Chemical communication in the honeybee (*Apis mellifera* L.): a review

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ABSTRACT: An important area of physiology of the honeybee (*Apis mellifera*) is chemical communication between individuals and castes in the swarm, which maintains its integrity and function. The highly complex social organization of honeybees is mediated through pheromones. Releaser pheromones cause rapid changes in the behaviour of the recipient, while primer pheromones have relatively slow and long-term effects on the physiology and behaviour of the recipient. Queen retinue pheromone (QRP) is a blend of the nine compounds (9-oxo-(E)-2-decenoic acid, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid, methyl p-hydroxybenzoate, 4-hydroxy-3-methyoxyphenylethanol, methyl oleate, coniferyl alcohol, palmityl alcohol, and linolenic acid) and acts as a releaser pheromone by attracting worker bees to the queen. QRP also acts as a primer pheromone by physiologically inhibiting the ovary development of worker bees. An essential component of QRP, 9-oxo-(E)-2-decenoic acid, acts as a long-distance sex pheromone. Defensive behaviour of honeybees is induced and modulated by alarm pheromones. The essential alarm pheromone component is isopentyl acetate (IPA). The unsaturated derivative of IPA, 3-methyl-2-buten-1-yl acetate, was found in colonies of Africanized honeybees. The Nasanov gland of worker bees produces a pheromone (a blend of nerol, geraniol, (E)- and (Z)-citral, nerolic acid, geranic acid and (E,E)-farnesol) that acts as an attracting signal. This pheromone is used for aggregation (during swarming). Adult worker bees also produce a substance, ethyl oleate, that has a priming effect. Ethyl oleate is produced by adult forager bees and acts as a chemical inhibitory factor to delay age at onset of foraging (the presence of older worker bees causes a delayed onset of foraging in younger individuals). Chemical cues on the surface of larvae called a brood pheromone (ethyl and methyl esters of palmitic, linoleic, linolenic, stearic, and oleic acids, E-β-ocimene) are important in the communication between brood and worker bees. This pheromone modulates the feeding behaviour of worker bees, inhibits the activation of the worker ovary, induces worker bees to cap brood cells, increases the activity of the hypopharyngeal glands of nurse bees and modulates the behavioural maturation of worker bees.

Keywords: pheromone; worker bee; drone; queen; brood; interaction

Contents

1. Introduction

The honeybee (*Apis mellifera*) belongs to the Hymenoptera insects that live in social groups with a fixed function of individuals (including their rotation during the ontogeny of individuals), resulting in a swarm, which works to some extent, as an organism. Social living bees have been domesticated to generate bee products in sufficient quantities; wild solitary Hymenoptera also collect honey, however, in amounts unusable for humans. Similar to the rearing of livestock honeybee

breeding has undergone a long evolution. Despite modernization it retains some specifics which remain unchanged – such as the acquisition of bee products being dependent on the free movement of worker bees over the landscape. Therefore, even domestic species, which is what the honeybee is regarded as today, require original instincts, including communication and orientation ability. A fundamental prerequisite for the integrity of swarms is communication between individuals. One of the main manners of communication is chemical communication. Detailed knowledge of the biology of this species is therefore an essential prerequisite for successful breeding, including health care. The issues in bee communication are many in number; this paper gives a basic overview.

2. Interactions between queens and worker bees

Slessor et al. (2005) reported many aspects of pheromone communication in the honeybee. The authors described in detail the components of the queen retinue pheromone (QRP) that is attractive to worker bees. It is known that QRP entices worker bees to lick and antennate the queen to gather a small sample of this attractive blend. The essential component of this blend is 9-oxo-(E)-2-decenoic acid (ODA). Other components include two enantiomers of ODA's biosynthetic precursor, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid (HDA), and two aromatic components methyl *p*-hydroxybenzoate (HOB) and 4-hydroxy-3-methyoxyphenylethanol (HVA). All these compounds are products of the mandibular glands and the blend is called the queen mandibular pheromone (QMP). Individual components are not by themselves attractive. Only when all five components are combined does the blend elicit the full retinue response. It has become evident that some strains of honeybees do not find synthetic QMP at all attractive; thus there exist additional substances involved in the retinue response. Methyl oleate, coniferyl alcohol, palmityl alcohol, and linolenic acid have been identified as further synergistic substances. Three new fatty-acid-derived constituents are not of mandibular gland origin, and therefore the terminology shift to QRP was necessary. The complete identity of the QRP is still not fully defined. Except for methyl oleate (a synergistic component of QRP) other queen esters (the palmitates, oleates, ethyl stearate, ethyl and methyl palmitoleate) have been found. These queen esters are distributed as passive chemical passengers in the queen bouquet because they are not attractive. They function as primer pheromones, which affect the physiology of the worker bees. For example, ethyl palmitate is apparently an active agent contributing to the queen's ability to inhibit worker ovarian development (Slessor et al., 2005). Primer pheromones are efficient means for maintaining social harmony in the colony and their effects are important. These pheromones act by affecting the physiology of the recipients with a subsequent shift in their behaviour (Le Conte and Hefetz, 2008). Many primer pheromones also have a releaser effect. For example, queen retinue pheromone (QRP) acts as a releaser pheromone by attracting worker bees to the queen and as a primer pheromone by physiologically inhibiting worker ovary development (Wanner et al., 2007). Primer functions are associated with brood and queen retinue pheromones (Pankiw, 2004a). The most abundant queen mandibular gland pheromone component, 9-keto-2-(E)-decenoic acid (9-ODA) and two aromatic components, 4-hydroxy-3-hydroxyphenylethanol (HVA) and methyl *p*-hydroxybenzoate (HOB) are similarly transmitted (Naumann et al., 1991, 1992). Thus, the queen mandibular gland pheromone complex is transferred through the nest as a unit. After being secreted onto the body surface of the queen it is removed by worker bees in the queen's retinue, especially those who come into contact with the queen through their mouthparts. Other worker bees acquire pheromones via direct contact with retinue bees or with other worker bees that have already acquired the queen pheromone. Grooming behaviour also contributes to the transfer of pheromone. Naumann (1991) showed in his study that self-grooming results in the translocation of synthetic queen mandibular gland pheromone from the mouthparts and head to the abdomen and limbs of honeybee workers. The queen mandibular gland pheromone can also reach worker bees through queen or worker "footprints" onto comb wax. Fischer and Grozinger (2008) tested the effects of queen mandibular pheromone (QMP) exposure on starvation resistance, lipid storage, and gene expression in the fat bodies of worker bees. QMP can indeed modify nutrient storage pathways, because QMP-treated bees survived much longer compared to control bees when starved and also had higher lipid levels. Expression of vitellogenin RNA, which

encodes a yolk protein, was also higher in the fat bodies of QMP-treated bees. Bees involved in brood care (nurses) have higher lipid stores than forager bees. QMP thus slows the transition from nursing to foraging. Queen mandibular pheromone (QMP) also regulates the timing of colony-level reproduction (swarming). This mechanism is described by Pankiw (2004a). QMP also inhibits queen rearing behaviours. When the titre of QMP decreases below inhibitory thresholds, worker bees initiate queen rearing. As colonies grow, the worker population increases in size and the amount of QMP which reaches individual bees decreases due to a dilution effect and restricted movement. Worker bees are released from the inhibitory effects of QMP on queen rearing and begin to rear queens in preparation for swarming. Queen pheromones regulate the reproductive division of labour; specifically the queens prevents the reproduction of workers. The presence of the brood has the same effect. Dietemann et al. (2006) found that an invasive lineage of parasitic Cape honeybee (*Apis mellifera capensis*) worker bees occurring in the range of *A. m. scutellata* has resistance to reproductive regulation by host queens. Worker reproduction in *A. m. capensis* is associated with the production of queen-like pheromones. *Apis mellifera scutellata* queens do not prevent the production of queen-like mandibular gland compounds by parasites. Parasitic worker bees produce these signals despite the presence of a queen. This mechanism allows *A. m. capensis* worker bees to usurp resources and reproduction in foreign colonies. In the study of Kocher et al. (2009) it was suggested that the queen pheromone blend is modulated by the reproductive status of the queens. Worker bees of appropriate bee colonies can detect these subtle differences and are more responsive to queens with higher reproductive potential. Queen honeybee pheromone modulates many aspects of worker physiology and behaviour and is critical for colony social organization. This pheromone is produced in the mandibular glands and it differs between virgin and mated, laying queens. In comparison with virgin queens, naturally mated queens, and queens experimentally inseminated with either semen or saline the following differences were found: naturally mated queens had the most activated ovaries and the most distinct chemical profile in their glands, while the ovary activation and chemical profiles of other experimental queens (instrumentally inseminated queens and virgins) were distinct. Pheromone samples were collected two days after mating or insemination. Natural mating probably has considerable importance, because the experimentally inseminated queens were intermediate between virgins and naturally mated queens, whereas no significant differences between semen- and saline-inseminated queens were found. Thus, the results suggest, that the insemination process or fluid volume is responsible for stimulating these early post-mating changes in honeybee queens. In all aculeate hymenopteran females the poison gland and the Dufour gland is associated with the sting apparatus (Abdalla and Cruz-Landim, 2001). Secretion from the Dufour gland is caste-regulated. Martin and Jones (2004) found that C28–C38 esters are associated with queens and alcohol eicosenol is associated with non-laying worker bees. Both esters and eicosenol are biochemically similar compounds (both are products of fatty acid biosynthesis). Egg-laying worker bees in queenless colonies produce both esters and eicosenol. Egg-laying anarchistic worker bees and parasitic Cape worker bees from queenright colonies even show the typical queen pattern (i.e., esters present and eicosenol absent). Dufour's gland pheromone operates apparently as a fertility signal. Dor et al. (2005) conducted experiments in which two worker bees were confined in a small arena. Between worker bees a hierarchy of reproductive dominance was established, i.e., one worker demonstrated greater ovarian development than her paired bee. Ovarian development was tightly linked to production of queen-like Dufour's gland secretion. There was a particular increase in the production of queen-like esters. Their occurrence can serve as a reliable fertility signal. Advertising ovarian status may recruit helper worker bees with less developed ovaries to assist their nestmates. Gilley et al. (2006) used the method of solid-phase microextraction (SPME, 65 μm PDMS-DVB fiber) to sample the volatile compounds emitted by live honeybee queens and workers. They detected nine compounds and four of these were present only in queens. One of these four queen-specific compounds, identified as E-β-ocimene, was expressed fully only in established mated queens and may signal the diploid egg-laying activity. The three remaining compounds (including one identified as 2-phenylethanol) were associated with unmated queens. The five compounds that the authors detected in both queens and workers were hydrocarbons and apparently function in social recognition.

3. Interactions between queens and drones

An elaborate system of chemical communication in honeybees has evolved primarily in the context of social behaviour and mating. Single components (or a mixture of components) of the queen mandibular gland secretion may have different functions. For example, the virgin queen uses the mandibular gland secretions to attract drones on her mating flights, whereas the mated queen uses mandibular gland secretions to signal her presence to worker bees in the hive (Brockmann et al., 2006). In the 1970s, 9-oxo-2-decenoic acid (9-ODA), the major component of the mandibular gland secretions, was shown to function as a sex pheromone. 9-ODA is viewed as the major long-distance sex attractant. Drones and virgin queens leave their colonies for mating flights. Drones gather at the drone congregation areas (estimated sizes range from 50 to 200 m in diameter) and here wait for virgin queens. Upon detection of the pheromone, drones initiate searching and chasing of the queen with only a few fast ones being successful (Brockmann et al., 2006). Wanner et al. (2007) identified an odorant receptor (Or) for the queen's 9-ODA in drone antennae. They assayed the pheromone responsiveness of four candidate receptors (AmOr10, -11, -18, and -170) by using *Xenopus* oocytes and electrophysiology. AmOr11 responded specifically to 9-ODA (EC_{50} = 280 ± 31 nM) and not to any of the other seven QRP components (9-hydroxy-2-decenoic acid, methyl *p*-hydroxybenzoate, 4-hydroxy-3-methyoxyphenylethanol, methyl oleate, coniferyl alcohol, 1-hexadecanol, and linolenic acid). 9-ODA is probably the only QRP component that acts as a long-distance sex pheromone (Wanner et al., 2007). Brockmann et al. (2006) suggested that other components of the queen's secretion play a role in the communication between sexes. (2E)-9-hydroxydecenoic acid (9-HDA) and (2E)-10-hydroxydecenoic acid (10-HDA) apparently act over a short range. These two compounds are not attractive to drones from a distance, but added to 9-ODA they increased the drone's contacts with a queen dummy. A similar increase in the number of drones making contact with the baited dummy was also found when tergite gland extracts were added to 9-ODA (Brockmann et al., 2006). The tergal gland secretion is composed of long chain fatty acids (major compound is (Z)-9-octadecenoic acid), long-chain esters (predominant decyl decanoate was detected in virgin queens) and a linear series of unsaturated hydrocarbons (Wossler and Crewe, 1999). Tergal gland alkenes probably do not function as sex pheromones. The production of queen tergal gland alkenes starts after mating. Smith et al. (1993) demonstrated in their experiments that the production of tergal gland alkenes is stimulated by natural mating and not by experimental insemination. It has long been recognized in the beekeeping industry that instrumentally inseminated queens are not as productive as naturally mated queens. Problems are observed with initial introduction and acceptance of the inseminated queens, rapid replacement of the introduced inseminated queen by a queen raised from her eggs and decreased brood production by inseminated queens. The tergal gland alkenes may play a key role in the care and acceptance of the queen and her eggs by worker bees in the hive (Smith et al., 1993). Rhodes et al. (2007) recorded changes in constituent levels from head extracts of queen with increasing age. Non-mated 7 day old queens had higher average levels of 9-HDA and 9-ODA and 10-HDA than mated seven day old queens. These results suggest that these particular three constituents have sex pheromone functions in the honeybee. 9-octadecenoic acid and decyl decanoate from the tergal gland may also participate in the communication between sexes.

4. Interactions between worker bees

One key advantage of eusociality is shared defence of the nest, brood, and stored food (Breed et al., 2004). Defence of the nest plays an important role in the biology of honeybees. Defensive behaviour is partly induced and modulated by pheromones. These alarm pheromones are produced in the mandibular gland and sting apparatus of worker bees (Pankiw, 2004a). Most honeybee alarm pheromone components are produced in the Koschewnikow gland and sting gland (Breed et al., 2004). Over 40 compounds (including precursor, intermediate and final biosynthetic products) have been identified from extracts of the worker sting apparatus (sting gland and Koschewnikow gland) (Pankiw, 2004a). About 15 components release one or more alarm behaviours (flying from the nest to locate the source of disturbance, pursuing, biting and stinging) (Pankiw, 2004a). Isopentyl acetate (isoamyl acetate, or IPA) was first identified as a defensive compound (Boch et al., 1962). IPA elicits more stinging activity than any of the other defen-

sive compounds and it also acts as a target-marking pheromone, guiding other defenders to the sting site (Pankiw, 2004a). Pickett et al. (1982) identified a less volatile component, (Z)-11-eicosenol, as another effective alarm pheromone component for inducing stinging behaviour. (Z)-11-eicosenol prolongs the activity of the more volatile IPA presumably by slowing down the evaporation of IPA. The blend of IPA and (Z)-11-eicosenol is active for a longer time than IPA alone (Pickett et al., 1982). Hunt et al. (2003) analyzed the alarm pheromone components from colonies of Africanized honeybees and they found an unsaturated derivative of IPA (3-methyl-2-buten-1-yl acetate, 3M2BA). This compound was present at levels of 0–38% the amount of IPA. Behavioural assays showed that 3M2BA recruited worker bees from hives at least as efficiently as IPA (Hunt et al., 2003). IPA and 3M2BA are synergistic in their natural ratios and a mixture of these two compounds recruited bees more efficiently than either of the compounds alone (Hunt et al., 2003). 3M2BA may be specific to certain populations of Africanized honeybees (Breed et al., 2004). The mandibular glands of worker bees also produce the alarm substance, 2-heptanone (2HPT; Pankiw, 2004a). This compound shows a much lower ability to attract guards from colony entrances and sting than IPA does (Breed et al., 2004). With increasing age of worker bees, the size of the mandibular gland and the amount of 2HPT progressively increases (Vallet et al., 1991). This means that the level of 2HPT is higher in foragers than in guard bees. It is therefore possible that 2HPT has other functions associated mainly with foraging behaviour. 2HPT showed a repulsive effect when added to sucrose solution which was visited by foragers and it may act as a repellent forage-marking scent (Vallet et al., 1991; Giurfa, 1993). Repellent scents are used to avoid the probing of flowers that have recently been depleted of nectar or pollen (Stout and Goulson, 2001). Worker bees strongly reject all flowers they have recently visited (Giurfa, 1993). Flowers just abandoned by the other worker are also rejected, in a lower although significant proportion (Giurfa, 1993). Differences in the response level of bees to their own marks or to partner's marks suggest that repellent scent-marks are primarily self-use signals (Giurfa, 1993). However, Stout and Goulson (2001) also observed interspecific interactions. Bumblebees (*Bombus lapidarius*, Apidae) avoided flowers recently visited by honeybees and vice versa. Honeybees rejected flowers that had previously been visited by bumblebees even more than those previously been visited by honeybees. The repellent forage-marking scents of bumblebees are tarsal secretions (long-chain alkanes and alkenes), which are less volatile than 2HPT (Goulson et al., 2000; Stout and Goulson, 2001). The molecular weight of 2HPT is 114, whereas bumblebee tarsal hydrocarbons have a molecular weight of ca. 300–400 (Stout and Goulson, 2001). Stout and Goulson (2001) also found that repellent forage-marking scents can be active for 40–60 min. This suggests that honeybees may use less volatile substances than 2HPT and it is possible that 2HPT is not the only repellent forage-marking scent that they use. If bumblebees and honeybees both use tarsal secretions as repellent scents, bumblebees, being larger than honeybees, may deposit larger quantities. This may cause a higher frequency of rejection of flowers previously visited by bumblebees. Foraging honeybees apparently also use long-term attractant scent marks. Stout and Goulson (2001) found that honeybees visited flowers that had been visited in the previous 24 h more often than flowers that had never been visited (the effect of repellent forage-marking scents disappeared and nectar was replenished in flowers). Honeybees apparently use scent marks – Nasanov secretions and (Z)-11-eicosenol – as attractants to mark rewarding flowers. The Nasanov gland of the worker honeybee is located just beneath the sixth intertergal membrane, near the dorsal surface of the abdomen (Wells et al., 1993). When a bee raises its abdomen and flexes the terminal segment, the intertergal membrane is exposed and volatile secretions of the Nasanov gland are released (Wells et al., 1993). Many of the components of the Nasanov pheromone are biochemically related (Slessor et al., 2005). In this mixture were identified nerol (Z)-3,7-dimethyl-2,6-octadien-1-ol), geraniol (E)-3,7-dimethyl-2,6-octadien-1-ol), (E) and (Z)-citral (E/Z)-3,7-dimethyl-2,6-octadienal), nerolic acid ((Z)-3,7-dimethyl-2,6-octadienoic acid), geranic acid (E)-3,7-dimethyl-2,6-octadienoic acid) and (E,E)-farnesol (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol; Free et al., 1981). These terpene derivatives contribute to the characteristic odour of several plant species (for example lemon-grass; Wells et al., 1993; Slessor et al., 2005). It was found that the Nasanov scent elicits clustering activity during swarming (Abdullah et al., 1990). When the swarm leaves the nest, the bees form an unstructured cloud that remains within 50 m of the old nest. Worker bees settle in various spots and form

small, incipient clusters until the queen joins one of the clusters. Worker bees rapidly crowd around the queen and emit attraction signals from their Nasanov glands, so that the cluster with the queen attracts bees from the other, queenless clusters (Janson et al., 2005) and the swarm gradually forms into a tight cluster. The Nasanov pheromone is associated with a variety of circumstances. It is used to recruit nestmates to a new nesting cavity in a swarming context, to mark the entrance of the nest (helping to orient lost or dislocated worker bees) and to mark profitable food and water sources (Wells et al., 1993; Sandoz et al., 2007). Honeybees are well known for their 'dance language' (Sandoz et al., 2007). Waggle dances are mechanical signals that are used by returning foragers to recruit other foragers to food, water, and nest cavities (von Frisch, 1967). In the communication of honeybees mechanical and chemical signals dominate, since they must be easily perceived by bees in the darkness that prevails inside the hive (Seeley, 1998). Thom et al. (2007) found that waggle-dancing bees produce and release four cuticular hydrocarbons (two alkanes, tricosane and pentacosane, and two alkenes, Z-(9)-tricosene and Z-(9)-pentacosene), from their abdomens into the air. These compounds are produced subcutaneously (they are not stored within a gland) and are present in only minute quantities on the surface of the cuticle in non-dancing worker bees (Thom et al., 2007). When these substances are injected into a hive, they significantly increase the number of foragers leaving the hive (Thom et al., 2007). This suggests that these compounds may play a pheromonal role in worker recruitment. A primary characteristic of eusocial life is a division of labour (Pankiw, 2004b). In a colony of honeybees there is a typical age-related division of labour among the worker bees, in which individuals perform different tasks at different ages (Robinson and Huang, 1998). Worker bees generally perform different tasks in the nest (i.e., cell cleaning, brood rearing, comb building, nectar ripening, caring for the queen and drones etc.) for the first three weeks of adult life and then venture outside to collect food and defend the nest when they get older (Kolmes et al., 1989; Huang and Robinson, 1992; Robinson and Huang, 1998). But division of labour in honeybee colonies is not rigid (Robinson and Huang, 1998; Leoncini et al., 2004). Worker bees can accelerate, delay, and even reverse their behavioural development in response to changes in colony or environmental conditions (Huang and Robinson, 1992, 1996). Removing older bees (foragers) from a colony accelerates the behavioural development of younger bees (some bees initiate foraging when they are as young as five days of age), while adding foragers delays behavioural development, and removing nurses reverses foragers to nursing tasks (Huang and Robinson, 1996; Leoncini et al., 2004; Pankiw, 2004b). A major influence in a worker's developmental rate is provided by her older sister foragers whose presence inhibits the behavioural maturation of younger bees (Huang and Robinson, 1996; Slessor et al., 2005). Leoncini et al. (2004) have identified a substance produced by adult forager honeybees, ethyl oleate (EO), which acts as a chemical inhibitory factor, delaying age of onset of foraging. EO was detected in different body parts (head, thorax, crop and the rest of the abdomen) of nurses and foragers (Leoncini et al., 2004). Foragers had approximately 30 times more EO in their crop (foregut specialized for storage of nectar and honey) than nurses did, despite comparable levels in the head, thorax, and the rest of the abdomen (Leoncini et al., 2004). This suggests that EO is transmitted via trophallaxis, the transfer of food by mouth from one individual to another (Crailsheim, 1998; Leoncini et al., 2004). Forager bees bring nectar, the main source of carbohydrates. They give the content of their crop preferentially to younger hive-mates, to food-storer bees that can again pass on a portion to other bees, but mainly deposit it into cells (Crailsheim, 1998). This nectar is processed into honey.

5. Interactions between adults and brood

In the communication between brood and worker bees a chemical cue on the surface of larvae called brood pheromone (BP) is important (Le Conte et al., 1995; Pankiw et al., 2008). BP is a blend of ten fatty-acid esters (methyl palmitate, methyl oleate, methyl stearate, methyl linoleate, methyl linolenate, ethyl palmitate, ethyl oleate, ethyl stearate, ethyl linoleate and ethyl linolenate; Le Conte et al., 1990; Le Conte et al., 2001). Some components are more active than others, but all ten individual compounds show some releaser pheromone effect on adult bees (Le Conte et al., 2001). The esters are present in different amounts and proportions as a function of caste and larval age (Le Conte et al., 1994/1995). Thus, nurses can recognize the various needs of larvae and provide them with optimal

care (Le Conte et al., 2006). Four esters, methyl linolenate, methyl linoleate, methyl oleate, and methyl palmitate, induce the worker bees to cap the cell with a thin cover of wax (Le Conte et al., 1994). They are produced in large quantities by the larvae during the capping (Le Conte et al., 1994, 1994/1995). Le Conte et al. (1995) tested BP as an additional chemical stimulus in the artificial rearing of the queens. They found that methyl stearate increases the acceptance of the queen cells, methyl linoleate enhances the amount of royal jelly deposited by the worker, and methyl palmitate increases the weight of the larvae. In addition to releaser effects on various aspects of brood care, BP also has primer effects (Le Conte et al., 2001, 2006). Methyl palmitate and ethyl oleate increase the activity of the hypopharyngeal glands, which produce proteinaceous material that is fed by nurse bees to larvae (Mohammedi et al., 1996; Le Conte et al., 2001). Brood pheromone also inhibits ovary development in worker bees similarly to the queen's pheromone (Mohammedi et al., 1998; Le Conte et al., 2001). It even seems that the presence of the unsealed brood provides an inhibitory signal stronger than the queen's pheromone (Kropacova and Haslbachova, 1971; Mohammedi et al., 1998). Mohammedi et al. (1998) showed that among the ten esters, ethyl palmitate and methyl linolenate are the compounds that are involved in the prevention of ovary development of bees. All of the ten esters (boiling point around 200 °C) generally known as brood pheromone are non-volatile and their movement is likely facilitated by worker to worker contact (Pankiw, 2004a; Maisonnasse et al., 2010). Very recently, a new highly volatile molecule, E-β-ocimene, has been identified in larvae (Maisonnasse et al., 2009). This brood pheromone component also acts as a primer pheromone with two actions on worker bee physiology: inhibition of worker ovaries (Maisonnasse et al., 2009) and acceleration of worker bee behavioural maturation (Maisonnasse et al., 2010). E-β-ocimene (boiling point 73 °C), which belongs to the terpene family, is volatile so and therefore has an aerial transmission and is easily dispersed within the colony (Maisonnasse et al., 2010). All worker bees in the nest can be in direct contact through this signal, such as the nurse (young bee), but also middle-aged bees, from ages 12–21 days, that specialize in nectar processing and nest maintenance but do not engage in the brood care (Johnson, 2010; Maisonnasse et al., 2010). E-β-ocimene could be the signal for the transition of middle-aged bees to foragers (Maisonnasse et al., 2010). Brood ester pheromone (the blend of 10 methyl and ethyl esters) also modulates the behavioural maturation of worker bees and its effects vary with dose (Le Conte et al., 2001; Maisonnasse et al., 2010). Low doses of brood ester pheromone accelerate foraging ontogeny, whereas high doses of this pheromone have the opposite effect (it slows down the progression of young bees towards the tasks typical of older bees; Le Conte et al. 2001; Maisonnasse et al., 2010). Young and old larvae emit different quantities of pheromones. E-β-ocimene is emitted principally by the young instars (L1, L2–3) while brood ester pheromone reaches a maximum value during the capping stage (L4–5; Maisonnasse et al., 2010). The young larvae (low need in nurses) promote foraging by emitting a low quantity of brood ester pheromone and a large amount of E-β-ocimene. In contrast, old larvae (high need in nurses), by producing a high quantity of brood ester pheromone, promote tending (keeping nurses in contact with them for a longer time; Maisonnasse et al., 2010).

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