament, S.A., Wang, Y., Robinson, G.E. (2010) Nutritional regulation of division of labor in honey bees: toward a systems biology perspective. WIREs Syst. Biol. Med. **2**(5), 566–576

Ament, S.A., Blatti, C.A., Alaux, C., Wheeler, M.M., Toth, A.L., et al. (2012a) New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. Proc. Natl. Acad. Sci. U. S. A. **109**(26), E1801–E1810

Ament, S.A., Wang, Y., Chen, C.C., Blatti, C.A., Hong, F., et al. (2012b) The transcription factor ultraspiracle influences honey bee social behavior and behavior-related gene expression. PloS Genet. **8**(3), e1002596

Barchuk, A.R., Cristino, A.S., Kucharski, R., Costa, L.F., Simoes, Z.L.P., et al. (2007) Molecular determinants of caste differentiation in the highly eusocial honeybee *Apis mellifera*. BMC Dev. Biol. **7**, 70. doi:10.1186/1471-213X-7-70

Behura, S.K., Whitfield, C.W. (2010) Correlated expression patterns of microRNA genes with age-dependent behavioural changes in honeybee. Insect Mol. Biol. **19**(4), 431–439

Ben-Shahar, Y. (2005) The foraging gene, behavioral plasticity, and honeybee division of labor. J. Comp. Physiol. A, Neuroethology, Sensory, Neural, and Behavioral Physiology **191**(11), 987–994

Bird, A. (2007) Perceptions of epigenetics. Nature **447**(7143), 396–398

Bonasio, R., Li, Q., Lian, J., Mutti, N.S., Jin, L., et al. (2012) Genome-wide and caste-specific DNA methylomes of the ants *Camponotus floridanus* and *Harpegnathos saltator*. Curr. Biol. **22**(19), 1755–1764

Cardoen, D., Wenseleers, T., Ernst, U.R., Danneels, E.L., Laget, D., et al. (2011) Genome-wide analysis of alternative reproductive phenotypes in honeybee workers. Mol. Ecol. **20**(19), 4070–4084

Cash, A.C., Whitfield, C.W., Ismail, N., Robinson, G.E. (2005) Behavior and the limits of genomic plasticity: power and replicability in microarray analysis of honeybee brains. Genes Brain Behav. **4**(4), 267–271

Chandrasekaran, S., Ament, S.A., Eddy, J.A., Rodriguez-Zas, S.L., Schatz, B.R., et al. (2011) Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. Proc. Natl. Acad. Sci. U. S. A. **108**(44), 18020–18025

Chen, K., Rajewsky, N. (2007) The evolution of gene regulation by transcription factors and microRNAs. Nat. Rev. Genet. **8**(2), 93–103

Chen, X., Hu, Y., Zheng, H.Q., Cao, L.F., Niu, D.F., et al. (2012) Transcriptome comparison between honey bee queen- and worker-destined larvae. Insect Biochem. Mol. **42**(9), 665–673

Cornman, S.R., Schatz, M.C., Johnston, S.J., Chen, Y.P., Pettis, J., et al. (2010) Genomic survey of the ectoparasitic mite *Varroa destructor*, a major pest of the honey bee *Apis mellifera*. BMC Genomics **11**, 602. doi:10.1186/1471-2164-11-602

Cornman, R.S., Tarpy, D.R., Chen, Y.P., Jeffreys, L., Lopez, D., et al. (2012) Pathogen webs in collapsing honey bee colonies. PloS ONE **7**(8), e43562. doi:10.1371/journal.pone.0043562

Cornman, R.S., Boncristiani, H., Dainat, B., Chen, Y.P., vanEngelsdorp, D., et al. (2013) Population-genomic variation within RNA viruses of the Western honey bee, *Apis mellifera*, inferred from deep sequencing. BMC Genomics **14**, 154. doi:10.1186/1471-2164-14-154

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., et al. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. Science **318**(5848), 283–287

Crews, D. (2008) Epigenetics and its implications for behavioral neuroendocrinology. Front. Neuroendocrinol. **29**(3), 344–357

Cristino, A.S., Nunes, F.M.F., Lobo, C.H., Bitondi, M.M.G., Simoes, Z.L.P., et al. (2006) Caste development and reproduction: a genome-wide analysis of hallmarks of insect eusociality. Insect Mol. Biol. **15**(5), 703–714

Daugherty, T.H.F., Toth, A.L., Robinson, G.E. (2011) Nutrition and division of labor: effects on foraging and brain gene expression in the paper wasp *Polistes metricus*. Mol. Ecol. **20**(24), 5337–5347

Dickman, M.J., Kucharski, R., Maleszka, R., Hurd, P.J. (2013) Extensive histone post-translational modification in honey bees. Insect Biochem. Mol. **43**(2), 125–137

Dyer, F.C. (2002) The biology of the dance language. Annu.Rev. Entomol. **47**, 917–949

Edgar, B.A. (2006) How flies get their size: genetics meets physiology. Nat. Rev. Genet. **7**(12), 907–916

Elsik, C.G., Mackey, A.J., Reese, J.T., Milshina, N.V., Roos, D.S., et al. (2007) Creating a honey bee consensus gene set. Genome Biol **8**(1)

Engel, P., Martinson, V.G., Moran, N.A. (2012) Functional diversity within the simple gut microbiota of the honey bee. Proc. Natl. Acad. Sci. U. S. A. **109**(27), 11002–11007

Erickson, D.L., Fenster, C.B., Stenoien, H.K., Price, D. (2004) Quantitative trait locus analyses and the study of evolutionary process. Mol. Ecol. **13**(9), 2505–2522

Evans, J.D., Schwarz, R.S. (2011) Bees brought to their knees: microbes affecting honey bee health. Trends Microbiol. **19**(12), 614–620

Evans, J.D., Wheeler, D.E. (2001) Expression profiles during honeybee caste determination. Genome Biol. **2**(1), research0001.1–research0001.6.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., et al. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Mol. Biol. **15**(5), 645–656

Ferreira, P.G., Patalano, S., Chauhan, R., Ffrench-Constant, R., Gabaldon, T., et al. (2013) Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes. Genome Biol. **14**(2), R20

Fischman, B.J., Woodard, S.H., Robinson, G.E. (2011) Molecular evolutionary analyses of insect societies. Proc. Natl. Acad. Sci. U. S. A. **108**, 10847–10854

Flores, K., Wolschin, F., Corneveaux, J.J., Allen, A.N., Huentelman, M.J., et al. (2012) Genome-wide association between DNA methylation and alternative splicing in an invertebrate. Bmc Genomics **13**, 480. doi:10.1186/1471-2164-13-480

Foret, S., Maleszka, R. (2006) Function and evolution of a gene family encoding odorant binding-like proteins in a social insect, the honey bee (*Apis mellifera*). Genome Res. **16**(11), 1404–1413

Foret, S., Wanner, K.W., Maleszka, R. (2007) Chemosensory proteins in the honey bee: insights from the annotated genome, comparative analyses and expressional profiling. Insect Biochem. Mol. Biol. **37**(1), 19–28

Foret, S., Kucharski, R., Pellegrini, M., Feng, S.H., Jacobsen, S.E., et al. (2012) DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. Proc. Natl. Acad. Sci. U. S. A. **109**(13), 4968–4973

Gallai, N., Salles, J.M., Settele, J., Vaissiere, B.E. (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. **68**(3), 810–821

Gempe, T., Stach, S., Bienefeld, K., Beye, M. (2012) Mixing of honeybees with different genotypes affects individual worker behavior and transcription of genes in the neuronal substrate. PloS ONE **7**(2), e31653. doi:10.1371/journal.pone.0031653

Gerhold, D., Caskey, C.T. (1996) It's the genes! EST access to human genome content. Bioessays **18**(12), 973–981

Gotzek, D., Ross, K.G. (2007) Genetic regulation of colony social organization in fire ants: an integrative overview. Q. Rev. Biol. **82**(3), 201–226

Gotzek, D., Ross, K.G. (2009) Current status of a model system: the gene Gp-9 and its association with social organization in fire ants. PloS ONE **4**(11), e7713. doi:10.1371/journal.pone.0007713

Graham, A.M., Munday, M.D., Kaftanoglu, O., Page, R.E., Amdam, G.V., et al. (2011) Support for the reproductive ground plan hypothesis of social evolution and major QTL for ovary traits of Africanized worker honey bees (*Apis mellifera* L.). BMC Evol. Biol. **11**, 95. doi:10.1186/1471-2148-11-95

Greenberg, J.K., Xia, J., Zhou, X., Thatcher, S.R., Gu, X., et al. (2012) Behavioral plasticity in honey bees is associated with differences in brain microRNA transcriptome. Genes Brain Behav. **11**(6), 660–670

Grozinger, C.M., Robinson, G.E. (2002) Microarray analysis of pheromone-mediated gene expression in the honey bee brain. Integr. Comp. Biol. **42**(6), 1237

Grozinger, C.M., Robinson, G.E. (2007) Endocrine modulation of a pheromone-responsive gene in the honey bee brain. J. Comp. Physiol. A. **193**(4), 461–470

Grozinger, C.M., Sharabash, N.M., Whitfield, C.W., Robinson, G.E. (2003) Pheromone-mediated gene expression in the honey bee brain. Proc. Natl. Acad. Sci. U. S. A. **100**, 14519–14525

Hartfelder, K. (2000) Insect juvenile hormone: from "status quo" to high society. Braz. J. Med. Biol. Res. **33**, 157–177

Herb, B.R., Wolschin, F., Hansen, K.D., Aryee, M.J., Langmead, B., et al. (2012) Reversible switching between epigenetic states in honeybee behavioral subcastes. Nat. Neurosci. **15**(10), 1371–1373

Hernandez, L.G., Lu, B.W., da Cruz, G.C.N., Calabria, L.K., Martins, N.F., et al. (2012) Worker honeybee brain proteome. J. Proteome Res. **11**(3), 1485–1493

Hogeweg, P. (2011) The roots of bioinformatics in theoretical biology. PloS Comput. Biol. **7**(3), e1002021. doi:10.1371/journal.pcbi.1002021

Hunt, G.J., Page, R.E., Fondrk, M.K., Dullum, C.J. (1995) Major quantitative trait loci affecting honeybee foraging behavior. Genetics **141**(4), 1537–1545

Hunt, G.J., Guzman-Novoa, E., Fondrk, M.K., Page, R.E. (1998) Quantitative trait loci for honey bee stinging behavior and body size. Genetics **148**(3), 1203–1213

Hunt, G.J., Amdam, G.V., Schlipalius, D., Emore, C., Sardesai, N., et al. (2007) Behavioral genomics of honeybee foraging and nest defense. Naturwissenschaften **94**(4), 247–267

Hunt, B.G., Wyder, S., Elango, N., Werren, J.H., Zdobnov, E.M., et al. (2010) Sociality is linked to rates of protein evolution in a highly social insect. Mol. Biol. Evol. **27**(3), 497–500

Johnson, B.R., Tsutsui, N.D. (2011) Taxonomically restricted genes are associated with the evolution of sociality in the honey bee. Bmc Genomics **12**, 164. doi:10.1186/1471-2164-12-164

Johnson, R.M., Evans, J.D., Robinson, G.E., Berenbaum, M.R. (2009) Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*). Proc. Natl. Acad. Sci. U. S. A. **106**(35), 14790–14795

Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. Nature **473**(7348), 478–483

Keller, L., Ross, K.G. (1999) Major gene effects on phenotype and fitness: the relative roles of Pgm-3 and Gp-9 in introduced populations of the fire ant *Solenopsis invicta*. J. Evol. Biol. **12**(4), 672–680

Kocher, S.D., Richard, F.J., Tarpy, D.R., Grozinger, C.M. (2008) Genomic analysis of post-mating changes in the honey bee queen (*Apis mellifera*). Bmc Genomics **9**, 232. doi:10.1186/1471-2164-9-232

Kocher, S.D., Ayroles, J.F., Stone, E.A., Grozinger, C.M. (2010a) Individual variation in pheromone response correlates with reproductive traits and brain gene expression in worker honey bees. PloS ONE **5**(2), e9116. doi:10.1371/journal.pone.0009116

Kocher, S.D., Tarpy, D.R., Grozinger, C.M. (2010b) The effects of mating and instrumental insemination on queen honey bee flight behaviour and gene expression. Insect Mol. Biol. **19**(2), 153–162

Kronforst, M.R., Gilley, D.C., Strassmann, J.E., Queller, D.C. (2008) DNA methylation is widespread across social Hymenoptera. Current Biology **18**(7): R287–R288.

Kucharski, R., Maleszka, R. (2002) Evaluation of differential gene expression during behavioral development in the honeybee using microarrays and northern blots. Genome Biol. **3**(2), RESEARCH0007.

Kucharski, R., Maleszka, J., Foret, S., Maleszka, R. (2008) Nutritional control of reproductive status in honeybees via DNA methylation. Science **319**(5871), 1827–1830

Le Conte, Y., Hefetz, A. (2008) Primer pheromones in social hymenoptera. Annu. Rev. Entomol. **53**, 523–542

Le Conte, Y., Mohammedi, A., Robinson, G.E. (2001) Primer effects of a brood pheromone on honeybee behavioural development. Proc. R. Soc. B.: Biol. Sci. **268**(1463), 163–168

Le Conte, Y., Alaux, C., Martin, J.F., Harbo, J.R., Harris, J.W., et al. (2011) Social immunity in honeybees (*Apis mellifera*): transcriptome analysis of *Varroa*-hygienic behaviour. Insect Mol. Biol. **20**(3), 399–408

Liang, Z.Z.S., Nguyen, T., Mattila, H.R., Rodriguez-Zas, S.L., Seeley, T.D., et al. (2012) Molecular determinants of scouting behavior in honey bees. Science **335**(6073), 1225–1228

Linksvayer, T.A., Rueppell, O., Siegel, A., Kaftanoglu, O., Page, R.E., et al. (2009) The genetic basis of transgressive ovary size in honeybee workers. Genetics **183**(2), 693–707

Liu, F., Peng, W., Li, Z., Li, W., Li, L., et al. (2012) Next-generation small RNA sequencing for microRNAs profiling in *Apis mellifera*: comparison between nurses and foragers. Insect Mol. Biol. **21**(3), 297–303

Lobo, N.F., Ton, L.Q., Hill, C.A., 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**(12), 2588–2593

Lutz, C.C., Rodriguez-Zas, S.L., Fahrbach, S.E., Robinson, G.E. (2012) Transcriptional response to foraging experience in the honey bee mushroom bodies. Dev. Neurobiol. **72**(2), 153–166

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**(11), e1000506. doi:10.1371/journal.pbio.1000506

McClung, C.A., Nestler, E.J. (2008) Neuroplasticity mediated by altered gene expression. Neuropsychopharmacol: Off. Publ. Am. Coll. Neuropsychopharmacol. **33**(1), 3–17

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., et al. (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PloS ONE **5**(3), e9754. doi:10.1371/journal.pone.0009754

Naug, D. (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biol. Conserv. **142**(10), 2369–2372

Navajas, M., Migeon, A., Alaux, C., Martin-Magniette, M.L., Robinson, G.E., et al. (2008) Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. BMC Genomics **9**, 301. doi:10.1186/1471-2164-9-301

Nelson, F.C. (1927) Adaptability of young bees under adverse conditions. Am. Bee J. **67**, 242–243

Nelson, C.M., Ihle, K., Amdam, G.V., Fondrk, M.K., Page, R.E. (2007) The gene *vitellogenin* has multiple coordinating effects on social organization. PLoS Biol. **5**, 673–677

Ometto, L., Shoemaker, D., Ross, K.G., Keller, L. (2011) Evolution of gene expression in fire ants: the effects of developmental stage, caste, and species. Mol. Biol. Evol. **28**(4), 1381–1392

Oxley, P.R., Thompson, G.J., Oldroyd, B.P. (2008) Four quantitative trait loci that influence worker sterility in the honeybee (*Apis mellifera*). Genetics **179**(3), 1337–1343

Oxley, P.R., Spivak, M., Oldroyd, B.P. (2010) Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). Mol. Ecol. **19**(7), 1452–1461

Page, R.E., Fondrk, M.K. (1995) The effects of colony level selection on the social-organization of honeybee (*Apis mellifera* L) colonies—colony level components of pollen hoarding. Behav. Ecol. Sociobiol. **36**(2), 135–144

Page, R.E., Rueppell, O., Amdam, G.V. (2012) Genetics of reproduction and regulation of honeybee (*Apis mellifera* L.) social behavior. Annu. Rev. Genet. **46**, 97–119

Pankiw, T., Huang, Z.Y., Winston, M.L., Robinson, G.E. (1998) Queen mandibular gland pheromone influences worker honey bee (*Apis mellifera* L.) foraging ontogeny and juvenile hormone titers. J. Insect Physiol. **44**(7–8), 685–692

Pelosi, P., Calvello, M., Ban, L.P. (2005) Diversity of odorant-binding proteins and chemosensory proteins in insects. Chem. Senses **30**, I291–I292

Qiu, P., Pan, P.C., Govind, S. (1998) A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis. Development **125**(10), 1909–1920

Rachinsky, A., Hartfelder, K. (1990) Corpora allata activity, a prime regulating element for caste-specific juvenile hormone titre in honey bee larvae (*Apis mellifera carnica*). J. Insect Physiol. **36**, 189–194

Rembold, H. (1987) Caste specific modulation of juvenile hormone titers in *Apis mellifera*. Insect Biochem. **17**, 1003–1006

Rembold, H., Czoppelt, C., Rao, P.J. (1974) Effect of juvenile-hormone treatment on caste differentiation in honeybee, *Apis mellifera*. J. Insect Physiol. **20**(7), 1193–1202

Richard, F.J., Holt, H.L., Grozinger, C.M. (2012) Effects of immunostimulation on social behavior, chemical communication and genome-wide gene expression in honey bee workers (*Apis mellifera*). BMC Genomics **13**, 558

Robinson, G.E. (1987) Regulation of honey bee age polyethism by juvenile hormone. Behav. Ecol. Sociobiol. **20**, 329–338

Robinson, G.E. (1992) Regulation of division of labor in insect societies. Annu. Rev. Entomol. **37**, 637–665

Robinson, G.E. (1999) Integrative animal behaviour and sociogenomics. Trends Ecol. Evol. **14**(5), 202–205

Robinson, G.E. (2002) Genomics and integrative analyses of division of labor in honeybee colonies. Am. Nat. **160**, S160–S172

Robinson, G.E., Page, R.E., Strambi, C., Strambi, A. (1989) Hormonal and genetic-control of behavioral integration in honey bee colonies. Science **246**(4926), 109–111

Robinson, G.E., Grozinger, C.M., Whitfield, C.W. (2005) Sociogenomics: social life in molecular terms. Nat. Rev. Genet. **6**(4), 257–270

Rosenkranz, P., Aumeier, P., Ziegelmann, B. (2010) Biology and control of *Varroa destructor*. J. Invertebr. Pathol. **103**, S96–S119

Rothenbuhler, W.C. (1964) Behaviour genetics of nest cleaning in honey bees. I. Responses of 4 inbred lines to disease-killed brood. Anim. Behav. **12**(4), 578

Rueppell, O. (2013) The architecture of the pollen hoarding syndrome in honey bees: implications for understanding social evolution, behavioral syndromes, and selective breeding. Apidologie. doi:10.1007/s13592-013-0244-3 (this issue)

Schena, M., Shalon, D., Davis, R.W., Brown, P.O. (1995) Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. Science **270**(5235), 467–470

Schneider, S.S., Lewis, L.A. (2004) The vibration signal, modulatory communication and the organization of labor in honey bees, *Apis mellifera*. Apidologie **35**(2), 117–131

Seeley, T.D. (1985) Honeybee ecology: a study of adaptation in social life. Princeton University Press, Princeton

Sen Sarma, M., Whitfield, C.W., Robinson, G.E. (2007) Species differences in brain gene expression profiles associated with adult behavioral maturation in honey bees. BMC Genomics **8**, 202.

Sen Sarma, M., Rodriguez-Zas, S.L., Hong, F., Zhong, S., Robinson, G.E. (2009) Transcriptomic profiling of central nervous system regions in three species of honey bee during dance communication behavior. PloS ONE **4**(7)

Sen Sarma, M., Rodriguez-Zas, S.L., Gernat, T., Nguyen, T., Newman, T., et al. (2010) Distance-responsive genes found in dancing honey bees. Genes Brain Behav. **9**(7), 825–830

Sinha, S., Ling, X., Whitfield, C.W., Zhai, C.X., Robinson, G.E. (2006) Genome scan for *cis*-regulatory DNA motifs associated with social behavior in honey bees. Proc. Natl. Acad. Sci. U. S. A. **103**(44), 16352–16357

Smith, C.R., Toth, A.L., Suarez, A.V., Robinson, G.E. (2008) Genetic and genomic analyses of the division of labour in insect societies. Nat. Rev. Genet. **9**(10), 735–748

Smith, C.R., Mutti, N.S., Jasper, W.C., Naidu, A., Smith, C.D., et al. (2012) Patterns of DNA methylation in development, division of labor and hybridization in an ant with genetic caste determination. PloS ONE **7**(8), e42433. doi:10.1371/journal.pone.0042433

Sullivan, J.P., Jassim, O., Fahrbach, S.E., Robinson, G.E. (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. Hormon. Behav. **37**, 1–14

Sumner, S., Pereboom, J.J.M., Jordan, W.C. (2006) Differential gene expression and phenotypic plasticity in behavioural castes of the primitively eusocial wasp, *Polistes canadensis*. Proc. R. Soc. B.: Biol. Sci. **273**(1582), 19–26

Thompson, G.J., Kucharski, R., Maleszka, R., Oldroyd, B.P. (2006) Towards a molecular definition of worker sterility: differential gene expression and reproductive plasticity in honey bees. Insect Mol. Biol. **15**(5), 637–644

Thompson, G.J., Kucharski, R., Maleszka, R., Oldroyd, B.P. (2008) Genome-wide analysis of genes related to ovary activation in worker honey bees. Insect Mol. Biol. **17**(6), 657–665

Toth, A.L., Robinson, G.E. (2005) Worker nutrition and division of labour in honeybees. Anim. Behav. **69**, 427–435

Toth, A.L., Robinson, G.E. (2007) Evo-devo and the evolution of social behavior. Trends Genet. **23**(7), 334–341

Toth, A.L., Kantarovich, S., Meisel, A.F., Robinson, G.E. (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees. J. Exp. Biol. **208**(24), 4641–4649

Toth, A.L., Varala, K., Henshaw, M.T., Rodriguez-Zas, S.L., Hudson, M.E., et al. (2010) Brain transcriptomic analysis in paper wasps identifies genes associated with behaviour across social insect lineages. Proc. R. Soc. B.: Biol. Sci. **277**(1691), 2139–2148

Tu, M.P., Yin, C.M., Tatar, M. (2005) Mutations in insulin signaling pathway alter juvenile hormone synthesis in *Drosophila melanogaster*. Gen. Comp. Endocrinol. **142**(3), 347–356

Viljakainen, L., Evans, J.D., Hasselmann, M., Rueppell, O., Tingek, S., et al. (2009) Rapid evolution of immune proteins in social insects. Mol. Biol. Evol. **26**(8), 1791–1801

Wang, Z., Gerstein, M., Snyder, M. (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. **10**(1), 57–63

Wang, Y., Kocher, S.D., Linksvayer, T.A., Grozinger, C.M., Page, R.E., et al. (2012) Regulation of behaviorally associated gene networks in worker honey bee ovaries. J. Exp. Biol. **215**(1), 124–134

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.C., et al. (2013) A Y-like social chromosome causes alternative colony organization in fire ants. Nature **493**(7434), 664–668

Weaver, D.B., Anzola, J.M., Evans, J.D., Reid, J.G., Reese, J.T., et al. (2007) Computational and transcriptional evidence for microRNAs in the honey bee genome. Genome Biol. **8**(6), R97.

Weiner, S.A., Galbraith, D.A., Adams, D.C., Valenzuela, N., Noll, F.B., et al. (2013) A survey of DNA methylation across social insect species, lifestages, and castes reveals abundant and caste-associated methylation in a primitively social wasp. Naturwissenschaften 100(8), 795–799. doi:10.1007/s00114-013-1064-z

Weinstock, G.M., Robinson, G.E., Gibbs, R.A., Weinstock, G.M., Weinstock, G.M., et al. (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature **443**(7114), 931–949

Wheeler, D.E., Buck, N., Evans, J.D. (2006) Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. Insect Mol. Biol. **15**(5), 597–602

Whitfield, C.W., Band, M.R., Bonaldo, M.F., Kumar, C.G., 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**(4), 555–566

Whitfield, C.W., Cziko, A.M., Robinson, G.E. (2003) Gene expression profiles in the brain predict behavior in individual honey bees. Science **302**(5643), 296–299

Whitfield, C.W., 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. U. S. A. **103**(44), 16068–16075

Wilson, E.O. (1971) The insect societies. Belknap Press of Harvard University Press, Cambridge

Wilson-Rich, N., Spivak, M., Fefferman, N.H., Starks, P.T. (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. Annu. Rev. Entomol. **54**, 405–423

Winston, M.L. (1987) The biology of the honey bee. Harvard University Press, Cambridge

Winston, M.L., Slessor, K.N. (1998) Honey bee primer pheromones and colony organization: gaps in our knowledge. Apidologie **29**(1–2), 81–95

Wolschin, F., Amdam, G.V. (2007) Plasticity and robustness of protein patterns during reversible development in the honey bee (*Apis mellifera*). Anal. Bioanal. Chem. **389**(4), 1095–1100

Woodard, S.H., Fischman, B.J., Venkat, A., Hudson, M.E., Varala, K., et al. (2011) Genes involved in

convergent evolution of eusociality in bees. Proc. Natl. Acad. Sci. U. S. A. **108**(18), 7472–7477

Wray, G.A. (2007) The evolutionary significance of *cis*-regulatory mutations. Nat. Rev. Genet. **8**(3), 206–216

Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., et al. (2011) The genome of the fire ant *Solenopsis invicta*. Proc. Natl. Acad. Sci. U. S. A. **108**(14), 5679–5684

Zhang, Y., Liu, X.J., Zhang, W.Q., Han, R.C. (2010) Differential gene expression of the honey bees *Apis mellifera* and *A. cerana* induced by *Varroa destructor* infection. J. Insect Physiol. **56**(9), 1207–1218