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Running title: Pheromones in a superorganism

Abstract

Analogous to the importance of hormones in controlling organism homoeostasis, pheromones play a major role in the regulation of group homoeostasis at the social level. In social insects, pheromones coordinate the association of "unitary" organisms into a coherent social unit or so called "superorganism". For many years, honey bees have been a convincing model for studying pheromone regulation of social life. In addition, with the recent sequencing of its

genome, a global view of pheromone communication is starting to emerge, and it is now possible to decipher this complex chemical language from the molecular to the social level. We review here the different pheromones regulating the main biological functions of the superorganism and detail their respective action on the genome, physiology and behavior of nestmates. Finally, we suggest some future research that may improve our understanding of the remarkably rich syntax of pheromone communication at the social level.

Key Words: Honey bee, division of labor, social regulation, gene expression, transcription factor, *cis*-regulatory element, juvenile hormone, vitellogenin

I. Introduction

Social insect colonies have often been referred as "superorganisms" in analogy to the functional organization of complex higher organisms being composed of numerous cells (Wilson and Sober, 1989). Indeed, the colony organization of insect societies reveals numerous analogies to multicellular organisms. The first analogy is that both are associations of single units, cells and individuals (organisms). Like cells, members of the colonies depend on the functioning of the higher unit and are unable to survive and develop outside this system. In the second analogy, the reproductive castes fulfill the role of the germ cells in organisms, and the sterile castes become analogous to the soma. Similar to somatic cells, sterile individuals follow organizational principles: the specialization into different functions and the coordination between the functional groups; this organization being governed by a well-developed and sophisticated communication.

In organisms the regulation of cell and organ activity is controlled by signals, like hormones, which elicit specific reactions in certain organs. Equivalent to the hormonal regulation in the individual organism, the colony is regulated chemically by pheromones (Fig. 1). Interactions between members of the society are mainly under the control of complex pheromone signaling, which spreads global information to further enable the colony homeostasis, provisioning, growth, defense and reproduction but also mediate social conflicts (see Le Conte and Hefetz (2008) for a review). Pheromones, defined also in the past as "ectohormones", are chemicals that are secreted externally and which produce dramatic and stereotyped changes in behavior and physiology of members of the same species (Karlson and Burtenandt, 1959). Pheromones are usually divided into two categories according to their effects: releaser pheromones induce an immediate behavioral response but primer pheromones alter behavioral repertoire through putative response threshold shifts (Wilson and Bossert, 1963). However, it has been shown recently that releaser pheromones can also modify response thresholds (Alaux and Robinson, 2007; Anderson *et al.*, 2007), which blurs the long-standing distinction between primer and releaser pheromone.

To function as coherent social units, social insects use a complex language based on specialized chemical signals that provide a remarkably rich syntax, which might be equivalent to the visual and auditory repertoire of higher vertebrates. The only social insect for which any primer pheromones have been identified is the honey bee (*Apis mellifera*), that has all the

characteristics of a superorganism (Moritz and Fuchs, 1998). Honey bees live in societies that are characterized by a reproductive division of labor between the queen and workers, and a division of labor among workers for tasks related to colony growth and development. Far from being rigid, this social organization is highly flexible and mainly pheromone guided. Actually, around 50 chemical substances are known to be essential to the functioning of the society (Slessor *et al.*, 2005). With their unusually well characterized behaviors and pheromones (Le Conte and Hefetz, 2008; Slessor *et al.*, 2005) and their newly annotated genome sequence (Honey Bee Genome Sequencing Consortium, 2006), honey bees are an emerging model for decoding the mechanism of pheromone language from molecular to social level. Here, we review, through a multilevel approach, this rapidly evolving field for honey bees that has been constantly fueled by the discovery of new pheromones and functions, and recently by the development of genomic tools. First, the main pheromones will be introduced by presenting their multiple effects on worker physiology and behavior. Then, we will detail the transcriptional changes occurring at the brain level upon pheromone perception and finally, we will explain how the pheromone language regulates social life.

II. Physiological and behavioral regulation

A. Reproduction

A hallmark of eusocial societies is the reproductive specialization, where the queen monopolizes reproduction and facultatively sterile workers contribute to the colony work force. In honey bees, despite workers being unable to mate, they have retained the capacity to develop ovaries and lay viable haploid eggs, which develop into males, since honey bees are haplo-diploid (females are derived from fertilized eggs produced by the queen). However, only 0.01% of workers have fully developed ovaries and 1% of male eggs are derived from workers (Ratnieks, 1993). Worker reproduction is inhibited by the presence of the queen and/or brood (larvae), since a loss of one of them leads to the development of ovaries by workers. For example, in the absence of the queen, from 5 to 24% of workers possess developed ovaries (Page and Erickson, 1988). With several thousands of workers composing the colony, the queen cannot physically control this strong reproductive bias. Worker reproduction is rather inhibited through a primer pheromone mechanism, but also via worker policing (workers prevent each other from reproducing by removing worker-laid eggs, see Le Conte and Hefetz (2008)).

After some controversal research toward the identification of the queen pheromone, an active blend produced in the queen mandibular gland and composed of 5 molecules ((E)-9-oxodec-2-enoic acid (9-ODA), both enantiomers of 9-hydroxydec-2-enoic acid (9-HDA), methyl p-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA)) was identified (Slessor et al., 1988). Later, Hoover and colleagues (2003) showed that this blend has a strong inhibitory effect on worker reproduction. Besides this queen mandibular pheromone (QMP), the brood produced by the queen also inhibits ovary development in workers by emitting two pheromones: the brood pheromone (BP), mainly composed of esters (Arnold *et al.*, 1994) and the highly volatile E-*β*-ocimene (Maisonnasse *et al.*, 2009). In reality, two esters from the BP have an effect on worker ovaries: ethyl palmitate and methyl linolenate (Mohammedi *et al.*, 1998). Interestingly, those inhibiting components produced by the brood were found to not be caste specific since the queen also produces the ethyl palmitate (Keeling and Slessor, 2005) and the E-*β*-ocimene (Gilley *et al.*, 2006). It is interesting to note that each of those compounds trigger a partial effect on ovary development (i.e. some workers still "escape" the reproductive control), which suggests synergistic effects between brood and queen pheromones on worker ovary inhibition.

In most insects, it has been documented that juvenile hormone (JH) controls ovary development by stimulating the production of vitellogenin (Vg, yolk protein precursor) in the fat body and its uptake by developing oocytes (Engelmann, 1983). In honey bees, QMP and BP inhibit JH biosynthesis by the corpora allata (Kaatz *et al.*, 1992; Le Conte *et al.*, 2001; Pankiw *et al.*, 1998), however, no direct effects of JH on ovary development has been found. Contrary to others insects, JH and Vg titers show an inverse pattern and behave antagonistically (Bloch *et al.*, 2002; Guidugli *et al.*, 2005), which explains the higher RNA levels of Vg induced by QMP (Fischer and Grozinger, 2008). Since Vg has multiple coordinating effects on social organization, it might regulate an alternative utilization of yolk protein other than oogenesis (Amdam *et al.*, 2003) (see below).

B. Task allocation

A common feature of higher organisms and honey bee societies is the specialization of cells and workers, respectively, into various tasks. The many tasks associated to the maintenance of the hive are divided among the workers giving a division of labor. In honey bees, this task division can arise from genetic variability between workers (Oldroyd and Fewell, 2007;

Robinson and Page, 1989). As a consequence, workers develop into specialists for certain tasks due to their low response threshold for this task. However, the main task division is age-related. During their life, workers undergo a behavioral maturation: they spend the first 2 to 3 weeks of their adult life working in the hive (feeding and taking care of the brood, building comb), and then the rest of their life outside of the hive (foraging for nectar and pollen to supply the colony growth). The nurse/forager ratio, crucial to the colony functioning, is not rigid, but highly plastic, depending on social environment and colony needs, which are communicated via pheromones.

The transition from nurse to forager is controlled by a colony-level network: the queen, brood and foragers regulate the progression of young bees towards the typical tasks of older bees. QMP delays the onset of foraging (Pankiw *et al.*, 1998), but the action of BP is dose dependant. Below a certain threshold, BP stimulates behavioral maturation, but high doses (i.e. a large number of larvae) induce a delay (Le Conte *et al.*, 2001). Workers also inhibit the nestmate transition from nurse to forager via a pheromone, ethyl oleate (EO), which is mainly produced by foragers (Leoncini *et al.*, 2004). EO, which is also produced by the queen (Keeling and Slessor, 2005) and the brood (Le Conte *et al.*, 1989), stimulates the growth of hypopharyngeal gland used by nurses for the brood nourishment (Mohammedi *et al.*, 1996).

The hormonal mechanisms underlying honey bee behavioral maturation are well-studied and reveal the major regulatory roles of JH and Vg (Bloch *et al*., 2002; Guidugli *et al.*, 2005). The transition to a forger state is mediated by an increase in JH and a decrease of Vg titer (Page and Amdam, 2007). The higher level of Vg in nurses might be used for brood food production in the hypopharyngeal glands rather than for egg production in the ovaries (Amdam *et al.*, 2003). Pheromones are expected to regulate the division of labor through the modification of endocrine levels of JH and Vg. This was tested by different studies, which showed that QMP and high doses of BP decrease JH levels in workers during their behavioral maturation (Kaatz *et al.*, 1992; Le Conte *et al.*, 2001; Pankiw *et al.*, 1998). In addition, QMP and low doses of BP increases and decreases, respectively, Vg production (Fischer and Grozinger, 2008; Smedal *et al.*, 2009), which is consistent with their effect on the onset of foraging. Finally, it has been shown that QMP and BP modulate the sucrose response threshold of workers (Pankiw and Page, 2003). Since there is a robust association between worker sucrose responsiveness and foraging behavior (i.e. bees with low response threshold to sucrose mature faster into forager) (Page *et al.*, 1998), the regulation of this behavioral module might directly

affect behavioral maturation. The EO regulation on worker physiology is not yet known, but it is likely similar to the effects of QMP and BP due to their convergent effects.

C. Defense

Another important trait of honey bee biology is the shared defense of the colony upon disturbance by intruders or potential enemies. A rapid defense response is launched by guard bees when they detect a danger at the colony entrance. Alarm pheromones are immediately released by the guards to signal a threat to the colony members and coordinate the defensive response, which is characterized by either a dispersal of individuals or an attack against the potential danger. In this defensive context, guards play an analogous role to the T cells of the vertebrate immune system, which search out invaders in the body and release cytokines to mediate the immune response. In honey bees, two main alarm pheromones have been identified: the sting alarm pheromone, which is mainly composed of isopentyl acetate (IPA) (Boch *et al.*, 1962) and 2-heptanone, produced in the mandibular glands (Shearer and Boch, 1965). If the role of IPA is well-known in mediating defensive behavior, the role of 2-heptanone in colony defense is less clear. 2-heptanone may act as a repellent scent-mark in the foraging context, because when applied by bees to flowers, subsequent floral visitors avoid these depleted flowers (Giurfa, 1993).

The primary role of IPA is to alert and recruit bees. However, defending bees need to localize the intruder, which would not be accurate if based on olfaction alone. Wager and Breed (2000) have shown that a second role of IPA is to release searching behavior and enhance response to moving targets. A third role of IPA in mediating defense response has been identified by rating sting extension in bees subjected to electrical shocks. Prior exposure to small doses of IPA increased the sting extension reflex to the electrical shocks, but higher doses resulted in decreased responsiveness (Nunez *et al.*, 1998). This reduction was antagonized by application of naloxone, a specific antagonist of opioids, indicating that large doses of IPA activated an endogenous opioid system leading to stress-induced analgesia (Nunez *et al.*, 1998). The opioid system is believed to inhibit pain to trigger alternative behavioral responses (Dyakonova, 2001). Therefore Nunez and colleagues (1998) suggested that the activation of opioid analgesia by IPA decreases the probability of withdrawal when facing an enemy thus increasing its defensive efficiency.

Besides inducing a quick hard-wired defensive response, IPA also affects longer-latency behavioral responsiveness. The colony responsiveness to IPA increases after subsequent exposures, meaning that a brief exposure to IPA without association to reward can modify the behavioral response threshold (Alaux and Robinson, 2007). It is possible that a shift in responsiveness to IPA enables the colony to respond more rapidly and vigorously to intruders that have been encountered previously. Such a phenomenon is analogous to the immunological memory of mammals, which is a fundamental feature of an adaptive immune system. In that context, previously aroused guards resemble memory T cells, by mounting a faster and stronger immune response toward foreign invaders that were encountered during a prior infection.

D. Longevity

In honey bees, adult lifespan can be influenced by the social environment via pheromones. QMP-treated bees live longer, when starved, compared to control bees (Fischer and Grozinger, 2008), but bees exposed to low doses of BP have a reduced lifespan (Smedal *et al.*, 2009). Despite QMP and BP having antagonistic effects on bee longevity, they share the same mechanism. Both affect Vg levels and nutrient storage capacity, which are known to increase worker survival (e.g. Vg protects bees from oxidative stress) (Corona *et al.*, 2007; Seehuus *et al.*, 2006). QMP increased and low doses of BP decreased both Vg levels and nutrient storage (Fischer and Grozinger, 2008; Smedal *et al.*, 2009). Since, Vg levels and nutrient storage are higher in nurses compared to foragers (Fluri *et al.*, 1982; Toth and Robinson, 2005), their regulation by pheromones are consistent with QMP and BP effects on worker division of labor. In that context, one would expect that high doses of BP, which delay the onset of foraging (Le Conte *et al.*, 2001), increase bee lifespan.

E. Learning

The olfactory system of honey bees undergoes significant maturation during early adult life, a process that is influenced by environmental stimuli (Gascuel and Masson, 1987), but also by the presence of the queen (Morgan et al., 1998). While the role of QMP in regulating olfactory maturation is not known, a recent series of studies have shown that QMP can affect olfactory learning and memory. Vergoz and colleagues (2007b) first showed that exposure to QMP significantly blocks aversive learning in young bees. QMP attracts young workers to the

queen and entices them to feed her, but also to lick and antennate her body, which allow a distribution of her QMP bouquet throughout the colony (Slessor *et al.*, 1988). Vergoz and colleagues (2007b) explained that the effects of QMP on aversive learning would increase the likelihood of workers attending the queen by preventing them from forming an aversion to her pheromones. This effect was mediated by a single component of QMP (HVA), which has a similar structure to a dopamine molecule and affects dopamine signaling in the brain of young bees (Beggs *et al.*, 2007). Finally, Beggs and Mercer (2009) found that HVA actually interacts with dopamine receptors in the bee brain, and thus targets directly the dopamine pathways, which play an essential role in aversive learning (Vergoz *et al.*, 2007a).

III. Gene regulation

Before eliciting a response, pheromone signals are processed in the brain. Identification of neural pathways and olfactory sensory maps in the honey bee brain has given new clues about pheromone processing and representation (see Sandoz *et al.* (2007) for a review). A complementary approach is to determine how pheromones are molecularly transduced in the brain. Indeed, molecular signaling pathways connect pheromone signals to the regulation of neural functions and, ultimately to behavioral and physiological responses. Since its genome has been sequenced (Honey Bee Genome Sequencing Consortium, 2006), it has been possible to analyze the pheromone-regulated transcription. And thanks to genomic approaches using microarrays, a global view of gene regulation by pheromone signaling is beginning to emerge. In this section, we will review how pheromones are orchestrating gene expression changes in the honey bee brain.

A. Long-term regulation

In order to better understand how pheromones affect neuronal responsiveness and behavioral state, studies started to trace the molecular changes that occur throughout the brain in response to pheromone perception. Tracking the long-term changes in brain gene expression is needed to reflect the long-term changes in physiology and behavior induced by primer pheromones. QMP influences behavior by affecting neural and endocrine systems that specify responsiveness to specific social and environmental stimuli (Beggs *et al.*, 2007; Morgan *et al.*,

1998; Pankiw *et al.*, 1998). Using cDNA microarray analysis, Grozinger and colleagues (2003) showed that QMP affects the expression levels of hundreds of genes in the brain of adult honey bees. A previous study reported widespread differences in gene expression between nurses and foragers, with some genes being more highly expressed in nurse brain compared to forager brain (nurse genes), and inversely others genes being more highly expressed in forager brain compared to nurse brain (forager genes) (Whitfield *et al.*, 2003). Comparing QMP-regulated genes to behavioral genes, Grozinger and colleagues (2003) found that QMP tends to activate genes in the brain that are upregulated in nurses but repress genes that are upregulated in foragers. Similarly, BP, when administered in high doses, leads to a delay in the onset of honey bee foraging (Le Conte *et al.*, 2001) and modifies the expression of genes in the honey bee brain (Alaux *et al.*, 2009a). BP tended to upregulate nurse genes but downregulate forager genes in the brain of young bees. Those findings were consistent with results for QMP and supported the idea that the effects of pheromones on behavior are due to effects on brain gene expression.

The effects of pheromones on behavioral genes is not hard-wired and independent of age but is modulated by maturational processes as shown by age-dependent effects of BP on the "molecular signature" of the brain (Alaux *et al.*, 2009a). On one hand, BP tended to repress forager genes in young honey bees, but, on the other hand, forager genes were stimulated in older honey bees. These differences might be related to the dose-dependent effects of BP on behavioral maturation (Le Conte *et al.*, 2001). Perhaps bees become less sensitive to BP with age and this leads to the differences in brain gene expression detected here; age-related changes in responsiveness to pheromones are well known (Grozinger and Robinson, 2007; Pham-Delegue *et al.*, 1993; Robinson, 1987a). The conclusion that pheromones modulate behavior by regulating the expression level of behavioral genes was further confirmed by the upregulation of the gene *malvolio* in old bees (Alaux *et al.*, 2009a). *malvolio* encodes a manganese transmembrane transporter and is upregulated in the forager brain compared with the nurse brain (Ben-Shahar *et al.*, 2004). In addition, manganese treatment causes an increase in sucrose responsiveness (Ben-Shahar *et al.*, 2004), which is associated with an earlier age at onset of foraging and a tendency to forage for pollen rather than nectar (Page *et al.*, 1998). The effects of *malvolio* are consistent with the dual effects of BP on the onset of foraging and the tendency to specialize on pollen foraging (Pankiw, 2004a; Pankiw, 2004b).

QMP and BP are two different chemical blends with pleiotropic effects but which have in common the effect of delaying the transition from nurse to foraging behavior and inhibiting worker ovary development. It is not clear why such redundancy exists in chemical communication, but this is a common theme in insect societies (Slessor *et al.*, 2005). Perhaps such redundancy leads to finer levels of control or increased resiliency in the event of a communication failure. Pheromones with overlapping functions could have evolved to converge on the same molecular targets in the brain, or they could engage parallel pathways. The very low number of genes regulated by both QMP and BP suggest that they both elicit effects on different sets of genes in the brain (Alaux *et al.*, 2009a). Cautious conclusion should be drawn from this comparison, because the two pheromones were analyzed with different microarray platforms (cDNA mircroarrays generated from brain expressed sequenced tags for QMP and oligonucleotide microarrays generated from the whole genome for BP). However, because they likely use different peripheral receptors, they are expected to affect different neural and molecular pathways. The weak overlap of brain gene expression could also be explained by the different behavioral and physiological processes regulated by QMP and BP. Indeed, BP also increases larval feeding (Le Conte *et al.*, 1995; Mohammedi *et al.*, 1996) and QMP stimulates "retinue" behavior (Slessor *et al.*, 1988).

Another main effect of QMP is the inhibition of worker ovary development (Hoover *et al.*, 2003). One prediction from the previously observed effect of QMP on behavioral genes is that QMP-regulated genes are associated with ovary development. However, by analyzing the differences in brain gene expression between reproductive and sterile workers, Grozinger and colleagues (2007) did not find a significant bias for genes upregulated in reproductive workers to be downregulated by QMP. This doesn't preclude the idea that pheromones regulate the behavior and physiology of workers by regulating behaviorally- and physiologically-relevant genes. Because the main effect observed here is at the level of ovaries, monitoring, in those tissues, the expression patterns of genes regulated by QMP might provide a better characterization of the pheromonal effect on worker reproduction.

B. Short-term regulation

It is well-established that short social interactions elicit strong genomic responses in the brain, suggesting that perception of social signal may modify the brain neurogenomic state to allow individuals to respond adaptively to subsequent social interactions (Clayton, 2000; Robinson

et al., 2008). Similar responses have been observed in honey bees stimulated by the stinging alarm pheromone. A brief exposure of workers to IPA causes, within 30 minutes, an increase in mRNA expression of the transcription factor, *c-Jun*, in the antennal lobes (main olfactory center in the brain) (Alaux and Robinson, 2007). This response is associated with an increase in colony responsiveness to subsequent exposure to alarm pheromone (Alaux and Robinson, 2007). Thus, honey bees have the capacity to mount a rapid genomic response to pheromone stimulation coupled with neural and behavioral plasticity. In addition, Alaux and colleagues (2009b) analyzed the transcriptional cascade activated by *c-Jun* in response to alarm pheromone and found that a one minute exposure affects brain expression of hundreds of genes one hour later. Even a quick behavioral response to a brief stimulus is likely to be associated with many changes in gene expression, thereby changing the experience of the organisms to the given stimulus.

Some genes involved in biogenic amine signaling (*Dopa decarboxylase* and *Tyramine receptor*) were upregulated by alarm pheromone in the honey bee brain (Alaux *et al.*, 2009b). This signaling pathway is implicated in the regulation of aggression in invertebrates (Dierick and Greenspan, 2007; Hunt *et al.*, 2007). In addition, the expression level of the gene encoding the corticotropin-releasing hormone-binding protein, a key protein involved in animals' "fight-or-flight" stress-response (Huising and Flik, 2005), was modified by this short exposure to alarm pheromone. This rapid genome-wide response to alarm pheromone showed a significant overlap with genes that are differentially expressed between the extremely aggressive Africanized honey bee (AHB) and the more docile European honey bees (EHB) (Alaux *et al.*, 2009b). The detection of alarm pheromone activates genes that are highly expressed in unexposed AHB and represses genes highly expressed in EHB suggesting that alarm pheromone causes an AHB-like gene expression profile.

Using a gene ontology analysis Alaux and colleagues (2009b) showed that functions involved in visual perception and the response to stimuli were significantly overrepresented in the gene set upregulated by alarm pheromone. This is consistent with the fact that aggressive behavior is visually guided and that alarm pheromone, besides recruiting nestmates, also increases flight activity and enhances the response to moving targets (Wager and Breed, 2000). It would be important to extend this type of genome-wide analysis to other releaser pheromones (like attraction and sex pheromones) to determine if the rapid induction of a genomic response in the brain, coupled with an increase in arousal, is a general response to pheromones.

C. Pheromone-regulated transcription factors

The high proportion of transcription factors (TFs) regulated by QMP and BP (Alaux *et al.*, 2009a; Grozinger *et al.*, 2003) suggests that they might be key mediators of pheromone signaling responses. TFs function as transcriptional activators or repressors by binding to sequence-specific enhancer or promoter regions of DNA adjacent to the genes that they regulate. Putative TF binding sites can be identified via bioinformatic methods and can provide additional information about the mechanisms of pheromone-regulated transcription. Alaux and colleagues (2009a) showed that the promoter regions of genes regulated by BP are enriched for putative TF-binding *cis*-regulatory motifs. An increase or a decrease in the behavioral response to the same pheromone is well-known in invertebrates (see Anton *et al.* (2007) for a review). This plasticity of pheromone response is usually influenced by endocrine factors like juvenile hormone (Anton and Gadenne, 1999; Grozinger and Robinson, 2007; Robinson, 1987b). However, as discussed above, BP induces "opposite" effects on brain gene expression depending on the age of individuals. The mechanisms underlying such changes remain to be elucidated, but the age specificity of the DNA motif-gene set associations provides some first insights about the mechanisms (Alaux *et al.*, 2009a). Among those, the *cis*-regulatory DNA motif *Adf1* was enriched in gene sets regulated by BP in young bees as compared to old bees and has been shown to be associated with genes regulated by juvenile hormone (Sinha *et al.*, 2006), which increases pheromone sensitivity (Anton and Gadenne, 1999). This motif could represent a key factor in the age-dependant sensitivity to BP discussed previously.

The *cis*-regulatory DNA motifs associated with QMP-regulated genes have not yet been identified, so it is not known whether they are shared by both pheromones. However, some studies have begun to provide new insights into the relationships between QMP and TF regulation in the inhibition of honey bee behavioral maturation. For example, Grozinger and colleagues (2003) found the honey bee ortholog of *Drosophila melanogaster Krüppel-homolog 1* (*Kr-h1*) to be strongly and chronically downregulated over several days by QMP. The regulation of this zinc finger TF by QMP is influenced by the endocrine status of the honey bee (Grozinger and Robinson, 2007) and a previous study indicated that it is more highly expressed in foragers compared to nurses (Whitfield *et al.*, 2003). *Kr-h1* may play a

role in orchestrating ecdysone-regulated transcriptional pathways and neuronal morphogenesis (Shi *et al.*, 2007).

All of these connections suggest a functional linkage between pheromone, behavior and molecular regulation. Due to known similarities in pheromone signaling across organisms, it is reasonable to assume that studies of model organisms, such as the honey bee, will have general significance. Also future genomic studies will undoubtedly continue to increase our knowledge of pheromone-regulated gene expression. For example, combining the standard chromatin immunoprecipitation assay with microarrays or high-throughput sequencing, will enable researchers to localize the binding sites of TFs in the genome and to establish a genomic landscape of pheromone-regulated TFs, similar to what has been done in hormone research (Cheung and Kraus, 2010).

IV. Social regulation

Collective decision-making requires an informational flow that is effective in reaching the majority of the workers. Contrary to volatile substances, which do not require close proximity between members of the society to be effective at the group level, non-volatile substances needs a continuous transport analogous to the transport of hormones by the blood in vertebrates. Workers can actively transport pheromones through trophallaxis and body contact. This quick and constant circulation of pheromones provides flow of information important to the maintenance of colony organization and functionality (e.g. reproduction and growth).

A. Reproduction

Honey bee colonies follow a yearly cycle starting with a "somatic" growth stage, which is characterized by an increase in worker number, and followed by colony reproduction (i.e. production of drones and queens). Reproduction in honey bee colonies occurs at two levels: individual and colonial, both being regulated by the same pheromone, QMP. This primary superorganismic pheromone inhibits the development of ovaries in all members of the colony as discussed above, but also prevents the construction of new queen cells and, thus, the rearing of new queens (Winston *et al.*, 1990). QMP is rapidly moved among members by messenger bees that have body contact with the queen and extensively lick her (Naumann *et*

al., 1991). These messenger bees contribute to the dispersal of QMP and thus the maintenance of colony stability. However, instability occurs when the colony population grows and the QMP distribution becomes less efficient (Naumann *et al.*, 1993). Because of this congestion, the QMP is diluted and fails to be transmitted. Workers behave as if they were queenless and begin to build new queen cells and larvae, the precursors of colony reproduction by swarming (Winston *et al.*, 1991).

The reproductive capability of workers is normally inhibited by primer pheromones (QMP and BP), which target ovary development. This suppression is essential to the colony stability and functionality given that reproductive workers do not work as hard as normal worker bees (Dampney *et al.*, 2004). The importance of pheromonal control in maintaining colony reproductive harmony is better appreciated in the cases in which it breaks down. A rare behavioral syndrome in European honey bee populations (*A. m. mellifera*) illustrates this breakdown. In some colonies, called "anarchistic" colonies, a large proportion of workers activate their ovaries and lay eggs, affecting colony functionality, despite the inhibitory presence of the queen and brood pheromone (Oldroyd *et al.*, 1999). The pheromonal control could be inefficient, however these "anarchistic" colonies are headed by queens who have normal QMP production (Hoover *et al.*, 2005a), suggesting that workers are less sensitive to the QMP or that its transmission or reception is less effective (Hoover *et al.*, 2005b). Also, anarchistic workers seem to have a higher threshold to brood signal compared to wild-type bees (Oldroyd *et al.*, 2001). In some extreme cases, the failure of the pheromonal control can lead to a destructive state of the colony, as is the case for the uncontrolled replication of *A. m. capensis* workers (Cape honey bee) in *A. m. scutellata* host colonies. *A. m. capensis* workers can parasitize *A. m. scutellata* colonies and reproduce via thelytokous parthenogenesis, by producing clones of themselves (Oldroyd, 2002). In a few weeks, replication of *A. m. capensis* workers leads to an increase in number of parasitic workers within the host colony but also to reduced foraging activity and food supplies caused by an underrepresentation of *A. m. capensis* in the foraging force, which can lead to the death of the colony (Martin *et al.*, 2002). The ability of *A. m. capensis* parasitic workers to activate their ovaries inside the host society suggests that they are not inhibited by the pheromones that usually repress oogenesis. Parasitic workers are actually able to produce queen-like pheromones (Simon *et al.*, 2001). Therefore *A. m. capensis* workers look like pseudoqueens, with regards to their reproductive capacities and pheromone bouquet, which suggests that they are not inhibited by their own pheromones, nor by the colony queen pheromones. The

breakdown of reproductive order in the superorganism is analogous to the spread of cancer in vertebrates with the uncontrolled replication of malignant cell and consequently has been assimilated to a social cancer (Oldroyd, 2002). Like cancerous cells, which escape from the immune system, parasitic workers have the potential to bypass the pheromonal control of the colony.

B. Colony growth

During the "somatic" growth of the colony, each worker needs to obtain information about the colony requirements and to respond accordingly. The sensitivity and the ability of the society to reallocate tasks in response to changing conditions is a key component of colony development (Robinson, 1992). In that context, the role of pheromones is analogous to the biochemicals that coordinate the functions of distinct cell subpopulation during the development of multicelullar organisms.

After having selected a nest site, the honey bee swarm needs to build up a new comb for brood rearing and storing food. At that time, the colony needs to produce a worker force for the development of the colony, instead of reproductive individuals. Similar to growth hormone in vertebrates, Ledoux and colleagues (2001) found that QMP actually stimulates the secretion of wax and the production of worker-size cells. Therefore, QMP regulates both the development of the nest and task allocation. This centralized control is operated by a single queen, who produces a baseline amount of pheromone. However, colony needs change with colony size and time of the year. Workers need to acquire this information, but whether the queen can modulate her pheromone output according to the colony requirement is not known.

The dynamic regulation of task allocation is decentralized, governed not by a single colony leader but rather by regular interactions between colony members (self-organization). For example, to optimize colony and brood development, an effective ratio of nurse to forager is needed. The colony response to an increased need for brood rearing (large amount of brood) should involve a lengthening of the nursing phase. On the other hand, a reduced amount of brood suggests that more food is needed for the colony to increase the rate of brood production. These colony requirements are communicated through BP production: high doses lengthen the nursing phase whereas low doses accelerate the transition from nurse to forager

phase (Le Conte *et al.*, 2001). In addition to its primer effects, BP can also induce a quick increase in pollen foraging within a few hours (Pankiw, 2007; Pankiw and Page, 2001). This decentralized control is mediated as well by interactions between adult workers. This was demonstrated by experimental manipulations of old or young bees population (Huang and Robinson, 1992). Later it has been found that chemical extracts of nurses have stimulating effects on the foraging behavior of young bees (Pankiw, 2004b), but the pheromone produced by foragers (ethyl oleate) inhibits the behavioral maturation of young bees (Leoncini *et al.*, 2004). Altogether these results suggest a feedback mechanism between nurses and foragers, particularly adapted to regulate the size of the colony foraging force. For example, an abrupt loss of foragers due to predation or bad weather might be communicated through a decreased amount of ethyl oleate and thus followed by a quick behavioral maturation of nurse bees. In summary, in the organism and superorganism, cells and honey bee workers, respectively coordinate their action through similar general mechanisms involving centralized control, self-organization and feedback loops with hormones and pheromones being the key coordinators.

V. Conclusions and future directions

Great progress has been made toward deciphering the chemical language. It is now known that pheromones in honey bees modulate individual interaction through their action on behavioral genes and individual physiology. However, by including the importance of synergy, dose and context, pheromone communication in honey bees appears to be remarkably complex (Slessor *et al.*, 2005) (Fig. 2). Pheromones have been well-studied independently, but they may interact to further regulate the social life. For example, different chemicals like QMP and BP may not interact to inhibit ovary development (Hoover *et al.*, 2005b) but may have a synergistic effect on development of the hypopharyngeal glands (Peters *et al.*, 2010). Individuals need to constantly integrate chemical information originating from their nestmates and answer in the correct way. Therefore, research integrating more than one pheromone promises to give a better representation of pheromone communication in this superorganism.

Pheromone signaling involves the secretion of chemical blends and their detection and processing by receivers. Although recent electrophysiological and molecular approaches have provided new insights into the mechanisms of pheromone integration and processing in the

bee brain, less is known about pheromone biosynthesis and receptors. In that context, encouraging progress has been made thanks to the first identification of genes regulating QMP biosynthesis (Malka *et al.*, 2009) and the 9-ODA receptor, the main compound of QMP (Wanner *et al.*, 2007). Analysis of the honey bee genome sequence highlighted a remarkable expansion of the odorant receptor (Or) family (170 Ors) relative to solitary insects (62 and 79 Ors for *Drosophila melanogaster* and *Anopheles gambiae,* respectively) (Robertson and Wanner, 2006). This high diversity in Ors perhaps allows the bees to recognize diverse floral odors and pheromone blends. Future studies on the biosynthetic processes that regulate pheromone production and the identification of pheromone receptors will improve our knowledge of chemical communication and open new avenues of investigation.

Finally, another promising area of research is to determine how chemical communication is affected by infectious disease of the colony. Since colonies are typically composed of thousands of bees with frequent interactions, living in groups increases risk of pathogens transmission, with each individual being a potential host. Experimental evidence in insects has shown that parasites can not only evade immune responses but also exploit the endocrine system of the host to favor their growth and reproduction (Hurd, 2009). In honey bees, studies revealed that the cuticular hydrocarbon profile involved in social recognition can be modified by the activation of the immune system (Richard *et al.*, 2008) and parasitization by the ectoparasitic mite (*Varroa destructor*) (Salvy *et al.*, 2001). However, it is not know whether, analogous to the modification of hormone signaling in the organism, pathogen infection would affect pheromone signaling in the colony. Disruption of pheromone communication could occur in two ways: either a pathogen could exploit the pheromone system of the colony to its own advantage or during an infection the maintenance of this system would be too costly for the colony. Either way, a progressive modification or loss of pheromone production and/or detection at the individual level could lead to the colony collapse. We believe that such findings would provide new insights into how pheromone signaling is integrated and operates a dynamic regulation of the different units of the superorganism.

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References

Alaux, C., Le Conte, Y., Adams, H. A., Rodriguez-Zas, S., Grozinger, C. M., Sinha, S., and Robinson, G. E. (2009a). Regulation of brain gene expression in honey bees by brood pheromone. *Genes Brain Behav.* **8,** 309-319.

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

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzman-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J. L., Southey, B. R., Rodriguez-Zas, S., and Robinson, G. E. (2009b). Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proc. Natl. Acad. Sci. USA* **106,** 15400-15405.

Amdam, G. V., Norberg, K., Hagen, A., and Omholt, S. W. (2003). Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. USA* **100,** 1799-1802.

Anderson, P., Hansson, B. S., Nilsson, U., Han, Q., Sjoholm, M., Skals, N., and Anton, S. (2007). Increased behavioral and neuronal sensitivity to sex pheromone after brief odor experience in a moth. *Chem. Senses* **32,** 483-491.

Anton, S., Dufour, M. C., and Gadenne, C. (2007). Plasticity of olfactory-guided behaviour and its neurobiological basis: lessons from moths and locusts. *Entomol. Exp. Appl.* **123,** 1-11.

Anton, S., and Gadenne, C. (1999). Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proc. Natl. Acad. Sci. USA* **96,** 5764-5767.

Arnold, G., Le Conte, Y., Trouiller, J., Hervet, H., Chappe, B., and Masson, C. (1994). Inhibition of worker honeybee ovaries development by a mixture of fatty acid esters from larvae. *C. R. Acad. Sci. Paris, Ser. III* **317,** 511-515.

Beggs, K. T., Glendining, K. A., Marechal, N. M., Vergoz, V., Nakamura, I., Slessor, K. N., and Mercer, A. R. (2007). Queen pheromone modulates brain dopamine function in worker honey bees. *Proc. Natl. Acad. Sci. USA* **104,** 2460-2464.

Beggs, K. T., and Mercer, A. R. (2009). Dopamine receptor activation by honey bee queen pheromone. *Curr. Biol.* **19,** 1206-1209.

Ben-Shahar, Y., Dudek, N. L., and Robinson, G. E. (2004). Phenotypic deconstruction reveals involvement of manganese transporter *malvolio* in honey bee division of labor. *J. Exp. Biol.* **207,** 3281-3288.

Bloch, G., Wheeler, D. L., and Robinson, G. E. (2002). Endocrine influences on the organization of insect societies. *In* "Hormones, Brain, and Behavior" (D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, and R. T. Rubin, Eds.), Vol. 3, pp. 195–236. Academic Press, New York.

Boch, R., Shearer, D. A., and Stone, B. C. (1962). Identification of iso-pentyl acetate as an active component in the sting pheromone of the honey bee. *Nature* **195,** 1018-1020.

Cheung, E., and Kraus, W. L. (2010). Genomic analyses of hormone signaling and gene regulation. *Ann.Rev. Physiol.* **72,** 5.1-5.28.

Clayton, D. F. (2000). The genomic action potential. *Neurobiol. Learn. Mem.* **74,** 185-216.

Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A., and Robinson, G. E. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* **104,** 7128-7133.

Dampney, J. R., Barron, A. B., and Oldroyd, B. P. (2004). Measuring the cost of worker reproduction in honeybees: work tempo in an "anarchic" line. *Apidologie* **35,** 83-88.

Dierick, H. A., and Greenspan, R. J. (2007). Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat. Genet.* **39,** 678-682.

Dyakonova, V. E. (2001). Role of opioid peptides in behavior of invertebrates. *J. Evol. Biochem. Physiol.* **37,** 335-347.

Engelmann, F. (1983). Vitellogenesis controlled by juvenile hormone. *In* "Endocrinology of insects" (R. G. H. Downer and H. Laufer, Eds.), pp. 259-270. Alan R. Liss, New York.

Fischer, P., and Grozinger, C. M. (2008). Pheromonal regulation of starvation resistance in honey bee workers (*Apis mellifera*). *Naturwissenschaften* **95,** 723-729.

Fluri, P., Luscher, M., Wille, H., and Gerig, L. (1982). Changes in the weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* **28,** 61-68.

Gascuel, J., and Masson, C. (1987). Influence of olfactory deprivation on synapse frequency in developing antennal lobe of the honeybee *Apis mellifera*. *Neurosci. Res. Comm.* **1,** 173-180.

Gilley, D. C., DeGrandi-Hoffman, G., and Hooper, J. E. (2006). Volatile compounds emitted by live European honey bee (*Apis mellifera* L.) queens. *J. Insect Physiol.* **52,** 520-527.

Giurfa, M. (1993). The repellent scent-mark of the honeybee *Apis mellifera ligustica* and its role as communication cue during foraging. *Insect. Soc.* **40,** 59-67.

Grozinger, C. M., Fan, Y., Hoover, S. E., and Winston, M. L. (2007). Genome-wide analysis reveals differences in brain gene expression patterns associated with caste and reproductive status in honey bees (*Apis mellifera*). *Mol. Ecol.* **16,** 4837-4848.

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

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

Guidugli, K. R., Piulachs, M. D., Belles, X., Lourenco, A. P., and Simoes, Z. L. P. (2005). Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* **579,** 4961-4965.

Honey Bee Genome Sequencing Consortium (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* **443,** 931-949.

Hoover, S. E. R., Keeling, C. I., Winston, M. L., and Slessor, K. N. (2003). The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90,** 477-480.

Hoover, S. E. R., Oldroyd, B. P., Wossler, T. C., and Winston, M. L. (2005a). Anarchistic queen honey bees have normal queen mandibular pheromones. *Insect. Soc.* **52,** 6-10.

Hoover, S. E. R., Winston, M. L., and Oldroyd, B. P. (2005b). Retinue attraction and ovary activation: responses of wild type and anarchistic honey bees (*Apis mellifera*) to queen and brood pheromones. *Behav. Ecol. Sociobiol.* **59,** 278-284.

Huang, Z. Y., and Robinson, G. E. (1992). Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proc. Natl. Acad. Sci. USA* **89,** 11726-11729.

Huising, M. O., and Flik, G. (2005). The remarkable conservation of corticotropin-releasing hormone (CRH)-binding protein in the honeybee (*Apis mellifera*) dates the CRH system to a common ancestor of insects and vertebrates. *Endocrinology* **146,** 2165-2170.

Hunt, G. J., Amdam, G. V., Schlipalius, D., Emore, C., Sardesai, N., Williams, C. E., Rueppell, O., Guzman-Novoa, E., Arechavaleta-Velasco, M., Chandra, S., Fondrk, M. K., Beye, M., and Page, R. E., Jr. (2007). Behavioral genomics of honeybee foraging and nest defense. *Naturwissenschaften* **94,** 247-267.

Hurd, H. (2009). Evolutionary drivers of parasite-induced changes in insect life-history traits from theory to underlying mechanisms. *Adv. Parasitol.* **68,** 85-110.

Kaatz, H. H., Hildebrandt, H., and Engels, W. (1992). Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J. Comp. Physiol. B* **162,** 588-592.

Karlson, P., and Burtenandt, A. (1959). Pheromones (ectohormones) in insects. *Ann. Rev. Entomol.* **4,** 39-58.

Keeling, C. I., and Slessor, K. N. (2005). A scientific note on the aliphatic esters in queen honey bees. *Apidologie* **36,** 559-560.

Kocher, S. D., Ayroles, J. F., Stone, E. A., and Grozinger, C. M. (2010). Individual variation in pheromone response correlates with reproductive traits and brain gene expression in worker honey bees. *PLoS One* **in press**.

Le Conte, Y., Arnold, G., Trouiller, J., Masson, C., Chappe, B., and Ourisson, G. (1989). Attraction of the parasitic mite *Varroa* to the drone larvae of honey bees by simple aliphatic esters. *Science* **245,** 638-639.

Le Conte, Y., and Hefetz, A. (2008). Primer pheromones in social Hymenoptera. *Ann. Rev. Entomol.* **53,** 523-542.

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

Le Conte, Y., Sreng, L., and Poitout, S. H. (1995). Brood pheromone can modulate the feeding behavior of *Apis mellifera* workers (Hymenoptera: Apidae). *J. Econ. Entomol.* **88,** 798-804.

Ledoux, M. N., Winston, M. L., Higo, H., Keeling, C. I., Slessor, K. N., and LeConte, Y. (2001). Queen and pheromonal factors influencing comb construction by simulated honey bee (*Apis mellifera* L.) swarms. *Insect. Soc.* **48,** 14-20.

Leoncini, I., Le Conte, Y., Costagliola, G., Plettner, E., Toth, A. L., Wang, M. W., Huang, Z., Becard, J. M., Crauser, D., Slessor, K. N., and Robinson, G. E. (2004). Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. *Proc. Natl. Acad. Sci. USA* **101,** 17559-17564.

Maisonnasse, A., Lenoir, J. C., Costagliola, G., Beslay, D., Choteau, F., Crauser, D., Becard, J. M., Plettner, E., and Le Conte, Y. (2009). A scientific note on E-ß-ocimene, a new volatile primer pheromone that inhibits worker ovary development in honey bees. *Apidologie* **40,** 562-564.

Malka, O., Karunker, I., Yeheskel, A., Morin, S., and Hefetz, A. (2009). The gene road to royalty--differential expression of hydroxylating genes in the mandibular glands of the honeybee. *FEBS J.* **276,** 5481-5490.

Martin, S. J., Beekman, M., Wossler, T. C., and Ratnieks, F. L. W. (2002). Parasitic Cape honeybee workers, *Apis mellifera capensis*, evade policing. *Nature* **415,** 163-165.

Mohammedi, A., Crauser, D., Paris, A., and Le Conte, Y. (1996). Effect of a brood pheromone on honeybee hypopharyngeal glands. *C. R. Acad. Sci. Paris, Ser. III* **319,** 769-772.

Mohammedi, A., Paris, A., Crauser, D., and Le Conte, Y. (1998). Effect of aliphatic esters on ovary development of queenless bees (*Apis mellifera* L.). *Naturwissenschaften* **85,** 455-458.

Morgan, S. M., Butz Huryn, V. M., Downes, S. R., and Mercer, A. R. (1998). The effects of queenlessness on the maturation of the honey bee olfactory system. *Behav. Brain Res.* **91,** 115-126.

Moritz, R. F. A., and Fuchs, S. (1998). Organization of honeybee colonies: characteristics and consequences of a superorganism concept. *Apidologie* **29,** 7-21.

Naumann, K., Winston, M. L., and Slessor, K. N. (1993). Movement of honey bee (*Apis mellifera* L.) queen mandibuar gland pheromone in populous and unpopulous colonies. *J. Insect Behav.* **6,** 211-223.

Naumann, K., Winston, M. L., Slessor, K. N., Prestwich, G. D., and Webster, F. X. (1991). Production and transmission of honey bee queen (*Apis mellifera* L.) mandibular gland pheromone. *Behav. Ecol. Sociobiol.* **29,** 321-332.

Nunez, J., Almeida, L., Balderrama, N., and Giurfa, M. (1998). Alarm pheromone induces stress analgesia via an opioid system in the honeybee. *Physiol. Behav.* **63,** 75-80.

Oldroyd, B. P. (2002). The Cape honeybee: an example of a social cancer. *Trends Ecol. Evol.* **17,** 249-251.

Oldroyd, B. P., and Fewell, J. H. (2007). Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* **22,** 408-413.

Oldroyd, B. P., Halling, L., and Rinderer, T. E. (1999). Development and behaviour of anarchistic honeybees. *Proc. R. Soc. B.* **266,** 1875-1878.

Oldroyd, B. P., Wossler, T. C., and Ratnieks, F. R. (2001). Regulation of ovary activation in worker honey-bees (*Apis mellifera*): larval signal production and adult response thresholds differ between anarchistic and wild-type bees. *Behav. Ecol. Sociobiol.* **50,** 366-370.

Page, R. E., and Erickson, E. H. (1988). Reproduction by worker honey bees (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* **23,** 117-126.

Page, R. E., Jr., and Amdam, G. V. (2007). The making of a social insect: developmental architectures of social design. *BioEssays* **29,** 334-343.

Page, R. E., Jr., Erber, J., and Fondrk, M. K. (1998). The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A* **182,** 489-500.

Pankiw, T. (2004a). Brood pheromone regulates foraging activity of honey bees (Hymenoptera: Apidae). *J Econ. Entomol.* **97,** 748-51.

Pankiw, T. (2004b). Worker honey bee pheromone regulation of foraging ontogeny. *Naturwissenschaften* **91,** 178-81.

Pankiw, T. (2007). Brood pheromone modulation of pollen forager turnaround time in the honey bee (*Apis mellifera* L.). *J. Insect Behav.* **20,** 173-180.

Pankiw, T., Huang, Z.-Y., Winston, M. L., and 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,** 685-692.

Pankiw, T., and Page, R. E., Jr. (2001). Brood pheromone modulates honeybee (*Apis mellifera* L.) sucrose response thresholds. *Behav. Ecol. Sociobiol.* **49**, 206-213.

Pankiw, T., and Page, R. E., Jr. (2003). Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A* **189,** 675-684.

Pankiw, T., Winston, M. L., and Slessor, K. N. (1994). Variation in worker response to honey bee (*Apis mellifera* L) queen mandibular pheromone (Hymenoptera: Apidae). *J. Insect Behav.* **7,** 1-15.

Peters, L., Zhu-Salzman, K., and Pankiw, T. (2010). Effect of primer pheromones and pollen diet on the food producing glands of worker honey bees (*Apis mellifera* L.). *J. Insect Physiol.* **56**, 132-137.

Pham-Delegue, M. H., Trouiller, J., Caillaud, C. M., Roger, B., and Masson, C. (1993). Effect of queen pheromone on worker bees of different ages: behavioural and eletrophysiological responses. *Apidologie* **24,** 267-281.

Ratnieks, F. L. W. (1993). Egg-laying, egg-removal, and ovary development by workers in queenright honey-bee colonies. *Behav. Ecol. Sociobiol.* **32,** 191-198.

Richard, F.-J., Aubert, A., and Grozinger, C. M. (2008). Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biol.* **6,** 50.

Robertson, H. M., and Wanner, K. W. (2006). The chemoreceptor superfamily in the honey bee, Apis mellifera: expansion of the odorant, but not gustatory, receptor family. *Genome Res.* **16,** 1395-1403.

Robinson, G. E. (1987a). Modulation of alarm pheromone perception in the honey bee: evidence for division of labor based on hormonal regulated response thresholds. *J. Comp. Physiol. A* **160,** 613-619.

Robinson, G. E. (1987b). 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. *Ann. Rev. Entomol.* **37,** 637-665.

Robinson, G. E., Fernald, R. D., and Clayton, D. F. (2008). Genes and social behavior. *Science* **322,** 896-900.

Robinson, G. E., and Page, R. E. (1989). Genetic basis for division of labor in an insect society. *In* "The genetics of social evolution" (M. D. Breed and R. E. Page, Eds.), pp. 61–81. West view press, Boulder.

Salvy, M., Martin, C., Bagneres, A. G., Provost, E., Roux, M., Le Conte, Y., and Clement, J. L. (2001). Modifications of the cuticular hydrocarbon profile of *Apis mellifera* worker bees in the presence of the ectoparasitic mite *Varroa jacobsoni* in brood cells. *Parasitology* **122,** 145-159.

Sandoz, J. C., Deisig, N., de Brito Sanchez, M. G., and Giurfa, M. (2007). Understanding the logics of pheromone processing in the honeybee brain: from labeled-lines to across-fiber patterns. *Front. Behav. Neurosci.* **1:5**.

Seehuus, S. C., Norberg, K., Gimsa, U., Krekling, T., and Amdam, G. V. (2006). Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc. Natl. Acad. Sci. USA* **103,** 962-967.

Shearer, D. A., and Boch, R. (1965). 2-heptanone in the mandibular gland secretion of the honey bee. *Nature* **206,** 530.

Shi, L., Lin, S., Grinberg, Y., Beck, Y., Grozinger, C. M., Robinson, G. E., and Lee, T. (2007). Roles of *Drosophila Kruppel-homolog 1* in neuronal morphogenesis. *Dev. Neurobiol.* **67,** 1614-26.

Simon, U. E., Moritz, R. F. A., and Crewe, R. M. (2001). The ontogenetic pattern of mandibular gland components in queenless worker bees (*Apis mellifera capensis*). *J. Insect Physiol.* **47,** 735-738.

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

Slessor, K. N., Kaminski, L.-A., King, G. G. S., Borden, J. H., and Winston, M. L. (1988). Semiochemical basis of the retinue response to queen honey bees. *Nature* **332,** 354-356.

Slessor, K. N., Winston, M. L., and Le Conte, Y. (2005). Pheromone communication in the honeybee (Apis mellifera L.). *J. Chem. Ecol.* **31,** 2731-2745.

Smedal, B., Brynem, M., Kreibich, C. D., and Amdam, G. V. (2009). Brood pheromone suppresses physiology of extreme longevity in honey bees (*Apis mellifera*). *J. Exp. Biol.* **212**, 3795-3801.

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

Vergoz, V., Roussel, E., Sandoz, J.-C., and Giurfa, M. (2007a). Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. *PLoS One* **2,** e288.

Vergoz, V., Schreurs, H. A., and Mercer, A. R. (2007b). Queen pheromone blocks aversive learning in young worker bees. *Science* **317,** 384-386.

Wager, B. R., and Breed, M. (2000). Does honey bee sting alarm pheromone give orientation information to defensive bees? *Ann. Entomol. Soc. Am.* **93,** 329-1332.

Wanner, K. W., Nichols, A. S., Walden, K. K., Brockmann, A., Luetje, C. W., and Robertson, H. M. (2007). A honey bee odorant receptor for the queen substance 9-oxo-2-decenoic acid. *Proc. Natl. Acad. Sci. USA* **104,** 14383-14388.

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

Wilson, D. S., and Sober, E. (1989). Reviving the superorganism. *J. Theor. Biol.* **136,** 337-356.

Wilson, E. O., and Bossert, W. H. (1963). Chemical communication amongs animals. *Recent Prog. Horm. Res.* **19,** 673-716.

Winston, M. L., Higo, H. A., Colley, S., Pankiw, T., and Slessor, K. N. (1991). The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera* L.) reproductive swarming (Hymenoptera: Apidae). *J. Insect Behav.* **4,** 649-660.

Winston, M.L., Higo, H.A. and Slessor K.N. (1990) Effect of various dosages of queen mandibular gland pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidae). *Entomol. Soc. Am.* **83**, 234-238.

Figure legends

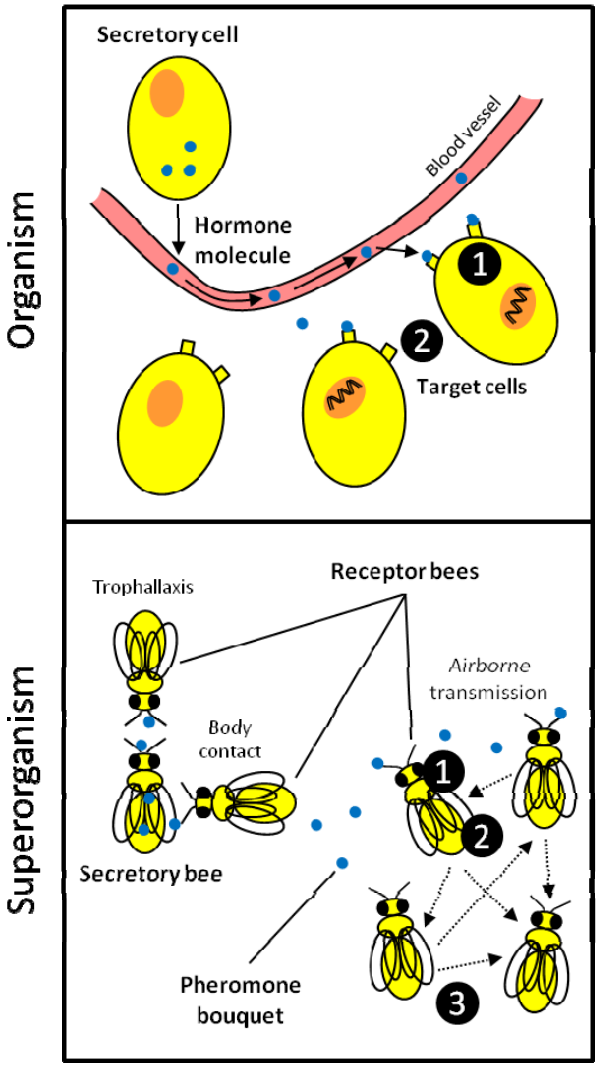
Figure 1: Analogy between organism hormones and superorganism pheromones.

In organisms, hormones are synthesized by secretory cells and transported via the blood flow to target cells. Analogous communication scheme is observed at the social level with pheromones. The pheromone message is transported outside of the body and delivered through trophallaxis (transfer of food between group members), body contact or airborne transmission. Similarly to the hormone response, reception of the semiochemicals results generally in transcriptional responses, followed by changes in hormone levels and/or behavior. However, a higher level of regulation is observed in the superorganism with a modification of social interactions.

Figure 2: Complexity of pheromone language in honey bees.

Pheromone language is remarkably complex since it is based on different levels of variation providing different nuances. First, there is a great diversity of pheromones produced by the different castes. Second, pheromone production is not rigid but dynamic and depends on the number of individuals, which follow temporal variation. Even if the single queen of the colony produces a baseline amount of QMP, its transmission is affected by the numbers of individual in the colony. Finally, variations might occur upon reception with different individuals having different response thresholds depending on their age (Pham-Delegue *et al.*, 1993) and genetic background (Kocher *et al.*, 2010; Pankiw *et al.*, 1994). This results in different genomic, physiological and behavioral responses, which will affect in return pheromone production.

Figure 1



Hormone regulation

1. Transcriptional regulation
2. Physiological and behavioral regulation

Pheromone regulation

1. Transcriptional regulation
2. Physiological and behavioral regulation
3. Social regulation