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REVIEW

Insights into the molecular basis of social behaviour from studies on the honeybee, *Apis mellifera*

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Abstract The honeybee, *Apis mellifera*, has been the most important insect species for the study of social behaviour. With the recent release of its genome sequence, the honeybee has emerged as an excellent model for molecular studies of social behaviour. A key feature of eusocial species is a complex division of labour. Adult honeybees perform a series of tasks in the hive when they are young and then shift to foraging for nectar or pollen outside the hive when they are 2–3 weeks of age. This transition from working in the hive to foraging involves changes in the expression of thousands of genes. In this review, we focus first on recent advances in understanding of the widespread changes in gene activity that accompany the transition to foraging. Thereafter, we examine three genes in particular, *foraging*, *malvolio* and *vitellogenin,* all implicated in this striking behavioural change in the life of the honeybee.

Keywords Honeybee · Foraging · Gene expression · Microarrays · RNA interference

Introduction

Social organisms behave in a way that depends not only on their own needs, but also on the requirements of group living. Their actions are subject to individual, environmental and social regulation, and as such must be flexible enough to reflect a complex interplay of influences. To understand the genetic foundations of social life, researchers have turned to the honeybee *Apis mellifera*. Honeybees live in complex societies, in which different bees play different roles in reproduction, brood care, hive maintenance and food collection in order to promote the overall functioning of the hive. These differences in behaviour result from differences in brain function, which in turn can be linked to the activity of genes.

The division of labour in honeybee societies can be separated into two broad categories. The first is a reproductive distinction, between the queen, the sole reproductive female of the hive, and the workers, facultatively sterile females performing a number of tasks essential for hive upkeep. The second relates to the types of tasks performed by the workers, and comprises both an age-related division of labour, or age polyethism, and foraging specializations. During the first 2–3 weeks of their adult lives, worker bees perform different tasks in the hive, including nursing, or caring for the young. After that, they become foragers, flying away from the hive to collect pollen and nectar, activities that will occupy the remaining 5–7 weeks of their lives. Finally, when the bees become foragers, they tend to specialize in the collection of either pollen or nectar, which creates a division of labour in the retrieval of these different kinds of food resources, pollen being a source of protein and nectar a source of carbohydrates (Winston 1987).

Ongoing research into the mechanisms underlying each of these kinds of division of labour in honeybee societies is shedding light into the biological basis of social organization. The publication of the honeybee genome sequence this past year will undoubtedly spur further advances (Honeybee

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Genome Sequencing Consortium 2006). We focus this review primarily on recent work investigating the genetics behind the nursing-to-foraging transition in honeybees, with some discussion of foraging specializations. Research into the transition to foraging is particularly well suited for the study of how changes in gene expression drive behavioural change, because the same bees behave as nurses and as foragers at different points in their lives. Although the individual genotypes of worker bees may influence the age at which they begin foraging (Calderone and Page 1988; Page et al. 1992), the shift is far from genetically predetermined. Rather, it depends on environmental factors: it can be delayed, accelerated or even reversed depending on the needs of the hive (Robinson 2002).

The transition from nursing to foraging in honeybees, then, is a flexible, socially responsive, yet well-defined change in behaviour. As such, it has become a model for the study of the molecular basis of social behaviour, tracing a path from genes to brain function and behaviour in an organism in which group-level factors modulate the actions of individuals. We discuss recent advances in understanding the widespread genetic changes that accompany the transition to foraging, and then focus on three genes in particular, *foraging*, *malvolio* and *vitellogenin*, which have all been implicated in this striking behavioural change in the life of the honeybee.

Large-scale genetic analyses of the transition to foraging

Large-scale analyses using the techniques of functional genomics have probed the pattern of genetic changes involved in the nurse-to-forager transition. These studies have revealed that this behavioural shift, mediated by exposure to pheromones and the activity of hormones, results in widespread changes in gene expression (Grozinger et al. 2003; Whitfield et al. 2003, 2006). The studies have also identified candidate genes for future research.

Microarray and protein studies

In one such analysis, Whitfield et al. (2003) compared gene expression in the brains of nurses and foragers in an attempt to identify genes that might contribute to this behavioural change. Their microarray analysis found that, of 6,878 cDNAs tested, estimated to represent ~5,500 genes, or ~40% of the honeybee genome (Whitfield et al. 2002), 39% showed significant differences between nurse and forager bees (Whitfield et al. 2003). This high percentage of genes with changing expression levels showed for the first time that gene expression in the honeybee brain is remarkably plastic. Also, these differences in gene expression, though highly significant, were small, more than 99% were less than a factor of 2, implying either relatively subtle expression changes over a wide area of the brain, or large changes in particular neurons. Future studies comparing gene expression changes in particular brain regions will undoubtedly help to distinguish between these two possibilities.

These same authors went on to show that the pattern of changes observed was better explained by nursing or foraging behaviour, which accounted for 49% of the variance between groups, than by age, which accounted for only 25% of the variance. This study does not establish whether these expression changes caused the behavioural shift or resulted from it, but it does show that the expression patterns were linked to foraging status and were not simply the products of aging. Finally, the authors showed that they could predict the behavioural role of an individual bee, nurse or forager, by examining that bee's individual gene expression profile. Seventeen of the most predictive cDNAs could be matched to functionally annotated *Drosophila melanogaster* genes, and included genes involved in axogenesis, intracellular signalling, transcription, synaptic plasticity and cell metabolism, all of which could conceivably contribute to structural brain changes mediating the observed change in behaviour (Whitfield et al. 2003).

Whitfield et al. (2006) built on these findings using microarray analysis to detect differences in honeybee gene expression patterns due to distinct effects of age, behaviour and environment. Principal component analysis revealed discrete trends due to age and behaviour (nursing or foraging). The age-related changes were essentially complete well before the bees began foraging and coincided with structural changes seen in the honeybee brain in that period, such as the growth and development of the mushroom bodies and expression of new neurotransmitter receptors (Farris et al. 2001; Guez et al. 2003). From data collected previously (Whitfield et al. 2003), the researchers selected the 100 genes that best classified an individual bee as either a nurse or a forager. They then treated the bees with methoprene (an analog of juvenile hormone (JH)), manganese, and cGMP, all of which have been found to accelerate the transition to foraging behaviour (Pankiw et al. 1998; Ben-Shahar et al. 2002, 2004). These treatments caused widespread changes in gene expression, mostly consistent with the pattern seen in the forager expression profile. However, the patterns of changes caused by each substance had relatively small overlap with one another, suggesting that they influenced bee behaviour through largely independent pathways. For instance, although methoprene and manganese treatment each resulted in the up- or down-regulation of hundreds of

genes, only 30 were up-regulated and 17 down-regulated by both substances (Whitfield et al. 2006).

Importantly, Whitfield et al. (2006) went on to address the question of whether gene expression patterns characterizing foragers arise from foraging experience; if this were the case, it would argue against a causal role for these genes in the transition to foraging. However, when foragers were compared to hive-restricted bees of the same age, only 11 of the 100 behavioural marker genes identified were significantly affected by foraging experience. Further studies will be needed to demonstrate causal relationships between different genes and the transition to foraging, but these results show that foraging-associated gene expression patterns can emerge even without foraging experience.

At a proteome level, Wolschin and Amdam (2007) have exploited the plastic nature of the nest bees-to-forager transition to identify proteins that characterize foragers and nest workers including nurses both before the initial age-related shift and after colony manipulations have reversed the development of some foragers, returning them to hive-based tasks. The researchers used a quantitative LC-MS/MS-based approach to identify proteins in the different groups of bees, matching the mass spectrometry data against an online *A. mellifera* sequence database. Out of 81 proteins tested, this study identified 22 proteins that showed significantly different levels in nest workers and foragers, both before and after reversion, firmly demonstrating the association between these genes and behaviour, as opposed to age. The reversion manipulation also rules out foraging experience as a factor in the expression of these proteins in foragers compared to nurses. In identifying genes that are both related to nest working or foraging behaviour and modulated by substances known to affect the transition between these behaviours, the above studies have also identified several promising candidate genes for future investigations into the molecular basis of socially regulated bee behaviour.

Variability in microarray studies

The attempt these researchers made to identify changes in gene expression caused by substances known to affect foraging behaviour represents an important first step in exploring the mechanism by which environmental factors interact with genes to change behaviour. However, it also raises a number of questions: What expression changes do substances like juvenile hormone cause directly, and what changes are downstream effects of the initial action? By what mechanisms do these initial changes and downstream effects occur? How might these changes be modulated by other environmental factors? Which changes in gene expression are essential for the behavioural change and which are merely correlative? And how do these changes in gene expression alter behaviour?

In order to begin to answer these kinds of questions, it is important first to know reliably what genes are up-regulated or down-regulated by the environmental factor in question. Grozinger et al. (2003) conducted a microarray analysis of gene expression changes related to queen mandibular pheromone (QMP), which delays the onset of foraging. They showed that depending upon the test conditions, there was considerable variability in the QMP-associated genes identified. For example, very different QMP-related gene expression patterns were found for bees raised in a cage, compared to those raised in a colony. The number of genes whose activity changed in colony bees was about a third of that observed in cage bees, and although over 2,500 cDNAs were significantly up- or down-regulated in response to QMP in cage bees, less than 150 cDNAs showed common up- or down-regulation in both cage and colony bees. The authors suggest that part of this discrepancy may be explained either by differences in QMP exposure in the cage versus the colony, or by the more complicated chemical environment of the colony. Still, this result raises the possibility that the effect of a single pheromone may change dramatically depending on the bee's environment, or that only a small subset of genes with changing expression patterns are critically related to the action of the pheromone.

This study also found that gene expression patterns relating to QMP change over time, with different genes being up- or down-regulated on each of the first 4 days after the initial exposure. Only 19 cDNAs were regulated in a sustained way by QMP, showing expression changes on the second, third, and fourth day; most (>99%) were regulated only transiently. Whether this reflects cascades of brief gene expression changes that operate sequentially to alter behaviour, variability in the effects of QMP or the analysis method is not yet known. These findings of environment- and time-dependent variability, however, must be remembered when interpreting the results of microarray studies that describe effects of behaviourally relevant substances without investigating the reliability of the results under different testing conditions. Understanding the sources of the kind of variability found by Grozinger et al. (2003) will help provide a more precise understanding of how genes are regulated by a given environmental signal and how gene expression alters depending on a bee's state and its surroundings.

Genome and quantitative trait loci scans

Working from the idea that important regulatory genes may be conserved across evolutionary history, Sinha et al.

(2006) scanned the recently-sequenced honeybee genome for 41 *cis*-regulatory motifs previously characterized in *Drosophila melanogaster*. They concentrated on the promoter regions of genes found to be differentially expressed in the honeybee brain in the context of the transition from nursing to foraging in the studies described above. The five motifs most significantly associated with the behaviourally related gene sets were Adf1, Hairy, Snail, Dri, and Cf1. The known functions of these fly transcription factors support the idea that their orthologues may act to regulate neural development or plasticity in the honeybee. Adf1, Hairy (Frankfort and Mardon 2002; Heng and Tan 2003) and Snail (Ashraf et al. 1999) proteins, for example, all contribute to the development of the nervous system in *Drosophila*. In the honeybee, Cf1 (also called "Ultraspiracle") interacts with the foraging-inducing hormone JH (Jones and Sharp 1997; Barchuk et al. 2004) and is highly expressed in the mushroom bodies of the bee brain, structures that play a central role in learning and memory in the honeybee (Velarde et al. 2006). The presence of binding sites in bee genes for three transcription factors, namely Hairy, Snail and GAGA, was found to predict significantly better than chance the classification of the genes into behavioural categories. For example, based on the presence or absence of the GAGA motif in a given gene, one could predict with 71% accuracy whether that gene was up-regulated or down-regulated in forager bees compared to nurse bees.

The strong association between these transcription factors and behaviourally-relevant genes make them prime candidates for further investigations into how social and environmental signals change the activity of large numbers of genes in order to coordinate behaviour. Studies that have identified quantitative trait loci (QTL) for behavioural phenotypes like age of foraging (Rueppell et al. 2004) and sucrose responsiveness (Rueppell et al. 2006) also provide excellent candidate regions for further investigation, especially since many of the QTLs identified seem to have pleiotropic behavioural effects. Microarray and QTL methods are complementary and can be used in tandem to identify and investigate genes regulating behaviour.

Genes regulating foraging behaviour

Large-scale gene analyses have shown that hundreds to thousands of genes show expression changes that correlate with behavioural changes and exposure to behaviourally relevant substances. To date, however, only a few honeybee genes have been shown to causally mediate the transition to foraging behaviour. The upcoming section will discuss three such genes: *foraging* (*Amfor*), *malvolio* (*Amvl*) and *vitellogenin* (*Vg*). Studies investigating these genes are beginning to yield insights into how genetic modulation of simple behaviours can contribute to a more complex behaviour change, how behaviourally-relevant gene expression changes are linked to neural changes, and how genes inherited from non-social evolutionary ancestors can be co-opted to mediate socially regulated behavioural changes.

The *foraging* gene affects phototaxis

The *foraging* gene was first described in *Drosophila*, a solitary insect, where it was found to influence feeding behaviour (Osborne et al. 1997). Different alleles resulted in flies that either searched large areas for food ("rovers") or restricted their search to a more confined space ("sitters"). The *foraging* gene codes for a cGMP-dependent protein kinase (PKG), and rovers showed higher levels of central nervous system *foraging* mRNA expression and PKG activity than sitters. cGMP signalling also affects feeding activity in the worm *Caenorhabiditis elegans* (Fujiwara et al. 2002) and the harvester ant *Pogonomyrmex barbatus*, although in ants, foragers have lower expression of the *foraging* gene than ants that work in the nest (Ingram et al. 2005).

Ben-Shahar et al. (2002) found that the honeybee *foraging* homologue *Amfor* plays a role in a bee's midlife transition from nursing to foraging. They showed that the transition to foraging is associated with an increased expression of *Amfor*, even in single-cohort colonies where there is no age difference between nurses and foragers. PKG activity was four times greater in foragers than in nurse bees. Ben-Shahar et al. (2002) established a causal relationship between PKG activity and foraging by showing that treating bees with 8-Br-cGMP elevated PKG levels and increased the likelihood of precocious foraging in a dose-dependent fashion. Using in situ hybridization, the researchers established that *Amfor* is expressed primarily in the optic lobes of the bee brain, and in the mushroom bodies, especially in the Kenyon cells. The mushroom bodies are the primary centre for crossmodal sensory processing in the honeybee brain. Kenyon cells receive input from visual and olfactory areas (Gronenberg 1986, 1999, 2001), and play an important role in learning and memory (Menzel 1999). This pattern of brain expression suggested that *Amfor* may contribute to a change in sensory processing, associated with foraging.

Support for the hypothesis that *Amfor* plays a role in visual processing was already in place from research showing that young bees avoided light (Southwick and Moritz 1987), while foragers were positively phototactic (Menzel and Greggers 1985) and tended to fly to well-lit places during foraging (Fry and Wehner 2002). This fits

well with the spatial separation of nurses and foragers within the hive. Nurses tend to be found in the dark, inner parts of the hive, while foragers tend to linger around the hive entrance (Seely 1995). More concrete evidence for an involvement of *Amfor* in phototaxis came from a study (Ben-Shahar et al. 2003) showing that "undertakers", bees that remove corpses from the hive and thus perform outdoor tasks, have levels of *Amfor* similar to those of foragers and greater than those of "food-handlers" bees that are the same age as undertakers but generally work in the interior of the hive. Stronger evidence still comes from the demonstration that cGMP treatment, previously shown to induce foraging behaviour (Ben-Shahar et al. 2002), also increases phototaxis (Ben-Shahar et al. 2003). However, the cGMP pathway likely plays a role in other kinds of sensory responses as well. For example, PKG activity is also associated with sucrose responsiveness in the fly (Scheiner et al. 2004b) and honeybee (Page et al. 2006).

In *Drosophila*, then, allelic variation in the *foraging* gene leads to different types of flies with different patterns of feeding behaviour, while in the honeybee, a midlife increase in *Amfor* expression leads to a change in foraging behaviour in each bee, mediating division of labour within the hive community. The *foraging* gene is an example of how genetic variations that may occur naturally across one population can be co-opted in another population into a system of orchestrated genetic changes that regulate complex behaviour across the lifespan.

The *malvolio* gene and sucrose responsiveness

Like the *foraging* gene, the *malvolio* gene *(mvl)* was first characterized in *Drosophila*, and based on its role in *Drosophila* feeding behaviour, was hypothesized to be involved in honeybee foraging behaviour. In the fly, *mvl* increases responsiveness not to light, but to sucrose (Rodrigues et al. 1995). It codes for a protein that transports manganese across the cell membrane (Supek et al. 1996; Orgad et al. 1998), and treatment with manganese was shown to rescue a sucrose response deficit observed in flies with *mvl* mutations (Orgad et al. 1998).

In honeybees, sucrose responsiveness is higher in foragers than in nurse bees, and is an indicator of the age at which honeybees start foraging (Pankiw and Page 2003). Sucrose responsiveness also indicates whether a foraging bee specializes in collecting pollen or nectar, with bees that collect pollen showing more sensitivity to sucrose (Pankiw and Page 1999). Sucrose responsiveness thus is associated with honeybee division of labour both between nest workers and foragers and within the foraging group itself.

Ben-Shahar et al. (2004) showed that mRNA expression of *Amvl* (the honeybee *mvl* homologue) is higher in pollen foragers than in nurses, with nectar foragers showing mRNA levels somewhere in between. Expression of *Amvl* was highest in the antennal lobes and the suboesophegeal ganglion of the bee brain but, in contrast to *Amfor*, was not especially high in the mushroom bodies. Manganese treatment both increased sucrose responsiveness and accelerated the transition to foraging. However, it did not consistently increase the number of bees foraging for pollen as opposed to nectar. This means that higher sucrose responsiveness does not necessarily translate into greater pollen foraging, and speaks of the need to understand how multiple interacting pathways may affect sensory tuning and behaviour.

Ben-Shahar et al. (2004) suggest that *Amvl* may work through a reward system to increase the bee's response to pleasurable stimuli, but this remains to be tested. Given the expression of *Amvl* in olfactory areas of the brain, it could also be fruitful to investigate the effect of manganese on olfactory responsiveness or learning, especially since manganese may have a general effect on synaptic transmission (Takeda 2003). The link between sucrose sensitivity and foraging also requires further investigation, as it is still unclear whether sucrose sensitivity itself promotes foraging behaviour, or whether this sensitivity is simply highly associated with other factors that lead to foraging. For example, sucrose responsiveness is correlated with sensitivities to pollen, odours and light (Scheiner et al. 2004a), as well as antennal scanning activity (Scheiner et al. 2005) and locomotor activity (Humphries et al. 2005).

In their study, Ben-Shahar et al. (2004) demonstrated increased expression of *Amvl* in forager brains compared to nurse brains and a causal role for manganese in the initiation of foraging, but an *Amvl* knockout might be a more direct way to test the involvement of *Amvl* in honeybee foraging behaviour. However, the effects of cGMP and manganese on foraging behaviour show how pathways affecting different stimulus sensitivities (here, sensitivities to light and sucrose) may together contribute to a more complicated behavioural change. The idea that stimulus sensitivity differences contribute to honeybee division of labour accords with the "response threshold model" of self-organizing insect societies proposed by Beshers and Fewell (2001).

New functions for the *vitellogenin* gene in the honeybee

The yolk precursor protein vitellogenin (Vg) plays a role in oocyte development in many insect species, but has been implicated in regulating a broader range of behaviours in the honeybee. Critically, it interacts with the juvenile hormone (JH), which is thought to play an important role in the transition to foraging (Page et al. 2006). This transition

is accompanied by increased levels of JH and reduced levels of Vg (Amdam and Omholt 2003). Treatment with methoprene, a JH analogue, hastens foraging onset (Bloch et al. 2002); JH depletion delays it (Schulz et al. 2002). Vg and JH have been shown to reciprocally inhibit one another (Amdam and Omholt 2003; Guidugli et al. 2005), which suggest that Vg, a protein that in most species is involved only in reproduction, has taken on additional regulatory roles in the honeybee.

Using RNA interference (RNAi) to inhibit the expression of specific genes by introducing double-stranded RNA (dsRNA) into cells, Nelson et al. (2007) demonstrated that the *vitellogenin* gene is causally involved in the regulation of foraging behaviour, suggesting a role for *Vg* in keeping bees in the nursing phase. They also found that silencing *Vg* using *Vg*-derived dsRNA accelerated the transition to foraging. Decreases in abdominal lipid as well as Vg have been found to be important factors in such nutritionally-induced shifts (Toth et al. 2005; Toth and Robinson 2005). This fits well with findings that nutritional depletion of a honeybee colony triggers foraging (Schulz et al. 1998). Nelson et al. (2007) also found that *Vg* knockdown increased the sizes of nectar loads collected by foraging bees.

However, in a prior RNAi study, Amdam et al. (2006b) showed that down-regulation of *Vg* increased sucrose responsiveness in 7-day-old workers. It seems paradoxical that silencing *Vg* could both increase nectar loads and increase sucrose responsiveness, since bees with high sucrose responsiveness at 7 days have been found to forage preferentially for pollen, not nectar, later in life (Pankiw and Page 2000). It is unlikely that methodological differences are responsible for the seemingly inconsistent findings, since the two authors used essentially the same RNAi procedures. Indeed, either finding could have been reasonably predicted. High Vg hemolymph titers early in the honeybee life are associated with increased pollen collection later on (Amdam et al. 2004), so a *Vg* knockdown could be expected to be associated with collecting more nectar. At the same time, *Vg* down-regulation by RNAi has been shown to increase levels of JH (Guidugli et al. 2005), which is associated with gustatory responsiveness (Pankiw and Page 2003) and pollen collection (Schulz et al. 2004).

Sucrose responsiveness is thought to be an indicator of foraging specialization not because it plays a causal role in determining the collection of either pollen or nectar (Page and Erber 2002; Scheiner et al. 2004a), but because it correlates with other measures of sensory sensitivity, including sensitivity to pollen (Scheiner et al. 2004a). The conflicting RNAi results could suggest that sucrose responsiveness is somehow dissociated from other sensory sensitivities in knockdown bees, or that sensory tunings in these bees change between day 7 and foraging onset. Another possible explanation is that factors other than sensory tuning determine foraging specialization in these bees. This could be the case if certain levels of Vg early in life are important for promoting pollen foraging later on, and can have a greater effect on foraging behaviour than sensory tuning. The notion that Vg in young bees lays the groundwork for future pollen foraging fits with the experimentally supported idea that nectar foragers have non-reproductive characteristics, including smaller ovaries (Amdam et al. 2006a) and lower Vg levels (see Page and Amdam 2007), while pollen foragers display a more maternal phenotype (Amdam et al. 2006a, 2004). This evidence supports the proposal that honeybee division of labour derives from a reproductive ground plan (Amdam et al. 2004; West-Eberhard 1996). Future studies can help to elucidate the long-term behavioural effects of the presence of different substances, such as JH and Vg, at specific time points in the development of the honeybee.

Although the interaction of Vg and JH is clearly important for honeybee maturation and behaviour, it is not yet clear how Vg inhibits JH or how their mutual regulation works to change sucrose responsiveness and foraging behaviour. There is evidence to suggest that JH may act at least in part by increasing levels of octopamine, which is expressed in the antennal lobes of foragers (Schulz et al. 2002), increases responsiveness to sucrose (Pankiw and Page 2003) and stimulates foraging activity (Schulz et al. 2002).

In non-social insects, JH is part of pathways affecting yolk protein production, ovarian physiology and sensory tuning (Amdam et al. 2004; Guidugli et al. 2005). In honeybees, upstream action of the insulin/insulin-like (IIS) signalling pathway helps to regulate the action of JH, influencing Vg synthesis and a host of functions from ovarian maturation to body growth (Page and Amdam 2007). Across animal species, the IIS pathway plays key regulatory roles in nutrition, fertility, aging and other processes (Nassel 2002; Toth and Robinson 2007). These functions may point to an important role for this pathway in the regulation of the foraging transition and foraging specializations.

In support of this idea, Hunt et al. (2007) found an association between pollen foraging and the IIS pathway in a study of gene expression. The authors mapped QTL regions associated with pollen foraging and used data from the draft honeybee genome sequence (Honeybee Genome Sequencing Consortium 2006) to identify predicted peptides in those regions, which were then evaluated for likely gene function. Significantly more of these pollen-associated genes than expected by chance were involved in IIS signalling and ovarian development (Hunt et al. 2007). In the wasp, insulin-related genes were among those found to

be differentially expressed in members of castes that show maternal care behaviour (workers and foundresses) compared to those that do not (queens and gynes; Toth et al. 2007), suggesting that reproductive regulatory pathways may be harnessed in social insects to govern division of labour, with the IIS pathway as a possible player.

The bi-directional regulatory relationship between Vg and JH seen in the honeybee is not widely observed in insects (Page et al. 2006). The involvement of *Vg* in the regulation of foraging is a surprising example of how a gene that in many species serves a strictly reproductive purpose has been co-opted to govern division of labour in facultatively sterile female honeybees.

Conclusion

Genetic studies of the transition from nursing to foraging in honeybees are beginning to build a picture of how the actions of genes regulate the division of labour in a society in which group factors influence individual behaviour. Microarray studies have shown that major changes in behaviour, like the transition to foraging, are accompanied by widespread changes in gene expression, while QTL mapping and genome scans have indicated that these changes may be orchestrated by the pleiotropic effects of a smaller number of critical loci. Transcription factors, like those identified by Sinha et al. (2006), which can up- or down-regulate many genes, are promising targets for future research. Studies of individual genes like *foraging* (Ben-Shahar et al. 2002), *malvolio* (Ben-Shahar et al. 2004) and *vitellogenin* (Nelson et al. 2007) have shown that increasing sensitivities to stimuli, like sucrose and light, may lie at the heart of the transition from nursing to foraging. The localization of behaviourally relevant gene expression to brain areas like the antennal lobes and mushroom bodies suggest that more focussed molecular studies of these areas may shed light into the neural circuits responsible for the behavioural change. Finally, this work in honeybees shows how genes like *vitellogenin* can at once be conserved and put to new use over the evolutionary history.

The recent publication of the *Apis mellifera* genome sequence (Honeybee Genome Sequencing Consortium 2006) will likely invigorate research into the genetic underpinnings of the honeybee society. This wealth of new information on a eusocial organism is a powerful and welcome tool for investigating mechanisms by which colony-level dynamics influence the diet, work and reproductive patterns of individuals. The finding that changes in honeybee behaviour are accompanied by widespread changes in gene expression (Whitfield et al. 2003, 2006) means that the honeybee could be a model organism for the study of how extensive and coordinated expression changes are orchestrated. This could involve the regulatory activity of non-gene elements, like microRNAs. With the genome sequence available and access to improved microarrays of honeybee genes, future research exploring such topics, such as how hormones and pheromones regulate gene expression, and how these expression changes alter behaviour through specific neural changes, will help to provide a working model for understanding socially-regulated behavioural changes.

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