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SHORT COMMUNICATION

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Mimicry of queen Dufour's gland secretions by workers of *Apis mellifera scutellata* and *A. m. capensis*

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Abstract The development of the Dufour's gland of workers of the two honey bee races *Apis mellifera scutellata* and *A. m. capensis* was measured. The Dufour's glands of *A. m. capensis* workers were longer and increased in length more rapidly than the glands of workers of *A. m. scutellata* at comparable ages. Analysis of the Dufour's gland secretions of workers and queens of both races revealed that there were caste and racial differences. Secretions of queenright *A. m. scutellata* workers were dominated by a series of long-chain hydrocarbons. In contrast the secretions of the *A. m. capensis* workers both under queenright and queenless conditions were a mixture of hydrocarbons and wax-type esters, as were those of queens. Multivariate analysis of the secretion profiles indicated that laying workers of both races mimic queens. The secretions of the *A. m. capensis* laying workers mimicked queen secretions most closely, enabling them to act as successful social parasites.

Introduction

In a queenless honey bee colony, workers can establish themselves as false queens, which express many of the attributes associated with the reproductive physiology of the queen caste (Sakagami 1958). *Apis mellifera capensis* workers appear to be particularly queenlike in that they have high ovariole numbers, larger sized spermathecae, a shorter larval development time than workers of other races, and a capacity to produce queenlike mandibular gland pheromones when they become queenless (Hemmling et al. 1979; Crewe and Velthuis 1980). In addition, the offspring of these workers are females, since they are the only honey bee race in which thelytokous parthenogenesis occurs.

The facility with which *A. m. capensis* workers become false queens has allowed them to act as social parasites in *A. m. scutellata* colonies and has given rise to the *capensis* problem (Allsopp 1992). During the last 10 years the *capensis* problem has resulted in the loss of many thousands of *A. m. scutellata* colonies in South African commercial apiaries. Workers of *A. m. capensis* invade colonies of *A. m. scutellata* and eventually monopolize reproduction. The *A. m. scutellata* colonies lose their queens in the process, and eventually the colony dwindles and dies. Kryger (2001) has shown that a single thelytokous worker gave rise to all the other workers that exhibit these traits of social parasitism. These bees are called the *capensis* pseudo-clone, since thelytokous parthenogenesis results in some crossing over during the production of diploid eggs (Kryger 2001). The *A. m. capensis* pseudo-clone workers are able to exhibit these traits even in queenright colonies of *A. m. scutellata*. In order to understand this phenomenon we chose to study the role of the Dufour's gland in establishing chemical mimicry of queens by these reproductive workers. The origin of the *A. m. capensis* pseudo-clone is currently under investigation (Neumann and Moritz 2002).

Caste specificity and biosynthetic plasticity are known to be properties of the mandibular gland secretions of honey bees (Crewe 1982), and this has been shown more recently to be the case for the Dufour's gland as well (Katzav-Gozansky et al. 1997). Under queenless conditions, egg-laying workers produce several esters typical of queen secretions, while in queenright colonies worker secretions consisted exclusively of hydrocarbons (Katzav-Gozansky et al. 1997). The queen's secretion is attractive to workers and was postulated to constitute a part of the complex queen signal (Katzav-Gozansky et al. 2002). The secretion of egg-laying workers, but not that of non-laying workers, is attractive to nest mates, albeit to a lesser extent than the queen's secretion (Katzav-Gozansky et al. 2001, 2002a, b).

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Table 1 Chemical composition of the Dufour's gland contents of individuals from two populations of African honey bees. *Queen scut* = *A. m. scutellata* queens, *Laying scut* = *A. m. scutellata* laying workers, *Laying cap* = *A. m. capensis* laying workers, *Non-laying scut* = non-reproductive *A. m. scutellata* workers and *Non-laying cap* = non-reproductive *A. m. capensis* workers. The symbol – indicates that component is missing, +, ++, +++ indicate the relative amount of each compound

Compound	Queen scut *n*=5	Laying scut *n*=2	Laying cap *n*=5	Non-laying scut *n*=9	Non-laying cap *n*=15
Eicosane	+	++	+	++	++
Heneicosane	+	+	++	++	++
Oleic acid	–	–	++	++	++
Docosane	–	++	+	++	++
Tricosane	+++	+++	+++	+++	+++
Tetracosane	–	–	–	+++	+++
Pentacosane	++	+++	++	+++	+++
Hexacosane	–	–	+	+++	+++
Heptacosane	++	++	++	+++	++
Octacosane	–	–	–	++	++
Nonacosane	++	++	+	++	++
Triacontane	–	–	–	++	++
Hentriacontane	–	+	+	++	++
Dotriacontane	++	++	+	–	–
Tritriacontane	++	++	–	–	–
Tetratriacontane	+	–	++	–	–
Tetradecyltetradecanoate	+++	+++	+++	–	++
Tetradecyl Z-9 hexadecenoate	++	++	++	–	++
Tetradecyl hexadecanoate + Hexadecyltetradecanoate	+++	++	+++	+	++
Z-9-Hexadecyl Z-9-hexadecenoate	+	+	++	–	–
Tetradecyl Z-9-octadecenoate	++	+	+	–	++
Hexadecyl Z-9- hexadecenoate	+	++	+	–	–
Hexadecyl hexadecanoate	++	++	–	–	–
Z-9 Hexadecenyl Z-9-octadecenoate	+	+	++	–	–
Hexadecyl Z-9-octadecenoate	+	+	++	–	–

Since the *A. m. capensis* pseudo-clone workers exhibit many of the queenlike traits even in queenright colonies, we studied whether Dufour's gland secretions conforms to this pattern. We also investigated whether the Dufour's glands secretion might contribute to pheromonal mimicry that may provide a mechanism for successful usurpation of a host colony.

Materials and methods

Apis mellifera scutellata workers, *A. m. capensis* pseudo-clone workers and brood frames were obtained from colonies on the Pretoria University farm and from a commercial apiary in the Pretoria region. The *A. m. capensis* pseudo-clone workers came from a colony without an *A. m. scutellata* queen, so only *A. m. capensis* pseudo-clone workers were emerging. In general it is easy to distinguish the *A. m. capensis* pseudo-clone workers based on their darker brown color versus the bright yellow banding of the *A. m. scutellata* workers. The brood frames were placed in an incubator kept at 34°C and the workers allowed to emerge. The workers that emerged were fed on a mixture of pollen, sugar water and water (Velthuis 1970).

Workers, of each population, that hatched on a single day were all marked with the same color Opalithplättchen (bee tags) and placed individually in 10×5×15 cm cages fitted with a piece of drawn comb. These bees were reared in the cages for a period until samples were frozen at 5, 15 and 20 days, respectively, and dissected for chemical analysis. Five laying queens of *A. m. scutellata* and one *A. m. capensis* queen were removed from their colonies and frozen for dissection and chemical analysis.

Dufour's glands of the workers were removed from the sting and the poison gland and their length was measured using an eyepiece micrometer. Between 15 and 20 individuals were dissected on each of the days on which samples were taken. Each gland was placed in 100 µl of dichloromethane (DCM) for subsequent chemical analysis.

All dissections included an assessment of ovary development. The ovaries were ranked from stage 1, showing no development, to stage 4, showing eggs in the ovarioles (Anderson et al. 1983).

Chemical analyses of Dufour's glands of workers of *A. m. capensis* and *A. m. scutellata* at different physiological stages (egg-laying and non-egg-laying) and queens of *A. m. scutellata* and *A. m. capensis* were first performed by combined gas chromatography/mass spectrometry (GC/MS), using a DB5 capillary column – temperature programmed from 60 to 300°C at 10°C/min. Compounds were identified by their fragmentation patterns and GC retention times as compared to synthetic reference samples and published spectra.

For quantification, 100 µl of the Dufour's gland extract in dichloromethane (DCM) was evaporated down to approximately 2 µl using a stream of nitrogen gas, to which 2 µl of *n*-tetradecane internal standard solution were added. Of the total 4 µl, half (2 µl) was injected into the GC equipped with a HP5 capillary column (25m×0.20 mm) and the oven temperature programmed from 70 to 300°C ramped at 6°C/min and held at 300°C for 10 min.

A *t*-test was used to analyze the length of the Dufour's gland of the individuals from the two bee populations. An unweighted pair group average analysis (UPGMA) was used to cluster individuals based on their Dufour's gland secretion profiles. The variables used were the chemical compounds in Table 1. The amount of each compound was quantified by computing the area of each peak as a proportion of the total area of the 24 chemical compounds that were identified. These values were arc-cosine transformed and used in a UPGMA analysis.

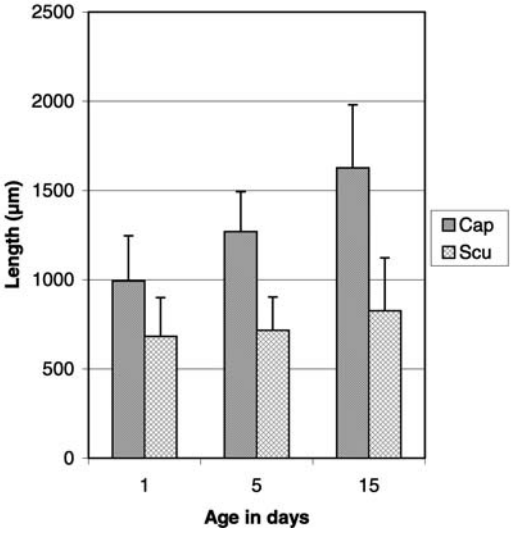

Fig. 1 Comparison of the change in Dufour's gland length with age in *Apis mellifera scutellata* and *Apis mellifera capensis* workers

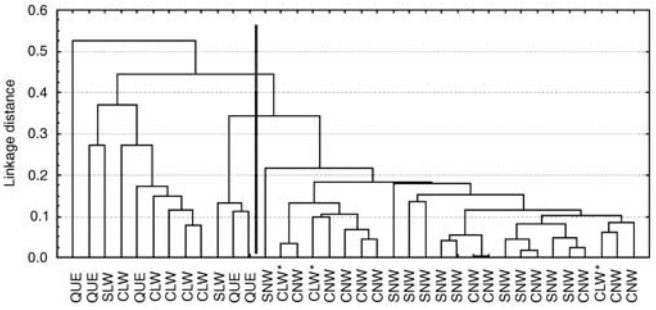

Fig. 2 UPGMA cluster analysis of the Dufour's gland secretion of queens and workers of *A. m. scutellata* and workers of *A. m. capensis*. The *A. m. scutellata* queens are identified by the abbreviation *QUE*, the *A. m. scutellata* laying workers by *SLW* and non-laying workers by *SNW* and the *A. m. capensis* laying workers by *CLW* and non-laying workers by *CNW*. The *asterisk* identifies the *A. m. capensis* laying workers that cluster together with the non-laying workers. To the *left of the vertical line* is sub-cluster 1 and to the *right* sub-cluster 2 (see text for reference)

Results and discussion

The Dufour's gland length was compared between each age group within each bee population as well as between populations. The Dufour's glands of *A. m. capensis* workers were longer and increased in length more rapidly than the glands of workers of *A. m. scutellata* at comparable ages (Fig. 1). The development of the Dufour's gland length was significantly different, with the *A.m. capensis* pseudo-clone being larger at all ages (1 day old, t=4.27, df=41; 5 days old, t=4.72, df=27; 15 days old, t=6.26, df=29: P<0.001 for all). This indicates that *A. m. capensis* pseudo-clone workers readily develop towards queen characteristics, which has been noted for a number of other traits (Hepburn and Crewe 1991).

The relative amounts of the compounds found in the Dufour's gland of the bees used in this study are indicated in Table 1. The glandular composition of the queens agrees with that published previously for *A. m. ligustica* queens. There were, however, quantitative differences between the single *A. m. capensis* and *A. m. scutellata* queens, the latter having higher proportions of the heavier esters. In workers, the saturated and unsaturated wax-type esters that predominate in queen secretions are found in both the *A. m. capensis* and the *A. m. scutellata* laying workers (those workers with stage 4 ovaries). Comparative analysis of secretion composition (Table 1) revealed that although laying *A. m. capensis* pseudo-clone workers have a higher number of hydrocarbons than the *A. m. scutellata* queens, they possess all the queen esters in similar concentrations as the *A. m. scutellata* queens. In laying *A. m. scutellata* workers, however, the amount of esters was rather low but permitted the unequivocal identification of all ten esters present in the queen. The most significant finding in these analyses was that the non-laying *A. m. capensis* workers possessed five out of the ten queenlike esters. These esters are, however, found in much lower concentrations than both the *A. m. scutellata* queens and *A. m. capensis* laying workers.

The proportions of each of 24 compounds, identified with GC, were used in a UPGMA analyses with data from 36 individuals (Fig. 2). The phenogram shows two major sub-clusters. Sub-cluster 1, indicated to the left of the line, consists of the laying individuals. Sub-cluster 2 is mainly made up of the non-layers. Within this sub-cluster we find three individuals, indicated with an asterisk (*), which despite having eggs in their ovaries still have the secretions of non-laying workers' Dufour's glands. The queens that were included in this analysis fall into sub-cluster 1, and indicate the similarity of laying *A. m. capensis* pseudo-clone and *A. m. scutellata* worker secretions to those of the queens.

Although it has been suggested that Dufour's gland esters are responsible for protecting eggs from policing by workers (Ratnieks 1995; Oldroyd et al. 2002), recent studies (Katzav-Gozansky et al. 2001, 2002; Martin et al. 2002) have shown that this is not the case. The attractiveness of the Dufour's gland's esters to workers lent credence to the idea that, along with the mandibular and tergal glands, they constitute part of the complex queen signal (Katzav-Gozansky et al. 2002b).

As is the case with the mandibular and tergal gland secretions, the *A. m. capensis* workers mimic *A. m. scutellata* queens, and this mimicry may allow them to act as pseudo-queens in the colonies of other populations of honey bees (Neumann et al. 2001). The simultaneous change in the composition of the secretions of three exocrine glands as the social conditions of an individual changes is documented here for honey bees. This suggests that the changes in gland secretions may be regulated by the hormonal changes associated with ovary activation (Hartfelder and Engels 1998). By producing queenlike secretions, the laying workers achieve higher reproductive success in two ways: the individual is recognized as a

reproductive and inhibits ovary activation in her nestmates, and a worker elicits retinue behavior in other workers, resulting in increased feeding.

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