ABSTRACTS:

Eusocial Insects: chemical ecology of social organisation at the genetic, behavioural and ecological levels:
Oral Presentations

In programme order

Session 3

The evolution of caste-specific chemical profiles and of queen pheromones in halictid bees

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In eusocial insects, chemical communication is crucial for mediating many aspects of social activities, especially the regulation of reproduction. Though queen signals are known to decrease ovarian activation of workers in highly eusocial species, little is known about their evolution. In contrast, some primitively eusocial species are thought to control worker reproduction through physical aggression by the queen rather than via pheromones, suggesting the evolutionary establishment of chemical signals with more derived sociality. However, studies supporting this hypothesis are largely missing. Socially polymorphic halictid bees, such as *Halictus rubicundus*, with social and solitary populations in both Europe and North America, offer excellent opportunities to illuminate the evolution of caste-specific signals. Here we compared the chemical profiles of social and solitary populations from both continents and tested whether (i) population or social level affect chemical dissimilarity and whether (ii) caste-specific patterns reflect a conserved queen signal. Our results demonstrate unique odor profiles of European and North American populations, mainly due to different isomers of n-alkenes and macrocyclic lactones; chemical differences may be indicative of phylogeographic drift in odor profiles. We also found common compounds overproduced in queens compared to workers in both populations, indicating a potential conserved queen signal. However, North American populations have a lower caste-specific chemical dissimilarity than European populations which raises the question if both use different mechanisms of regulating reproductive division of labor. Therefore, our study gives new insights into the evolution of eusocial behavior and the role of chemical communication in the inhibition of reproduction.

Keywords: Halictid bee, social behavior, chemical communication, regulation of reproduction, population dialect

Reproductive signaling in bees – The mighty queen, the power of peers and the role of brood

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Pheromones inducing sterility in the worker caste of social insects are one of the greatest puzzles in social evolution since they seemingly negate the worker reproductive interests and evolved in the absence of a shared interest between the producer and the receiver of the signals - a situation which calls for additional mechanisms ensuring signal honesty. While the role of the queen in regulating worker reproduction has grabbed most of the attention in selected model organisms, the roles of chemical signals produced by other players, such as the brood and nestmates, were poorly studied.

Bumble bees are primitively eusocial insects that go through several solitary and social phases, allowing the unique opportunity to study changes in regulatory patterns of reproduction as the colony develops. In my talk I will discuss some of latest progress in the study of reproductive division of labor in bumble bees. Particularly, the regulation of reproduction by multiple players in the colony (queen, nestmates and brood), the interplay between the use of behavioral and chemical means to control reproduction, the importance of social context in preventing cheating, and the genetic mechanisms underlying sterility-inducing pheromones.

Keywords: Pheromones, Reproduction, Social context, Mechanisms, Bumble bees

Reproductive dominance and the role of multiple pheromones sources in honey bees.

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Honey bee workers are potentially reproductive females and they can display both behavioural and physiological traits similar to those of the queen caste. Such false-queens are frequent in African subspecies of *Apis mellifera* and very common in the Cape honey bee making reproductive workers an ideal model system to investigate the role of pheromonal signals during the competition for reproduction. One of the challenges is that the same pheromones that help an individual gain reproductive dominance are used by the individual's opponents to subdue it. We have shown that exogenously applied pheromones on workers stimulates them to become a false-queen rather than inhibiting them. Here we present evidence of the role of multiple signals originating from different glandular sources and the role of diet on gaining reproductive dominance. Not only is the perception of the pheromones context dependent, we also showed that pheromones from mandibular, tergal and Dufour's gland working in synergy to allow individual workers (false queens) to establish reproductive dominance. The complex role of pheromonal signals in honey bees is used to investigate the evolution of this pheromone based communication system. Furthermore, understanding the role of pheromones in establishing dominance hierarchies in social insects like the honey bee deepens our understanding of the ultimate and proximate drivers shaping eusociality.

Keywords: competition; context dependent; communication; false-queens; hierarchies

The role of the host queen in regulating reproductive parasitism in *Apis mellifera* capensis laying workers

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The honeybee queen mandibular gland pheromone (QMP) maintains reproductive dominance by inhibiting ovary activation and secretion of queen-like pheromones in workers. Biosynthesis of the mandibular gland (MG) fatty acids starts with stearic acid which undergoes caste-selective hydroxylation and oxidation to produce the queen-associated (*E*)-9-oxodec-2-enoic acid (9-ODA) and (*E*)-9-hydroxydec-2-enoic acid (9-HDA), and the worker-associated 10-hydroxy-decanoic acid (10-HDAA) and (*E*)-10-hydroxydec-2-enoic acid (10-HDA). In a comparative study, we measured the fatty acid profiles, ovary activation and expression of genes encoding two Cytochrome P450s responsible for the caste-specific hydroxylation of acylated stearic acid, and also genes encoding the enzyme alcohol dehydrogenase (Adh), in the intraspecific socially parasitic *Apis mellifera capensis* workers ("clones") infesting *A. m. scutellata* colonies that were either queen-right (QR) or queen-less (QL). We show that clones infesting QL colonies primarily secreted the queen-associated 9-ODA and 9-HDA, had relatively low expression of the worker-associated Cytochrome P450s and fully activated ovaries. QR clones had an accumulation of 9-HDA and 10-HDA, relatively high expression of worker-associated Cytochrome P450 and inactive ovaries. This shows that while the QMP produced by *A. m. scutellata* queens chiefly inhibits dominance in *scutellata* workers at the level of hydroxylation of stearic acid, laying workers can bypass this inhibition. QMP acts on the hydroxylation products from these parasitic workers by inhibiting the oxidation of 9-HDA into the "queen substance" 9-ODA, shown by the significantly lower transcript levels of the enzyme alcohol dehydrogenase. This study contributes to our understanding of the evolution of reproductive division of labour in social insects.

Keywords: African honeybees; communication; mandibular gland; pheromones; social parasitism

Novelty seeking, attention and collective behavior in the honeybee, *Apis mellifera*

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Individual differences in learning can influence how animals respond to and communicate about their environment, which may nonlinearly shape how a social group accomplishes a collective task. There are few empirical examples of how differences in collective dynamics emerge from variation among individuals in cognition. Here, we use a naturally variable and heritable learning behavior called latent inhibition (LI) to show that interactions among individuals that differ in this cognitive ability drive collective foraging behavior in honeybee colonies. We artificially selected two distinct phenotypes: high-LI bees that ignore previously familiar stimuli in favor of novel ones and low-LI bees that learn familiar and novel stimuli equally well. We then provided colonies differentially composed of different ratios of these phenotypes with a choice between familiar and novel feeders. Colonies of predominantly high-LI individuals preferred to visit familiar food locations, while low-LI colonies visited novel and familiar food locations equally. Interestingly, in colonies of mixed learning phenotypes, the low-LI individuals showed a preference to visiting familiar feeders, which contrasts with their behavior when in a uniform low-LI group. We show that the shift in feeder preference of low-LI bees is driven by foragers of the high-LI phenotype dancing more intensely and attracting more followers. Our results reveal that cognitive abilities of individuals and their social interactions, which we argue relate to differences in attention, drive emergent collective outcomes.

Queen pheromone(s) in higher termites: a long quest with a happy-end

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Queens of advanced social insects maintain their reproductive dominance over sterile colony members using queen pheromones. While the empiric support for queen pheromone existence in termites is available for over half a century, little is known on the chemistry of these putative pheromones: the queen pheromone identified in 2010 in the lower termite *Reticulitermes speratus* remains the only termite queen pheromone characterized as yet. Current knowledge suggests that in small societies of socially primitive termites, the information on queen presence and fertility may be mediated via queen-specific signatures of cuticular hydrocarbons on the body surface of queens. By contrast, in socially advanced species, including higher termites, the queen pheromone is expected to be volatile so as to ensure the queen monopoly in the populous colonies inhabiting large nests. Indeed, the queen pheromone of *R. speratus*, living in large colonies, is a volatile blend. In my talk, I will overview our search for queen pheromones in higher termites, the most diversified and advanced clade of termites, during the past decade. I will show evidence of queen-specific volatiles in multiple South-American species of higher termites, including identification and synthesis of these queen pheromone candidates. I will show that these volatiles are secreted by the queens in high enantiomeric purity and in quantities correlated with the level of their fertility. And finally, I will report on the first experimental evidence that queen-specific volatiles in higher termites really do act as queen pheromones and inhibit the reproductive potential of nestmates.

Keywords: Termitidae; primer pheromone; queen dominance; (3*R*,6*E*)-nerolidol; (5*Z*,9*S*)-tetradec-5-en-9-olide

Behavioral and social context is necessary for the full effect of queen semiochemicals in *Bombus impatiens*

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Regulation of reproduction via chemical signaling has been shown in a number of social insect species. But few chemicals with this capacity have been identified and it remains largely unknown how semiochemicals are regulated by the environment and the social context in which they operate. We previously showed that in *Bombus impatiens,* the queens' strategy to monopolize worker reproduction changes with life stage, shifting from overt aggression to chemical signaling as the queen gets older. Particularly, old egg-laying queens exhibited a higher ratio of short to long cuticular hydrocarbons (CHCs). Here we investigated whether CHCs alone are able to inhibit worker reproduction and whether this effect depends on the context provided by the queen's behavior and the presence of eggs she lays. We examined the effect of the queen's CHC secretion on worker reproduction in three scenarios: (1) without a behavioral context; (2) with a behavioral context of a free-moving virgin queen, and (3) at the presence of free-moving virgin queen and newly laid eggs. Our results indicate that queen CHCs affect worker reproduction only when combined with a behavioral context and that the presence of eggs is indispensable for the full effect of the queen's signal. Our findings highlight the complexity of queen-worker interactions in *B. impatiens* and the role of social context in reproductive signaling.

Keywords: pheromones, social insects, social context; queen-worker interactions; honest signal

Eavesdropping into host communication: the bee louse *Braula coeca* selects its host using kairomones

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The bee louse Braula coeca had until recently a global distribution that coincided with its host the Western honey bee Apis mellifera L. The adult fly usually attaches itself to a worker and steals food out of the host's mouth. However, not all worker bees carry Braula and the mechanism used by the bee louse to select a particular host is poorly known. We sampled and analysed using gas chromatography, the mandibular gland secretions (MDG) of worker bees that were carrying and those not carrying Braula from queenright colonies of A. m. scutellata. MDG profiles were qualitatively identical containing the five main MDG components, but workers carrying Braula had proportionately more methyl p-hydroxybenzoate (HOB) and the queen substance 9-oxo-2(E)-decenoic acid (9-ODA). Quantitatively, bees with Braula had higher amounts of the pheromones with a mean of 6.02 µg per bee, compared to 3.62 µg per bee for those not carrying Braula. A multiple comparison between all the components in the MDG profiles shows that, irrespective of the colony sampled, bees carrying and those not carrying Braula are different in both the proportions and concentrations of pheromones except for the worker component 10-hydroxy decanoic acid (10-HDAA). Braula is thus capable of using kairomones as cue that allows it to benefit from throphallactic dominance by selecting individuals that have a higher probability of being fed so as to get enhanced access to food.

Keywords: Bee louse, pheromonal communication, Mandibular gland secretions, honeybee

Insights into reproductive processes and hierarchies within social insect societies

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The stability and integrity of the social insects' colony is established through effective pheromonal communication to regulate reproductive behaviour and dominance. Under normal conditions, the queen is the only one reproducing with group of workers that are functionally sterile. However, some honey bee workers do escape the reproductive regulatory mechanism and become reproductively active. This study investigated how honey bee workers that becomes reproductively active, exploit pheromonal communication to their advantage and how pheromones contribute to reproductive dominance and reproductive hierarchies. The results provide evidence for establishment of reproductive dominance through the use of pheromones from diverse glandular secretions acting synergistically or additively to regulate various processes in the colony. These complex interplay of pheromonal signals from different exocrine glands have both primer and releaser effects among the honey bee groups. This study provides additional understanding into how pheromones from various glandular secretions contribute to the evolution of reproductive dominance and reproductive division of labour within social insect societies.

Keywords: honey bees; behaviour; exocrine; evolution; secretions

ABSTRACTS:

Eusocial Insects: chemical ecology of social organisation at the genetic, behavioural and ecological levels:
Poster Presentations

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Poster Session 1

Avoidance response to low-quality pollen in foraging honeybees

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Deterrent substances present in food, like toxic and/or bitter compounds, can exert repelling responses. Pollen, the main protein resource for *Apis mellifera*, may present compounds that induce distasteful and/or malaise experiences. Although honeybee colonies avoid collecting some low-quality pollens, evidence supports that foragers themselves are not able to make foraging decisions based on pollen composition at the food sources. We hypothesize that assessment occurs after pollen is processed inside the nest, likely mediated by young bees. To unveil the mechanisms that enable foragers to avoid low-quality pollens, we performed dual-choice experiments with flying bees confined in cages (9x3x2m). We compared foragers' preferences for two monofloral-pollen sources before and after one of them was adulterated with amygdalin. The adulterated pollen was offered either: i) to all the bees inside the hive; ii) to foragers at the pollen source or iii) to young bees transiently isolated from the colony during the treatment. Controls with unadulterated pollens were included. Foragers significantly reduced their preferences for pollens that had been experienced as adulterated inside the hive (i). Interestingly, they could not avoid the adulterated pollen experienced directly at the food source (ii), but they did after the pollen was incorporated into the nest. Experienced young bees could not modify responses of inexperienced foragers (iii). Altogether, results suggest that pollen assessment requires the resource to be processed in the colony and rule out that experienced young bees alone could bias foraging preferences.

Keywords: amygdalin; *Apis mellifera*; foraging preferences; in-hive experiences; pollen avoidance

Heterogeneous chemical profiles of *Vespa velutina nigrithorax* alarm pheromone

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As major communication signals, pheromones have long been studied. In colonial organisms such as social insects, volatile compounds play a crucial role in interspecific interactions by helping regulate colony behavior. In certain circumstances, it is desirable to detect or suppress insect populations, as invasive species. Management's techniques have included lures, baits, and traps. Synthetic or natural insect pheromones are widely in use around the world for pest control. Such molecules can be used for pest management and or control strategies. Because of their success in establishment and ecological dominance, Asian hornets are considered as severe economic and ecological pests. Native to Southeast Asia *Vespa velutina nigrithorax* has spread throughout Europe. To identify the composition of the species' alarm pheromone and assess differences in chemical profiles among queens, foundresses, gynes, and workers, we employed gas chromatography-mass spectrometry. Twenty six compounds were identified in the venom gland (chain lengths: C_8 to C_{12}), the organ that produces the alarm pheromone. Venom gland composition differed quantitatively among the females (workers, gynes and queens). These results could help in the development of a pheromone-based trap for the control of this invasive hornet species.

Keywords: Yellow-legged hornet; Invasive species; Chemical communication; Vespidae; Defence