# The pheromones of laying workers in two honeybee sister species: *Apis cerana* and *Apis mellifera*

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## Abstract

When a honeybee colony loses its queen, workers activate their ovaries and begin to lay eggs. This is accompanied by a shift in their pheromonal bouquet, which becomes more queen like. Workers of the Asian hive bee *Apis cerana* show unusually high levels of ovary activation and this can be interpreted as evidence for a recent evolutionary arms race between queens and workers over worker reproduction in this species. To further explore this, we compared the rate of pheromonal bouquet change between two honeybee sister species of *Apis cerana* and *Apis mellifera* under queenright and queenless conditions. We show that in both species, the pheromonal components HOB, 9-ODA, HVA, 9-HDA, 10-HDAA and 10-HDA have significantly higher **amounts** in laying workers than in non-laying workers. In the queenright colonies of *A. mellifera* and *A. cerana* the ratios (9-ODA)/ (9-ODA+9-HDA+10-HDAA+10-HDA) are not significantly different between the two species, but in queenless *A. cerana* colonies the ratio is significant higher than in *A. mellifera*, suggesting that in *A. cerana*, the workers' pheromonal bouquet is dominated by the queen compound, 9-ODA. The amount of 9-ODA in laying *A. cerana* workers increase by over 585% compared to the non-laying workers, that is 6.75 times higher than in *A. mellifera* were laying workers only had 86% more 9-ODA compared to non-laying workers.

**Keywords:** *Apis cerana* – *Apis mellifera* – pheromonal bouquet – laying worker –queenless

## Introduction

In honeybee colonies, the queen is the only female individual capable of sexual reproduction. Pheromones secreted by the queens' mandibular gland can have an inhibitory affect on the ovarian activation of workers (Slessor, Kaminski et al. 1988; Winston, Slessor et al. 1989; Winston and Slessor 1992). The major components are 9-ODA, 9-HDA, 10-HDA, 10-HDAA and HOB (Crewe 1982; Plettner et al. 1996, 1997). However, when a colony loses its queen, the subsequent lack of queen pheromones allows many workers to activate their ovaries and lay unfertilized eggs, which normally develop into haploid males (drones) (Crozier 1975).

The proximate mechanisms that regulate worker ovary activation in the honey bees are well understood. Queens produce a variety of pheromones from multiple sources whose combined action inhibits ovary activation in workers. Chief among these are the pheromones secreted by the queen's mandibular glands (Butler 1959). Mandibular gland secretions of queens have a high proportion of 9-ODA, whereas the glands of workers produce secretions that are richer in 10-HDA (Plettner, Sutherland et al. 1995; Plettner, Slessor et al. 1996). Thus the ratio of 9-ODA/(9-ODA+10-HDA+10-HDAA) is a measure of how "queen-like" a mandibular gland bouquet is (Moritz, Simon et al. 2000; Hoover, Oldroyd et al. 2005; Schäfer et al. 2006), and the more 9-ODA circulating in a colony, the less likely *A. mellifera* workers are to activate their ovaries (Hoover, Keeling et al. 2003).

Ovary activation in workers appears to be accompanied by subtle changes in exocrine gland secretions. Dufour's gland secretions of laying workers differ from those of non-laying workers, especially in wax-type esters (Katzav-Gozansky, Soroker et al. 1997, 2001; Sole, Kryger et al. 2002). Furthermore, in some ants (Peeters, Monnin et al. 1999; Liebig, Peeters et al. 2000) and wasps (Sledge, Boscaro et al. 2001), ovary activation is correlated with changes in cuticular hydrocarbon profiles and may be used as cues by which workers recognize ovarian activation in other workers (Liebig, Peeters et al. 2000). However, this seems unlikely since ovarian activation and pheromones only covary (Hepburn et al. 1988; Plettner et al. 1993). False queens are laying workers that show queen-like characteristics in that they do not work and attract a court of workers as a queen would (Sakagami 1958).

The chemical complexity of the pheromone bouquets of queens and workers has been suggested as evidence that an evolutionary arms race for reproductive dominance has occurred (Katzav-Gozansky 2006). Often, laying workers produce a mandibular gland secretion with a composition very similar to that of the queen (Crewe and Velthuis 1980). As a consequence, these workers elicit behavioural patterns and prime

physiological responses in other subordinate workers that otherwise would only be observed in response to the presence of the queen (Velthuis, Ruttner et al. 1990). These workers are known as 'false queens'(Sakagami 1958) or 'pseudoqueens' (Velthuis, Ruttner et al. 1990). Pseudoqueens can suppress ovary activation in other workers, elicit retinue behaviour and escape worker policing. They also suppress the production of a queen-like pheromone signal in other workers (Moritz, Simon et al. 2000). Pseudoqueen development is particularly frequent in the Cape honeybee , *Apis mellifera capensis* (Neumann and Moritz 2002). Workers of this subspecies may develop a queen-like mandibular gland secretion, which, within a few days, is dominated by the queen substance (9-ODA)(Simon, Moritz et al. 2001). Since both queens and workers use the same biochemical pathways to produce either queen (9-ODA) or the worker substances (10-hydroxydecanoic acid, 10-HDAA; and 10-hydroxy-(E) 2-decenoic acid, 10-HDA) (Plettner et al. 1996, 1997), a worker's likelihood to be reproductive is detectable at the level of pheromone production. Reproductively dominant workers have been shown to swiftly develop a 9-ODA-dominated mandibular gland pheromone (Moritz, Simon et al. 2000; Zheng et al. 2010).

Curiously, however, workers of the Eastern hive bee *A. cerana* which is the honeybee sister species of *A. mellifera* have been reported to have high rates of ovary activation even under queenright conditions (Tan, Yang et al. 2009). Sakagami and Akahra (1958) reported that 10–20% of *A. cerana* workers in queenright colonies contained mature eggs in their ovaries (Sakagami and Akahra 1958). In queenless colonies, there are reports of up to 70% of workers having activated ovaries (Blanford 1923; Tan, Yang et al. 2009), much higher than workers of *A. mellifera* (about 40%: Page and Erickson 1988). Furthermore, unlike *A. mellifera*, *A. cerana* workers may continue to lay after the introduction of a new queen (Sakagami 1958).

The apparently high degree of reproductive conflict in *A. cerana* provides a valuable opportunity to further investigate the evolutionary arms race between queens and workers over worker reproduction in *Apis*. We compared the changes of pheromonal bouquet composition in both sister species and under queenright and queenless conditions. We predict that on average the *A. cerana* workers have a more queen-like pheromonal bouquet than *A. mellifera*. This would also provide evidence of an ongoing evolutionary arms race for reproductive dominance in this species.

## Materials and methods

### Study site

The experiments were conducted at the test apiary of Yunnan Agricultural University, Kunming, China in the spring of 2009. Pheromone composition analysis was conducted at Pretoria University, South Africa.

**Colonies**

Three colonies of *Apis cerana* and three *A. mellifera* colonies containing each two frames of brood and two of honey and pollen were used in the experiment. Six workers from all 6 colonies were collected and the ovarian status evaluated, confirming that these workers had ovaries of stage 1 (Hess 1942; Pirk, Boodhoo et al. 2010; Pirk et al. 2011). The heads of these workers were extracted in 200 µl dichloromethane (Sigma HPLC Grad, ≥ 99.9%) in GC bottles. Then these colonies were de-queened and checked daily for the appearance of queencells, which were removed. This continued until the workers began to lay and multiple eggs began to appear in individual cells. From each colony about 100 indoor workers were sampled randomly and dissected to check for ovary activation until 6 worker bees with fully developed ovaries, indicating that they were actively laying individuals. The heads of these workers were removed and kept as above process.

**Pheromones**

These samples were stored in 200 µL dichloromethane for at least 24 h to extract compounds of the mandibular gland. Half of the extracts were then evaporated to dryness under a stream of nitrogen, the other half was stored as a backup. The residues were redissolved in 10 µL of an internal standard (octanoic acid and tetradecane in dichloromethane) and 10 µL of bistrimethylsilyltrifluoroacetamide (Sigma). One microlitre of this solution was injected into an HP 6890 gas chromatograph fitted with a split-splitless inletand a 25 m x 0.32 mm methyl silicone-coated fused silica capillary (HP 1). Helium was used as carrier gas at a flow rate of 1 mL per min. The temperature of the oven was maintained at 60°C for 1 min, and then increased to 100°C (at a rate 50°C per min) and then to 220°C at a rate of 3°C per min. The final temperature was maintained for 10 min. Chromatograms were recorded and peak areas determined by using HP CHEMSTATION software (March 2006).

The mandibular gland compounds were identified based on the retention times of synthetic compounds and on their retention times compared with the internal standards. The 'queen substance' (9-ODA) and the 'worker substance' (10-HDA) were quantified by using peak areas and the relative mass ratios calculated relative to tetradecane. A standard solution containing the 9-ODA and 10-HDA were run daily to ensure that relative mass ratios were within the limit of the variability found in the series of standard runs. We calculated the following quantitative ratios: Ratio = (9-ODA)/(9-ODA+9-HDA+10-HDAA+10-HDA) (Moritz, Simon et al. 2000; Hoover, Oldroyd et al. 2005; Schäfer, Dietemann et al. 2006).

**Statistics**

Independent t-tests were used to test for differences in the absolute amounts (µg) of the constituents in the pheromones between non-laying workers and laying workers of *A. mellifera* and *A. cerana*. Homogeneity of variances and normality of the data were examined using Levene's test and Shapiro-Wilk's test (Johnson and Wichern 2002).

Heterogeneity was stabilized after a square-root transformation of the data. All tests were performed using Statistica© (StatSoft 2009).

## Results

18 samples of laying workers (LW) and non-laying workers (NLW) were tested in *A. cerana* for its pheromone, which included HOB, 9-ODA, HVA, 9-HDA, 10-HDAA and 10-HDA. Highly significant differences in the absolute **amounts** (ug) of most pheromonal compounds were observed between LW and NLW (*t*-test: HOB: $t$=4.942, $p$<0.001; 9-ODA: $t$=2.700, $p$=0.011; HVA: $t$=3.037, $p$=0.004; 10-HDAA: $t$=3.873, $p$<0.001 and 10-HDA: $t$=2.238, $p$=0.031), except 9-HDA ($t$=1.488, $p$=0.146) (Table 1).

**Table 1** Mean amount (ug) and standard errors (mean±se) of six major mandibular gland compounds, Total amount and Ratio = (9-ODA)/(9-ODA+9-HDA+10-HDAA+10-HDA) of NLW and LW in *A. cerana*

| | **NLW** | **LW** | | |
|---|---|---|---|---|
| | mean±se | mean±se | *t* | *p* |
| HOB | 0.017±0.006 | 0.160±0.038 | 4.942 | < 0.001 |
| 9-ODA | 0.014±0.003 | 0.096±0.034 | 2.700 | 0.011 |
| HVA | 0.003±0.001 | 0.026±0.010 | 3.037 | 0.004 |
| 9-HDA | 0.188±0.035 | 0.525±0.168 | 1.488 | 0.146 |
| 10-HDAA | 0.002±0.001 | 0.063±0.020 | 3.873 | < 0.001 |
| 10-HDA | 0.086±0.024 | 0.238±0.059 | 2.238 | 0.031 |
| **Amount** | **0.309±0.050** | **1.103±0.232** | **3.513** | **0.001** |
| **Ratio** | **0.097±0.034** | **0.089±0.022** | **0.177** | **0.861** |

**Table 2** Mean amount (ug) and standard errors (mean±se) of six major mandibular gland compounds, Total amount and Ratio = (9-ODA)/(9-ODA+9-HDA+10-HDAA+10-HDA) of NLW and LW in *A. mellifera*

| | **NLW** | **LW** | | |
|---|---|---|---|---|
| | mean±se | mean±se | t | p |
| HOB | 0.320±0.133 | 0.049±0.020 | 2.177 | 0.037 |
| 9-ODA | 0.068±0.009 | 0.127±0.017 | 2.842 | 0.001 |
| HVA | 0.005±0.004 | 0.057±0.025 | 2.660 | 0.012 |
| 9-HDA | 1.213±0.237 | 2.044±0.312 | 2.124 | 0.042 |
| 10-HDAA | 0.422±0.160 | 3.250±1.007 | 2.785 | 0.009 |
| 10-HDA | 0.558±0.254 | 5.204±1.837 | 2.562 | 0.015 |
| **Amount** | 2.587±0.622 | 10.733±2.995 | 2.647 | 0.013 |
| **Ratio** | **0.056±0.014** | **0.033±0.006** | **1.536** | **0.135** |

In 18 *A. mellifera* samples, all absolute **amounts** (ug) of mandibular gland compounds were significantly different between LW and NLW (*t*-test: HOB: $t$=2.177, $p$=0.037; 9-ODA: $t$=2.842, $p$=0.001; HVA: $t$=2.660, $p$=0.012; 9-HDA: $t$=2.124, $p$=0.042; 10-HDAA: $t$=2.785, $p$=0.009 and 10-HDA: $t$=2.562, $p$=0.015) (Table 2). The increase of 9-HDA when comparing non laying workers with laying workers in *A. cerana* is 179.3% whereas it is only 68.5% in *A. mellifera*. Similar for 9-ODA, where *in A. cerana* it increased by 585.7% and only 86.8% in *A mellifera* (Table 1 & 2). The ratio (9-ODA)/(9-ODA+9-HDA+10-HDAA+10-HDA) between NLW and LW in *A. cerana* was not significantly different ($t$=0.177, $p$=0.861), nor in *A. mellifera* ($t$=1.536, $p$=0.135) (Tables 1 and 2). There was no significant difference in the ratio of NLW between *A. cerana* and *A. mellifera* ($t$=1.045, $p$=0.304), but the ratio of LW of *A. cerana* was significantly higher than *A. mellifera* ($t$=2.447, $p$=0.019) (Fig. 1).

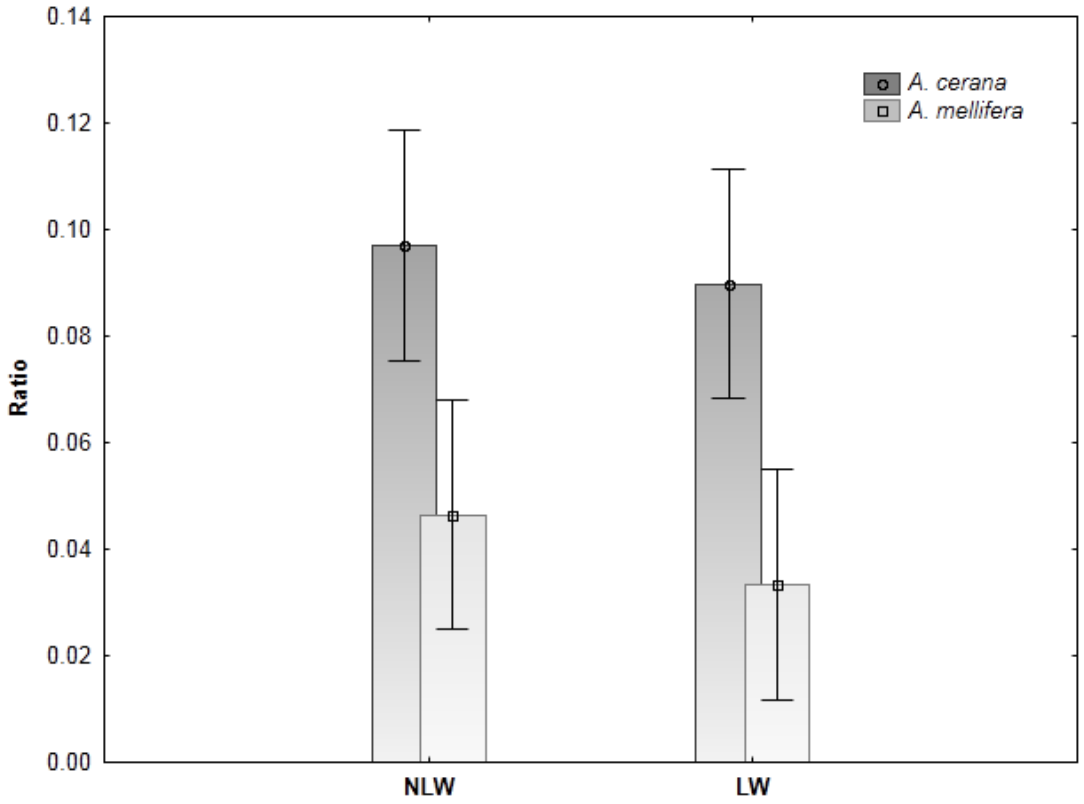


**Fig. 1** Mean and standard errors (mean±se) of the ratio 9-ODA/(9-ODA+9-HAD+10-HDA+10-HDAA) in non-laying workers (NLW) and laying workers (LW) of *A. cerana* and *A. mellifera*

## Discussion

Our results show that in both species, certain pheromone bouquet components have

significantly higher total amounts in LW compared to NLW. In particular, the queen compounds 9-ODA and 9-HDA of *A. cerana* were about 7 and 3 times higher respectively, while in *A. mellifera* these compounds were only about twice as high in LW compared with NLW (refer to Tables 1 and 2). This is consistent with previous studies which showed that *A. cerana* has a significantly higher queen bias (9-ODA presence) than *A. mellifera* (Keeling, Otis et al. 2001; Pirk, Sole et al. 2011 for *A. cerana*; Crewe and Velthuis 1980 for *A. mellifera*). Comparing NLW with LW in both species, *A. cerana* and *A. mellifera,* showed no significant differences in the ratio (9-ODA)(9-ODA+9-HDA+10-HDAA+10-HDA), in both species the 9-ODA compound amount increased significantly in the LW, as did most of other pheromone components.

Similarly to previous studies (Moritz, Lattorff et al. 2004), our results suggest that the compounds used to calculate the ratios are indeed sensitive indicators of the biosynthetic investment in queen substance. In queenless colonies of both *A. mellifera* and *A. cerana* some workers become highly reproductive (Sakagami 1954), and produce queen-like pheromones (Sole, Kryger et al. 2002). These pheromones inhibit reproductive development of other workers. The importance of these pheromones is founded on their multiple functions in signalling reproductive status and allowing individuals to prevent reproduction by their nestmates (Velthuis, Ruttner et al. 1990; Moritz, Simon et al. 2000; Simon, Moritz et al. 2005; Dietemann, Neumann et al. 2007).

Our study is consistent with previous work showing that social regulation in *A. cerana* is more easily disturbed than in European *A. mellifera* (Tan, Yang et al. 2009). In queenright European *A. mellifera* colonies, less than 0.8% of workers had activated ovaries; this increased to 39.4% in queenlees and broodless colonies (Tan, Yang et al. 2009). The queen dominates reproduction and the vast majority of workers are unable to attempt reproduction when she is present. In contrast, over 4.6% of workers in queenright *A. cerana* colonies had active ovaries and this increased to 72.1% in queenlees and broodless colonies (Tan, Yang et al. 2009). We suggest that the fast activation of queen-like pheromonal bouquet of some *A. cerana* workers, comparable to *A. m. capensis* (Zheng, Dietemann et al. 2010) allows them to compete with each other in egg reproductive dominance in queenless colony. Workers of a queenless colony not only compete with fellow nestmates but also non-nestmates (Nanork, Chapman et al. 2007). Therefore workers are selected to became reproductive dominant comparable to *A. m. capensis* (Moritz, Pirk et al. 2008) to out-compete fellow workers when it is time to reproduce. Indeed that could explain why the pheromonal production is significantly higher in *A. cerana* compared to the European *A. mellifera*, the former workers have to “control” more.

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## References


Blanford E (1923) Chinese bees as we find them. Bee World 5:104-106

Butler CG (1959) The source of the substance produced by a queen honeybee (*Apis mellifera*) which inhbits development of the workers of her colony. Proceedings of the Royal Entomological Society of London 34:137-138

Crewe RM (1982) Compositional variability: the key to social signals produced by the honeybee mandibular glands. In: Breed MD, Michener GD, Evans HE (eds) The Biology of Social Insects. Westview Press, London, pp 318–325

Crewe R, Velthuis H (1980) False queens: a consequence of mandibular gland signals in worker honeybees. Naturwissenschaften 67(9):467-469

Crozier RH (1975) Animal Cytogenetics. Bornträger, Berlin, Germany

Dietemann V, Neumann P, et al. (2007) Pheromonal dominance and the selection of a socially parasitic honeybee worker lineage (*Apis mellifera capensis* Esch.). Journal of Evolutionary Biology 20(3):997-1007

Hepburn HR, Nefdt RJC, Whiffler LA (1988) Queen loss in the Cape honeybee: the interactions of brood, laying workers (false queens?) and queen cells. South African Journal of Science 84:778-780

Hess G (1942) Über den Einfluß der Weisellosigkeit und des Fruchtbarkeitsvitamins E auf die Ovarien der Bienenarbeiterin Ein Beitrag zur Frage der Regulationen im Bienenstaat. Beihefte zur Schweizerischen Bienen-Zeitung 2(1):33-111

Hoover SER, Keeling CI, et al. (2003) The effect of queen pheromones on worker honey bee ovary development. Naturwissenschaften 90(10):477-480

Hoover SER, Oldroyd BP, et al. (2005) Anarchistic queen honey bees have normal queen mandibular pheromones. Insectes Sociaux 52(1):6-10

Johnson RA, Wichern DW (2002) Applied Multivariate Statistical Analysis, 5th edn. Prentice Hall, Upper Saddle River, New Jersey

Katzav-Gozansky T (2006) The evolution of honeybee multiple queen-pheromones a consequence of a queen-worker arms race. Braz J Morphol Sci 23:287-294

Katzav-Gozansky T, Soroker V, et al. (1997) Plasticity of caste-specific Dufour's gland secretion in the honey bee (*Apis mellifera* L.). Naturwissenschaften 84(6):238-241

Katzav-Gozansky T, Soroker V, et al. (2001) Dufour's gland secretion of the queen honeybee (Apis mellifera): an egg discriminator pheromone or a queen signal? Behavioral Ecology and Sociobiology 51(1):76-86

Keeling CI, Otis GW, et al. (2001) Mandibular gland component analysis in the head extracts

of Apis cerana and Apis nigrocincta. Apidologie 32(3):243-252

Liebig J, Peeters C, et al. (2000) Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant Harpegnathos saltator? Proceedings of the National Academy of Sciences 97(8):4124

Moritz RFA, Simon UE, et al. (2000) Pheromonal contest between honeybee workers (*Apis mellifera capensis*). Naturwissenschaften 87(9):395-397

Moritz RFA, Lattorff HMG, et al. (2004) Honeybee workers (*Apis mellifera capensis*) compete for producing queen ¡®like pheromone signals. Proceedings of the Royal Society of London. Series B: Biological Sciences 271(3): S98

Moritz R, Pirk C, et al. (2008) Short-sighted evolution of virulence in parasitic honeybee workers (*Apis mellifera capensis* Esch.). Naturwissenschaften 95(6):507-513

Nanork P, Chapman NC, et al. (2007) Social parasitism by workers in queenless and queenright *Apis cerana* colonies. Molecular Ecology 16(5):1107-1114

Neumann P, Moritz R (2002) The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? Behavioral Ecology and Sociobiology 52(4):271-281

Page R, Erickson E (1988) Reproduction by worker honey bees (*Apis mellifera* L.). Behavioral Ecology and Sociobiology 23(2):117-126

Peeters C, Monnin T, et al. (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proceedings of the Royal Society of London. Series B: Biological Sciences 266(1426):1323

Pirk CWW, Boodhoo C, et al. (2010) The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). Apidologie 41(1):62-72

Pirk CWW, Sole CL, et al. (2011) Pheromones. In: H. R. Hepburn HR, Radloff SE (eds) Honeybees of Asia. Springer, Berlin Heidelberg, pp 207-214

Plettner E, Slessor K, et al. (1993) Mandibular gland components and ovarian development as measures of caste differentiation in the honey bee (*Apis mellifera* L.). Journal of Insect Physiology 39(3):235-240

Plettner E, Sutherland GRG, et al. (1995) Why not be a queen? Regioselectivity in mandibular secretions of honeybee castes. Journal of Chemical Ecology 21(7):1017-1029

Plettner E, Slessor KN, et al. (1996) Caste-selective pheromone biosynthesis in honeybees. Science 271:1851-1853

Plettner E, Otis GW, Winmalaratne PDC, Winston ML, Slessor KN, Pankiw T, Punchihewa PWK (1997) Species- and caste-determined mandibular gland signals in honeybees (*Apis*). Journal of Chemical Ecology 23:363–377

Sakagami SF (1954) Occurrence of an aggressive behaviour in queenless hives, with considerations on the social orgainization of honeybees. Insects Sociaux 1:331-343

Sakagami SF (1958) The false-queen: fourth adjustive response in dequeened honeybee colonies. Behaviour 13(3/4): 80-296

Sakagami SF, Akahra Y (1958) Comparison of ovarian size and number of ovrioles between workers of Japanese and European honeybees. Kontyo 26:103-107

Schäfer MO, Dietemann V, et al. (2006) Individual versus social pathway to honeybee worker reproduction (*Apis mellifera*): pollen or jelly as protein source for oogenesis? Journal of Comparative Physiology A 192(7): 61-768

Simon UE, Moritz RFA, et al. (2001) The ontogenetic pattern of mandibular gland components in queenless worker bees (*Apis mellifera capensis* Esch.). Journal of Insect Physiology 47(7): 735-738

Simon UE, Moritz RFA, et al. (2005) Reproductive dominance among honeybee workers in experimental groups of *Apis mellifera capensis*. Apidologie 36(3):413

Sledge MF, Boscaro F, et al. (2001) Cuticular hydrocarbons and reproductive status in the social wasp Polistes dominulus. Behavioral Ecology and Sociobiology 49(5):401-409

Slessor, KN, Kaminski LA, et al. (1988) Semiochemical basis of the retinue response to queen honey bees. Nature 332(6162): 354-356

Sole CL, Kryger P, et al. (2002) Mimicry of queen Dufour's gland secretions by workers of *Apis mellifera scutellata* and *A. m. capensis*. Naturwissenschaften 89:561-564

StatSoft Inc. (2009) STATISTICA, version 9.1, www.statsoft.com

Tan K, Yang M, et al. (2009) Worker reproduction in mixed-species colonies of honey bees. Behavioral Ecology 20(5):1106-1110

Velthuis HHW, Ruttner F, Crewe RM. (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: Engels W (ed) Social Insects: an Evolutionary Approach to Castes and Reproduction. Springer Verlag, Berlin, pp 231-243

Winston ML, Slessor KN (1992) The essence of royalty: honey bee queen pheromone. American Scientist 80:375-385

Winston ML, Slessor KN, et al. (1989) The influence of queen mandibular pheromones on worker attraction to swarm clusters and inhibition of queen rearing in the honey bee (*Apis mellifera* L.). Insectes Sociaux 36(1):15-27

Zheng H-Q, Dietemann V, et al. (2010) Pheromonal predisposition to social parasitism in the honeybee *Apis mellifera capensis*. Behavioral Ecology 21(6):1221-1226