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Queen mandibular pheromone: questions that remain to be resolved

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Abstract – The discovery of ‘queen substance’, and the subsequent identification and synthesis of key components of queen mandibular pheromone, has been of significant importance to beekeepers and to the beekeeping industry. Fifty years on, there is greater appreciation of the importance and complexity of queen pheromones, but many mysteries remain about the mechanisms through which pheromones operate. The discovery of sex pheromone communication in moths occurred within the same time period, but in this case, intense pressure to find better means of pest management resulted in a remarkable focusing of research activity on understanding pheromone detection mechanisms and the central processing of pheromone signals in the moth. We can benefit from this work and here, studies on moths are used to highlight some of the gaps in our knowledge of pheromone communication in bees. A better understanding of pheromone communication in honey bees promises improved strategies for the successful management of these extraordinary animals.

queen mandibular pheromone / *Apis mellifera* / olfactory system / biogenic amines / juvenile hormone / ecdysteroids

For more than five decades, researchers have sought to understand and to appreciate fully the actions of the complex array of chemicals recognized initially as ‘queen substance’ (Butler 1954). At the time when Butler used this term, the concept of pheromones (chemicals that trigger behavioural and/or physiological responses in members of the same species, Karlson and Luscher 1959) had yet to be clearly defined, but over the years the importance of chemical communication systems in insects has become well-known, and improvements in chemical detection techniques have enabled many, although probably not all, of the chemicals signals produced by honey bee queens (and workers) to be identified. In this brief review, we focus on queen mandibular pheromone (QMP; Slessor et al.

1988). The key components of this complex mixture of compounds are shown in Figure 1. While QMP has many effects on the behaviour and physiology of adult worker bees (reviewed by Slessor et al. 2005), and significant effects also on levels of gene expression in the brain (Grozingier et al. 2003), relatively little is known as yet about the mechanisms that support the actions of this important multicomponent pheromone. Here, we take advantage of extensive studies of pheromone processing in moths to highlight gaps in our knowledge that need to be addressed in order to understand the actions of QMP.

1. QMP'S ACTIONS AS A SEX PHEROMONE

Virgin queens like many female insects attract males by releasing a strong attractant, often referred to as a sex pheromone (Free

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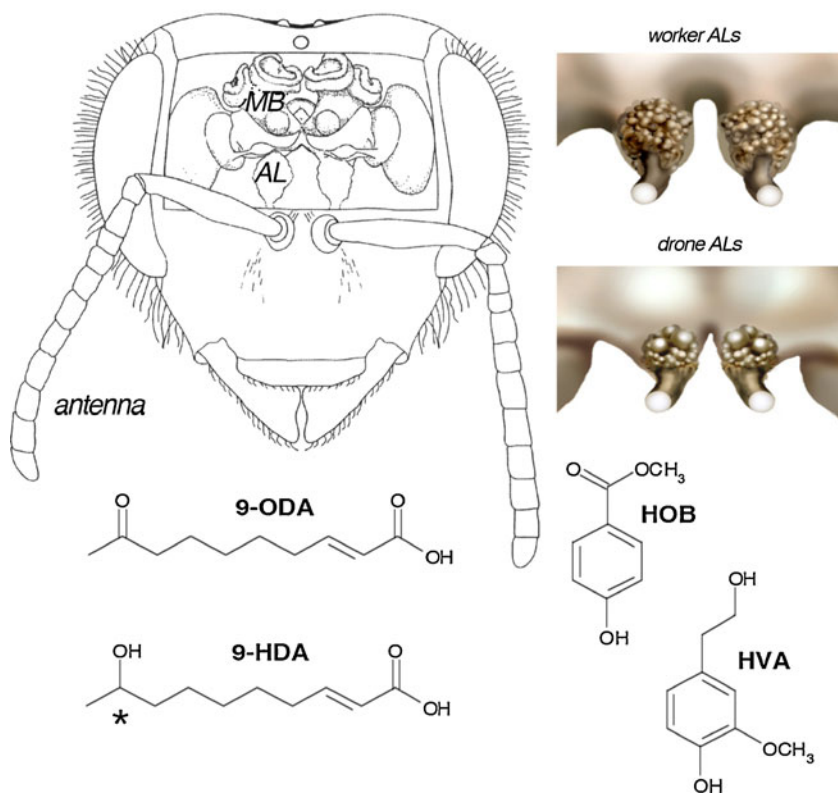


Figure 1. Key components of queen mandibular pheromone detected by the olfactory system of the bee. *Top left* Frontal view of the head of a bee showing the main olfactory organs (antennae), primary olfactory processing centres of the brain (antennal lobes) and higher-order sensory integration centres, the mushroom bodies. *Top right* Anatomy of drone and worker antennal lobes. Note the existence of larger glomeruli in the antennal lobe of the drone. *Bottom* Key components of queen mandibular pheromone. The asterisk indicates the existence of two enantiomers of 9HDA. *AL* antennal lobe, *HOB* methyl-*p*-hydrobenzoate, *HVA* 4-hydroxy-3-methoxyphenylethanol, *MB* mushroom bodies, *9HDA* 9-hydroxy-2-decenoic acid, *9ODA* 9-oxo-2-decenoic acid.

1987; Gary 1962). The first major component of QMP to be identified and characterized, 9-oxo-2-decenoic acid (9ODA; see Figure 1), plays this role. 9ODA is an effective attractant over large distances and elicits highly predictable responses in flying drones (Brockmann et al. 2006; Free 1987; Winston and Slessor 1992). Additional components, both enantiomers of 9-hydroxy-2-decenoic acid (respectively + and -9HDA, Figure 1) and 10-hydroxy-2-decenoic acid, 10HDA, also produced by the mandibular gland of the queen, synergize with 9ODA to increase male attraction at close range. 9ODA is detected by olfactory receptor neurons (ORNs) located in

the antennae of the bee (Figure 1). Honey bee antennae are not solely olfactory organs, but rather multifunctional structures that house a diverse array of sensory structures (sensilla). Sensilla placodea (pore plates), sensilla trichodea (hair-like structures) and sensilla basiconica (peg-like structures) are all olfactory sensilla in which ORNs have been identified (Esslen and Kaissling 1976).

2. AMOR11 IS THE OLFACTORY RECEPTOR THAT DETECTS 9ODA

How does 9ODA generate a response in bees? Olfactory sensilla have small pores that

allow odour molecules to diffuse through the cuticle of the antenna and into the fluid (lymph) within each olfactory sensillum. Here, the pheromone binds to carrier proteins that help transport the pheromone to olfactory receptors (ORs) located in the ORN membrane (Laughlin et al. 2008; Vogt and Riddiford 1981). The candidate carrier protein in the drone antenna is ASP1 which contains a hydrophobic domain that is able to bind the apolar components of 9ODA (Pesenti et al. 2008). The pheromone/carrier protein complex is then thought to interact with ORs that respond specifically to 9ODA. The identification of specific ORs in bees was advanced significantly with the sequencing of the honey bee genome (Honey Bee Genome Sequencing Consortium 2006) as sequence comparisons enabled researchers to identify honey bee orthologues of OR genes already identified in other insect species (Robertson and Wanner 2006). Robertson and colleagues identified four honey bee ORs expressed at a higher levels in males than in females (Wanner et al. 2007). Importantly, one of the four receptors identified, *AmOR11*, responds specifically to 9ODA (Wanner et al. 2007).

AmOR11 is found in all castes but is expressed at a higher levels in drone antennae (~13-fold higher) than in the antennae of workers or queens, most probably reflecting the important role that this pheromone plays in sexual communication. Interestingly, activation of *AmOR11* by 9ODA requires the presence of a second transmembrane protein, *Amel\Orco* (previously *AmOR2*; Vosshall and Hansson 2011), the honey bee orthologue of the *Drosophila* olfactory receptor, *DmelOrco* (Wanner et al. 2007). Binding of 9ODA to *AmOR11* alters the excitability of ORNs expressing this receptor protein. As a result, signals are conveyed via *AmOR11*-expressing ORNs to primary olfactory centres of the brain, the antennal lobes (ALs, Figure 1). ALs are the equivalent of vertebrate olfactory bulbs (Hildebrand and Shepherd 1997) and like olfactory bulbs, ALs are organised into spheroidal subunits known as 'glomeruli' (see ALs, Figure 1). Within the

glomeruli, ORNs make synaptic contact with local antennal-lobe neurons (LNs) and projection (output) neurons (PNs) that may process information entering the AL before it is conveyed (by PNs) to higher centres of the brain (Fonta et al. 1993; Gascuel and Masson 1991; Sun et al. 1993). Activity at this level can also be influenced by modulatory neurons (for example neurons that release dopamine, octopamine or serotonin) that project into the ALs from other parts of the brain (Hammer 1993; Kirchhof et al. 1999; Kreissl et al. 1994; Mercer et al. 1983; Rehder et al. 1987; Schäfer and Rehder 1989).

3. SEXUAL DIMORPHISM EXISTS IN OLFACTORY PATHWAYS OF THE BEE

Many of the glomeruli found in the ALs of the honey bee are readily identifiable from one individual to the next (Arnold et al. 1985; Flanagan and Mercer 1989; Galizia et al. 1999a). It is common for insect species that rely on olfaction for sexual communication to exhibit sexual dimorphism both, at the level of the antennae and the ALs (Hansson and Anton 2000; Rospars 1988). In the moth, *Antheraea polyphemus*, for example, the antenna of the male houses about 70,000 sensilla compared to about 13,000 sensilla in the antenna of the female (Boeckh et al. 1960; Meng et al. 1989). This difference is explained by the large number of sensilla dedicated to sex pheromone detection in male moths. Glomeruli receiving input from sex pheromone receptor neurons tend to be larger than glomeruli that respond to plant odours ('ordinary glomeruli') because they receive input from a larger number of ORNs. Honey bees also show sexual dimorphism in olfactory pathways. Drone antennae lack sensilla basiconica, but they have many more pore plates than the antennae of workers (18,600 vs 2,600), suggesting a role for pore plate sensilla in the detection of queen pheromone and in particular, 9ODA. ORNs that respond with high sensitivity to 9ODA have been identified (Kaissling and Renner 1968; Vareschi 1971), and measurements of global responses of

antennal receptor neurons ('electroantennograms') suggest that drone antennae are more sensitive to 9ODA than the antennae of worker bees (Brockmann et al. 1998). At the level of the ALs, drone bees possess four male-specific macroglomeruli (MG1-4, Arnold et al. 1985), three of which are shown in Figure 1 (compare drone and worker ALs).

4. HOW ARE 9ODA SIGNALS PROCESSED IN THE BRAIN?

The function of each glomerulus is defined by the type of ORN that projects into the glomerulus and more specifically, the ORs located in the ORN membrane. Generally speaking, in insects (as in vertebrates) each subtype of ORN expresses only one type of OR (insects: Krieger et al. 2002; Sakurai et al. 2004; Vosshall et al. 1999; vertebrates: Ressler et al. 1993; Vassar et al. 1993), and ORNs expressing the same OR converge onto the same glomerulus (Fishilevich and Vosshall 2005; Vosshall et al. 2000). As four ORs have been identified that are expressed at a higher level in males than in females (Wanner et al. 2007), it is tempting to speculate that the four male-specific macroglomeruli in drones process olfactory signals detected by ORN subtypes expressing these four OR proteins. However, this has yet to be confirmed. Optical imaging studies have revealed that ORNs expressing *AmOR11*, the OR that detects 9ODA (Wanner et al. 2007) converge onto the large male-specific glomerulus, MG2 (Sandoz 2006). Although worker bees are sensitive also to the effects of this pheromone, the location of the glomerulus (or glomeruli) responsive to 9ODA in worker ALs has yet to be identified (see Sandoz 2006). The identification in drones of a specific glomerulus (MG2) responsive to 9ODA could indicate that information about this pheromone is conveyed to higher centres of the brain via a so called 'labelled line' (Christensen and Hildebrand 2002). But is this the case, or is there processing of pheromonal signals at the level of the ALs?

Cross talk between ORNs, LNs and PNs can lead to processing of signals entering the ALs before they are conveyed to higher centres of the brain. Local antennal-lobe interneurons (LNs), for example, can spread information from one glomerulus to another, and projection (output) neurons (PNs) can convey information to higher brain centres from one, or more glomeruli. Consistent with these possibilities, LNs generally extend processes to many glomeruli within the AL (Fonta et al. 1993; Linster et al. 2005; Sun et al. 1993) and PNs in the honey bee vary in the number of glomeruli they innervate (Abel et al. 2001; Brandt et al. 2005; Kirschner et al. 2006; Müller et al. 2002). Uniglomerular PNs (uPNs), which send projections into a single glomerulus could convey information specific to one pheromone component, whereas PNs projecting to multiple glomeruli (multiglomerular PNs, mPNs) might instead integrate information originating from multiple glomeruli. Indeed, mPNs could potentially convey to higher centres of the brain information about the entire pheromone blend. In moths it is clear that uPNs and mPNs are involved extensively in the processing of sex pheromone signals at the level of the ALs. For example, moth uPNs, although responding predominantly to one component of the sex pheromone blend, are usually more generalist than the ORNs they synapse with (Christensen and Hildebrand 1987; Hansson et al. 1994, 1991; Jarriault et al. 2010, 2009; Mustaparta 1996). In contrast, some PNs in the moth (including some identified as mPNs) respond only when all components of the pheromone blend are presented (Anton et al. 1997; Christensen et al. 1995; Hansson et al. 1994). Interestingly, instances have been described in moths of mismatching between glomerular arborisations and response specificity of PNs (Anton and Hansson 1999; Vickers et al. 1998), which emphasises the complexity of processing that occurs already at this level of the brain. Taken together these observations support the idea of a combinatorial labelled-line system. Recent studies of olfactory information processing in honey bees, conducted using optical imaging techniques, have provided considerable insight into the combinatorial aspect

of odour representation not only in the ALs (Galizia et al. 1999b; Joerges et al. 1997; Sachse et al. 1999), but also at the next level of integration, the Kenyon cells of the mushroom bodies of the brain (Szyszka et al. 2005). Generally speaking, however, these studies have described the coding of floral odours rather than pheromones, leaving a large gap in our understanding of the neural bases of pheromone-elicited behaviours in the honey bee.

The relatively large number of macroglomeruli in drone bees is intriguing. It is possible that one or more of these specialised structures is involved in the processing of pheromone components released by queens from other species (Butler et al. 1967; Plettner et al. 1997). In moths for example, pheromonal chemicals, called behavioural antagonists, can contribute to the reproductive isolation of some species and macroglomeruli devoted to the processing of such signals are found in other closely related species (Baker et al. 1998; Hansson et al. 1995, 1992). Whether this occurs in bees also has yet to be determined. Indeed, our knowledge of how pheromones other than 9ODA are detected in the bee remains rudimentary. For example, despite behavioural evidence showing that other components of QMP act synergistically with 9ODA to enhance male attraction (Brockmann et al. 2006), it is unclear how this occurs. Interestingly, Sandoz (2006) found that the QMP components methyl-*p*-hydrobenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA) activated small ('ordinary') glomeruli in the AL of the drone. While this possibly highlights a functional difference between the macroglomerular complex of male moths and that of drone honey bees, there is no evidence currently that either, HVA or HOB play a role in sexual communication in the bee. These aromatic compounds are known, however, to play an important role in queen-worker interactions (Slessor et al. 1988, 2005).

5. QUEEN-WORKER INTERACTIONS

Primitively, mate attraction might have been the principal role of honey bee queen pheromone, as is the case for pheromones produced

by many non-social insects. However, a mated, egg-laying queen is essential for the survival of the whole colony and components of QMP, including 9ODA, play a critical role also in regulating the behaviour and physiology of worker bees (Slessor et al. 2005). Changes in the chemical composition of QMP after mating turn the queen's sex appeal into an olfactory aura that has a significant impact on workers and particularly, on young worker bees (reviewed by Slessor et al. 2005). The behavioural and physiological effects of this pheromone are well documented (Free 1987; Slessor et al. 2005; Winston and Slessor 1992) and are described in recent reviews (Alaux et al. 2010; Slessor et al. 2005). QMP as a blend acts as an attractant that plays a role in eliciting retinue behaviour in young worker bees (Slessor et al. 1988; Figure 2). The queen bee relies on workers to feed and groom her and young bees attracted to the queen by her bouquet of pheromones also lick and antennate her body (Naumann 1991). These young workers, which are not only receivers but also carriers of the queen's pheromonal messages, play an important role in distributing the queen's pheromones throughout the colony via antennal contacts and trophallaxis. As a result of such exchanges, even workers that do not come into direct contact with the queen are affected by her presence. There are many important consequences of this including, inhibition of swarming behaviour, the rearing of new queens and ovary development in worker bees (reviewed by Slessor et al. 2005). Removal of the queen and her pheromone signals has immediate effects and within 12–24 h, triggers the rearing of new queens (Pettis et al. 1995; Winston et al. 1990). As a general rule, aging of the queen and changes in her pheromone production lead to the rearing of new queens prior to reproductive swarming. As there appears to be no correlation between queen pheromone production and the initiation of swarming (Seeley and Fell 1981), it has been suggested that swarming behaviour might instead be explained by reduced dispersal of queen pheromone in populous colonies (Naumann et al. 1993; Winston et al. 1991), or

Figure 2. Retinue behaviour in a honey bee colony. Young worker bees feed and groom the queen. Her pheromone motivates them to antennate and lick her body. Photograph courtesy of Fanny Mondet.



by changes in the worker response threshold to QMP (Pankiw et al. 2000).

Slessor and colleagues (1988) identified five QMP components that play a role in eliciting retinue behaviour; 9ODA, -9HDA and +9HDA, which are involved also in mating behaviour (see above), and the aromatic compounds HOB and HVA (see Figure 1). Workers have the ability to produce these active chemicals also, but modifications of the biosynthetic pathways, possibly via modulation of gene expression in presence of the QMP, alter the resulting blend (Hasegawa et al. 2009; Malka et al. 2009; Plettner et al. 1996). 10HDA, which is produced in higher quantity by virgin queens than mated queens and is important in mating (Brockmann et al. 2006), appears not to participate in queen–worker interactions (Slessor et al. 1988).

Small but consistent differences in responses of workers to the 5-component QMP blend compared to queen extract indicated to Slessor

and colleagues that additional components must be involved. Some of these components have since been identified and found to be produced in locations other than in the mandibular glands (Keeling et al. 2003; see also Katzav-Gozansky et al. 2001; Wossler and Crewe 1999). Recently, Maisonnasse et al. (2010) found that demandibulated queens with no detectable 9ODA were as attractive to workers as sham-operated queens. This is interesting because it reveals that the ability to elicit retinue behaviour is not a property unique to QMP.

6. PHEROMONE EFFECTS ON BEHAVIOUR AND PHYSIOLOGY—HOW ARE THEY MEDIATED?

In 1992, Kaatz and colleagues reported that 9ODA reduces the rate of juvenile hormone (JH) biosynthesis in the bee (see also Pankiw et

al. 1998). This important finding provides a clue as to how pheromones might effect behavioural and physiological changes in the bee. As JH plays a critical role not only in metamorphosis but also in the behavioural and physiological development of the bee (Fluri et al. 1982; Huang et al. 1991; Robinson 1992), pheromone modulation of JH titres would be predicted to have significant effects at both a behavioural and physiological level. How does exposure to 9ODA trigger these effects? As outlined above, pheromone signals detected by ORNs located in the antennae are conveyed from the ALs to higher centres such as the mushroom bodies of the brain (Figure 1). Immediately behind the mushroom bodies are the cell bodies of large neurosecretory cells that project to endocrine organs located behind the brain. These include the corpora allata, organs that release JH in response to signals from the brain (Rachinsky and Hartfelder 1990; Tobe and Stay 1985). Although the neural circuitry involved remains unclear, it appears that pheromone signals originating in the ALs lead, either directly or indirectly to changes in the activity of these neurosecretory cells. Pheromone signals conveyed from the ALs to mushroom bodies of the brain may also influence ecdysteroid signalling in the bee as recent studies have revealed that intrinsic mushroom body neurons express genes for ecdysteroid signalling (Paul et al. 2006, 2005; Takeuchi et al. 2007; Yamazaki et al. 2006) and that the steroid hormone, ecdysone, is synthesised and secreted by the brain (Yamazaki et al. 2011). Studies in the fruit fly, *Drosophila melanogaster*, have revealed an astonishingly complex interplay between JH, ecdysteroids and biogenic amines that appears to be intimately involved in development and behaviour regulation (reviewed by Gruntenko and Rauschenbach 2008). The involvement of biogenic amines as mediators of development and behavioural plasticity is well established and has received a great deal of attention. Biogenic amines act as neurotransmitters, neuromodulators and neurohormones and in bees, amines such as dopamine (DA), octopamine (OA) and serotonin (5HT) have been strongly implicated in learning

and memory (Blenau and Baumann 2001; Hammer 1993; Mercer and Menzel 1982), recruitment behaviour (Barron et al. 2007; Bozic and Woodring 1998), division of labour (Schulz and Robinson 1999, 2001; Taylor et al. 1992; Wagener-Hulme et al. 1999), foraging behaviour (Barron and Robinson 2005; Barron et al. 2002), locomotor activity (Mustard et al. 2010) and ovary development (Dombroski et al. 2003; Harris and Woodring 1995; Hoover et al. 2003, Vergoz et al., in preparation).

JH titres (Fluri et al. 1982; Huang et al. 1991) and levels of biogenic amines in the worker brain (Schulz et al. 2002; Taylor et al. 1992), increase with age and a growing body of evidence suggests these two events are linked (Scheiner et al. 2006). For example, QMP not only reduces the rate of JH biosynthesis causing delays in the ontogeny of foraging behaviour (Kaatz et al. 1992; Pankiw et al. 1998; Slessor et al. 2005), but in young bees it can also reduce levels of DA in the brain (Beggs et al. 2007). Nurses and foragers have different gene expression profiles (Whitfield et al. 2003), and QMP treatments have been found to activate nurse genes and to repress forager genes in the brain of worker bees (Grozinger et al. 2003). One of these genes is the vitellogenin gene (Vg) the product of which, among several pleiotropic effects, regulates the nutritional stores to produce brood food (Nelson et al. 2007). QMP treatment increases Vg RNA expression levels in the fat bodies of young bees (Fischer and Grozinger 2008; Nelson et al. 2007). Vitellogenin and JH apparently interact via a regulatory feedback loop with vitellogenin inhibiting JH production (Guidugli et al. 2005) and JH inhibiting Vg synthesis (Fahrbach and Robinson 1996; Pinto et al. 2000). It has been suggested that the slow fall in Vg titres below a critical threshold may allow JH titres to rise and trigger neurochemical changes that lead to the initiation of foraging behaviour (Amdam and Omholt 2002). Interestingly, 3',5'-cyclic guanosine monophosphate has recently been found to inhibit the QMP-mediated increase in Vg RNA levels in the fat bodies of the worker bees (Fussnecker et al. 2011).

Pheromone signals, other than those mediated by 9ODA, are also likely to be conveyed from the ALs to higher centres of the brain and may, like 9ODA, target cells involved in hormone signalling, or signalling via modulators such as the biogenic amines. Indeed measurements of global responses of receptor neurons in the antennae of the bee have shown that antennal receptors are responsive to all five of the key components of QMP (Brockmann et al. 1998). Evidence suggests, however, that the aromatic compounds HOB and HVA may have additional roles as HVA has recently been found to selectively activate the honey bee DA receptor, *AmDOP3* (Beggs and Mercer 2009).

7. QMP AFFECTS DA SIGNALLING IN THE BEE

The aromatic compounds, HOB and HVA, are similar in structure to biogenic amines and one in particular, HVA, bears a striking structural resemblance to DA. Harris and Woodring found that one consequence of removing the queen from a honey bee colony was that brain DA levels in young worker bees increase (Harris and Woodring 1995). QMP, and HVA alone, have subsequently been shown to reduce DA levels in young worker bees and QMP transiently alters levels of DA receptor gene expression in the brain (Beggs et al. 2007). In an experiment in which DA receptors were expressed *in vitro*, HVA was found to selectively activate *AmDOP3* receptors while having no effect on the two other honey bee DA receptors, *AmDOP1* and *AmDOP2* (Beggs and Mercer 2009). As the dose at which HVA showed an effect on heterologously expressed *AmDOP3* receptors was rather high (~10 μM range) in comparison to the concentration detected in QMP, further studies are required to confirm that *AmDOP3* receptors *in vivo* are activated by the pheromone. While it would not be surprising to find that the sensitivity of *AmDOP3* receptors *in vivo* differs from that observed *in vitro*, it is possible also that HVA works synergistically with other components of the queen pheromone. Examples of synergistic

activation of olfactory receptors by pheromone blends have been described in the moth *Trichoplusia ni* (O'Connell et al. 1986; Mayer and Doolittle 1995). If HVA targets *AmDOP3* receptors *in vivo*, *AmDOP3* receptor activation could potentially contribute to effects of QMP on the behaviour and physiology of young worker bees. All three of these DA receptors are expressed in the antennae (Vergoz et al. 2009) as well as in the brain of the bee (Beggs et al. 2005; Kurshan et al. 2003; Humphries et al. 2003; Blenau et al. 1998). HVA activation of *AmDOP3* at the level of the antennae could potentially alter signals conveyed from the antennae to ALs of the brain. Studies in moths, for example, have shown that the physiology of ORNs can be modulated by biogenic amines acting at this level. In some moth species, it has been found that injections of OA lead to increased excitability of ORNs in response to pheromones (Grosmaître et al. 2001; Pophof 2002). Moreover, OA receptors identified in these species were found to be located at the base of pheromone and non-pheromone sensitive sensilla and in neuronal-shaped cells (Brigaud et al. 2009; Von Nickisch-Roseneck et al. 1996). It will be interesting to examine the distribution of OA and DA receptors in the antennae of the bee as modulation of responses at this level has the potential to have a profound effect on many aspects of bee behaviour. As retinue bees also lick the queen as they groom her, however, we cannot rule out the possibility that at least some of her pheromones may have targets other than ORs located in the antennae of the bee. The *AmDOP3* receptor, for example, is expressed not only in the antennae but also in the brain, but whether QMP components such as HVA are ingested and cross the blood–brain barrier has yet to be determined.

Interestingly, bees exposed to QMP early in adult life tend to have lower levels of *Amdop1* expression in the antennae and brain than bees that have never been exposed to this pheromone (Beggs et al. 2007; Vergoz et al. 2009). Moreover, young workers showing strong attraction to QMP have been found to have higher

Amdop3 transcript levels, and levels of transcript for the octopamine receptor, *Amoa1*, than bees not strongly attracted to this pheromone. Levels of *Amdop3* expression in the antennae decrease rapidly during the first week of adult life perhaps contributing to the well-documented decline in responsiveness to QMP with age. Vergoz et al. (2007b) found that bees exposed to QMP for 4 to 6 days from the time of adult emergence were not able to associate an odour with an aversive stimulus suggesting that their ability to predict punishment was blocked. Interestingly, bees treated in the same way retained their ability to form appetitive olfactory memories. HVA's ability to activate the DA receptor *AmdOP3* may contribute to these effects. Evidence that aversive learning in insects involves DA signalling is compelling, particularly in fruit flies where DA-releasing neurons have been shown to convey the negative reinforcing properties of punishment signals (Riemensperger et al. 2005; Schroll et al. 2006; Schwaerzel et al. 2003). Consistent with this model, inhibition of DA signalling with DA receptor antagonists has been shown to selectively impair aversive learning in bees (Vergoz et al. 2007a).

HVA is an important component of QMP in *Apis mellifera*, but it is not present in the pheromone blend of all *Apis* species. What might be the adaptive advantage of selection for this pheromone? One possible benefit to the queen of being able to block aversive learning in the young workers is that they will not associate the queen with any unpleasant effects of high concentrations of her pheromone. In contrast to young bees, bees of foraging age appear to be repelled by QMP (Vergoz et al. 2007b), and potentially also by nurses (see Fan et al. 2010) and perhaps even the queen herself. Fan and colleagues have recently shown that QMP exposure alters patterns of cuticular hydrocarbons in worker bees (Fan et al. 2010) and that nurses and foragers differ in their cuticular hydrocarbon profiles, probably because they are exposed to different levels of QMP. This is interesting because it may help to explain why bees of foraging age tend to remain towards the periphery of the hive (Winston

1987). Nestmate recognition is crucial in honey bee colonies as it helps bees identify parasites and conspecific intruders (Breed 1998; Breed and Buchwald 2009). Members of a colony form a memory of the colony odour during the first days after emergence based on the environmental odours including the odours of nestmates. Once this memory is established they tend to show aggression towards individuals with cuticular hydrocarbon profiles that do not match. As this profile is affected not only by genotype, but also by diet, colony environment and an individual's physiological condition (Howard and Blomquist 2005), it is not surprising that in bees this profile changes over time (Richard et al. 2008). The mechanism explaining the effect of QMP on cuticular hydrocarbon profiles remains unknown, but it would be interesting to investigate QMPs effects on N-acetyldopamine, which is a sclerotizing agent of the insect cuticle (Karlson and Sekeris 1962).

8. DOES QMP AFFECT THE QUEEN?

Whether the queen is affected by her own pheromone is unclear. Queens re-absorb a third of their own daily QMP secretion, probably through their cuticle and, as a thousandth of their gland extract is present on their body at any time (Naumann et al. 1991), they are constantly exposed to the highest possible levels of QMP. Interestingly, this does not inhibit ovary development in the queen suggesting either, that QMP does not induce the same physiological changes in queens as it does in workers, or that the queens' sensitivity to one or more components of QMP differs markedly from that of workers. Aspects of queen behaviour and physiology suggest that queens may, however, be affected by their own pheromones. For example, in contrast to workers, JH titres remain low throughout the adult lifetime of the queen. As QMP production during the first 2 days of the queen's adult life is relatively low but then rises and remains high until the end of her lifetime it has been suggested that JH levels in queens may be influenced by QMP (Pankiw et al. 1996; Slessor et al. 1990). Similarly, brain DA levels, which have been found in young workers

to be lowered by the QMP component HVA, are lower in mated queens (which produce HVA) than in virgin queens (Harano et al. 2005). Queens are also less mobile after mating (Winston 1987), a potential effect of QMP that would parallel effects of the pheromone on members of the queen's retinue. Consideration of the possibility that queen pheromones may affect the queen herself, in addition to other members of the colony, has interesting implications in terms of the evolution of queen pheromones. Whether queens use their pheromones to exert control over workers, or as honest signals of queen fecundity (Keller and Nonacs 1993) remains a matter of great interest and debate (see reviews, e.g. by Le Conte and Hefetz 2008; Keller 2009; Kocher and Grozinger 2011). While detailed consideration of this issue lies beyond the scope of the current review, it is interesting to note that female pheromone autodetection has been documented in some moth species (Ochieng et al. 1995; Schneider et al. 1998). Intriguingly, ALs of queen bees contain a glomerulus of larger size, which could be a female macroglomerulus (Arnold et al. 1988). Future studies will reveal whether this large glomerulus is dedicated to processing the queen's own pheromone and/or some other species-specific signal.

9. CONCLUSION

Chemical signalling is the principal means by which a queen bee can influence the development of a colony and QMP, even on its own, is remarkable for the complexity of behaviours that it regulates. The intensive efforts that have been made to identify components of this pheromone and to describe their effects on the behaviour and physiology of bees makes this an attractive model for studies of the neural bases of pheromonal regulation in insects. Recent advances have come not only from the sequencing of the honey bee genome and the identification of the 9ODA receptor, *AmOR11*, but also from the application of optical imaging techniques, which have revealed where in the brain pheromone signals are processed. This work represents an important foothold that will assist researchers in the task of identifying the central mechanisms through

which QMP components operate, filling gaps in our knowledge between the peripheral detection of pheromone and its physiological and behavioural consequences. Evidence suggesting that some pheromones have targets other than, or in addition to, olfactory receptors also warrants further attention as a comprehensive understanding of pheromonal communication systems in honey bees will undoubtedly suggest new strategies for the successful management of these extraordinary animals.

La phéromone mandibulaire de la reine: encore des questions à résoudre.

phéromone mandibulaire / reine / *Apis mellifera* / système olfactif / amines biogéniques / hormone juvénile / ecdystéroïde

Das Mandibelpheromon der Königin: Welche Fragen sind noch offen?

Königinnen Mandibelpheromon / *Apis mellifera* / olfaktorisches System / Biogene Amine / Juvenilhormon / Ecdysteroidhormone

Abbreviation

AL	Antennal lobe
cGMP	3',5'-Cyclic guanosine monophosphate
DA	Dopamine
9HDA	9-Hydroxy-2-decenoic acid
10HDA	10-Hydroxy-2-decenoic acid
HOB	Methyl- <i>p</i> -hydrobenzoate
5HT	Serotonin
HVA	4-Hydroxy-3-methoxyphenylethanol
JH	Juvenile hormone
LN	Local neuron
MB	Mushroom bodies
MG1-4	Macroglomeruli 1-4
mPN	Multiglomerular projection neuron
OA	Octopamine
9ODA	9-Oxo-2-decenoic acid
OR	Olfactory receptor
ORN	Olfactory receptor neuron
PN	Projection neuron
QMP	Queen mandibular pheromone
uPN	Uniglomerular projection neuron

REFERENCES

- Abel, R., Rybak, J., Menzel, R. (2001) Structure and response patterns of olfactory interneurons in the honeybee, *Apis mellifera*. *J. Comp. Neurol.* **437**, 363–383
- Alaux, C., Maisonnasse, A., Le Conte, Y., Gerald, L. (2010) Pheromones in a superorganism: from gene to social regulation. *Vit. Horm.* **83**, 401–423
- Amdam, G.V., Omholt, S.W. (2002) The regulatory anatomy of honeybee lifespan. *J. Theor. Biol.* **216**, 209–228
- Anton, S., Hansson, B. (1999) Physiological mismatching between neurons innervating olfactory glomeruli in a moth. *Proc. Royal Soc. London B* **266**, 1813–1820
- Anton, S., Löfstedt, C., Hansson, B.S. (1997) Central nervous processing of sex pheromones in two strains of the european corn borer *Ostrinia nubilalis* (*Lepidoptera: Pyralidae*). *J. Exp. Biol.* **200**, 1073–1087
- Arnold, G., Masson, C., Budharugsa, S. (1985) Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (*Apis mellifera*). *Cell Tissue Res.* **242**, 593–605
- Arnold, G., Budharugsa, S., Masson, C. (1988) Organization of the antennal lobe in the queen honey bee, *Apis mellifera* L. (*Hymenoptera: Apidae*). *Int. J. Insect Morph. Emb.* **17**, 185–195
- Baker, T.C., Cosse, A.A., Todd, J.L. (1998) Behavioral antagonism in the moth *Helicoverpa zea* in response to pheromone blends of three sympatric heliothine moth species is explained by one type of antennal neuron. *Ann. N. Y. Acad. Sci.* **855**, 511–513
- Barron, A.B., Robinson, G.E. (2005) Selective modulation of task performance by octopamine in honey bee (*Apis mellifera*) division of labour. *J. Comp. Physiol. A.* **191**, 659–668
- Barron, A.B., Schultz, D.J., Robinson, G.E. (2002) Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *J. Comp. Physiol. A* **188**, 603–610
- Barron, A.B., Maleszka, R., Vander Meer, R.K., Robinson, G.E. (2007) Octopamine modulates honey bee dance behavior. *Proc. Natl. Acad. Sci. USA* **104**, 1703–1707
- Beggs, K.T., Mercer, A.R. (2009) Dopamine receptor activation by honey bee queen pheromone. *Curr. Biol.* **19**, 1206–1209
- Beggs, K.T., Hamilton, I.S., Kurshan, P.T., Mustard, J.A., Mercer, A.R. (2005) Characterization of a D2-like dopamine receptor (*AmDOP3*) in honey bee, *Apis mellifera*. *Insect Biochem. Molec. Biol.* **35**, 873–882
- Beggs, K.T., Glendining, K.A., Marechal, N.M., Vergoz, V., Nakamura, I., Slessor, K.N., Mercer, A.R. (2007) Queen pheromone modulates brain dopamine function in worker honey bees. *Proc. Natl. Acad. Sci. USA* **104**, 2460–2464
- Blenau, W., Baumann, A. (2001) Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arch. Insect Biochem. Physiol.* **48**, 13–38
- Blenau, W., Erber, J., Baumann, A. (1998) Characterization of a dopamine D1 receptor from *Apis mellifera*: cloning, functional expression, pharmacology, and mRNA localization in the brain. *J. Neurochem.* **70**, 15–23
- Boeckh, J., Kaissling, K.E., Schneider, D. (1960) Sensillen und Bau der Antennengeißel von *Telega polyphemus* (Vergleiche mit weiteren Saturniden: *Antheraea*, *Platysamia* und *Philosamia*). *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **78**, 559
- Bozic, J., Woodring, J. (1998) Variations of brain biogenic amines in mature honeybees and induction of recruitment behavior. *Comp. Biochem. Physiol. A.* **120**, 737–744
- Brandt, R., Rohlfing, T., Rybak, J., Kroficzek, S., Maye, A., Westerhoff, M., Hege, H.C., Menzel, R. (2005) Three-dimensional average-shape atlas of the honeybee brain and its applications. *J. Comp. Neurol.* **492**, 1–19
- Breed, M.D. (1998) Recognition pheromones of the honey bee. *Bioscience* **48**, 463–470
- Breed, M.D., Buchwald, R. (2009) Cue diversity and social recognition. Harvard University Press, Cambridge
- Brigaud, I., Grosmaître, X., François, M.C., Jacquinjoly, E. (2009) Cloning and expression pattern of a putative octopamine/tyramine receptor in antennae of the noctuid moth *Mamestra brassicae*. *Cell Tissue Res.* **335**, 455–463
- Brockmann, A., Brückner, D., Crewe, R.M. (1998) The EAG response spectra of workers and drones to queen honeybee mandibular gland components: the evolution of a social signal. *Naturwissenschaften* **85**, 283–285
- Brockmann, A., Dietz, D., Spaethe, J., Tautz, J. (2006) Beyond 9-ODA: sex pheromone communication in the European honey bee *Apis mellifera*. *J. Chem. Ecol.* **32**, 657–667
- Butler, C. (1954) The method and importance of the recognition by a colony of honeybees (*Apis mellifera*) of the presence of its queen. *Trans. R. Entomol. Soc. Lond.* **105**, 11–29
- Butler, C., Calam, D., Callow, R. (1967) Attraction of *Apis mellifera* drones by the odours of the queens of two other species of honeybees. *Nature* **213**, 423–424
- Christensen, T.A., Hildebrand, J.G. (1987) Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth, *Manduca sexta*. *J. Comp. Physiol. A.* **160**, 553–569
- Christensen, T.A., Hildebrand, J.G. (2002) Pheromonal and host-odor processing in the insect antennal lobe: how different? *Curr. Opin. Neurobiol.* **12**, 393–399
- Christensen, T.A., Mustaparta, H., Hildebrand, J.G. (1995) Chemical communication in heliothine

- moths. VI. Parallel pathways for information processing in the macroglomerular complex of the male tobacco budworm moth *Heliothis virescens*. *J. Comp. Physiol. A.* **177**, 545–557
- Dombroski, T., Simões, Z.L.P., Bitondi, M.M.G. (2003) Dietary dopamine causes ovary activation in queenless *Apis mellifera* workers. *Apidologie* **34**, 281–290
- Esslen, J., Kaissling, K.-E. (1976) Zahl und Verteilung antennaler Sensillen bei der Honigbiene (*Apis mellifera*). *Zoomorphology* **83**, 227–251
- Fahrbach, S.E., Robinson, G.E. (1996) Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Dev. Neurosci.* **18**, 102–114
- Fan, Y.L., Richard, F.J., Rouf, N., Grozinger, C.M. (2010) Effects of queen mandibular pheromone on nestmate recognition in worker honeybees, *Apis mellifera*. *Anim. Behav.* **79**, 649–656
- Fischer, P., Grozinger, C.M. (2008) Pheromonal regulation of starvation resistance in honey bee workers (*Apis mellifera*). *Naturwissenschaften* **95**, 723–729
- Fishilevich, E., Voshall, L.B. (2005) Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr. Biol.* **15**, 1548–1553
- Flanagan, D., Mercer, A.R. (1989) An atlas and 3-D reconstruction of the antennal lobes in the worker honey bee, *Apis mellifera* L. (*Hymenoptera: Apidae*). *Int. J. Insect Morphol. Embryol.* **18**, 145–159
- Fluri, P., Lüscher, M., Wille, H., Gerig, L. (1982) Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* **28**, 61–68
- Fonta, C., Sun, X.-J., Masson, C. (1993) Morphology and spatial distribution of bee antennal lobe interneurons responsive to odours. *Chem. Senses* **18**, 101–119
- Free, J.B. (1987) Pheromones of social bees. Chapman and Hall
- Fussnecker, B.L., McKenzie, A.M., Grozinger, C.M. (2011) cGMP modulates responses to queen mandibular pheromone in worker honey bees. *J. Comp. Physiol. A.* 1–10
- Galizia, C.G., McIlwrath, S.L., Menzel, R. (1999a) A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy. *Cell Tissue Res.* **295**, 383–394
- Galizia, C.G., Sachse, S., Rappert, A., Menzel, R. (1999b) The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nat. Neurosci.* **2**, 473–478
- Gary, N.E. (1962) Chemical mating attractants in the queen honey bee. *Science* **136**, 773–774
- Gascuel, J., Masson, C. (1991) Developmental study of afferented and deafferented bee antennal lobes. *J. Neurobiol.* **22**, 795–810
- Grosmaître, X., Marion-Poll, F., Renou, M. (2001) Biogenic amines modulate olfactory receptor neurons firing activity in *Mamestra brassicae*. *Chem. Senses* **26**, 653–661
- Grozinger, C.M., Sharabash, N.M., Whitfield, C.W., Robinson, G.E. (2003) Pheromone-mediated gene expression in the honeybee brain. *Proc. Natl. Acad. Sci. USA* **100**, 14519–14525
- Gruntenko, N.E., Rauschenbach, I.Y. (2008) Interplay of JH, 20E and biogenic amines under normal and stress conditions and its effect on reproduction. *J. Insect Physiol.* **54**, 902–908
- Guidugli, K.R., Nascimento, A.M., Amdam, G.V., Barchuk, A.R., Omholt, S., Simões, Z.L.P., Hartfelder, K. (2005) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* **579**, 4961–4965
- Hammer, M. (1993) An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366**, 59–63
- Hansson, B.S., Anton, S. (2000) Function and morphology of the antennal lobe: new developments. *Annu. Rev. Entomol.* **45**, 203–231
- Hansson, B.S., Christensen, T.A., Hildebrand, J.G. (1991) Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *J. Comp. Physiol. A.* **312**, 264–278
- Hansson, B.S., Ljungberg, H., Hallberg, E., Lofstedt, C. (1992) Functional specialization of olfactory glomeruli in a moth. *Science* **256**, 1313–1315
- Hansson, B.S., Anton, S., Christensen, T.A. (1994) Structure and function of antennal lobe neurons in the male turnip moth, *Agrotis segetum*. *J. Comp. Physiol. A.* **175**, 547–562
- Hansson, B.S., Almaas, T.J., Anton, S. (1995) Chemical communication in heliothine moths. V. Antennal lobe projection patterns of pheromone-detecting olfactory receptor neurons in the male *Heliothis virescens* (*Lepidoptera: Noctuidae*). *J. Comp. Physiol. A.* **177**, 535–543
- Harano, K., Sasaki, K., Nagao, T. (2005) Depression of brain dopamine and its metabolite after mating in European honeybee (*Apis mellifera*) queens. *Naturwissenschaften* **92**, 310–313
- Harris, J.W., Woodring, J. (1995) Elevated brain dopamine levels associated with ovary development in queenless worker honey bees (*Apis mellifera* L.). *Comp. Biochem. Physiol.* **111**, 271–279
- Hasegawa, M., Asanuma, S., Fujiyuki, T., Kiya, T., Sasaki, T., Endo, D., Morioka, M., Kubo, T. (2009) Differential gene expression in the mandibular glands of queen and worker honeybees, *Apis mellifera* L.: Implications for caste-selective aldehyde and fatty acid metabolism. *Insect Biochem. Molec. Biol.* **39**, 661–667
- Hildebrand, J.G., Shepherd, G.M. (1997) Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu. Rev. Neurosci.* **20**, 595–631
- Honey Bee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* **443**, 931–949

- Hoover, S.R., Keeling, C., Winston, M., Slessor, K. (2003) The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**, 477–480
- Howard, R.W., Blomquist, G.J. (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* **50**, 371–393
- Huang, Z.-Y., Robinson, G.E., Tobe, S.S., Yagi, K., Strambi, C., Strambi, A., Stay, B. (1991) Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *J. Insect Physiol.* **37**, 733–741
- Humphries, M.A., Mustard, J.A., Hunter, S.J., Mercer, A., Ward, V., Ebert, P.R. (2003) Invertebrate D2 type dopamine receptor exhibits age-based plasticity of expression in the mushroom bodies of the honeybee brain. *J. Neurobiol.* **55**, 315–330
- Jarriault, D., Gadenne, C., Rospars, J.-P., Anton, S. (2009) Quantitative analysis of sex-pheromone coding in the antennal lobe of the moth *Agrotis ipsilon*: a tool to study network plasticity. *J. Exp. Biol.* **212**, 1191–1201
- Jarriault, D., Gadenne, C., Lucas, P., Rospars, J.P., Anton, S. (2010) Transformation of the sex pheromone signal in the noctuid moth *Agrotis ipsilon*: from peripheral input to antennal lobe output. *Chem. Senses* **35**, 705–715
- Joerges, J., Kuettner, A., Galizia, C.G., Menzel, R. (1997) Representations of odors and odor mixtures visualized in the honeybee brain. *Nature* **387**, 285–288
- Kaatz, H.-H., Hildebrandt, H., Engels, W. (1992) Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J. Comp. Physiol. B.* **162**, 588–592
- Kaissling, K.E., Renner, M. (1968) Antennale Rezeptoren für queen substance und sterzelduft bei der Honigbiene. *J. Comp. Physiol. A.* **59**, 357–361
- Karlson, P., Luscher, M. (1959) ‘Pheromones’: a new term for a class of biologically active substances. *Nature* **183**, 55–56
- Karlson, P., Sekeris, C.E. (1962) N-acetyl-dopamine as sclerotizing agent of the insect cuticle. *Nature* **195**, 183–184
- Katzav-Gozansky, T., Soroker, V., Ibarra, F., Francke, W., Hefetz, A. (2001) Dufour’s gland secretion of the queen honeybee (*Apis mellifera*): an egg discriminator pheromone or a queen signal? *Behav. Ecol. Sociobiol.* **51**, 76–86
- Keeling, C.I., Slessor, K.N., Higo, H.A., Winston, M.L. (2003) New components of the honey bee (*Apis mellifera* L.) queen retinue pheromone. *Proc. Natl. Acad. Sci. USA* **100**, 4486–4491
- Keller, L. (2009) Adaptation and the genetics of social behaviour. *Philosophical Trans. R. Soc. B: Biol. Sci.* **364**, 3209–3216
- Keller, L., Nonacs, P. (1993) The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav.* **45**, 787–794
- Kirchhof, B.S., Homberg, U., Mercer, A.R. (1999) Development of dopamine-immunoreactive neurons associated with the antennal lobes of the honey bee, *Apis mellifera*. *J. Comp. Neurol.* **411**, 643–653
- Kirschner, S., Kleineidam, C.J., Zube, C., Rybak, J., Grünewald, B., Rössler, W. (2006) Dual olfactory pathway in the honeybee, *Apis mellifera*. *J. Comp. Neurol.* **499**, 933–952
- Kocher, S.D., Grozinger, C.M. (2011) Cooperation, conflict, and the evolution of queen pheromones. *J. Chem. Ecol.* 1–13
- Kreissl, S., Eichmüller, S., Bicker, G., Rapus, J., Eckert, M. (1994) Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. *J. Comp. Neurol.* **348**, 583–595
- Krieger, J., Raming, K., Dewer, Y.M.E., Bette, S., Conzelmann, S., Breer, H. (2002) A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. *Eur. J. Neurosci.* **16**, 619–628
- Kurshan, P.T., Hamilton, I.S., Mustard, J.A., Mercer, A. R. (2003) Developmental changes in expression patterns of two dopamine receptor genes in mushroom bodies of the honeybee, *Apis mellifera*. *J. Comp. Neurol.* **466**, 91–103
- Laughlin, J.D., Ha, T.S., Jones, D.N.M., Smith, D.P. (2008) Activation of pheromone-sensitive neurons is mediated by conformational activation of pheromone-binding protein. *Cell* **133**, 1255–1265
- Le Conte, Y., Hefetz, A. (2008) Primer pheromones in social hymenoptera. *Annu. Rev. Entom.* **53**, 523–542
- Linster, C., Sachse, S., Galizia, C.G. (2005) Computational modeling suggests that response properties rather than spatial position determine connectivity between olfactory glomeruli. *J. Neurophysiol.* **93**, 3410–3417
- Maisonnette, A., Alaux, C., Beslay, D., Crauser, D., Gines, C., Plettner, E., Le Conte, Y. (2010) New insights into honey bee (*Apis mellifera*) pheromone communication. Is the queen mandibular pheromone alone in colony regulation? *Front. Zool.* **7**, 18
- Malka, O., Karunker, I., Yeheskel, A., Morin, S., 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
- Mayer, M., Doolittle, R. (1995) Synergism of an insect sex pheromone specialist neuron: implications for component identification and receptor interactions. *J. Chem. Ecol.* **21**, 1875–1891
- Meng, L.Z., Wu, C.H., Wicklein, M., Kaissling, K.E., Bestmann, H.J. (1989) Number and sensitivity of three types of pheromone receptor cells in *Antheraea pernyi* and *A. polyphemus*. *J. Comp. Physiol. A.* **165**, 139–146
- Mercer, A., Menzel, R. (1982) The effects of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honey bee *Apis mellifera*. *J. Comp. Physiol. A.* **145**, 363–368

- Mercer, A.R., Mobbs, P.G., Davenport, A.P., Evans, P.D. (1983) Biogenic-amines in the brain of the honeybee, *Apis mellifera*. *Cell Tissue Res.* **234**, 655–677
- Müller, Abel, Brandt, Zöckler, Menzel (2002) Differential parallel processing of olfactory information in the honeybee, *Apis mellifera* L. *J. Comp. Physiol. A.* **188**, 359–370
- Mustaparta, H. (1996) Central mechanisms of pheromone information processing. *Chem. Senses* **21**, 269–275
- Mustard, J.A., Pham, P.M., Smith, B.H. (2010) Modulation of motor behavior by dopamine and the D1-like dopamine receptor *AmDOP2* in the honey bee. *J. Insect Physiol.* **56**, 422–430
- Naumann, K. (1991) Grooming behaviors and the translocation of queen mandibular gland pheromone on worker honey-bees (*Apis mellifera* L.). *Apidologie* **22**, 523–531
- Naumann, K., Winston, M.L., Slessor, K.N., Prestwich, G.D., Webster, F.X. (1991) Production and transmission of honey-bee queen (*Apis mellifera* L.) mandibular gland pheromone. *Behav. Ecol. Sociobiol.* **29**, 321–332
- Naumann, K., Winston, M.L., Slessor, K.N. (1993) Movement of honey-bee (*Apis mellifera* L.) queen mandibular gland pheromone in populous and unpopulous colonies. *J. Insect Behav* **6**, 211–223
- Nelson, C.M., Ihle, K.E., Fondrk, M.K., Page, R.E., Amdam, G.V. (2007) The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol.* **5**, 673–677
- O'Connell, R.J., Beauchamp, J.T., Grant, A.J. (1986) Insect olfactory receptor responses to components of pheromone blends. *J. Chem. Ecol.* **12**, 451–467
- Ochieng, S.A., Anderson, P., Hansson, B.S. (1995) Antennal lobe projection patterns of olfactory receptor neurons involved in sex pheromone detection in *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Tissue Cell* **27**, 221–232
- Pankiw, T., Winston, M.L., Plettner, E., Slessor, K.N., Pettis, J.S., Taylor, O.R. (1996) Mandibular gland components of European and Africanized honey bee queens (*Apis mellifera* L.). *J. Chem. Ecol.* **22**, 605–615
- Pankiw, T., Huang, Z.Y., Winston, M.L., Robinson, G.E. (1998) Queen mandibular gland pheromone influences worker honey bee (*Apis mellifera* L.) foraging ontogeny and juvenile hormone titers. *J. Insect Physiol* **44**, 685–692
- Pankiw, T., Winston, M.L., Fondrk, M.K., Slessor, K.N. (2000) Selection on worker honeybee responses to queen pheromone (*Apis mellifera* L.). *Naturwissenschaften* **87**, 487–490
- Paul, R.K., Takeuchi, H., Matsuo, Y., Kubo, T. (2005) Gene expression of ecdysteroid-regulated gene E74 of the honeybee in ovary and brain. *Insect Mol. Biol.* **14**, 9–15
- Paul, R.K., Takeuchi, H., Kubo, T. (2006) Expression of two ecdysteroid-regulated genes, Broad-Complex and E75, in the brain and ovary of the honeybee (*Apis mellifera* L.). *Zool. Sci.* **23**, 1085–1092
- Pesenti, M.E., Spinelli, S., Bezirard, V., Briand, L., Pernollet, J.-C., Tegoni, M., Cambillau, C. (2008) Structural basis of the honey bee PBP pheromone and pH-induced conformational change. *J. Mol. Biol.* **380**, 158–169
- Pettis, J.S., Winston, M.L., Collins, A.M. (1995) Suppression of queen rearing in European and Africanized honey-bees *Apis mellifera* L. by synthetic queen mandibular gland pheromone. *Insectes Soc.* **42**, 113–121
- Pinto, L.Z., Bitondi, M.M.G., Simões, Z.L.P. (2000) Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxifen. *J. Insect Physiol.* **46**, 153–160
- Plettner, E., Slessor, K.N., Winston, M.L., Oliver, J.E. (1996) Caste-selective pheromone biosynthesis in honeybees. *Science* **271**, 1851–1853
- Plettner, E., Otis, G.W., Wimalaratne, P.D.C., Winston, M.L., Slessor, K.N., Pankiw, T., Punchiheva, P.W. K. (1997) Species- and caste-determined mandibular gland signals in honeybees (*Apis*). *J. Chem. Ecol.* **23**, 363–377
- Pophof, B. (2002) Octopamine enhances moth olfactory responses to pheromones, but not those to general odorants. *J. Comp. Physiol. A* **188**, 659–662
- Rachinsky, A., Hartfelder, K. (1990) Corpora allata activity, a prime regulating element for caste-specific juvenile hormone titre in honey bee larvae. *J. Insect Physiol.* **36**, 189–194
- Rehder, V., Bicker, G., Hammer, M. (1987) Serotonin-immunoreactive neurons in the antennal lobes and suboesophageal ganglion of the honeybee. *Cell Tissue Res.* **247**, 59
- Ressler, K.J., Sullivan, S.L., Buck, L.B. (1993) A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* **73**, 597
- Richard, F.-J., Aubert, A., Grozinger, C. (2008) Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biol.* **6**, 50
- Riemensperger, T., Voller, T., Stock, P., Buchner, E., Fiala, A. (2005) Punishment prediction by dopaminergic neurons in *Drosophila*. *Curr. Biol.* **15**, 1953–1960
- Robertson, H.M., 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. (1992) Regulation of division of labor in insect societies. *Ann. Rev. Entomol.* **37**, 637–665
- Rospars, J.P. (1988) Structure and development of the insect antennodeutocerebral system. *Int. J. Insect Morphol. Embryol.* **17**, 243–294

- Sachse, S., Rappert, A., Galizia, C.G. (1999) The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. *Eur. J. Neurosci.* **11**, 3970–3982
- Sakurai, T., Nakagawa, T., Mitsuno, H., Mori, H., Endo, Y., Tanoue, S., Yasukochi, Y., Touhara, K., Nishioka, T. (2004) Identification and functional characterization of a sex pheromone receptor in the silkwmoth *Bombyx mori*. *Proc. Natl. Acad. Sci. USA* **101**, 16653–16658
- Sandoz, J.-C. (2006) Odour-evoked responses to queen pheromone components and to plant odours using optical imaging in the antennal lobe of the honey bee drone *Apis mellifera* L. *J. Exp. Biol.* **209**, 3587–3598
- Schäfer, S., Rehder, V. (1989) Dopamine-like immunoreactivity in the brain and suboesophageal ganglion of the honeybee. *J. Comp. Neurol.* **280**, 43–58
- Scheiner, R., Baumann, A., Blenau, W. (2006) Aminergic control and modulation of honeybee behaviour. *Curr Neuropharm* **4**, 259–276
- Schneider, D., Schulz, S., Priesner, E., Ziesmann, J., Francke, W. (1998) Autodetection and chemistry of female and male pheromone in both sexes of the tiger moth *Panaxia quadripunctaria*. *J. Comp. Physiol A* **182**, 153–161
- Schroll, C., Riemensperger, T., Bucher, D., Ehmer, J., Völler, T., Erbguth, K., Gerber, B., Hendel, T., Nagel, G., Buchner, E. (2006) Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr. Biol.* **16**, 1741–1747
- Schulz, D.J., Robinson, G.E. (1999) Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol A* **184**, 481–488
- Schulz, D.J., Robinson, G.E. (2001) Octopamine influences division of labor in honey bee colonies. *J. Comp. Physiol. A* **187**, 53–61
- Schulz, D.J., Sullivan, J.P., Robinson, G.E. (2002) Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. *Horm. Behav.* **42**, 222–231
- Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., Heisenberg, M. (2003) Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J. Neurosci.* **23**, 10495–10502
- Seeley, T.D., Fell, R.D. (1981) Queen substance production in honey bee (*Apis mellifera*) colonies preparing to swarm (*Hymenoptera: Apidae*). *J. Kansas Entomol. Soc.* **54**, 192–196
- Slessor, K.N., Kaminski, L.A., King, G., Borden, J.H., Winston, M.L. (1988) Semiochemical basis of the retinue response to queen honey bees. *Nature* **332**, 354–356
- Slessor, K.N., Kaminski, L.A., King, G.G.S., Winston, M.L. (1990) Semiochemicals of the honeybee queen mandibular glands. *J. Chem. Ecol.* **16**, 851–860
- Slessor, K.N., Winston, M.L., Le Conte, Y. (2005) Pheromone communication in the honeybee (*Apis mellifera* L.). *J. Chem. Ecol.* **31**, 2731–2745
- Sun, X.-J., Fonta, C., Masson, C. (1993) Odour quality processing by bee antennal lobe interneurons. *Chem. Senses* **18**, 355–377
- Szyszka, P., Ditzen, M., Galkin, A., Galizia, C.G., Menzel, R. (2005) Sparsening and temporal sharpening of olfactory representations in the honeybee mushroom bodies. *J. Neurophysiol.* **94**, 3303–3313
- Takeuchi, H., Paul, R.K., Matsuzaka, E., Kubo, T. (2007) EcR-A expression in the brain and ovary of the honeybee (*Apis mellifera* L.). *Zool. Sci.* **24**, 596–603
- Taylor, D.J., Robinson, G.E., Logan, B.J., Laverty, R., Mercer, A.R. (1992) Changes in brain amine levels associated with the morphological and behavioral development of the worker honeybee. *J. Comp. Physiol. A* **170**, 715–721
- Tobe, S.S., Stay, B. (1985) Structure and regulation of the corpus allatum. In *Advances in Insect Physiology*, vol. 18, pp. 305–432. Academic Press
- Vareschi, E. (1971) Duftunterscheidung bei der Honigbiene — Einzelzell-Ableitungen und Verhaltensreaktionen. *Z. vgl. Physiol.* **75**, 143–173
- Vassar, R., Ngai, J., Axel, R. (1993) Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* **74**, 309–318
- Vergoz, V., Roussel, E., Sandoz, J.C., 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., Mercer, A.R. (2007b) Queen pheromone blocks aversive learning in young worker bees. *Science* **317**, 384–386
- Vergoz, V., McQuillan, H.J., Geddes, L.H., Pullar, K., Nicholson, B.J., Paulin, M.G., Mercer, A.R. (2009) Peripheral modulation of worker bee responses to queen mandibular pheromone. *Proc. Natl. Acad. Sci. USA* **106**, 20930–20935
- Vickers, N.J., Christensen, T.A., Hildebrand, J.G. (1998) Combinatorial odor discrimination in the brain: attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. *J. Comp. Neurol.* **400**, 35–56
- Vogt, R.G., Riddiford, L.M. (1981) Pheromone binding and inactivation by moth antennae. *Nature* **293**, 161–163
- Von Nickisch-Roseneck, E., Krieger, J., Kubick, S., Laage, R., Strobel, J., Strotmann, J., Breer, H. (1996) Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). *Insect Biochem. Molec. Biol.* **26**, 817–827
- Vosshall, L.B., Hansson, B.S. (2011) A unified nomenclature system for the insect olfactory coreceptor. *Chem. Senses* **36**, 497

- Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., Axel, R. (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**, 725–736
- Vosshall, L.B., Wong, A., Axel, R. (2000) An olfactory sensory map in the fly brain. *Cell* **102**, 147
- Wagener-Hulme, C., Kuehn, J.C., Schulz, D.J., Robinson, G.E. (1999) Biogenic amines and division of labor in honey bee colonies. *J. Comp. Physiol. A* **184**, 471–479
- Wanner, K.W., Nichols, A.S., Walden, K.K.O., Brockmann, A., Luetje, C.W., 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., Robinson, G.E. (2003) Gene expression profiles in the brain predict behavior in individual honey bees. *Science* **302**, 296–299
- Winston, M.L. (1987) *The biology of the honey bee*. Harvard University Press
- Winston, M.L., Slessor, K.N. (1992) The essence of royalty - honey bee queen pheromone. *Am. Sci.* **80**, 374–385
- Winston, M.L., Higo, H.A., 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*). *Ann. Entomol. Soc. Am.* **83**, 234–238
- Winston, M.L., Higo, H.A., Colley, S.J., Pankiw, T., 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
- Wossler, T.C., Crewe, R.M. (1999) The releaser effects of the tergal gland secretion of queen honeybees (*Apis mellifera*). *J. Insect Behav.* **12**, 343–351
- Yamazaki, Y., Shirai, K., Paul, R.K., Fujiyuki, T., Wakamoto, A., Takeuchi, H., Kubo, T. (2006) Differential expression of HR38 in the mushroom bodies of the honeybee brain depends on the caste and division of labor. *FEBS Lett.* **580**, 2667–2670
- Yamazaki, Y., Kiuchi, M., Takeuchi, H., Kubo, T. (2011) Ecdysteroid biosynthesis in workers of the European honeybee *Apis mellifera* L. *Insect Biochem. Molec. Biol.* **41**(5), 283–293